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Social Issues Roundtable—4:00 p.m.	
514. Health Care Economics and the Brain *Sponsored by:* Social Issues Committee of the Society for Neuroscience S.F. WITSELSON, Chairperson	No Abstract

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515. Structure, Function, and Regulation of Glutamate Receptors *Chaired by:* R.L. HUGANIR	1263
516. General Anesthetic Effects on Somatomotor Processing *Chaired by:* P. MASON	1263
Warner-Lambert Lecture—11:15 a.m.	
517. Ins and Outs of Programmed Cell Death M. RAFF	No Abstract
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Symposia—1:00 p.m.	
598. The Cell Biology of Secretion Vesicles *Chaired by:* K.J. SKINNER	1463
599. Neuropeptide Regulation of Brain Development: From Cells to Animals *Chaired by:* E.M. DICICCO-BLOOM and J.P. SCHWARTZ	1463
Special Lecture—1:00 p.m.	
600. Brain Maps for Movement R.H. WURTZ	No Abstract
Special Lecture—4:15 p.m.	
601. Muscimol, Memory, and Motivation in the Basal Ganglia O. HIKOSAKA	No Abstract
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697. Aging process	1708
698. Synaptic structure and function III	1711
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701. Calcium channels VII	1718
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280.	Cell lineage and determination I	Poster		Tue PM			
446.	Cell lineage and determination II	Poster			Wed PM		
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614.	Cell migration and differentiation III	Poster				Thu PM	
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682.	Cell migration and differentiation V	Slide					Fri AM
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543.	Cerebral cortex and limbic system II	Poster				Thu AM	
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117.	Development of chemical senses	Poster	Mon PM				
695.	Development of retinofugal projection and targets	Poster					Fri AM
456.	Development of the auditory and vestibular systems	Poster			Wed PM		
98.	Development of visual cortex I	Slide	Mon PM				
203.	Development of visual cortex II	Poster		Tue AM			
457.	Development of visual cortex III	Poster			Wed PM		
346.	**Early Development and Regionalization of the Mouse Brain**	**SYMP**			**Wed AM**		
281.	External influences on neuritic outgrowth	Poster		Tue PM			
269.	Formation and specificity of synapses I	Slide		Tue PM			
363.	Formation and specificity of synapses II	Poster			Wed AM		
450.	Formation and specificity of synapses III	Poster			Wed PM		
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21.	Genesis of neurons and glia I	Poster	Mon AM				
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613.	Genesis of neurons and glia III	Poster				Thu PM	
442.	Glia and other non-neuronal cells	Slide			Wed PM		
294.	Glia: differentiation and cell biology	Poster		Tue PM			
616.	Glia: glial response to injury, toxins, and disease	Poster				Thu PM	
365.	Glia: microglia and ensheathing cells	Poster			Wed AM		
201.	Hormones and development I	Poster		Tue AM			
202.	Hormones and development II	Poster		Tue AM			
364.	Hormones and development III	Poster			Wed AM		
545.	Influences on axonal regeneration	Poster				Thu AM	
687.	Influences on sprouting in situ	Poster					Fri AM
533.	Mechanisms of axon guidance	Poster				Thu AM	

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542.	Molecular and pharmacological correlates of development	Poster				Thu AM	
296.	Molecular correlates of central injury	Poster		Tue PM			
617.	Molecular correlates of peripheral axonal regeneration	Poster				Thu PM	
3.	**Molecular Mechanisms of Axon Guidance**	**SYMP**	**Mon AM**				
27.	Motor system I	Poster	Mon AM				
116.	Motor system II	Poster	Mon PM				
112.	Neuronal death I	Poster	Mon PM				
113.	Neuronal death II	Poster	Mon PM				
114.	Neuronal death III	Poster	Mon PM				
188.	Neuronal death IV	Slide		Tue AM			
290.	Neuronal death V	Poster		Tue PM			
291.	Neuronal death VI	Poster		Tue PM			
292.	Neuronal death VII	Poster		Tue PM			
599.	**Neuropeptide Regulation of Brain Development: From Cells to Animals**	**SYMP**				**Thu PM**	
22.	Neurotransmitter systems and channels: amino acids	Poster	Mon AM				
689.	Neurotransmitter systems and channels: channels	Poster					Fri AM
535.	Neurotransmitter systems and channels: other transmitters	Poster				Thu AM	
193.	Neurotrophic factors: biological effects I	Slide		Tue AM			
285.	Neurotrophic factors: biological effects II	Poster		Tue PM			
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287.	Neurotrophic factors: biological effects IV	Poster		Tue PM			
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289.	Neurotrophic factors: biological effects VI	Poster		Tue PM			
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452.	Neurotrophic factors: biological effects IX	Poster			Wed PM		
453.	Neurotrophic factors: biological effects X	Poster			Wed PM		
454.	Neurotrophic factors: biological effects XI	Poster			Wed PM		
455.	Neurotrophic factors: biological effects XII	Poster			Wed PM		
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24.	Neurotrophic factors: receptors and cellular mechanisms II	Poster	Mon AM				
25.	Neurotrophic factors: receptors and cellular mechanisms III	Poster	Mon AM				
26.	Neurotrophic factors: receptors and cellular mechanisms IV	Poster	Mon AM				
109.	Neurotrophic factors: receptors and cellular mechanisms V	Slide	Mon PM				
536.	Neurotrophic factors: expression and regulation I	Poster				Thu AM	
537.	Neurotrophic factors: expression and regulation II	Poster				Thu AM	
538.	Neurotrophic factors: expression and regulation III	Poster				Thu AM	
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540.	Neurotrophic factors: expression and regulation V	Poster				Thu AM	
541.	Neurotrophic factors: expression and regulation VI	Poster				Thu AM	
610.	Neurotrophic factors: expression and regulation VII	Slide				Thu PM	
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690.	Other factors and trophic agents I	Poster					Fri AM
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693.	Pattern formation, compartments, and boundaries III	Poster					Fri AM

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97.	Process outgrowth I	Slide	Mon PM				
607.	Process outgrowth II	Slide				Thu PM	
531.	Process outgrowth and adhesion molecules	Poster				Thu AM	
532.	Process outgrowth and cytoskeleton	Poster				Thu AM	
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448.	Process outgrowth and second messengers	Poster			Wed PM		
447.	Process outgrowth in retina and retinal prosection sites	Poster			Wed PM		
520.	Regeneration	Slide				Thu AM	
544.	Retinal development	Poster				Thu AM	
295.	Sequelae to central injury	Poster		Tue PM			
694.	Somatosensory development	Poster					Fri AM
204.	Successful axonal regeneration	Poster		Tue AM			
458.	Transplant- and prosthesis-assisted regeneration	Poster			Wed PM		
11.	Transplantation I	Slide	Mon AM				
118.	Transplantation II	Poster	Mon PM				
205.	Transplantation III	Poster		Tue AM			
367.	Transplantation IV	Poster			Wed AM		
546.	Transplantation V	Poster				Thu AM	
696.	Transplantation VI	Poster					Fri AM
605.	Visual system development and plasticity	Slide				Thu PM	
	THEME B: CELL BIOLOGY						
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460.	Cytoskeleton transport and membrane targeting: axon and dendrite cytoskeleton	Poster			Wed PM		
547.	Cytoskeleton transport and membrane targeting: axoplasmic transport and synapses	Poster				Thu AM	
29.	Gene structure and function I	Poster	Mon AM				
30.	Gene structure and function II	Poster	Mon AM				
100.	Gene structure and function III	Slide	Mon PM				
270.	Gene structure and function IV	Slide		Tue PM			
619.	Gene structure and function V	Poster				Thu PM	
300.	Membrane composition and surface macromolecules: membrane lipids and receptors	Poster		Tue PM			
206.	Membrane composition and surface macromolecules: synapses and glia	Poster		Tue AM			
299.	Neuroglia and myelin I	Poster		Tue PM			
369.	Neuroglia and myelin II	Poster			Wed AM		
459.	Neuroglia and myelin III	Poster			Wed PM		
618.	Neuroglia and myelin IV	Poster				Thu PM	
297.	Staining, tracing, and imaging techniques I	Poster		Tue PM			
298.	Staining, tracing, and imaging techniques II	Poster		Tue PM			
598.	**The Cell Biology of Secretion Vesicles**	**SYMP**				**Thu PM**	
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35.	Calcium channels II	Poster	Mon AM				
268.	Calcium channels III	Slide		Tue PM			
374.	Calcium channels IV	Poster			Wed AM		
375.	Calcium channels V	Poster			Wed AM		

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701.	Calcium channels VII	Poster					Fri AM
700.	Chloride and other channels	Poster					Fri AM
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306.	Ion channels: cell function I	Poster		Tue PM			
627.	Ion channels: cell function II	Poster				Thu PM	
463.	Ligand-gated ion channels I	Poster			Wed PM		
603.	Ligand-gated ion channels II	Slide				Thu PM	
464.	Ligand-gated ion channels: alcohol modulation	Poster			Wed PM		
622.	Long-term potentiation: pharmacology I	Poster				Thu PM	
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119.	Long-term potentiation: physiology I	Poster	Mon PM				
195.	Long-term potentiation: physiology II	Slide		Tue AM			
302.	Long-term potentiation: physiology III	Poster		Tue PM			
354.	Long-term potentiation: physiology IV	Slide			Wed AM		
373.	Long-term potentiation: physiology V	Poster			Wed AM		
551.	Long-term potentiation: physiology VI	Poster				Thu AM	
93.	**Molecular Mechanisms Controlling K+ Channel Diversity and Distribution in the Nervous System**	**SYMP**	**Mon PM**				
347.	**Molecular Plasticity in Epilepsy and Ischemia**	**SYMP**			**Wed AM**		
183.	**New Approaches to Understanding Dendrites**	**SYMP**		**Tue AM**			
624.	Pharmacology of synaptic transmission I	Poster				Thu PM	
699.	Pharmacology of synaptic transmission II	Poster					Fri AM
279.	Postsynaptic mechanisms I	Slide		Tue PM			
372.	Postsynaptic mechanisms II	Poster			Wed AM		
620.	Postsynaptic mechanisms III	Poster				Thu PM	
621.	Postsynaptic mechanisms IV	Poster				Thu PM	
36.	Potassium channels I	Poster	Mon AM				
304.	Potassium channels II	Poster		Tue PM			
305.	Potassium channels III	Poster		Tue PM			
362.	Potassium channels IV	Slide			Wed AM		
625.	Potassium channels V	Poster				Thu PM	
626.	Potassium channels VI	Poster				Thu PM	
199.	Presynaptic mechanisms I	Slide		Tue AM			
371.	Presynaptic mechanisms II	Poster			Wed AM		
462.	Presynaptic mechanisms III	Poster			Wed PM		
549.	Presynaptic mechanisms IV	Poster				Thu AM	
550.	Presynaptic mechanisms V	Poster				Thu AM	
32.	Presynaptic mechanisms: calcium	Poster	Mon AM				
31.	Presynaptic mechanisms: vesicles	Poster	Mon AM				
33.	Sodium channels I	Poster	Mon AM				
303.	Sodium channels II	Poster		Tue PM			
301.	Synaptic structure and function I	Poster		Tue PM			
370.	Synaptic structure and function II	Poster			Wed AM		
461.	Synaptic structure and function III	Poster			Wed PM		
698.	Synaptic structure and function IV	Poster					Fri AM
	THEME D: NEUROTRANSMITTERS, MODULATORS, TRANSPORTERS, AND RECEPTORS						
208.	Acetylcholine receptors: muscarinic molecular biology	Poster		Tue AM			
209.	Acetylcholine receptors: muscarinic pharmacology	Poster		Tue AM			
207.	Acetylcholine receptors: muscarinic signal transduction	Poster		Tue AM			

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
351.	Acetylcholine receptors: nicotinic	Slide			Wed AM		
465.	Acetylcholine receptors: nicotinic development	Poster			Wed PM		
468.	Acetylcholine receptors: nicotinic function	Poster			Wed PM		
466.	Acetylcholine receptors: nicotinic molecular biology	Poster			Wed PM		
467.	Acetylcholine receptors: nicotinic pharmacology	Poster			Wed PM		
37.	Acetylcholine: anatomy/imaging	Poster	Mon AM				
38.	Acetylcholine: lesions	Poster	Mon AM				
39.	Acetylcholine: molecular biology	Poster	Mon AM				
40.	Acetylcholine: regulation	Poster	Mon AM				
221.	Anatomical localization of peptides and their receptors: peptides	Poster		Tue AM			
41.	Anatomical localization of peptides and their receptors: receptors	Poster	Mon AM				
222.	Anatomical localization of peptides and their receptors: regulated expression of peptides	Poster		Tue AM			
104.	Behavioral pharmacology I	Slide	Mon PM				
557.	Behavioral pharmacology II	Poster				Thu AM	
641.	Behavioral pharmacology III	Poster				Thu PM	
558.	Behavioral pharmacology: dopamine	Poster				Thu AM	
16.	Catecholamine receptors I	Slide	Mon AM				
223.	Catecholamine receptors II	Poster		Tue AM			
224.	Catecholamine receptors III	Poster		Tue AM			
275.	Catecholamine receptors IV	Slide		Tue PM			
377.	Catecholamine receptors V	Poster			Wed AM		
555.	Catecholamine receptors VI	Poster				Thu AM	
12.	Catecholamines	Slide	Mon AM				
126.	Catecholamines: dopamine release	Poster	Mon PM				
473.	Catecholamines: measurements, spect, lesions—immediate early genes	Poster			Wed PM		
225.	Catecholamines: noradrenergic systems—neuronal circuitry	Poster		Tue AM			
125.	Catecholamines: tyrosine hydroxylase and other synthesizing enzymes	Poster	Mon PM				
108.	Excitatory amino acid receptors I	Slide	Mon PM				
210.	Excitatory amino acid receptors II	Poster		Tue AM			
211.	Excitatory amino acid receptors III	Poster		Tue AM			
309.	Excitatory amino acid receptors IV	Poster		Tue PM			
310.	Excitatory amino acid receptors V	Poster		Tue PM			
214.	Excitatory amino acid receptors: expression and splice variants	Poster		Tue AM			
213.	Excitatory amino acid receptors: localization	Poster		Tue AM			
212.	Excitatory amino acid receptors: metabotropic receptors	Poster		Tue AM			
312.	Excitatory amino acid receptors: posttranslational modification	Poster		Tue PM			
311.	Excitatory amino acid receptors: topology	Poster		Tue PM			
123.	Excitatory amino acids: anatomy and physiology I	Poster	Mon PM				
124.	Excitatory amino acids: anatomy and physiology II	Poster	Mon PM				
120.	Excitatory amino acids: excitotoxicity I	Poster	Mon PM				
121.	Excitatory amino acids: excitotoxicity II	Poster	Mon PM				
122.	Excitatory amino acids: excitotoxicity III	Poster	Mon PM				
198.	Excitatory amino acids: excitotoxicity IV	Slide		Tue AM			
278.	Excitatory amino acids: excitotoxicity V	Slide		Tue PM			
685.	Excitatory amino acids: excitotoxicity VI	Slide					Fri AM

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
628.	Excitatory amino acids: excitotoxicity and NMDA receptors	Poster				Thu PM	
629.	Excitatory amino acids: excitotoxicity and non-NMDA receptors	Poster				Thu PM	
96.	Excitatory amino acids: pharmacology I	Slide	Mon PM				
308.	Excitatory amino acids: pharmacology II	Poster		Tue PM			
469.	Excitatory amino acids: pharmacology III	Poster			Wed PM		
470.	Excitatory amino acids: pharmacology IV	Poster			Wed PM		
702.	Excitatory amino acids: pharmacology V	Poster					Fri AM
630.	Excitatory amino acids: pharmacology and non-NMDA receptors	Poster				Thu PM	
307.	Excitatory amino acids: pharmacology, metabotrophic receptors	Poster		Tue PM			
218.	GABA receptors: development	Poster		Tue AM			
216.	GABA receptors: function I	Poster		Tue AM			
217.	GABA receptors: function II	Poster		Tue AM			
703.	GABA receptors: function III	Poster					Fri AM
215.	GABA receptors: function—modulation	Poster		Tue AM			
13.	GABA receptors: molecular biology I	Slide	Mon AM				
219.	GABA receptors: molecular biology II	Poster		Tue AM			
639.	Neurotransmitter interactions	Poster				Thu PM	
638.	Neurotransmitter interactions: cholinergic/GABA	Poster				Thu PM	
637.	Neurotransmitter interactions: serotonin	Poster				Thu PM	
636.	Neurotransmitters and other amines/purines	Poster				Thu PM	
42.	Neurotransmitters in invertebrates I	Poster	Mon AM				
226.	Neurotransmitters in invertebrates II	Poster		Tue AM			
378.	Neurotransmitters in invertebrates III	Poster			Wed AM		
526.	Neurotransmitters in invertebrates IV	Slide				Thu AM	
640.	Neurotransmitters: glutamate/nitric oxide	Poster				Thu PM	
524.	Neurotransmitters: neurotransmitter interactions	Slide				Thu AM	
316.	Opioids: anatomy and physiology I	Poster		Tue PM			
707.	Opioids: anatomy and physiology II	Poster					Fri AM
708.	Opioids: anatomy and physiology III	Poster					Fri AM
611.	Opioid receptors	Slide				Thu PM	
706.	Opioid receptors: cell biology and effector mechanisms	Poster					Fri AM
705.	Opioid receptors: localization	Poster					Fri AM
313.	Opioid receptors: molecular biology	Poster		Tue PM			
315.	Opioid receptors: physiology	Poster		Tue PM			
314.	Opioid receptors: sigma receptors	Poster		Tue PM			
704.	Opioid receptors: tolerance and regulation	Poster					Fri AM
20.	Peptide receptor structure and function I	Slide	Mon AM				
220.	Peptide receptor structure and function II	Poster		Tue AM			
376.	Peptide receptor structure and function III	Poster			Wed AM		
552.	Peptide receptor structure and function IV	Poster				Thu AM	
196.	Peptides: biosynthesis and metabolism	Slide		Tue AM			
472.	Peptides: biosynthesis and processing	Poster			Wed PM		
471.	Peptides: biosynthesis, metabolism, and biochemical characterization I	Poster			Wed PM		
631.	Peptides: biosynthesis, metabolism, and biochemical characterization II	Poster				Thu PM	
356.	Peptides: physiological effects I	Slide			Wed AM		
553.	Peptides: physiological effects II	Poster				Thu AM	
554.	Peptides: physiological effects III	Poster				Thu AM	
384.	Receptor modulation up- and down-regulation I	Poster			Wed AM		

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
440.	Receptor modulation up- and down-regulation II	Slide			Wed PM		
437.	Regional localization of receptors and transmitters I	Slide			Wed PM		
478.	Regional localization of receptors and transmitters II	Poster			Wed PM		
479.	Regional localization of receptors and transmitters III	Poster			Wed PM		
190.	Second messengers I	Slide		Tue AM			
433.	Second messengers II	Slide			Wed PM		
228.	Second messengers: G protein-mediated	Poster		Tue AM			
480.	Second messengers: kinases and phosphatases	Poster			Wed PM		
556.	Second messengers: NO, CO, and gene regulation	Poster				Thu AM	
383.	Second messengers: phospholipase C, phospholipase A2	Poster			Wed AM		
229.	Second messengers: protein kinase C—calcium	Poster		Tue AM			
128.	Serotonin	Poster	Mon PM				
127.	Serotonin: behavior	Poster	Mon PM				
519.	Serotonin receptors	Slide				Thu AM	
474.	Serotonin receptors: 5-HT3	Poster			Wed PM		
634.	Serotonin receptors: behavior	Poster				Thu PM	
475.	Serotonin receptors: effector mechanisms	Poster			Wed PM		
635.	Serotonin receptors: localization	Poster				Thu PM	
476.	Serotonin receptors: molecular biology	Poster			Wed PM		
633.	Serotonin receptors: pharmacology	Poster				Thu PM	
632.	Serotonin receptors: physiology	Poster				Thu PM	
477.	Serotonin receptors: regulation	Poster			Wed PM		
379.	Storage, secretion, and metabolism	Poster			Wed AM		
515.	**Structure, Function, and Regulation of Glutamate Receptors**	**SYMP**				**Thu AM**	
267.	Uptake and transporters	Slide		Tue PM			
380.	Uptake and transporters: GABA, glycine, and other amino acids	Poster			Wed AM		
227.	Uptake and transporters: acetylcholine	Poster		Tue AM			
381.	Uptake and transporters: catecholamines	Poster			Wed AM		
382.	Uptake and transporters: glutamate	Poster			Wed AM		
129.	Uptake and transporters: serotonin	Poster	Mon PM				
THEME E: ENDOCRINE AND AUTONOMIC REGULATION							
263.	**Autonomic Integration in the Control of Food Intake**	**SYMP**		**Tue PM**			
352.	Cardiovascular regulation	Slide			Wed AM		
132.	Cardiovascular regulation: dorsal vagal complex	Poster	Mon PM				
484.	Cardiovascular regulation: medullary reticular responses	Poster			Wed PM		
559.	Cardiovascular regulation: pre- and postganglionic control	Poster				Thu AM	
131.	Cardiovascular regulation: system organization	Poster	Mon PM				
51.	Cardiovascular regulation: system pharmacology	Poster	Mon AM				
50.	Cardiovascular regulation: system physiology	Poster	Mon AM				
516.	**General Anesthetic Effects on Somatomotor Processing**	**SYMP**				**Thu AM**	
276.	Hypothalamic-pituitary-adrenal axis regulation I	Slide		Tue PM			
386.	Hypothalamic-pituitary-adrenal axis regulation II	Poster			Wed AM		
436.	Hypothalamic-pituitary-gonadal regulation	Slide			Wed PM		
43.	Hypothalamic-pituitary-gonadal regulation: GnRH I	Poster	Mon AM				
272.	Hypothalamic-pituitary-gonadal regulation: GnRH II	Slide		Tue PM			

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387.	Hypothalamic-pituitary-gonadal regulation: GnRH release	Poster			Wed AM		
709.	Hypothalamic-pituitary-gonadal regulation: hypophysiotrophic area	Poster					Fri AM
45.	Hypothalamic-pituitary-gonadal regulation: pituitary	Poster	Mon AM				
44.	Hypothalamic-pituitary-gonadal regulation: steroid hormones	Poster	Mon AM				
385.	Hypothalamic-pituitary-adrenal axis regulation: stress studies	Poster			Wed AM		
392.	Neural-immune interactions: cytokine effects on the nervous system	Poster			Wed AM		
130.	Neural-immune interactions: cytokine expression in neural tissues	Poster	Mon PM				
390.	Neural-immune interactions: hormonal regulation of immune function	Poster			Wed AM		
49.	Neural-immune interactions: neural regulation of immune function	Poster	Mon AM				
48.	Neural-immune interactions: neurotransmitter mechanisms	Poster	Mon AM				
391.	Neural-immune interactions: responses to immunologic challenge	Poster			Wed AM		
389.	Neural-immune interactions: stress and other behavioral models	Poster			Wed AM		
47.	Neuroendocrine regulation: estradiol	Poster	Mon AM				
388.	Neuroendocrine regulation: glucocorticoids	Poster			Wed AM		
481.	Neuroendocrine regulation: magnocellular system	Poster			Wed PM		
46.	Neuroendocrine regulation: neuropeptides	Poster	Mon AM				
361.	Neuroendocrine regulation: other I	Slide			Wed AM		
482.	Neuroendocrine regulation: other II	Poster			Wed PM		
106.	Osmoregulation and cardiovascular regulation	Slide	Mon PM				
642.	Osmotic regulation	Poster				Thu PM	
561.	Regulation of autonomic functions: CNS pathways and transmitters	Poster				Thu AM	
560.	Regulation of autonomic functions: cardiovascular	Poster				Thu AM	
562.	Regulation of autonomic functions: gastrointestinal	Poster				Thu AM	
52.	Regulation of autonomic functions: reproductive tract	Poster	Mon AM				
53.	Regulation of autonomic functions: urinary tract	Poster	Mon AM				
393.	Respiratory regulation: chemoreceptive mechanisms	Poster			Wed AM		
230.	Respiratory regulation: developmental mechanisms	Poster		Tue AM			
394.	Respiratory regulation: effects of hypoxia or hypercapnia	Poster			Wed AM		
133.	Respiratory regulation: generation and modulation of respiratory pattern	Poster	Mon PM				
563.	Respiratory regulation: human studies	Poster				Thu AM	
231.	Respiratory regulation: transmitter mechanisms	Poster		Tue AM			
483.	Thermoregulation and fever	Poster			Wed PM		
THEME F: SENSORY SYSTEMS							
400.	Auditory systems: central anatomy I	Poster			Wed AM		
401.	Auditory systems: central anatomy II	Poster			Wed AM		
62.	Auditory systems: central physiology—brainstem	Poster	Mon AM				
141.	Auditory systems: central physiology—cortex I	Poster	Mon PM				

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
142.	Auditory systems: central physiology—cortex II	Poster	Mon PM				
140.	Auditory systems: central physiology—midbrain and thalamus	Poster	Mon PM				
399.	Auditory systems: cochlea	Poster			Wed AM		
398.	Auditory, vestibular, and lateral line: hair cells	Poster			Wed AM		
606.	Chemical senses	Slide				Thu PM	
138.	Extrastriate visual cortex: functional organization I	Poster	Mon PM				
186.	Extrastriate visual cortex: functional organization II	Slide		Tue AM			
350.	Extrastriate visual cortex: functional organization III	Slide			Wed AM		
710.	Extrastriate visual cortex: functional organization IV	Poster					Fri AM
434.	Extrastriate visual cortex: inferior temporal areas	Slide			Wed PM		
324.	Extrastriate visual cortex: parietal areas I	Poster		Tue PM			
525.	Extrastriate visual cortex: parietal areas II	Slide				Thu AM	
402.	Gustatory and related chemical senses	Poster			Wed AM		
677.	**Neural Coding of Visual Space: Visual Mechanisms and Multimodal Integration**	**SYMP**					**Fri AM**
144.	Olfactory senses: central processing and behavior	Poster	Mon PM				
143.	Olfactory senses: olfactory bulb	Poster	Mon PM				
63.	Olfactory senses: peripheral mechanisms	Poster	Mon AM				
14.	Pain	Slide	Mon AM				
318.	Pain: central mechanisms	Poster		Tue PM			
234.	Pain: dorsal horn pharmacology	Poster		Tue AM			
233.	Pain: dorsal horn physiology	Poster		Tue AM			
320.	Pain: models of nociception	Poster		Tue PM			
319.	Pain: peripheral mechanisms	Poster		Tue PM			
59.	Pain modulation: pharmacology I	Poster	Mon AM				
135.	Pain modulation: pharmacology II	Poster	Mon PM				
235.	Pain modulation: pharmacology III	Poster		Tue AM			
321.	Pain modulation: pharmacology IV	Poster		Tue PM			
396.	Pain modulation: pharmacology V	Poster			Wed AM		
568.	Pain modulation: pharmacology VI	Poster				Thu AM	
58.	Pain pathways I	Poster	Mon AM				
232.	Pain pathways II	Poster		Tue AM			
644.	Pain pathways III	Poster				Thu PM	
60.	Retina and photoreceptors I	Poster	Mon AM				
99.	Retina and photoreceptors II	Slide	Mon PM				
236.	Retina and photoreceptors III	Poster		Tue AM			
322.	Retina and photoreceptors IV	Poster		Tue PM			
397.	Retina and photoreceptors V	Poster			Wed AM		
569.	Retina and photoreceptors VI	Poster				Thu AM	
645.	Retina and photoreceptors VII	Poster				Thu PM	
54.	Sensory systems: spinal cord I	Poster	Mon AM				
134.	Sensory systems: spinal cord II	Poster	Mon PM				
565.	Somatic and visceral afferents: anatomy and physiology of mechanoreception	Poster				Thu AM	
643.	Somatic and visceral afferents: nociception	Poster				Thu PM	
564.	Somatic and visceral afferents: thermoreception	Poster				Thu AM	
395.	Somatic and visceral afferents: visceral afferents	Poster			Wed AM		
57.	Somatosensory cortex and thalamocortical relationships I	Poster	Mon AM				
317.	Somatosensory cortex and thalamocortical relationships II	Poster		Tue PM			
566.	Somatosensory cortex and thalamocortical relationships III	Poster				Thu AM	

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
567.	Somatosensory cortex and thalamocortical relationships IV	Poster				Thu AM	
56.	Subcortical somatosensory pathways: brainstem and spinal cord	Poster	Mon AM				
55.	Subcortical somatosensory pathways: thalamus	Poster	Mon AM				
10.	Subcortical visual pathways: LGN I	Slide	Mon AM				
61.	Subcortical visual pathways: LGN II	Poster	Mon AM				
485.	Subcortical visual pathways: midbrain	Poster			Wed PM		
323.	Subcortical visual pathways: thalamus and midbrain	Poster		Tue PM			
429.	**Toward a Neurobiology of Visual Consciousness**	**SYMP**			**Wed PM**		
136.	Visual cortex: striate I	Poster	Mon PM				
137.	Visual cortex: striate II	Poster	Mon PM				
266.	Visual cortex: striate III	Slide		Tue PM			
349.	Visual cortex: striate IV	Slide			Wed AM		
608.	Visual cortex: striate V	Slide				Thu PM	
646.	Visual cortex: striate VI	Poster				Thu PM	
139.	Visual psychophysics and behavior I	Poster	Mon PM				
647.	Visual psychophysics and behavior II	Poster				Thu PM	
679.	Visual psychophysics and behavior III	Slide					Fri AM
	THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION						
7.	Basal ganglia and thalamus I	Slide	Mon AM				
145.	Basal ganglia and thalamus II	Poster	Mon PM				
237.	Basal ganglia and thalamus III	Poster		Tue AM			
238.	Basal ganglia and thalamus IV	Poster		Tue AM			
327.	Basal ganglia and thalamus V	Poster		Tue PM			
328.	Basal ganglia and thalamus VI	Poster		Tue PM			
406.	Basal ganglia and thalamus VII	Poster			Wed AM		
407.	Basal ganglia and thalamus VIII	Poster			Wed AM		
486.	Basal ganglia and thalamus IX	Poster			Wed PM		
17.	Cerebellum	Slide	Mon AM				
712.	Cerebellum: behavioral neurophysiology and clinical studies	Poster					Fri AM
711.	Cerebellum: cellular neurophysiology	Poster					Fri AM
713.	Cerebellum: neuroanatomy	Poster					Fri AM
714.	Cerebellum: pharmacology	Poster					Fri AM
18.	Circuitry and pattern generation I	Slide	Mon AM				
491.	Circuitry and pattern generation II	Poster			Wed PM		
577.	Circuitry and pattern generation III	Poster				Thu AM	
652.	Circuitry and pattern generation IV	Poster				Thu PM	
718.	Circuitry and pattern generation V	Poster					Fri AM
146.	Control of posture and movement I	Poster	Mon PM				
147.	Control of posture and movement II	Poster	Mon PM				
241.	Control of posture and movement III	Poster		Tue AM			
331.	Control of posture and movement IV	Poster		Tue PM			
357.	Control of posture and movement V	Slide			Wed AM		
408.	Control of posture and movement VI	Poster			Wed AM		
490.	Control of posture and movement VII	Poster			Wed PM		
575.	Control of posture and movement VIII	Poster				Thu AM	
576.	Control of posture and movement IX	Poster				Thu AM	
653.	Invertebrate motor function	Poster				Thu PM	
404.	Motor cortex: anatomy	Poster			Wed AM		
570.	Motor cortex: biophysics, models, plasticity	Poster				Thu AM	

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
405.	Motor cortex: cingulate and prefrontal cortex	Poster			Wed AM		
403.	Motor cortex: cortical physiology	Poster			Wed AM		
571.	Motor cortex: human studies	Poster				Thu AM	
648.	Motor systems and sensorimotor integration: reflex function I	Poster				Thu PM	
715.	Motor systems and sensorimotor integration: reflex function II	Poster					Fri AM
194.	Motor systems: cortex	Slide		Tue AM			
719.	Muscle: electrophysiology	Poster					Fri AM
654.	Muscle: fiber types and endplates	Poster				Thu PM	
492.	Muscle: force, frequency, and fatigue	Poster			Wed PM		
574.	Oculomotor: blinking, vengence, and torsion	Poster				Thu AM	
572.	Oculomotor: brainstem and muscle	Poster				Thu AM	
489.	Oculomotor: clinical studies	Poster			Wed PM		
65.	Oculomotor: cortex, thalamus, pretectum	Poster	Mon AM				
573.	Oculomotor: performance and modeling of saccades	Poster				Thu AM	
107.	Oculomotor: saccades	Slide	Mon PM				
64.	Oculomotor: superior colliculus	Poster	Mon AM				
359.	Oculomotor: vestibuloocular reflex and smooth pursuit I	Slide			Wed AM		
488.	Oculomotor: vestibuloocular reflex and smooth pursuit II	Poster			Wed PM		
649.	Spinal cord and brainstem: anatomy	Poster				Thu PM	
330.	Spinal cord and brainstem: cellular neurophysiology	Poster		Tue PM			
329.	Spinal cord and brainstem: functional neurophysiology	Poster		Tue PM			
717.	Spinal cord and brainstem: neonates	Poster					Fri AM
716.	Spinal cord and brainstem: pattern generation	Poster					Fri AM
651.	Spinal cord and brainstem: pharmacology	Poster				Thu PM	
66.	Spinal cord and brainstem: response to injury	Poster	Mon AM				
650.	Spinal cord and brainstem: transmitter localization	Poster				Thu PM	
240.	Vestibular: pharmacology	Poster		Tue AM			
239.	Vestibular: vestibuloocular reflex in humans	Poster		Tue AM			
487.	Vestibular: vestibuloocular reflex physiology	Poster			Wed PM		
THEME H: OTHER SYSTEMS OF THE CNS							
578.	Association cortex and thalamocortical relations	Poster				Thu AM	
242.	Brain metabolism and blood flow: basic mechanisms I	Poster		Tue AM			
581.	Brain metabolism and blood flow: basic mechanisms II	Poster				Thu AM	
720.	Brain metabolism and blood flow: disease mechanisms	Poster					Fri AM
518.	Brain metabolism and blood flow: general	Slide				Thu AM	
67.	Brain metabolism and blood flow: imaging techniques	Poster	Mon AM				
410.	Brain metabolism and blood flow: nitric oxide	Poster			Wed AM		
580.	Comparative neuroanatomy: chemical anatomy	Poster				Thu AM	
579.	Comparative neuroanatomy: sensory systems	Poster				Thu AM	
409.	Comparative neuroanatomy: sex, evolution, and the forebrain	Poster			Wed AM		
148.	Limbic system and hypothalamus I	Poster	Mon PM				
149.	Limbic system and hypothalamus II	Poster	Mon PM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
150.	Limbic system and hypothalamus III	Poster	Mon PM				
151.	Limbic system and hypothalamus IV	Poster	Mon PM				
	THEME I: NEURAL BASIS OF BEHAVIOR						
165.	Aging I	Poster	Mon PM				
166.	Aging II	Poster	Mon PM				
167.	Aging III	Poster	Mon PM				
197.	Biological rhythms	Slide		Tue AM			
497.	Biological rhythms: lesions pathologies	Poster			Wed PM		
722.	Biological rhythms: light	Poster					Fri AM
72.	Biological rhythms: limbic system and reproduction	Poster	Mon AM				
723.	Biological rhythms: models	Poster					Fri AM
498.	Biological rhythms: molecular regulation	Poster			Wed PM		
588.	Biological rhythms: pineal and melatonin	Poster				Thu AM	
587.	Biological rhythms: SCN, activation, and anatomy	Poster				Thu AM	
70.	Biological rhythms: sleep I	Poster	Mon AM				
496.	Biological rhythms: sleep II	Poster			Wed PM		
71.	Biological rhythms: suprachiasmatic nucleus	Poster	Mon AM				
243.	Cognition I	Poster		Tue AM			
411.	Cognition II	Poster			Wed AM		
412.	Cognition III	Poster			Wed AM		
582.	Cognition IV	Poster				Thu AM	
521.	Cognition: human I	Slide				Thu AM	
678.	Cognition: human II	Slide					Fri AM
9.	Cognition: imaging I	Slide	Mon AM				
152.	Cognition: imaging II	Poster	Mon PM				
189.	Cognition: imaging III	Slide		Tue AM			
420.	Drugs of abuse: CNS stimulants—behavior	Poster			Wed AM		
663.	Drugs of abuse: CNS stimulants—cellular and molecular mechanisms	Poster				Thu PM	
419.	Drugs of abuse: CNS stimulants—toxicity	Poster			Wed AM		
684.	Drugs of abuse: addiction/tolerance	Slide					Fri AM
360.	Drugs of abuse: alcohol I	Slide			Wed AM		
659.	Drugs of abuse: alcohol II	Poster				Thu PM	
660.	Drugs of abuse: alcohol III	Poster				Thu PM	
661.	Drugs of abuse: alcohol IV	Poster				Thu PM	
662.	Drugs of abuse: alcohol V	Poster				Thu PM	
657.	Drugs of abuse: alcohol and benzodiazepines	Poster				Thu PM	
658.	Drugs of abuse: alcohol and opioids	Poster				Thu PM	
529.	Drugs of abuse: amphetamines and CNS stimulants	Slide				Thu AM	
502.	Drugs of abuse: behavior I	Poster			Wed PM		
503.	Drugs of abuse: behavior II	Poster			Wed PM		
101.	Drugs of abuse: cocaine	Slide	Mon PM				
248.	Drugs of abuse: cocaine—behavior	Poster		Tue AM			
249.	Drugs of abuse: cocaine—cell biology	Poster		Tue AM			
250.	Drugs of abuse: cocaine—development	Poster		Tue AM			
664.	Drugs of abuse: cocaine—dopamine	Poster				Thu PM	
665.	Drugs of abuse: cocaine—dopamine receptors	Poster				Thu PM	
666.	Drugs of abuse: cocaine—non-dopaminergic systems	Poster				Thu PM	
251.	Drugs of abuse: cocaine—peripheral effects	Poster		Tue AM			
504.	Drugs of abuse: metabolism—pharmacology	Poster			Wed PM		
501.	Hormonal control of reproductive behavior: mating	Poster			Wed PM		
247.	Hormonal control of reproductive behavior: other I	Poster		Tue AM			

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
725.	Hormonal control of reproductive behavior: other II	Poster					Fri AM
591.	Hormonal control of reproductive behavior: parental/aggressive	Poster				Thu AM	
161.	Hormonal control of reproductive behavior: steroid receptors	Poster	Mon PM				
245.	Ingestive behaviors I	Poster		Tue AM			
246.	Ingestive behaviors II	Poster		Tue AM			
338.	Ingestive behaviors III	Poster		Tue PM			
499.	Ingestive behaviors IV	Poster			Wed PM		
500.	Ingestive behaviors V	Poster			Wed PM		
528.	Ingestive behaviors VI	Slide				Thu AM	
686.	Ingestive behaviors VII	Slide					Fri AM
105.	Invertebrate learning and behavior I	Slide	Mon PM				
244.	Invertebrate learning and behavior II	Poster		Tue AM			
337.	Invertebrate learning and behavior III	Poster		Tue PM			
443.	Invertebrate learning and behavior IV	Slide			Wed PM		
325.	Invertebrate sensory systems I	Poster		Tue PM			
326.	Invertebrate sensory systems II	Poster		Tue PM			
68.	Learning and memory: pharmacology—acetylcholine	Poster	Mon AM				
416.	Learning and memory: pharmacology—excitatory amino acids	Poster			Wed AM		
69.	Learning and memory: pharmacology—monoamines	Poster	Mon AM				
417.	Learning and memory: pharmacology—others I	Poster			Wed AM		
721.	Learning and memory: pharmacology—others II	Poster					Fri AM
332.	Learning and memory: physiology I	Poster		Tue PM			
333.	Learning and memory: physiology II	Poster		Tue PM			
334.	Learning and memory: physiology III	Poster		Tue PM			
335.	Learning and memory: physiology IV	Poster		Tue PM			
153.	Learning and memory: systems and functions I	Poster	Mon PM				
154.	Learning and memory: systems and functions II	Poster	Mon PM				
155.	Learning and memory: systems and functions III	Poster	Mon PM				
187.	Learning and memory: systems and functions IV	Slide		Tue AM			
413.	Learning and memory: systems and functions V	Poster			Wed AM		
414.	Learning and memory: systems and functions VI	Poster			Wed AM		
415.	Learning and memory: systems and functions VII	Poster			Wed AM		
444.	Learning and memory: systems and functions VIII	Slide			Wed PM		
493.	Learning and memory: systems and functions IX	Poster			Wed PM		
494.	Learning and memory: systems and functions X	Poster			Wed PM		
495.	Learning and memory: systems and functions XI	Poster			Wed PM		
530.	Learning and memory: systems and functions XII	Slide				Thu AM	
339.	Monoamines and behavior: catecholamines I	Poster		Tue PM			
340.	Monoamines and behavior: catecholamines II	Poster		Tue PM			
341.	Monoamines and behavior: catecholamines III	Poster		Tue PM			
342.	Monoamines and behavior: catecholamines IV	Poster		Tue PM			
162.	Monoamines and behavior: serotonin	Poster	Mon PM				
336.	Motivation and emotion: biochemistry and pharmacology	Poster		Tue PM			
156.	Motivation and emotion: non-primates	Poster	Mon PM				
157.	Motivation and emotion: primates including humans	Poster	Mon PM				
158.	Motivation and emotion: self-stimulation	Poster	Mon PM				
583.	Neural plasticity I	Poster				Thu AM	
584.	Neural plasticity II	Poster				Thu AM	
585.	Neural plasticity III	Poster				Thu AM	

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
586.	Neural plasticity IV	Poster				Thu AM	
73.	Neuroethology: bird song I	Poster	Mon AM				
74.	Neuroethology: bird song II	Poster	Mon AM				
159.	Neuroethology: electric fish	Poster	Mon PM				
75.	Neuroethology: general	Poster	Mon AM				
418.	Neuroethology: invertebrates	Poster			Wed AM		
163.	Neuropeptides and behavior: CCK, TRH, and vasopressin	Poster	Mon PM				
441.	Neuropeptides and behavior: CRF and oxytocin	Slide			Wed PM		
656.	Neuropeptides and behavior: other	Poster				Thu PM	
164.	Psychotherapeutic drugs: antidepressants and anxiolytics	Poster	Mon PM				
726.	Psychotherapeutic drugs: antipsychotics	Poster					Fri AM
103.	Psychotherapeutic drugs: antipsychotics and antidepressants	Slide	Mon PM				
667.	Psychotherapeutic drugs: atypical antipsychotics	Poster				Thu PM	
727.	Psychotherapeutic drugs: other	Poster					Fri AM
589.	Stress: neurochemistry and behavior I	Poster				Thu AM	
724.	Stress: neurochemistry and behavior II	Poster					Fri AM
160.	Stress: neuroendocrine and c-*fos* studies	Poster	Mon PM				
15.	Stress: neurotransmitter and endocrine studies	Slide	Mon AM				
590.	Stress: neurotransmitter studies	Poster				Thu AM	
655.	Suprachiasmatic nucleus: neuropeptides	Poster				Thu PM	
184.	**The Amygdala: From Circuits and Synapses to Emotional Memory**	**SYMP**		**Tue AM**			
	THEME J: DISORDERS OF THE NERVOUS SYSTEM						
191.	Degenerative disease: Alzheimer's—beta amyloid I	Slide		Tue AM			
252.	Degenerative disease: Alzheimer's—beta amyloid II	Poster		Tue AM			
253.	Degenerative disease: Alzheimer's—beta amyloid III	Poster		Tue AM			
271.	Degenerative disease: Alzheimer's—beta amyloid IV	Slide		Tue PM			
355.	Degenerative disease: Alzheimer's—beta amyloid V	Slide			Wed AM		
435.	Degenerative disease: Alzheimer's—beta amyloid VI	Slide			Wed PM		
507.	Degenerative disease: Alzheimer's—beta amyloid VII	Poster			Wed PM		
508.	Degenerative disease: Alzheimer's—beta amyloid VIII	Poster			Wed PM		
597.	Degenerative disease: Alzheimer's—beta amyloid IX	Poster				Thu AM	
612.	Degenerative disease: Alzheimer's—beta amyloid X	Slide				Thu PM	
669.	Degenerative disease: Alzheimer's—beta amyloid XI	Poster				Thu PM	
681.	Degenerative disease: Alzheimer's—beta amyloid XII	Slide					Fri AM
510.	Degenerative disease: Alzheimer's—cellular mechanisms of degeneration	Poster			Wed PM		
77.	Degenerative disease: Alzheimer's: cognitive function—imaging and cognitive function	Poster	Mon AM				
192.	Degenerative disease: Alzheimer's: cognitive function—visual and motor skills	Slide		Tue AM			
254.	Degenerative disease: Alzheimer's: neuropathology and neurotransmitters—cholinergic systems	Poster		Tue AM			

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
509.	Degenerative disease: Alzheimer's: neuropathology and neurotransmitters—other systems	Poster			Wed PM		
78.	Degenerative disease: Alzheimer's: neuropharmacology and neurotransmitters—cholinergic systems	Poster	Mon AM				
421.	Degenerative disease: Alzheimer's—mechanisms of cellular injury I	Poster			Wed AM		
683.	Degenerative disease: Alzheimer's—mechanisms of cellular injury II	Slide					Fri AM
522.	Degenerative disease: Alzheimer's—mechanisms of degeneration	Slide				Thu AM	
728.	Degenerative disease: Alzheimer's—models, assessment, and treatment	Poster					Fri AM
422.	Degenerative disease: Alzheimer's—tau phosphorylation and neurofibrillary degeneration	Poster			Wed AM		
277.	Degenerative disease: Alzheimer's—tau protein	Slide		Tue PM			
445.	Degenerative disease: other I	Slide			Wed PM		
671.	Degenerative disease: other II	Poster				Thu PM	
511.	Degenerative disease: other—neurotoxic effects and Huntington's	Poster			Wed PM		
176.	Degenerative disease: other—diabetes, EAE, and viruses	Poster	Mon PM				
602.	Degenerative disease: Parkinson's	Slide				Thu PM	
79.	Degenerative disease: Parkinson's—model and transplantation	Poster	Mon AM				
729.	Degenerative disease: Parkinson's—human studies	Poster					Fri AM
175.	Degenerative disease: Parkinson's—neurochemistry	Poster	Mon PM				
670.	Degenerative disease: Parkinson's—rodent and primate studies	Poster				Thu PM	
505.	Developmental disorders: cortical injury models	Poster			Wed PM		
168.	Developmental disorders: toxins, autoimmunity, and autism	Poster	Mon PM				
668.	Epilepsy: anticonvulsant drugs—other neurotransmitter receptors	Poster				Thu PM	
506.	Epilepsy: anticonvulsant drugs—AMPA and NMDA receptor	Poster			Wed PM		
169.	Epilepsy: basic mechanisms I	Poster	Mon PM				
273.	Epilepsy: basic mechanisms II	Slide		Tue PM			
170.	Epilepsy: basic mechanisms—circuitry I	Poster	Mon PM				
171.	Epilepsy: basic mechanisms—circuitry II	Poster	Mon PM				
172.	Epilepsy: basic mechanisms—genetic models	Poster	Mon PM				
173.	Epilepsy: basic mechanisms—kainate and pilocarpine models	Poster	Mon PM				
174.	Epilepsy: basic mechanisms—kindling	Poster	Mon PM				
592.	Epilepsy: human studies and animal models I	Poster				Thu AM	
593.	Epilepsy: human studies and animal models II	Poster				Thu AM	
594.	Epilepsy: human studies and animal models III	Poster				Thu AM	
680.	Epilepsy: human studies and animal models IV	Slide					Fri AM
596.	Epilepsy: human studies and animal models—kindling	Poster				Thu AM	
595.	Epilepsy: human studies and animal models—morphological changes	Poster				Thu AM	
4.	**Genes With Triplet Repeats in Neuropsychiatric Illness**	**SYMP**	**Mon AM**				
8.	Genetic models	Slide	Mon AM				

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76.	Genetic models: learning, memory, and epilepsy	Poster	Mon AM				
343.	Genetic models: transgenics, trisomics, and mutants	Poster		Tue PM			
512.	Infectious disease	Poster			Wed PM		
425.	Ischemia: apoptosis	Poster			Wed AM		
256.	Ischemia: gene transcription	Poster		Tue AM			
255.	Ischemia: inflammation	Poster		Tue AM			
423.	Ischemia: mechanisms I	Poster			Wed AM		
424.	Ischemia: mechanisms II	Poster			Wed AM		
438.	Ischemia: mechanisms III	Slide			Wed PM		
102.	Ischemia: miscellaneous	Slide	Mon PM				
257.	Ischemia: molecular	Poster		Tue AM			
80.	Ischemia: models	Poster	Mon AM				
258.	Ischemia: neonatal	Poster		Tue AM			
259.	Ischemia: nerve	Poster		Tue AM			
609.	Ischemia: protection	Slide				Thu PM	
81.	Ischemia: treatment I	Poster	Mon AM				
82.	Ischemia: treatment II	Poster	Mon AM				
83.	Ischemia: treatment III	Poster	Mon AM				
177.	Ischemia: white matter	Poster	Mon PM				
19.	Mental illness	Slide	Mon AM				
730.	Mental illness: depression, anxiety, and schizophrenia	Poster					Fri AM
261.	Mental illness: schizophrenia I	Poster		Tue AM			
513.	Mental illness: schizophrenia II	Poster			Wed PM		
344.	Neuro-oncology: cell biology	Poster		Tue PM			
428.	Neuro-oncology: therapy I	Poster			Wed AM		
731.	Neuro-oncology: therapy II	Poster					Fri AM
260.	Neuromuscular disease: ALS	Poster		Tue AM			
86.	Neuromuscular disease: muscle	Poster	Mon AM				
87.	Neuromuscular disease: neurons	Poster	Mon AM				
92.	**Neuroscience Implications of Inborn Errors of Metabolism**	**SYMP**	**Mon PM**				
274.	Neurotoxicity	Slide		Tue PM			
426.	Neurotoxicity: EAA I	Poster			Wed AM		
427.	Neurotoxicity: EAA II	Poster			Wed AM		
672.	Neurotoxicity: drugs	Poster				Thu PM	
673.	Neurotoxicity: metals	Poster				Thu PM	
676.	Neurotoxicity: miscellaneous	Poster				Thu PM	
675.	Neurotoxicity: oxidation	Poster				Thu PM	
674.	Neurotoxicity: striatum	Poster				Thu PM	
353.	Trauma	Slide			Wed AM		
178.	Trauma: cord	Poster	Mon PM				
180.	Trauma: miscellaneous	Poster	Mon PM				
85.	Trauma: models	Poster	Mon AM				
84.	Trauma: treatment	Poster	Mon AM				
179.	Trauma: white matter	Poster	Mon PM				
OTHER							
88.	History of neuroscience	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM
90.	Teaching of neuroscience: computer programs	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM
91.	Teaching of neuroscience: curriculum development	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM
89.	Teaching of neuroscience: laboratory courses and exercises	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM

383.21

Characterization of a novel phospholipase A_2 activity in autopsied human brain. B.M. Ross*, D.K. Kim, J.V. Bonventre and S.J. Kish, University of Toronto, Ontario and Harvard Medical School, Boston.

Phospholipases A_2 (PLA_2), the enzymes which catalyze the hydrolysis of the *sn*-2 fatty acid residue esterified to a variety of phospholipid species, were studied in preparations of autopsied human cerebral cortex. A greater amount of PLA_2 activity was found in the membrane (particulate) fractions when compared with the cytosolic fraction. The particulate and cytosolic enzymes possessed similar characteristics, including having a pH optimum of 8.5, and being maximally active in the presence of millimolar concentrations of calcium ions. The particulate enzyme could be solubilized by 1M potassium chloride, and was capable of hydrolyzing phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), with a preference being displayed for PI and PE over PC. However the enzyme displayed only a small preference for arachidonic over linoleic acid residues at the *sn*-2 position of PE. When the solubilized particulate enzyme was subjected to gel filtration chromatography, PLA_2 activity eluted as a single peak of relative molecular mass greater than 67kDa. PLA_2 activity was dithiothreitol insensitive, but was inhibited by brief heat treatment, bromophenacylbromide, $AACOCF_3$ (the tri-fluoroketone derivative of arachidonic acid) and γ-linolenoyl amide. Thus, while the human brain enzyme displays many of the characteristics of low molecular weight platelet PLA_2, it differs in several key features, suggesting that human brain may contain a novel form(s) of PLA_2.

RECEPTOR MODULATION UP- AND DOWN-REGULATION I

384.1

DOCA-INDUCED CHANGES IN SEPTO-PREOPTIC NEURON SENSITIVITY TO ANG II AND LOSARTAN. F.P. Martial*, S.N. Thornton, F. Liénard, M.-C. Mousseau, and S. Nicolaïdis. CNRS URA 1860, Neurobiologie des Régulations, Collège de France, 11 pl. M. Berthelot, 75231 Paris Cedex 05, France.

Angiotensin II (AII) and aldosterone are known to act in synergy at the central level to produce a robust sodium appetite. Our previous electrophysiological results have shown that neurons in the septo-preoptic area are influenced by these hormones. We have used iontophoresis of AII and Losartan, a non-peptide AT1 antagonist, to investigate the effect of DOCA pretreatment on the action of AII on these neurons.

Thirty-six male Wistar rats were used in the study. A multibarrelled iontophoretic electrode attached to an extracellular recording electrode was advanced into the medial septal/median preoptic area where unit activity was recorded.

In the studied area, iontophoretic application of AII induced activation on 17 (23.3%) and inhibition on 4 (5.5%) of the spontaneous activity of the 73 neurons tested for this peptide in the control animals. In the DOCA pretreated rats, AII induced activation on 31 (35.6%) and inhibition on 7 (8%) of the spontaneous activity of the 87 neurons tested. These numbers give a ratio of excitatory/inhibitory responses to AII of 81/19 for control and of 82/18 for DOCA. Iontophoretic application of Losartan by itself in this same forebrain region induced activation on 6 (13%) and inhibition on 6 (13%) of the spontaneous activity of the 46 neurons tested in the control animals. In the DOCA group, Losartan induced activation on 14 (26.9%) and inhibition on 1 (1.9%) of the spontaneous activity of the 52 neurons tested, giving an excitatory/inhibitory ratio of 50/50 for control and of 93/7 for DOCA.

These results show that AII can be excitatory or inhibitory on neurons of the same region. The number of responses to AII is enhanced by DOCA pretreatment but the shift in excitatory/inhibitory ratio for Losartan, but not for AII suggests that several types of receptors are involved in these DOCA-induced modifications.

(Supported by MH 43787 & Evian C°)

384.2

ADRENERGIC REGULATION OF *B*-ADRENERGIC RECEPTOR AND ICER (INDUCIBLE cAMP EARLY REPRESSOR) mRNA LEVELS IN C6 GLIOMA CELLS. L. Rydelek-Fitzgerald* and R.S. Duman, Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

We have shown previously that exposure of C6 glioma cells to isoproterenol decreases the transcription rate of *B*1- and *B*2-adrenergic receptor (*B*1AR, *B*2AR) mRNA. This decrease in transcription is sensitive to inhibition of protein synthesis suggesting that isoproterenol induces a transcriptional repressor. A potential mediator of the repression is ICER, a member of the CREM (CRE modulator) family of transcription factors, which is induced by activation of the cAMP system, can bind to the CRE (cAMP responsive element), and antagonize the stimulatory effects of CREB (CRE binding protein) (Stehle et al., Nature '93). Since a CRE is present in both *B*1AR and *B*2AR promoters, the effects of isoproterenol on ICER regulation in C6 glioma cells were investigated. RNAse protection assays reveal that exposure of C6 glioma cells to isoproterenol or forskolin for 1 hour induces levels of ICER, as well as CREM, mRNA approximately 10-fold. ICER and CREM mRNA levels return to control levels following 24 hours of exposure to agonist. The induction of ICER mRNA levels by isoproterenol is not blocked by inhibition of protein synthesis. Moreover, ICER mRNA levels are more highly induced in the presence of protein synthesis inhibitors, consistent with reports that ICER can inhibit its own production. Electrophoretic mobility shift assays show that isoproterenol and forskolin induce at least two CRE binding complexes, both of which migrate faster than the CREB containing complex. Supershift assays with a CREB antibody demonstrate that these complexes do not contain CREB and thus may contain ICER. Additional studies will be performed to fully characterize the regulation of ICER and to investigate its potential role in the repression of *B*1AR and *B*2AR gene transcription.

384.3

AGONIST TREATMENT DOWN-REGULATES LEVELS OF CRF-STIMULATED ADENYLYL CYCLASE, RECEPTOR BINDING AND mRNA LEVELS IN A LOCUS COERULEUS-LIKE (LC-LIKE) CELL LINE. P.A. Iredale*, H.J. Strausbaugh and R.S. Duman. Lab. of Molecular Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Corticotropin-releasing factor (CRF) is involved in regulation of the pituitary-adrenal axis as well as the CNS response to stress. The CRF receptor is positively coupled to adenylyl cyclase, however the mechanisms which control CRF receptor expression are poorly understood. In this study we used an immortalized locus coeruleus-like (LC-like) cell line, previously shown to contain CRF receptors, as a model system to study regulation of CRF receptor coupling to adenylyl cyclase, ligand binding and mRNA levels.

CRF treatment resulted in a rapid, time-dependent down-regulation of CRF-stimulated adenylyl cyclase and CRF receptor ligand binding, which was maximal after 20 min, and was observed for up to 2-4 hrs. However, after 20 hrs of CRF treatment there was a further decrease of approximately 20-30 %. Incubation of LC cells also decreased levels of CRF receptor mRNA but with a different time-course: agonist incubation decreased levels of receptor mRNA by 30 % after 4 hrs and by 60 % after 24 hrs. The results suggest that agonist regulation of receptor expression is mediated by several processes. The rapid down-regulation is probably mediated by receptor sequestration and internalisation as described for adrenergic receptors, and decreased levels of receptor mRNA probably contributes to the more long-term decrease observed after 24 hrs of treatment.

384.4

CHRONIC MORPHINE TREATMENT DECREASES MELANOCORTIN 4-RECEPTOR mRNA EXPRESSION IN RAT FRONTAL CORTEX. J.D. Alvaro*, E.J. Nestler, and R.S. Duman. Neuroscience Program, Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Med., New Haven, CT 06508.

Previously we described the cloning and characterization of a rat melanocortin 4-receptor (MC4-R) (Soc. Neurosci. Abst.19, 634.9). This receptor is 996bp in length and is 95% identical to the amino acid sequence of the published human MC4-R. In order to study the regulation of MC4-R by psychotropic drugs, we have investigated the possible effects of morphine addiction on melanocortin receptors. Rats were administered 75mg morphine pellets (s.c.) once daily for five days, and on the sixth day several brain regions from morphine- and sham-treated animals were dissected. Using an MC4-R specific riboprobe in an RNase protection assay, we found that MC4-R mRNA levels in the frontal cortex were consistently down-regulated in morphine-treated animals. To determine whether this effect was specific to MC4-R, the RNase protection assay was repeated using a riboprobe for MC3-R. Down-regulation in the frontal cortex was not seen, but rather a tendency toward up-regulation was noted. The effect of morphine treatment on melanocortin receptor RNA will be examined in the other dissected brain regions by the RNase protection assay, and *in situ* hybridization will be used to localize more discrete regions of receptor regulation. Regulation of MC4-R expression could contribute to the neurochemical adaptations underlying the long-term actions of opiates in the brain.

384.5

EFFECT OF SEROTONERGIC DRUGS ON THE UP-REGULATION OF DOPAMINE2 RECEPTOR INDUCED BY HALOPERIDOL

T. Ishikane*, I. Kusumi, R. Matsubara, S. Matsubara and T. Koyama.
Dept. of Psychiatry, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

We examined the modulatory effect of serotonin(5-HT) on haloperidol-induced up-regulation of dopamine(D)2 receptor in order to elucidate the pharmacological characteristics of atypical antipsychotic drugs. Chronic treatment of rats with haloperidol (HPD, 0.1-0.5mg/kg, i.p., 3weeks) increased the number of D2 receptors in the striatum, while no increase was observed by that with clozapine (1mg/kg) and ORG5222 (0.25mg/kg), atypical antipsychotic drug and its candidate which have high affinity at 5-HT2 receptor sites with lower affinity at D2 sites. Chronic treatment with MK212, a nonselective 5-HT agonist (2.5mg/kg), or with citalopram, a 5-HT reuptake inhibitor (10mg/kg), both of which had no effect on the number of D2 receptor sites by themselves, potentiated the up-regulation of D2 receptor sites when coadministrated with HPD (0.5mg/kg). Coadministration of ritanserin(RIT), a 5-HT2/5-HT1c antagonist (1mg/kg), with HPD (0.5mg/kg), had no influence on the HPD-induced increase of D2 receptor sites, but that with smaller dose of HPD(0.1mg/Kg) attenuated the D2 up-regulation. These results suggest that serotonergic activity may have a complex modulatory influence on the up-regulation of D2 receptor sites induced by HPD. We are now examining the effect of 8-OH-DPAT, a selective 5-HT1A agonist (0.1mg/kg), on the HPD-induced D2 up-regulation.

384.6

EFFECT OF PRENATAL DOPAMINERGIC DRUG TREATMENTS ON THE DEVELOPMENT OF DOPAMINE D-2 RECEPTORS IN RAT BRAIN.

P. Park, J.J. Liang, R. Quirion, and L. Srivastava*. *Douglas Hospital Research Centre, Departments of Psychiatry and Neurology and Neurosurgery, McGill University, Montreal (Que), Canada, H4H 1R3.*

Studies on the postnatal development of dopamine receptors have shown a maturation of the receptors between the second and the third postnatal weeks. It has been suggested that the presence or absence of dopamine during prenatal or early postnatal periods may affect subsequent development of dopamine D-1 and D-2 receptors. In the present study we examined the prenatal effects of a dopamine D-2 agonist (quinpirole) and of an antagonist (raclopride) on the density and mRNA levels of dopamine D-2 receptors. Quinpirole (1 mg/kg, s.c.) and raclopride (0.5 mg/kg, s.c) were administered daily to pregnant Sprague-Dawley rats from gestation day 10-11 till birth of the pups. Pups were sacrificed at different postnatal ages and their brains were removed and sectioned for anatomical studies. [^{3}H]Spiperone and a D-2 selective 39-mer oligonucleotide, recognizing both alternatively spliced forms of the receptor, were used in receptor binding studies and *in situ* hybridization, respectively. In accordance with previous results, the maximum density of the D-2 receptor and its mRNA were observed at postnatal day 28. However, the striatal D-2 receptor density and mRNA levels were significantly elevated at postnatal day 28 in rats prenatally treated with quinpirole. No significant changes in D-2 mRNA levels were evident in the raclopride treated rats of the same age group. These results suggest that prenatal stimulation of dopamine D-2 (and/or D-3 and D-4 that have high affinity for quinpirole) receptors has long-term consequences for postnatal dopamine receptor development. (Supported by the Medical Research Council of Canada).

384.7

ASTROGLIAL OXYTOCIN RECEPTOR DOWN-REGULATION: MODULATION BY PROTEIN(S) KINASE(S) C.

D Di Scala-Guenot, Ch. Kelche* and M.Th. Strosser. Institut de Physiologie, URA 1446, 21 rue René Descartes, 67000 Strasbourg France.

Specific oxytocin (OT) receptors have been previously characterized on hypothalamic cultured astrocytes and intracellular Ca^{2+} mobilization by the agonist has been demonstrated. The involvement of phospho-inositide hydrolysis which generates IP_3 and diacylglygerol, an activator of protein kinases C (PKC), could be hypothetized. As stimulation of PKC appears to be an important event in transduction mechanisms and receptor regulation, the role of PKC was studied on hypothalamic cultured astrocytes. Binding studies were performed on cells pretreated with a phorbol ester (phorbol-12 myristate, 13-acetate, PMA). Short-term exposure (up to 30 min) to PMA (10^{-7}M) or OT(10^{-9}M) alone was without effect on ligand binding whereas simultaneous addition of OT and PMA significantly decreased the binding and this effect was reversed by staurosporin. PMA long-term treatment is thought to desensitize PKC and in our system, 18 hours treatment with PMA or OT alone decreased ligand binding and simultaneous application of the drugs potentiated this effect which could not be reversed by staurosporin. In conclusion, the first step in agonist- and phorbol ester-mediated ligand binding inhibition, depends on PKC activation whereas down-regulation induced by long-term treatment is independent from PKC.

384.8

OXYTOCIN BINDING IN HIPPOCAMPUS, VMH AND AMYGDALA: EFFECTS OF STRESS AND GLUCOCORTICOIDS.

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Neuroanatomical studies reveal that central oxytocin receptors are concentrated in major regions of the limbic system: hippocampus, olfactory nucleus, amygdala, BNST and hypothalamus. We had previously shown that oxytocin receptor binding in hippocampus is regulated by the level of circulating glucocorticoid hormones, using adrenalectomy and corticosterone replacement model. In the current study we examined the effects of non habituating stress and high dose corticosterone implants on oxytocin binding in non adrenalectomized animals. Seventeen Sprague-Dawley male rats (250 g) were divided into 3 groups: Controls, Stressed and Corticosterone implanted (6,6 and 5 animals respectively). Animals were sacrificed 1 week after the implantation of two 100mg 100% corticosterone pellets, or 24h after the seventh day of exposure to non habituating stress (swim; cold room; restrain and ether anesthesia - two stressors/day) Oxytocin receptor binding autoradiography results suggest that non habituating stress significantly increased oxytocin receptor binding in Amygdala ($p<.05$) with trend to increase receptor binding in other areas examined (VMH, Dorsal hippocampus and Ventral Hippocampus). High dose corticosterone implants increased oxytocin receptor binding in Dorsal hippocampus and showed trend in Ventral hippocampus. The results of this study further support our early findings regarding the effects of glucocorticoids on oxytocin bindings. They provide first preliminary evidence for the effects of non habituating stress on central oxytocin binding and suggest that glucocorticoids play a role in mediating this effect in hippocampus.

384.9

OLIGODEOXYNUCLEOTIDES TO THE CLONED DELTA OPIATE RECEPTOR, DOR-1:UPTAKE, STABILITY AND REGULATION OF RECEPTOR GENE EXPRESSION

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Previously, we reported that phosphodiester antisense oligodeoxynucleotides (ODNs) directed towards various domains of the coding region of the cloned mouse delta receptor, DOR-1, reduced delta receptor binding *in vivo* and *in vitro*. Additionally, an antisense directed toward the amino terminus of DOR-1 (Antisense A) blocked DPDPE and deltorphin II-mediated spinal analgesia with no effect on mu- or kappa-mediated spinal analgesia. Subsequently, Porrecca demonstrated that the same antisense also blocked supraspinal deltorphin II-mediated analgesia but not that of DPDPE. In this study we examined the ability of Antisense A to enter NG108-15 neuroblastoma cells and mouse spinal cord, and its stability therein. Radiolabeled Antisense A (250 nM) was taken up into cells in a time-dependent fashion, reaching a plateau after 4 hours that extended to several days. Solution hybridization assays using a riboprobe transcribed from the coding sequence of DOR-1 were used to measure levels of spinal cord mRNA after antisense A treatment. The decrease in spinal cord mRNA levels were consistent with a loss in binding levels.

384.10

DIFFERENTIAL EFFECT OF LONG-TERM ANTIDEPRESSANT TREATMENT ON 5-HT_{2A} VERSUS 5-HT_{2C} RECEPTOR-MEDIATED HYPERTHERMIA IN FAWN-HOODED RATS.

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We have recently demonstrated that hyperthermia induced by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and m-chlorophenylpiperazine (m-CPP) is mediated by selective stimulation of 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Mazzola-Pomietto et al., 1993; Murphy et al., 1993). Furthermore, hyperthermia induced by either DOI (Aulakh et al., in press) or m-CPP (Mazzola-Pomietto et al., 1993) was found to be significantly less in Fawn-Hooded (FH) rats (a rat strain suggested to represent a genetic model of depression) relative to Wistar rats. In the present study, we studied the effects of long-term antidepressant treatment on DOI (2.5 mg/kg)-induced and m-CPP (2.5 mg/kg)-induced hyperthermia in male FH rats. Long-term (21 days) treatment with the tricyclic antidepressants, imipramine or clomipramine (each 5 mg/kg/day) attenuated DOI-induced hyperthermia, while m-CPP-induced hyperthermia was accentuated. On the other hand, long-term (21 days) treatment with the monoamine oxidase type-A inhibiting antidepressant, clorgyline (1 mg/kg/day) did not modify m-CPP-induced hyperthermia, but significantly attenuated DOI-induced hyperthermia. These findings demonstrate a differential effect of long-term antidepressant treatment on 5-HT_{2A} versus 5-HT_{2C} receptor-mediated hyperthermia in a genetic animal model of depression.

384.11

CHRONIC TREATMENT WITH THE 5-HT-1A RECEPTOR LIGAND LESOPITRON ALTERS LEVELS OF TYROSINE HYDROXYLASE IN THE RAT LOCUS COERULEUS. X.Guitart*, R.Sanchez-Arroyos and A.J.Farré. Dept. Pharmacology-CNS, Research Center Lab. Esteve. Barcelona. Spain.

During the last decade, several non-benzodiazepine drugs that display high affinity for the 5-HT-1A receptor subtype, including buspirone and gepirone, have been shown to be effective in the treatment of anxiety. Lesopitron is a new pyrimidinyl-piperazine that reduces behavioral responses to aversive situations in animal models of anxiety and that shows high selectivity and specificity for the 5-HT-1A receptor. On the other hand, different lines of pharmacological evidence have led to the hypothesis that the locus coeruleus (LC), the largest noradrenergic nucleus of the rat brain, is a mediator of anxiety and its behavioral manifestations. Moreover, in vivo experiments have shown that serotonin can attenuate LC activity, and serotonin-containing terminals have been described in this brain area. The current study examined the possibility that chronic lesopitron might alter levels of tyrosine hydroxylase (TH) in the LC. Rats were administered lesopitron for 14 days (15 mg/kg), and on day 15 levels of TH immunoreactivity were quantified by immunoblotting. Chronic lesopitron treatment decreased levels of TH immunoreactivity in the LC by 40%. Our results suggest a relation ship between lesopitron and the noradrenergic system that could be linked to its mechanism of action.

384.12

COCAINE-INDUCED CHANGES IN BZD RECEPTORS: EFFECTS OF TREATMENT DURATION AND DRUG WITHDRAWAL. **A.L.Gorman* and N.E.Goeders. Dept of Pharmacology and Therapeutics, LSU-MC, Shreveport, LA 71130-3932.**

Previously, we reported that chronic cocaine administration induced changes in benzodiazepine (BZD) receptor binding in brain areas associated with the mesocorticolimbic dopaminergic system, and possibly involved in the development of behavioral sensitization. Adult male Sprague Dawley rats were injected once daily with cocaine (5 or 20 mg/kg ip) or saline for 2, 4, or 8 weeks, and sacrificed 24 hrs, 14 days, or 28 days post-treatment to determine the effects of withdrawal on BZD receptor binding. BZD receptors were visualized autoradiographically using [^{3}H]Ro 15-1788. Chronic cocaine produced minimal changes in BZD receptors 24 hrs post-cocaine, yet these rats exhibited significant increases in stereotypy after 2 weeks of cocaine, suggesting that cocaine-induced alterations in BZD receptors were not essential for the development of behavioral sensitization. BZD receptors were increased in the rostral nucleus accumbens, and in cortical areas 14 days post-cocaine, but returned to control values 28 days post-cocaine. Cocaine-induced increases in BZD receptors in these rats were related to the dose of drug, the length of exposure, and the time point of drug withdrawal. In addition, the effects of cocaine on BZD receptors may differ between Wistar and Sprague Dawley strains of rats.

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384.13

MODULATION OF GLYCINE RECEPTOR MEDIATED CHLORIDE RESPONSES BY PROTEIN KINASE C. Y. Gu[1]* and L.-Y.M. Huang[1,2] Marine Biomedical Institute[1] and Department of Physiology and Biophysics[1,2], University of Texas Medical Branch, Galveston, Texas 77555-0483.

Glycine is a major inhibitory transmitter that mediates synaptic transmission in the spinal and medullary dorsal horns. We have shown previously that protein kinase A potentiates glycine-activated Cl^- conductance by increasing the probability of channel openings (Nature 348:242, 1990). To determine whether other second messengers also modulate the glycine responses, we studied the effects of protein kinase C (PKC) on the glycine-activated Cl^- in isolated trigeminal neurons. The currents were recorded using the whole cell patch-clamp recording technique. Glycine was applied to the recorded cells with a fast perfusion method. The protein kinase C and protein kinase inhibitor (PKCI) were applied intracellularly through a plastic tube inserted into the patch pipet.

PKC was found to increase the glycine-induced Cl^- currents up to 2 fold. This enhancing effect of PKC was blocked when PKCI(19-31), apseudosubstrate competing for the subtrate recognition site of PKC, was introduced into the cells. PKC did not change the affinity of glycine to its receptors. The apparent dissociation constants for glycine were 26.4μM in control and 25.8μM in PKC. To determine the mechanism of PKC action on glycine-activated Cl^- channels, we examined the current-voltage relations of the currents in control and in PKC. PKC changed neither the kinetics nor the voltage dependence of the glycine responses. The reversal potentials of the glycine-activated Cl^- currents remained unchanged after PKC treatment. The effects of PKC on the channel conductance and on the probability of channel openings are currently under investigation. (The work is supported by NIH grants NS30045, NS23061 and NS11255).

384.14

NON-COMPETITIVE INHIBITION BY DESIPRAMINE, PROPRANOROL AND ALPRENOROL OF [3H]1,3-DI-O-TOLYLGUANIDINE (DTG) DEFINED SIGMA BINDING SITES IN RAT BRAIN. Y. Shirayama, T. Nishikawa and K. Takahashi*. Div. Mental Disorder Res., Natl. Inst. Neurosci., NCNP, Tokyo, Japan.

[3H]DTG (1,3-di-o-tolylguanidine) binding to the homogenate of rat cerebral cortex was competitively inhibited by a variety of neurotropic drugs such as pentazocine, haloperidol, clomipramine and imipramine, which increased the Kd value with no changes in the Bmax value of the [3H]DTG defined sigma binding site. In contrast, a classical antidepressant desipramine reduced Bmax of the [3H]DTG binding in a concentration-dependent fashion without effects on its Kd. This non-competitive inhibition is unlikely to be related to the blocking action of desipramine at the N-methyl-D-aspartate receptor because selective competitive (CGS19755) and non-competitive (MK-801) antagonists of the excitatory amino acid receptor failed to mimic the effects of desipramine. Furthermore, potent antagonists of the beta-adrenergic receptors, (-)-propranorol and (-)-alprenorol also caused a non-competitive inhibition of the [3H]DTG binding. Together with the potent blocking action of desipramine at norepinephrine uptake, the present results suggest that noradrenergic systems might allosterically interact with the cerebral sigma site or that the sigma macromolecule could have certain allosteric regulation sites which would be affected by desipramine, propranorol and alprenorol.

384.15

REGULATION OF nAChR SUBUNIT EXPRESSION IN CHICK SYMPATHETIC NEURONS BY PRE- AND POSTGANGLIONIC PARTNERS P. Devay*, D. McGehee, S. Peng and L. Role. Dept. of Anat. and Cell Biol. & Cntr. for Neurobiol. & Behav., Columbia Univ.,722 W 168th Str., NY, NY 10032

During embryonic development in vivo ACh sensitivity of chick sympathetic neurons increases. Concomitant with these changes we find an elevated expression of α3, α5, α7 and β4 AChR subunits mRNAs (Devay et al '94). Since both pre- and postsynaptic contacts are established during this time, it is difficult to dissect which of these interactions regulates nAChR expression

To separate the role of pre- from postsynaptic influences on nAChRs we examined sympathetic neurons in vitro alone or in the presence of either the pre- or the postsynaptic partners. The level of nAChR subunit expression was mesured in individual cells with established pre- or postsynaptic contacts. Single channel recording studies of innervated neurons and neurons contacting target were performed as well, for comparison of changes in nAChR expression vs. nAChR-channel properties. To quantitatively evaluate the subunit expression levels, we developed a PCR assay. In contrast to the upregulation of α3, α5 and α7 with both input and target contact, target contact alone decreases the expression of α3 while increasing that of α5 and α7 mRNAs. Along with these changes in nAChR subunit gene expression with target contact, all three of the nAChR-channels expressed by neurons prior to innervation or target contact are suppressed and a single class of large conductance (~60-70pS) is expressed. Since innervation by preganglionic neurons upregulates the expression of AChR subunits and the number of channels expressed by sympathetic neurons, these experiments suggest that input and target contact collaborate in the regulation of the number and properties of mature nAChRs.

384.16

CASTRATION CAUSES A SIGNIFICANT INCREASE IN NMDA RECEPTOR BINDING IN THE HIPPOCAMPUS OF MALE RATS. L.Kus* [1], R.I. Handa [2], J.M. Hautman[1], and A.J. Beitz [1]. Dept. Vet. Pathobiology[1], Univ. of Minnesota, St. Paul, MN 55108 and Dept. Cell Biology, Neurobiology and Anatomy [2], Loyola Univ. Chgo., Maywood, IL 60153.

The hippocampus contains high levels of both NMDA and non-NMDA receptors. Previous studies have shown the CA1 region of the hippocampus to be particularly sensitive to ischemia-induced neuronal damage. Based on the observation that androgen receptor concentration is higher in CA1 than other hippocampal regions this study examined the effect of castration and castration with dihydrotestosterone propionate (DHTP) replacement on [^{125}I]MK801 binding in the dorsal hippocampus. Adult Sprague-Dawley male rats were castrated and implanted subcutaneously with either a 2.5 cm Silastic capsule that contained DHTP (n= 3) or an empty capsule (n=5). Control rats (n=8) were left intact. Animals were sacrificed 21 days after castration. In intact control rats [^{125}I]MK801 binding was highest in stratum oriens and radiatum of CA1 as well as in the molecular layer of the dentate gyrus. In castrated rats, [^{125}I]MK801 binding was significantly increased in the pyramidal cell layer, stratum oriens and radiatum of CA1 compared to intact controls. This increase in [^{125}I]MK801 binding was prevented by treatment of castrated rats with DHTP. [^{125}I]MK801 binding in the hippocampus of castrate-DHTP treated rats did not differ from [^{125}I]MK801 binding in intact rats in any region of the hippocampus examined. These data suggest that androgen receptor stimulation may influence excitatory amino acid neurotransmission within the hippocampus. (NSF BNS 9109226, NIH DA06687, DE06682, DC01086, AA08696)

384.17

MODULATION OF NMDA CURRENT BY FATTY ACIDS IN MOUSE CORTICAL NEURONS. S.P. Yu*, L.L. Dugan and D.W. Choi, Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

We previously showed that fatty acid-induced changes in neuronal membrane fluidity were associated with alterations in NMDA-evoked $^{45}Ca^{2+}$ accumulation and death (Dugan *et al.*, Soc. Neurosci. Abs. 18:756, 1992). Modulation of NMDA current (I_{NMDA}) by fatty acids was studied in cultured mouse cortical neurons using whole-cell voltage clamp at 33°C. Each fatty acid was bath-applied at 50 μM together with 50 μM bovine serum albumin. I_{NMDA} in untreated cells was 1.19 ± 0.11 nA (SEM, n=20), and showed no obvious run-down (ATP was included in the recording pipette). Palmitic acid (16:0) which decreases membrane fluidity, reversibly blocked 52 ± 11% of the I_{NMDA} (n=4) without changing the reversal potential. Fatty acid-induced suppression of I_{NMDA} was attenuated with increasing degrees of unsaturation. Oleic acid (18:1) blocked 36% (n=6) of the I_{NMDA}, while linoleic acid (18:2) and docosahexaenoic acid (22:6) had little effect. In contrast, arachidonic acid (20:4) which increases membrane fluidity, enhanced I_{NMDA} (143 ± 9% increase, n=6). The lipoxygenase inhibitor NDGA (10 μM), cyclooxygenase inhibitor ibuprofen (50 μM), mixed inhibitor ETYA (20 μM), or the kinase inhibitor, staurosporine (1 μM) failed to prevent arachidonic acid-induced potentiation. Diacylglycerol (50 μM; n=3) which decreases membrane fluidity, blocked 31% of I_{NMDA}. We are attracted to the idea that degree of unsaturation or impact on membrane fluidity may be important determinants of the effect of fatty acids on NMDA current. Fatty acid-induced modulation of NMDA receptor current may influence the function of this receptor in physiological or disease states. Supported by NIH grants NS 30337 (DWC) and AG00599-01A1 (LLD).

384.18

INCREASED EXPRESSION OF NMDA RECEPTOR SUBUNIT mRNA AFTER MK-801 TREATMENT IN NEONATAL RATS. M.A. Wilson*, S.L. Kinsman and M.V. Johnston. Neuroscience Laboratory, Kennedy Krieger Research Institute and Dept. of Neurology, Johns Hopkins University, Baltimore, MD 21205

Treatment with excitatory amino acid receptor antagonists has been shown to reduce neuronal damage in experimental models of brain injury. However, pretreatment of rats with the NMDA antagonist MK-801 causes a subsequent increase in vulnerability to excitotoxic injury and an increase in NMDA receptor binding (McDonald et al, *Neurosci.* 38:103), suggesting an underlying alteration in receptor regulation. Kinetic analysis of this phenomenon in cultured cortical neurons indicates that a change in receptor density occurs after MK-801 exposure (Williams et al., *Mol. Pharm.* 42:147). We have used *in situ* hybridization methods to determine whether mRNA for the NMDA receptor subunits is increased after MK-801 treatment. Rat pups were treated with MK-801 (1 mg/kg, s.c.) on postnatal day 6 and killed 24 hrs later or treated on P7 and killed 2 hrs later. *In situ* hybridization with oligonucleotide probes for NR1, NR2A, NR2B, NR2C, and NR2D was used to evaluate expression in cortex, hippocampus and striatum. Two hours after MK-801 treatment, NR1 increased approximately 15-20%, NR2A increased 10-40%, and NR2B increased 0-20% in the areas examined. NR2C is present in limited zones of the cerebellum at this age, and was not analyzed quantitatively. These results indicate that the alteration in binding to NMDA receptors observed after antagonist treatment is due, at least in part, to altered gene expression. Expression of NMDA receptor subunit mRNA increases within 2 hours of MK-801 treatment, and remains elevated 24 hours later. These findings should be considered in devising interventions to prevent or reduce neuronal injury in clinical settings. (Support: NICHD T32 HD07414, NINDS NS28208, NINDS K08 NS01455)

384.19

***N*-METHYL-D-ASPARTATE RECEPTORS IN THE RAT BRAIN FOLLOWING OLFACTORY BULBECTOMY: AUTORADIOGRAPHIC AND BEHAVIORAL STUDIES.** T. Dennis*, V. Beauchemin and N. Lavoie, Neurobiological Psychiatry Unit, McGill University, Montréal, Canada.

Olfactory bulbectomy (OBX) in rodents induces a variety of neurochemical and behavioral alterations unrelated to anosmia. We have demonstrated that OBX induces an antidepressant-reversible up-regulation of cortical $GABA_A$ receptors. Recent observations have shown that increases in NMDA receptor activity may regulate $GABA_A$ receptor subunit mRNA expression and binding levels. Such evidence suggests that the $GABA_A$ up-regulation observed after OBX could be secondary to enhanced glutamatergic activity.

We investigated the time course of the effects of OBX on [^{125}I]iodo-MK-801 binding to NMDA receptor complex in brain tissue slices of rats 1, 2, 3 and 4 weeks after OBX using quantitative autoradiography. Persistent increases were observed in the prefrontal and piriform cortices, anterior cortical and lateral amygdaloid nuclei and anteroventral thalamic nucleus throughout the time period investigated. Significant decreases were also observed 3 weeks after OBX in frontal and parietal cortices, posteromedial cortical and dorsal endopiriform amygdaloid nuclei, lateral hypothalamus and hippocampus. Behavioral studies of open-field activity showed that the administration of MK-801 (0.2 mg/kg, i.p.) to naive rats induces hyperactivity, circling and hyperthermia. One month after surgery, OBX rats displayed a markedly decreased responsiveness to MK-801 for both behavioral activation and hyperthermia. This decreased responsiveness to MK-801 is consistent with the apparent decreases in the number of NMDA receptors disclosed in our autoradiographic studies. This could result in a reduced synaptic plasticity and may be responsible for the impaired learning displayed by OBX rats.

384.20

DIFFERENTIAL ALTERATIONS OF IGF-I, IGF-II AND INSULIN BINDING SITES IN THE HIPPOCAMPAL FORMATION FOLLOWING SYSTEMIC INJECTION OF KAINIC ACID TO NEWBORN RATS. N.P.V. Nair*, S. Doré, S. Kar, and R. Quirion. Douglas Hosp. Res. Ctr., McGill University, Montréal, Québec, Canada, H4H 1R3.

The insulin-like growth factors (IGF-I and IGF-II) and insulin are localized in distinct brain regions and their respective functions are mediated by specific receptors. High concentrations of binding sites for these growth factors are discretely distributed throughout the brain, including the hippocampal formation. Functionally, IGFs and insulin, in addition to their growth promoting actions, are considered to play important roles in normal cell functions as well as in response to pharmacological or surgical manipulations. Previously, we have shown that systemic injection of kainic acid (KA) to adult rats altered IGF binding sites in discrete layers of the hippocampal formation (Srivastava *et al*, Soc. Neurosci. Abst, 1993). Since IGFs and insulin play important roles during development, the present study was designed to evaluate the response of IGF and insulin binding sites at different times following systemic injection of KA to newborn rats. KA was injected to post-natal day 1 pups (5mg/kg; i.p.) and ^{125}I-IGF-I, ^{125}I-IGF-II and ^{125}I-insulin binding sites were studied at different time periods (7, 14, 21, 28 and 35 days) using quantitative receptor autoradiography. In normal neonatal hippocampus, ^{125}I-IGF-I binding sites are concentrated primarily in the dentate gyrus (DG) and the CA2-CA3 sub-fields whereas ^{125}I-IGF-II binding sites are discretely localized to the pyramidal layer and the granular layer of the DG. ^{125}I-insulin binding sites are mostly present in the molecular layer of the DG and the CA1 sub-field. Following KA injection, the level of IGF-I binding sites was increased on days 14 and 21 before returning to normal values at later times. In contrast, IGF-II and insulin binding sites decreased from day 7 to 35 in post-natally treated rats. Taken together, these results provide further evidence for the distinct existence of IGF-I, IGF-II and insulin binding sites in the rat hippocampal formation. The transient increase in IGF-I binding sites at days 14 and 21 may relate to glial cell proliferation caused by the KA treatment. (Supported by MRCC and The Alzheimer Society of Canada)

384.21

EFFECT OF OVARIECTOMY AND ESTRADIOL REPLACEMENT THERAPY IN RATS ON SUBSTANTIA NIGRA $GABA_A$ RECEPTOR BINDING. R. Bossé and T. Di Paolo* . School of Pharmacy, Laval University, Québec, Québec G1K 7P4 and Department of Molecular Endocrinology, Laval University Medical Center, Sainte-Foy, Québec, G1V 4G2, CANADA

Many of the basal ganglia neurons are GABAergic and are probably implicated in the development of dyskinesia. GABAergic systems, are also known to be modulated by estrogens. Furthermore, tardive dyskinesia is more important in women than men and significantly so in the age group over 50 years. To investigate the possible involvement of $GABA_A$ receptors in the behavioral changes occuring at menopause, we investigated long-term ovariectomy (LT-OVX) as a model of decreased gonadal function associated with menopause. The effect of short-term ovariectomy (ST-OVX, 2 weeks) and long-term (LT-OVX, 3 months) and its possible antagonism with a 17-ß estradiol treatment (10 μg E_2, b.i.d., 2 weeks) on substantia nigra $GABA_A$ receptors in rats was investigated. Female Sprague-Dawley rats were divided into 5 groups: (1) intact rats at random stages of the estrous cycle (CTRL), (2) ST-OVX,(3) ST-OVX+E_2, (4) LT-OVX and (5) LT-OVX+E_2.Quantitative autoradiography of 10 μm brain slices at two rostro-caudal levels was performed using [^{3}H]Flunitrazepam (10 nM) to label the benzodiazepine site associated with $GABA_A$ receptors. ST-OVX and LT-OVX led to an increase of respectively 20 and 50 % of $GABA_A$ receptors compared to intact controls (330-460 fmol/mg tissue). A two week E_2 treatment before sacrifice lowered the $GABA_A$ receptor densities toward control levels, reversing the ST-OVX as well as the LT-OVX. These results suggest supersensitivity of the substantia nigra to GABAergic inputs as a result of gonadal hormone withdrawal which may predispose to dyskinesia. *Supported by the M.R.C. of Canada.*

384.22

NEURAL PLASTICITY INDUCED BY EPILEPTIFORM ACTIVITY OF THE RAT MOTOR CORTEX INJECTED WITH TETANUS TOXIN: MAPPING OF CaM KINASE II, GAD, $GABA_A$ RECEPTOR SUBUNIT mRNAs AND *C-FOS* IMMUNOREACTIVITY. F. Liang* and E.G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717

To study activity-dependent plasticity in response to epileptiform activity, tetanus toxin (2-6 ng) was injected into the forelimb area of the rat motor cortex. One to 14 days later, the animals were perfused and sections of brain processed by *in situ* hybridization for Ca^{2+}/calmodulin-dependent protein kinase II α subunit (CaM kinase IIα), glutamic acid decarboxylase (GAD), $GABA_A$ receptor subunit (α1, β2, & γ2) mRNAs, and by immunocytochemistry for *c-fos* oncoprotein. CaM kinase IIα mRNA was down-regulated in the injected cortex, whereas GAD mRNA was up-regulated in bilateral motor cortices, as were the α1 and γ2 subunit mRNAs of the $GABA_A$ receptor. No change in mRNA level of β2 subunit of the receptor was detected. *C-fos* immunoreactivity was markedly up-regulated in bilateral motor cortices. Primary and secondary somatosensory cortices, rostral forelimb motor area, cingulate and insular cortices also showed intense *c-fos* labeling bilaterally, especially in layers II, III and VI. Subcortical structures exhibiting enhanced *c-fos* immunoreactivity included hippocampus, dentate gyrus, caudate-putamen, anterior olfactory nucleus, amygdala, pontine nuclei and cerebellar cortex (granular layer). No *c-fos* labeling was found in the thalamic reticular, ventrolateral and ventromedial nuclei. These results indicate that tetanus toxin induced epilepsy promotes differential plastic changes in multiple form of gene expression affecting both pre- and post-synaptic components of distributed brain structures and neural circuits. Supported by NIH grant NS21377.

384.23

DOPAMINE (DA) RECEPTOR-C-FOS COUPLING IN THE DA DEAFFERENTED AMYGDALOID COMPLEX. T.Ely*, R. Gross and C. Kilts, Emory University School of Medicine, Atlanta, GA 30322.

The autoradiographic localization of DA receptors in the rat amygdaloid complex indicated a topographic, nonoverlaping distribution of the D_1 and D_2 receptor subtypes in its component nuclei and subnuclear zones (Synapse 11:146, 1992). The functional compartmentation of amygdaloid DA receptors and their comparison to striatal DA receptors was further assessed by the effect of a unilateral 6-OHDA lesion of the MFB on the response of fos-LI in central (Ce) and basolateral (BL) amygdaloid nuclei to D_1, D_2 or D_1/D_2 receptor activation. The D_1 agonist SKF 38393 (3 mg/kg sc) produced a marked increase in fos-LI in the medial and dorsolateral caudate nucleus; the effect of SKF 38393 was enhanced greatly by the coadministration of quinpirole (0.3 mg/kg). Quinpirole did not induce fos-LI when administered alone. Though quantitatively smaller, similar effects of D_1, D_2 and D_1/D_2 receptor activation were observed in the core and shell of the nucleus accumbens and olfactory tubercle ipsilateral to the side of the MFB lesion. A strikingly different pattern of effects of activation of DA receptor subtypes was observed in the DA deafferented amygdaloid complex. Specifically, the administration of apomorphine (0.3 mg/kg), SKF 38393, or the combination of quinpirole and SKF 38393 increased fos-LI in BL, though no differences in drug effects were noted between the ipsilateral and contralateral (intact) BL. These preliminary results suggest that D_1 receptors in BL induce fos expression by an intracellular pathway that is not upregulated in response to deafferentation. The apparent lack of effect of D_2 receptor activation on the D_1 response is consistent with the negligible localization of D_2 receptors in BL. In contrast, the increase in fos-LI in Ce resulting from D_1/D_2 receptor activation was greater in the lesioned versus intact Ce.

384.24

Down-regulation of neurotransmitter receptors and up-regulation of neurofilament phosphorylation in the lateral geniculate nucleus of the adult cats after visual deafferentation. Y.L. Liu*, R. Douglas and M.S. Cynader. Department of Ophthalmology, University of British Columbia, 2550 Willow Street, Vancouver, B.C., Canada. V5Z,3N9

Several progressive neurodegenerative disorders such as Parkinson's disease and Alzheimer disease have been associated with abnormal neurotransmitter receptor expression and neurofilament distribution. To test the hypothesis that the redistribution and regulation of neurotransmitter receptors and cytoskeleton protein phosphorylation may trigger early neurodegenerative process induced by input deprivation, we investigated the response of several signal transduction and cytoskeletal markers to removal of retinal input in the cat lateral geniculate nucleus. Nine neurotransmitter receptors, including α and ß-adrenergic receptors, muscarinic acetylcholine receptors, as well as L-type calcium channel, protein kinase C and phosphorylated neurofilaments were examined autoradiographically and immunocytochemically in the lateral geniculate nucleus(L.G.N) of eight adult cats at various times after monocular visual deafferentation. While α and ß-adrenergic receptors, and muscarinic acetylcholine receptors were down-regulated in the deafferented layers of the L.G.N. as early as 8 days after enucleation, phosphorylated neurofilaments were increased in the same layers as early as 4 days after enucleation. These results may suggest that the early changes in density of adrenergic and muscarinic acetylcholine receptors and phosphorylated neurofilament are involved in the neurodegenerative process induced by input restriction, and may be useful as markers for neurodegeneration.

384.25

ANALYSIS OF PHOSPHORYLATION SITES INVOLVED IN THE DEPOLARIZATION-DRIVEN INACTIVATION OF MYOGENIN. C.-T. Su, C.-F. Huang and J. Schmidt*. Dept. of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY 11794.

Activation of nuclear protein kinase C (PKC) is an integral element in the signaling pathway coupling the depolarization of the plasma membrane to the inactivation of genes coding for acetylcholine receptor (AChR) subunits. Since PKC activation is immediately followed by gene inhibition we assume that a transcription factor is directly targeted by the kinase. The muscle-specific expression of AChR and the presence and functional significance of E boxes in AChR subunit promoters strongly suggest that myogenic determination factors are involved. Among them myogenin is a good candidate because its activation and inactivation kinetics resemble that of the endogenous transactivator. Threonine 87 in mouse myogenin has been shown to be a target for PKC, but since it is part of the DNA binding domain it may not be accessible to the kinase in the DNA-bound factor. To determine the phosphorylation site responsible for the rapid inactivation by PKC, we have systematically replaced all eight potential PKC targets in the chicken myogenin molecule and analyzed the PKC sensitivity of the mutant factors. Results of this analysis will be presented.

HYPOTHALAMIC-PITUITARY-ANDRENAL AXIS REGULATION: STRESS STUDIES

385.1

STRESS-INDUCED TRANSCRIPTIONAL ACTIVATION OF THE CORTICOTROPIN-RELEASING FACTOR GENE PRECEDES IMMEDIATE-EARLY GENES RESPONSES IN THE PARAVENTRICULAR NUCLEUS. K.J. Kovacs* and P.E. Sawchenko. Institute of Experimental Medicine, Budapest, H-1083 Hungary, and The Salk Institute, La Jolla, CA 92037

Exposure to ether vapor for 5 min results in rapid activation of the hypothalamo-pituitary-adrenal (HPA) axis in rats, with peak circulating levels of ACTH and corticosterone seen at 5-10 min and 15-30 min, respectively, after the challenge. This model was used to provide insight into the sequence of molecular events underlying the stress-induced enhancement of corticotropin-releasing factor (CRF) expression in hypophysiotropic neurons of the paraventricular nucleus of the hypothalamus (PVH). Animals were sacrificed at intervals ranging between 5 min and 4 hr after exposure to ether, or no manipulation (control). Series of sections through the hypothalamus from each animal were prepared for hybridization histochemical localization of mRNAs encoding the immediate-early genes, *c-fos* and NGFI-B, and heteronuclear RNA (hnRNA) encoding CRF, all using ^{35}[S]-labeled cRNA probes. An additional series from each animal was prepared for immunoperoxidase localization of Fos protein. Non-stressed control rats displayed no consistent expression of any of the markers of interest in the PVH. A 5 min exposure to ether resulted in robust induction of both *c-fos* and NGFI-B mRNAs whose expression was largely limited to the dorsal medial parvocellular part of the nucleus. Both immediate-early gene mRNA responses were detectable at 30 min, and maximal at 60 min, following the challenge. Induction of a Fos protein response was predictably delayed, appearing initially at 1 hr and attaining peak levels by 2 hr, following exposure to ether. By contrast, nuclear signal for CRF hnRNA was seen as early as 5 min after stress in the parvocellular PVH. The most robust response, in terms of both signal intensity and cell number, was seen at 30 min following exposure to ether, and no specific signal was detected in rats sacrificed 1 hr after the challenge. The fact that the ether-induced transcriptional activation of the CRF gene precedes the maximal immediate-early gene responses suggests that these transcription factors are not directly involved in initiating stress-induced up-regulation of CRF expression in hypophysiotropic neurons.

385.2

HIPPOCAMPAL CHOLINERGIC BLOCKADE ELEVATES STRESS-INDUCED CORTICOSTERONE (CORT) SECRETION. S. Bhatnagar*, J.W. Smythe and M.J. Meaney. Depts. of Neurology & Neurosurgery, and Psychiatry, Douglas Hosp. Res. Ctr., McGill Univ., Montreal, Quebec, Canada H4H 1R3.

The hippocampal formation (HPC) regulates stress-induced hypothalamic-pituitary-adrenal (HPA) activity. Lesions of the HPC result in hypersecretion of ACTH and CORT in response to a variety of stressors, suggesting that the HPC plays an inhibitory role in the regulation of HPA activity. Septal cholinergic projections innervate all fields and subfields of the HPC and increase HPC excitability. These cholinergic inputs are thought to regulate sensory processing of stressful environmental events, as suggested by studies on HPC electrophysiology. We investigated whether HPC cholinergic systems might underlie HPC control of HPA function. Adult rats were implanted bilaterally with cannulas aimed at the molecular layer of the dentate gyrus. Scopolamine HCl (SCOP;10 ug/ul; 3 ul total) was administered into the HPC 20 min prior to onset of restraint stress. Plasma CORT levels were measured immediately following and up to 2 hours following termination of restraint. Rats administered SCOP (n=6) hypersecreted CORT at 0 min and showed elevated levels 2 hours following termination of restraint, relative to vehicle injected rats (n=6). These data suggest that HPC cholinergic systems actively inhibit HPA activity. Thus, compromising HPC cholinergic inputs may interfere with sensory processing and alter the ability of the HPC to exert negative-feedback control of HPA activity in response to stressful stimuli. (Supported by MRCC and FRSQ)

385.3

A CENTRAL NITRIC OXIDE SIGNALING MECHANISM MEDIATES STRESS-INDUCED CORTICOSTERONE RELEASE. M. Rackover*, D. Funk and S. Amir. CSBN, Concordia University, Montreal, Quebec, Canada.

Nitric Oxide synthase (NOS) containing neurons have been localized in the hypothalamic paraventricular nucleus (PVN), and in vitro studies suggest that nitric oxide (NO) is involved in the release of corticotrophin releasing hormone (CRH) from PVN neurons. Because CRH plays a role in the stressed-induced release of pituitary adrenocoticotrophin (ACTH) and corticosterone (CORT) secretion from the adrenal cortex, we investigated the effects of blockers of NO synthesis on plasma CORT levels following immobilization stress in rats. Immobilization stress for 60 min caused a significant increase in plasma CORT levels in saline-pretreated rats (1 ml/kg i.p., 45 minutes prior to restraint), and was associated with induction of c-fos protein in a proportion of NOS-containing (NADPH diaphorase-stained) PVN cells. Pretreatment with the NOS blocker N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg i.p.), but not with the inactive isomer N^G-nitro-D-arginine (D-NAME, 10 mg/kg i.p.) significantly attenuated the stress-induced rise in plasma CORT. Similarly, pretreatment with 7-Nitroindalzole (10 mg/kg i.p.), an NO synthesis blocker devoid of peripheral cardiovascular actions attenuated the stress-induced increase in plasma CORT. Finally, injection of L-NAME (100μg) into the third cerebral ventricle attenuated the stress-induced rise in plasma CORT. The results suggest a role of a central NO signaling mechanism in the neuroendocrine response to stress.

385.4

INVOLVEMENT OF C-FOS IN DEVELOPMENTAL REGULATION OF CRH GENE EXPRESSION BY COLD-STRESS. S.-J. Yi, P.S. Gott and T.Z. Baram. Neurology, Childrens Hospital Los Angeles and University of Southern California, Los Angeles, CA 90027.

C-fos expression is induced by a variety of stimuli, including stress, to regulate transcription of target genes. These genes often contain cyclic AMP-responsive elements (CREs). The corticotropin releasing hormone (CRH) gene promoter contains a CRE, and is regulated by the cAMP-dependent cascade. We postulated that cAMP-mediated induction of CRH gene promoter involved upregulation of c-fos. We previously found that cold-stress induction of CRH gene expression is developmentally regulated: CRH-mRNA abundance increased by 4 h after cold-stress on postnatal day 9 (PND9) or 16, but not on PND6. In this study we tested the hypothesis that cAMP infusion on PND6 would induce both c-fos and CRH gene expression. Rats were subjected to cold stress-anesthesia, and implanted unilaterally with a cannula directed to the paraventricular nucleus (PVN). Rats were sacrificed 4h after saline or dibutyryl cAMP (db-cAMP, 1umole) infusion via the cannula. Fos-like immunoreactivity (Fos-IR) and CRH-mRNA abundance were determined in PVN and limbic structures involved in stress mediation. PVN-CRH-mRNA abundance was enhanced by cold stress alone or with db-cAMP on PND9. On PND6, db-cAMP infusion increased CRH-mRNA, while cold alone did not. In hippocampus, CRH-gene expression was upregulated by db-cAMP+cold, but not by cold alone in both ages. CRH-mRNA abundance in the central amygdaloid nucleus (ACE) was increased by db-cAMP infusion on PND9. Cold stress induced hippocampal Fos-IR, with a further increase by db-cAMP in both ages. Fos-IR in ACE was enhanced following db-cAMP infusion only on PND9. An additive effect of cold stress and db-cAMP on plasma corticosterone occurred on both PND6 and PND9. These data support a role for immediate early genes and cAMP-in stress-induced upregulation of CRH-gene expression in limbic structures.

385.5

DIFFERENTIAL REGULATION OF EGF AND TGFα mRNA IN RAT PITUITARY AND HYPOTHALAMUS INDUCED BY STRESSES. X. Fan, G. T. Nagle, T. J. Collins and G. V. Childs*. Department of Anatomy and Neurosciences, University of Texas Medical Branch at Galveston, Galveston, TX 77555

Evidence has shown that both epidermal growth factor (EGF) and transforming growth factor α (TGFα) are present in the anterior pituitary and hypothalamus, and EGF can influence the function of pituitary cells, particularly corticotropes in vivo and in vitro. However, little is known about their exact functional roles in the pituitary and hypothalamus. This study was designed to determine if EGF and TGFα mRNA are expressed in the rat pituitary and hypothalamus and how stress conditions such as cold, ether or restraint affect their expression. A sensitive mRNA detection method, ribonuclease protection assay (RPA), detected both EGF and TGFα mRNA in the rat pituitary and hypothalamus. Reverse transcriptase-polymerase chain reaction (RT-PCR) also showed the presence of EGF and TGFα mRNA in these and several other rat tissues (submandibular gland, liver, kidney, lung, cerebral cortex and testis). No TGFα mRNA was found in the kidney however. The EGF mRNA is upregulated in the pituitary after 30 min acute cold stress (CS) and rat holder-restraint stress (RS) but not after 30 min ether stress (ES), novelty stress (NS) or tape-restraint stress (TS). Longer cold exposures showed that expression of EGF mRNA was downregulated after 60 min cold stress and then upregulated after 180 min cold exposure. In contrast, EGF mRNA in the hypothalamus is not responsive to either acute stresses or longer periods of cold exposures. No significant change in TGFα mRNA expression was detected in both pituitary and hypothalamus after acute and longer cold stresses. The results suggested that the change in pituitary EGF mRNA in response to stresses varies according to the type of stress and may be under the influence of glucocorticoid feedback. Preliminary evidence suggests that the corticotrope may be one of the cells that express EGF mRNA in the pituitary. Our data further support that EGF is a stimulator of HPA axis in primates. These stress-induced changes in EGF mRNA suggest that it may play an autocrine role during some stresses. Supported by NSF # IBN-9117897.

385.6

MODULATION OF CELLULAR CARDIOVASCULAR COMPONENTS AND THE HPA AXIS IN CONGENITAL LEARNED HELPLESS RATS EXPOSED TO EARLY STRESS. J. A. King*[1], E. Edwards[2], S. L. Abend[1] and J.C.S. Fray[3] Depts. Psychiatry[1] and Physiology[3], Univ Massachusetts Med Ctr, Worcester, MA 01655, Dept. Pharmacology[2], Univ. Maryland, Baltimore, MD 21201

Learned helpless behavior has been successfully bred in rats and designated as an animal model of human depression and/or anxiety. Since congenital learned helpless (cLH) animals have an altered response to stress in adulthood, we examined the effects of early stressors (at postnatal day 7, 14, & 21) on the hypothalamic pituitary adrenal (HPA) axis and the cardiovascular system. The functioning of the HPA axis was monitored through changes in adrenocorticotropin (ACTH) plasma levels in the adult animals after acute exposure to maternal deprivation (MD) stress. Cardio-vascular functioning was assessed by plasma renin activity(PRA) and renin secretory competence at the intracellular level. Unstressed cLH animals had basal levels of ACTH and PRA that were similar to control animals (Sprague Dawley animals not stressed during development). However, the renin specific activity was higher in the secretion-ready vesicles of the cLH rats suggesting that even in the absence of stressors cLH rats have a system that is primed for cardiovascular responsiveness to stress, without activation of the HPA axis. cLH animals (adults) that were MD on postnatal day 14 had lower plasma levels of ACTH than control, while exhibiting a 98% increase in PRA, again pointing to a readiness potential in terms of cardiovascular functioning. The most robust effect of MD on the cLH adult animals was apparent after acute stress on postnatal day 14 as opposed to postnatal days 7 and 21. In contrast, there was a step-wise increase in ACTH plasma levels in the congenital non learned helpless (cNLH) rats with age of acute presentation of MD stress. The above results suggest that there are longterm changes in both the HPA axis and the cardiovascular system in cLH animals after acute exposure to a postnatal stressor.

385.7

IN VIVO QUIPAZINE INCREASES GLUCOCORTICOID RECEPTOR EXPRESSION IN THE HIPPOCAMPUS OF NEONATAL PIGS. S. Weaver[1], D. O'Donnell[2], A. Schaefer[1], L. Thibault*[3], and M.J. Meaney[2]. [1]Dept. Animal Sci., Univ. of Alberta, Edmonton, AB, Canada. [2]Douglas Hosp. Res. Ctr., Montreal, PQ, Canada. [3]Sch. Diet/Hum. Nutr., McGill Univ, Montreal, PQ, Canada.

The glucocorticoid receptor (GR) in the hippocampus is involved in the negative feedback effect of glucocorticoids in rats. Neonatal handling results in increased GR binding capacity in the hippocampus and frontal cortex and an attenuation of the stress response in rats. The postulated mechanism is via increased serotonin (5-HT) turnover in the hippocampus (Mitchell et al., 1990, J. Neurosci. **10**:1745). Little is known about the presence or development of GR in porcine brain. Stress is associated with dramatic costs in the swine industry due to impaired animal growth, reproduction, and meat quality and reducing its impact represents significant savings. In the current study we examined the effect of the 5-HT agonist quipazine on GR levels in specific brain sites using Western blotting with a commercially available GR antibody (Affinity BioReagents). Pigs were injected with 1 mg/kg quipazine dissolved in saline (1 ml/kg) or saline from postnatal day 1 to 14. The animals were sacrificed on day 13, and the pituitary gland, frontal cortex, hypothalamus, and hippocampus were collected. Increased levels of immunoreactive GR were detected in the hippocampus of quipazine versus saline treated pigs. We are currently analyzing the remaining tissues to determine whether the changes in GR expression are confined to the hippocampus and frontal cortex as is the case for neonatally handled rats.

385.8

PRENATAL STRESS-INDUCED CHANGES IN THE ACTIVITY OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS OF ADULT OFFSPRING DEPEND ON MATERNAL CORTICOSTERONE. S. Maccari*, A. Barbazanges, P.V. Piazza, H. Simon, M. Le Moal. Psychobiologie des Comportements Adaptatifs, INSERM U.259, Université de Bordeaux II, 33077 Bordeaux Cedex, France.

The development of the organism is subjected to critical influences during the perinatal period. Prenatal stress can have long-term behavioral effects, such as an increased emotional reactivity and a higher vulnerability to self-administer drugs. Changes in the activity of the hypothalamo-pituitary-adrenal (HPA) axis could be one of the biological substrates of the long-term effects of prenatal stress. We have previously shown that, in adult male rats, prenatal stress prolongs stress-induced corticosterone-secretion. Such an effect is probably mediated by a decrease in the number of central corticosteroid receptors. The mechanisms by which prenatal stress could exercise its long term effects on the activity of the HPA axis are unknown. In the present experiment we tested if stress-induced increase in maternal glucocorticoids, which cross the placental and blood-brain barriers reaching the foetus, may play a role. For this purpose we investigated the effects of adrenalectomy and/or corticosterone injections to the mother on the outcome of prenatal stress on the activity of the HPA axis of adult (3 months of age) male and female offspring. Repeated restraint during the last week of pregnancy was used as prenatal stress. In adult male and female offspring of mothers with an intact HPA axis, prenatal stress prolonged stress-induced corticosterone-secretion and decreased central corticosteroid receptors. Such effects of prenatal stress depended on maternal corticosterone-secretion: 1) maternal adrenalectomy protected the offspring from the effects of prenatal stress on both corticosterone-secretion and corticosteroid receptors; 2) injections of corticosterone to the mother had the same effects of prenatal stress in adult offspring. In conclusion, stress-induced increase in maternal levels of corticosterone may be one of the mechanisms by which prenatal stress exercises its long-term effects on the activity of the HPA axis.

385.9

EFFECTS OF STRESS ON CRF AND ITS RECEPTOR GENE EXPRESSION IN THE BRAIN OF SPONTANEOUSLY HYPERTENSIVE RATS. Guy Drolet* and Serge Rivest, Laboratoires de Neurobiologie & d'Hypertension et d'Endocrinologie Moléculaire, Centre de Recherche du CHUL, Université Laval, Québec, Canada, G1V 4G2.

The present study investigated the influence of immobilization stress on corticotropin-releasing factor (CRF) system in the brain of spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. Male rats (13 weeks) were rapidly anesthetized and perfused with 4% paraformaldehyde before stress, and 30, 60, and 180 minutes after the beginning of the immobilization session which lasted 60 min. Brains were cut in 30 μm sections and mounted on poly-L-lysine-coated slides. mRNAs encoding CRF and CRF-receptor (CRF-R, kindly provided by Dr. W. Vale, The Salk Institute) were assayed by *in situ* hybridization using a ^{35}S-labeled riboprobes. Strong basal levels of CRF mRNA were found consistently in both strains in the parvocellular division of the paraventricular nucleus (PVN), the central nucleus of the amygdala (CEA), the bed nucleus of the stria terminalis (BST), the Barrington/lateral dorsotegmental (Bar/LDT) area, and the ventrolateral medulla (VLM). However, higher basal expression of CRF mRNA was detected in the PVN, CEA and BST of SHR rats. Immobilisation stress induced notable increase of CRF transcripts in the PVN, CEA, BST of WKY, but this phenomenon was not observed in SHR rats. In this strain only, stress raised the levels of CRF mRNA in VLM and Bar/LDT. Although the PVN did not exhibit detectable levels of CRF-R mRNA before immobilization, 2 hrs after the stress session, a positive signal for CRF-R mRNA was observed in the dorsomedial parvocellular division of this nucleus in both strains. Interestingly, stress-induced CRF-R transcription in the PVN seemed lower in SHR than in WKY rats.

These results provide evidence that central CRF system may respond differently to stress in various sites, particularly in the PVN of SHR and WKY rats. These functional differences may contribute to the physiopathology of hypertension of the SHR rats. Support: MRC, HSFQ and FRSQ.

385.10

IMMUNE CHALLENGE INDUCES CRF RECEPTOR GENE EXPRESSION IN THE RAT PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. Serge Rivest*, Laboratoire d'endocrinologie moléculaire, Centre de recherche du CHUL, Québec, Canada, G1V 4G2.

The present study investigated the effect of intraperitoneal (i.p.) administration of the endotoxin lipopolysaccharide (LPS) on the genetic expression of CRF receptor (CRF-R) in the brain of conscious Sprague-Dawley rats. One, 3, 6, 9, and 12 hrs after a single i.p. injection of either the LPS (250 μg/100 g of BW) or the vehicle solution, adult male rats were anesthetized and rapidly perfused with 4 % paraformaldehyde. Frozen brains were mounted on a microtome and were cut from the olfactory bulb to the medulla in 30 μm coronal sections. mRNA encoding the rat CRF-R was assayed by *in situ* hybridization histochemistry using a ^{35}S-labeled riboprobe. *The rat CRF-R cDNA in Bluescript vector (prCRF-R PP1.3-BS, 4.3 kb) was kindly provided by Dr. W. Vale (The Salk Institute, La Jolla, CA).* A strong basic levels of CRF-R transcripts were observed in several define regions of the brain such as the red nucleus, the pontine gray, the cerebellum, the laterodorsal tegmental nucleus, the caudal division of the zona incerta, the endopiriform nucleus and in various layers of the cortex. A low to moderate signal was also detected in multiple sites including the medial septal nucleus, the nucleus of the diagonal band, the supraoptic nucleus, the nucleus prepositus, and the medial vestibular nucleus. While vehicle-treated rats did not exhibit detectable signal of CRF-R mRNA in the paraventricular nucleus (PVN), CRF-R gene transcription was highly stimulated by LPS administration in this hypothalamic structure. Indeed, the CRF-R mRNA signal was positive in the dorso-medial parvocellular PVN 3 hrs after LPS injection, strong and maximum at 6 hrs postinjection, and declined 9 and 12 hrs after the treatment. Systemic endotoxin did not appear to modulate the expression of CRF-R gene in other regions suggesting that this type of stress can induce a selective activation of CRF-R within the parvocellular PVN. It is thus possible that CRF plays a role in the control of the biosynthesis and/or the release of neuroendocrine CRF during immune challenge. *(Supp. by the FRSQ)*

385.11

DEVELOPMENTAL ONSET OF HYPOTHALAMO-PITUITARY-ADRENAL AXIS RESPONSIVENESS TO NGF IN RATS. G.Taglialatela* and J.R.Perez-Polo, Dept. of HBC&G, UTMB Galveston, Texas.

Nerve growth factor (NGF) is involved in the regulation of the hypothalamo-pituitary-adrenal axis (HPAA). Peripherally-injected NGF stimulates the activity of the HPAA, resulting in a long-lasting increase in plasma corticosteroid levels, whereas serum NGF levels increase in response to stressful stimuli. Autoradiographic studies after injection of ^{125}I-NGF indicated that a possible site for this NGF action may be the hippocampus, a limbic structure involved in the modulation of the HPAA. Since newborn rats acquire a full stress response capability after 14 days of age, consistently with completeness of hippocampal maturation, we studied the effect of injected NGF on HPAA activity in pups at different days of postnatal life. We did not observed any NGF-induced increase in plasma corticosterone levels in pups of 3, 8, and 11 days of age, while a significant response of the HPAA to NGF was attained in 15 and 22 day old pups as well as in adult rats. On the other hand, both 8 day old pups and 2.5 month old animals responded to exogenously administered CRH.

These data would suggest that the onset of the HPAA responsiveness to NGF takes place concomitantly to complete maturation of the hippocampus and therefore they support the hypothesis that serum NGF participate in the stress activation of the HPAA by acting directly at the hippocampus. (This is publication 18A from USPHS grant PO1 AG10514 awarded by NIA).

385.12

Hypercaloric intake decreases both sympathetic nervous system (SNS) and hypothalamo-pituitary-adrenal (HPA) responses to acute restraint stress. S.F. Akana*, C.J. Horsley, and A.M. Strack, Dept Physiol, UCSF, San Francisco, CA 94143

SNS and HPA activity both increase in response to stress. Brown adipose tissue (BAT) activity, which promotes nonshivering thermogenesis, is an index of SNS activity . We hypothesized that a hypercaloric load would separate basal activity in the two systems and tested whether both stress responses were facilitated due to increased tone in SNS. Therefore, rats were implanted with thermister probes under BAT and were fed ad lib chow and water only (control) or chow & 30% sucrose to drink (sugar) in addition to water. On d5, subgroups of uninstrumented rats were subjected to 30m restraint before decapitation. The instrumented rats were subjected to restraint on d10 while BAT temperature was recorded every 60s. On d5 & 10, sugar rats did not differ from controls in basal activity of the HPA axis, insulin and glucose. On d5, there was no sugar effect on the responses of ACTH or B to restraint. On d10, there was no difference in ACTH response, but a smaller B response at 30m as well as lower integrated B. Both control and sugar rats increased BAT temperature with restraint but sugar rats had a smaller increase than controls. SNS (BAT) activity responds to sugar with chronic elevation of SNS tone similar to HPA responses to chronic stress. In contrast to our initial hypothesis, the chronically sugar-activated SNS hyporesponds to acute restraint in parallel with the HPA hyporesponse in ACTH and B secretion. We conclude that a hypercaloric load acts to switch SNS and HPA responses to acute restraint from high to low amplitude.

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385.13

ADRENALECTOMY DOES NOT ALTER STRESS-INDUCED C-FOS EXPRESSION. D.L. Helmreich*, W.E. Cullinan, S.J. Watson, Mental Health Research Institute, Univ. of Michigan, Ann Arbor MI, 48109

Previously, we determined the pattern of c-fos mRNA expression throughout the brain after stress in order to gain further insight into the identification of the neural circuits mediating stress-induced activation of the hypothalamic-pituitary-adrenal axis. In the present study, to determine what portion, if any, of this widespread expression results from rapid effects of increased glucocorticoid levels after stress, stress-induced c-fos expression was characterized in adrenalectomized (ADX) animals. Male rats underwent ADX (n=4) or sham-surgery (n=4), and 5 days later were subjected to 10 min swim stress. Animals were sacrificed 30 min post-stress onset, and brains were quickly removed and frozen. *In-situ* hybridization histochemistry was used to detect c-fos mRNA throughout the brain, using a 680 nt cRNA probe. We found that the pattern of c-fos induction in the ADX animals was similar to that observed in the sham operated animals; c-fos expression was detected in multiple areas, including the ventral subdivision of the lateral septum, the medial bed nucleus of the stria terminalis, the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN), the dorsomedial nucleus of the hypothalamus, the medial nucleus of the amygdala, and limbic cortices. Additionally, densometric measurements were made to quantify the c-fos response in the PVN and the CA1-2 region of the hippocampus, areas that are believed to be targets of circulating glucocorticoids. We found that ADX did not alter the magnitude of the c-fos response to stress in these areas. In sum, these results suggest that the pattern of c-fos expression observed 30-min post stress is independent of stress-induced increases in circulating glucocorticoid concentrations. This work was supported by grant MH-42251.

385.14

IMMEDIATE EARLY GENE INDUCTION IN RAT FOREBRAIN GABAERGIC NEURONS IN RESPONSE TO AN ACUTE STRESS. W.E. Cullinan* and S.J. Watson, Mental Health Research Institute, University of Michigan, Ann Arbor MI, 48109-0720.

Previous data have suggested that the hypothalamic paraventricular nucleus (PVN) receives a prominent GABAergic input; this innervation has recently been shown to emanate in part from various hypothalamic nuclei and the bed nucleus of the stria terminalis (BST) (Roland and Sawchenko, 1993; Cullinan et al., 1993). These areas have also recently been shown to express immediate early genes in response to acute stress. In the present experiment we examined whether GABAergic neurons in these regions expressed immediate early genes (c-fos, zif/268) following acute swim stress. Rats were subjected to 10' forced swim at 37°C, and were sacrificed 30' post-stress by rapid decapitation. Brains were quickly removed, frozen, and subsequently sectioned. Tissue was processed for dual (nonradioactive-radioactive) *in situ* hybridization histochemistry. GABAergic neurons were marked using digoxigenin-labeled cRNA probes (210 and 564 nts.) for either the M_r 67,000 or M_r 65,000 isoform of glutamic acid decarboxylase (GAD), and immediate early genes were detected with ^{35}S-labeled riboprobes for c-fos (680 nt) or zif/268 (588 nt). Among areas investigated, immediate early genes were induced in GABAergic neurons in the anterior hypothalamic area, dorsomedial hypothalamic nucleus, rostromedial zona incerta, as well as additional hypothalamic loci and the BST. Experiments are underway which combine these data with retrograde neuronal tract-tracing from the PVN.

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385.15

WESTERN BLOT ANALYSIS OF CYTOSOLIC AND NUCLEAR TYPE II CORTICOSTEROID RECEPTOR LEVELS IN RAT BRAIN. R.L. Spencer*, A. H. Miller, H. Moday, B.S. McEwen. Rockefeller Univ. and Mt. Sinai Sch Med, NY, NY 10021.

In vitro studies support a model in which the unoccupied corticosteroid receptor is present in the cytosolic cellular fraction, whereas the hormone activated receptor is present in the nuclear fraction. We have used the western blot procedure to measure cytosolic and nuclear levels of Type II corticosteroid receptors in rat tissues. For western blot analysis, proteins from cytosol or immunoprecipitants of cytosol and nuclear extracts were separated by PAGE and transferred to nitrocellulose. Blots were probed with Type II corticosteroid receptor reactive antibodies (GR#57 or BUGR. Affinity BioReagents), and immunoreactivity was visualized anc quantitated by a chemiluminescence method (ECL, Amersham). Cytosolic Type II receptor binding level and western blot immunoreactivity were measured in cortex or hippocampus from rats that were sacrificed (in the AM) under 4 hormone conditions: 1) adrenal-intact, no stress, 2) 18 h ADX, 3) 5 d ADX, 4) adrenal-intact + 2 h CORT. Both receptor binding and western blot analysis detected parallel changes in Type II receptor levels with treatment. Relative to the no-stress group, there was no change with 18 h ADX, a 60% increase with 5 d ADX (receptor upregulation), and a 70-90% decrease with acute CORT treatment (receptor activation). In recent studies a large increase in nuclear receptor signal was detected by western blot in CORT treated animals. These studies indicate that the western blot procedure can be used to directly measure both the cytosolic and nuclear level of corticosteroid receptors in rat neural tissues, and that cytosolic changes with hormone manipulations parallel those seen with receptor binding. (Supported by MH47674, MacArthur Foundation)

385.16

ACUTE AND CHRONIC INTERMITTENT FOOTSHOCK DIFFERENTIALLY INDUCE FOS EXPRESSION IN THE PARAVENTRICULAR NUCLEUS AND ITS EXTRAHYPOTHALAMIC AFFERENTS. H.-Y. Li*, C.A. Arias and P.E. Sawchenko. The Salk Institute, La Jolla, Ca 92037

Immunolocalization of Fos protein was coupled with immuno- and hybridization histochemistry and/or axonal transport techniques in order to characterize the neural circuits underlying the acute and chronic effects of intermittent footshock on stress-related neuroendocrine neurons. Exposure to a single 30 min footshock session induced maximal Fos-ir in the paraventricular nucleus of the hypothalamus (PVH) at 2 hr after the challenge; at this time point, Fos induction in the parvocellular division of the PVH was localized principally to cells hybridized for corticotropin-releasing factor mRNA, while that in the magnocellular neurosecretory system was detected predominantly in oxytocinergic neurons. Relative to sham-operated controls, adrenalectomized rats replaced with low levels of corticosterone showed enhanced Fos induction in response to acute footshock in all PVH compartments. Extrahypothalamic cell groups displaying prominent Fos induction in response to acute footshock included catecholaminergic neurons in the nucleus of the solitary tract and ventrolateral medulla, the lateral parabrachial nucleus, the ventrolateral periaqueductal gray, and circumscribed aspects of the amygdala, bed nucleus of the stria terminalis, lateral septal nucleus and infra- and prelimbic cortical areas. Rats bearing retrograde tracer deposits in the PVH and sacrificed 2 hr after a single footshock session displayed Fos induction in retrogradely labeled neurons found principally in medullary catecholaminergic cell groups, and secondarily in the lateral septal and medial amygdaloid nuclei. Rats subjected to chronic intermittent stress (2 X 30 min sessions/day for 7 days), and sacrificed 2 hr after the final footshock session, displayed patterns of Fos expression that were similar to those seen acutely. Fos expression in chronically stressed animals sacrificed on the day following the final footshock session was not distinguishable from non-shocked control levels. These results suggest that the patterns of cellular activation seen in rats exposed acutely and chronically to a footshock challenge are similar. Medullary catecholaminergic cell groups are most strongly implicated as candidate afferent mediators of this stressor's effects on endocrine hypothalamic mechanisms.

385.17

MODULATION OF 5-HT$_{2C}$ RECEPTOR mRNA EXPRESSION IN THE HIPPOCAMPUS DURING THE CIRCADIAN RHYTHM AND FOLLOWING STRESS. M.C. Holmes*, K.L. French and J.R. Seckl. Dept. Med., Univ. of Edinburgh, Western Gen. Hosp., Edinburgh, UK.

Serotonin has been implicated in the generation and maintenance of circadian rhythms, as well as the modulation of HPA axis activity to certain stressors. 5-HT$_2$-type (2A and/or 2C) receptors in the hippocampus and hypothalamus may mediate both actions. Using *in situ* hybridization, we examined 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor mRNA expression in the rat hippocampus at the peak and nadir of the glucocorticoid diurnal rhythm. At 08.00h (lights on 07.00-19.00h) 5-HT$_{2C}$ receptor mRNA expression throughout the hippocampus was significantly greater than at 20.00h (CA1 oriens, 184%; CA2, 106%; ventral CA1 147%). 5-HT$_{2A}$ receptor mRNA expression was unchanged. Receptor gene expression was also determined following acute (6h after laparotomy) and chronic (15 days adjuvant-induced arthritis) stress. 6h after acute stress (20.00h), 5-HT$_{2C}$ receptor mRNA expression was significantly elevated in CA2 neurons (86% greater than controls). Chronic arthritis elevated plasma corticosterone, abolishing the circadian rhythm, and 5-HT$_{2C}$ receptor mRNA expression was significantly decreased in CA1 (55%), CA2 (50%) and ventral CA1 (45%) at 08.00h, giving levels of expression similar to the normal diurnal glucocorticoid peak (20.00h). Again, 5-HT$_{2A}$ receptor gene expression was unaltered. Our previous data showed that glucocorticoids decrease hippocampal 5-HT$_{2C}$ receptor gene expression after adrenalectomy. However, the increase in 5-HT$_{2C}$ receptor mRNA expression following acute stress suggests that other factors (perhaps 5-HT) are also important short-term regulators of expression of this gene.

385.18

ANTIDEPRESSANT DRUG TREATMENT REVERSES HPA FEEDBACK *DEFICITS* AND BASAL HPA HYPERACTIVITY IN THE AGED-IMPAIRED RAT. A. Steverman, M. Walker, W. Rowe, S. Sharma*, N. Barden, J.R. Seckl & M.J. Meaney. Douglas Hosp. Res. Ctr., Dept. Psychiatry, McGill Univ., Montreal H4H 1R3 Canada., Dept. Medicine, Univ. Edinburgh, Western Gen. Hosp., Edinburgh, Scotland EH4 2XU, UK and Ctr. Hosp. Univ. Laval, Québec Canada.

Aged, cognitively-impaired (AI) rats show increased basal HPA activity in comparison to both young (Y) and aged, cognitively-unimpaired (AU) rats. These findings are consistent with the idea that glucocorticoids can compromise both the function and survival of hippocampal neurons. In this study AI, AU and Y rats were treated with the antidepressant, desipramine, for 4 weeks following which time we examined basal HPA activity and delayed feedback sensitivity to elevated glucocorticoid levels.

Antidepressant drug treatment reduced PM basal levels of both ACTH and corticosterone (B) in all animals, without affecting AM hormone levels. The effect was greatest in the AI rats, such that differences in PM levels of ACTH and B observed in controls were absent following chronic desipramine treatment. In controls, a bolus injection of B 3h prior to restraint stress completely abolished ACTH responses in AU and Y rats, while in AI rats ACTH responses were only partially reduced. Following chronic desipramine treatment ACTH responses to restraint were completely abolished in AI as well as AU and Y rats. These data suggest that chronic antidepressant drug treatment enhances glucocorticoid negative feedback sensitivity and reduces PM basal HPA activity in AI rats.

These results are consistent with previous findings in young adult animals and point to potential therapeutic approaches in the treatment of HPA dysfunction in the aged. Supported by MRCC and NIA AG90488.

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION II

386.1

DECREASED HIPPOCAMPAL NORADRENALINE DOES NOT AFFECT ELECTRICALLY STIMULATED CORTICOSTERONE RELEASE. W.M.U. Daniels, A. Jaffer, V.A. Russell, S. Daya* and J.J.F. Taljaard Dept. Chem. Path., Univ. Stellenbosch, Tygerberg Hosp., P.O. Box 19113, Tygerberg, 7505, RSA.

Bipolar electrodes were implanted into the CA1 pyramidal cells of the dorsal hippocampus and the effect of electrical stimulation of these cells on corticosterone secretion was investigated in freely moving rats. Histology showed that the electrodes were positioned in close proximity to the CA1 pyramidal cells. Rats that were subjected to electrical stimulation did not only behave differently to their sham stimulated controls, but also had significantly higher plasma corticosterone levels. Prior treatment of rats with DSP$_4$ significantly reduced hippocampal noradrenaline content, but had no effect on electrically stimulated corticosterone release. These data suggested that excitation of CA1 pyramidal cells may lead to increased corticosterone release and that a significant decrease in hippocampal noradrenaline concentration was not able to alter this corticosterone response.

386.2

NEUROTOXIN-STIMULATED HYPOTHALAMO-PITUITARY-ADRENAL (HPA) AXIS FUNCTION IN MICE. M. Carino, B. Giovanbatista, R. Hilal and E. Spinedi*. Neuroendocrine Unit, Department of Neurosciences, IMBICE; 1900 La Plata, Argentina.

The aim of the present study was to elucidate whether snake venom (SV; Sigma Chem. Co., V-7125) is able to stimulate the HPA axis activity when administered ip (25 µg/animal) in adult BALB/c mice of both sexes. Mice were killed at 0.5, 1, 2 or 4 h after SV or vehicle (sample time zero h) administration. Plasma glucose (G), ACTH and corticosterone (B) concentrations were measured. The results indicated that, SV treatment induced a significant ($P<0.05$ vs. 0 h) hyperglycemia at all periods studied with maximal values attained at 2 h after treatment, regardless the sex; the neurotoxin-elicited G release in plasma was significantly ($P<0.05$) higher in females than in males at 0.5, 1 and 2 h after administration. Neurotoxin injection sgnificantly ($P<0.05$ vs. 0 h) enhanced ACTH release in plasma at 0.5 and 1 h after treatment in animals of both sexes, with 1 h-values significantly ($P<0.05$) higher in males than in females; then, plasma ACTH levels recovered the baseline at 2 h in both sexes. SV treatment also significantly ($P<0.05$ vs. 0 h) stimulated B release in plasma in a sexual dimorphic manner at 0.5, 1 and 2 h after treatment; being female values significantly ($P<0.05$) higher than male values. Finally, this dose of SV resulted lethal at 4 h for a 50 % of males and for a 30 % of females. These results indicate that the neurotoxic shock enhances glucose plasma levels and HPA axis activity in a sexual dimorphic fashion. It remains to be determined the mechanisms whereby SV induced such responses, as well as which steps are modulated by endogenous sex hormones. (Supported by CONICET 252 11 35 91).

386.3

HYPOTHALAMIC GALANIN-LIKE IMMUNOREACTIVITY AND ITS GENE EXPRESSION IN RELATION TO CIRCULATING CORTICOSTERONE. A. Akabayashi*, H.J. Chae and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021

The hypothalamic peptides, neuropeptide Y (NPY) and galanin (GAL), known to stimulate feeding behavior, have differential effects on the release of the adrenal steroid, corticosterone (CORT), with NPY enhancing and GAL inhibiting its secretion. In terms of the feedback effects of CORT on endogenous peptide, other studies have shown this steroid to potentiate the production of neuropeptide Y (NPY) in specific hypothalamic areas, namely, the paraventricular (PVN) and arcuate (ARC) nuclei, as well as the locus coeruleus (LC). This investigation examined changes in GAL gene expression and peptide levels in relation to circulating CORT in male, Sprague-Dawley rats. Using radioimmunoassay (n=30, 10/group) and in situ hybridization (n=18, 6/group) techniques, this peptide was examined in 3 groups of rats that received either SHAM surgery, adrenalectomy (ADX), or ADX + CORT replacement. The results showed a clear, site-specific change in GAL in relation to circulating CORT. A loss of CORT after ADX caused a dramatic decline in GAL peptide (-60%, $p < 0.05$) and mRNA levels (-35%, $p < 0.05$) in the ARC and also peptide levels in the median eminence (-40%, $p < 0.05$). Other hypothalamic areas, including the PVN, showed no change. In the brainstem, a similar effect after ADX was seen in the dorsal raphe nucleus, but not the LC. The GAL peptide and mRNA levels in these brain areas of ADX rats were restored by s.c. CORT implants, which had no impact on GAL in other brain sites. These findings show GAL-synthesizing neurons to respond differently from NPY neurons in relation to circulating CORT.

386.4

LONG-TERM FLUOXETINE, PHENELZINE AND IDAZOXAN TREATMENT INCREASES LEVELS OF POMC AND NPY mRNA LEVELS IN THE HYPOTHALAMIC ARCUATE NUCLEUS. R.A. Baker*, M. Herkenham and L.S. Brady. Section on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Antidepressant drug treatments have been shown to decrease corticotrophin releasing hormone (CRH) mRNA levels in the hypothalamic paraventricular nucleus (PVN) with a delayed time course that is relevant to their clinical efficacy (Br.Res., '92, 572:117). The question of which neural inputs to the PVN may mediate this decrease remains unanswered. Inputs to the PVN from the arcuate nucleus, particularly the NPY and POMC arcuato-paraventricular projections, are candidates for mediating the effects of antidepressant drugs on CRH mRNA levels. *In situ* hybridization histochemistry was used to assess arcuate peptide mRNA levels in 4 groups of rats following daily administration of fluoxetine, phenelzine, idazoxan, or saline for 2 or 8 weeks. Long-term treatment with each of the 3 antidepressants significantly increased arcuate POMC and NPY mRNA levels, ranging from 34% to 72% above control levels. In contrast, following 2 week treatment, arcuate NPY mRNA levels were significantly increased by 31% over control levels in the phenelzine treated group, but were unchanged in the fluoxetine and idazoxan groups. Following the 2 week treatment, levels of POMC mRNA were unchanged in all 3 treatment groups. These findings suggest that NPY and POMC mRNA-containing projections from the arcuate to the PVN may contribute to the decreased levels of CRH mRNA found following long-term (8 week) but not short-term (2 week) antidepressant drug treatment.

386.5

CHANGES IN HIPPOCAMPAL CORTICOSTEROID RECEPTOR GENE EXPRESSION FOLLOWING 5,7-DHT LESIONS AND 8-OHDPAT ADMINISTRATION. J.R.Seckl* and J.L.W.Yau. Dept. Med. Western Gen. Hosp. Edinburgh, U.K.

Serotonergic denervation attenuates hippocampal mineralocorticoid (MR) and glucocorticoid (GR) receptor gene expression, whereas 5-HT increases hippocampal neuronal GR, at least in primary culture. The 5-HT receptor subtype(s) involved in these effects are unknown. We examined the effects of the 5-HT_{1A} receptor agonist, 8-OHDPAT, upon hippocampal MR and GR gene expression in rats bearing central 5,7-DHT lesions and controls. Rats, pretreated with desipramine, were given 5,7-DHT icv and 12 days later 8-OHDPAT (1 mg/kg.dy^{-1}, sc) or saline for 2 days. Controls (vehicle icv) were given saline or 8-OHDPAT. 5,7-DHT lesions decreased hippocampal MR (~20% fall in CA1-3) and GR (31-40% fall in the dentate gyrus, CA1 and CA2) mRNA expression. In 5,7-DHT-lesioned rats, 8-OHDPAT administration reversed the fall in hippocampal MR mRNA expression, most notably in CA1. 8-OHDPAT had no effect on MR gene expression in controls. By contrast, 8-OHDPAT led to further decreases in hippocampal GR mRNA expression in 5,7-DHT lesioned rats (dentate gyrus, 42% fall; CA1-2, 52%). Moreover in controls, 8-OHDPAT also decreased GR mRNA expression in CA1 (31% fall) and CA2 (39%). Thus, the attenuation of hippocampal MR gene expression by 5-HT denervation can be partly reversed by $5HT_{1A}$ receptor agonists, whereas the attenuation of GR gene expression does not appear to be due to loss of $5HT_{1A}$ receptor activation. Whether the 8-OHDPAT-related decrease in GR mRNA expression is directly mediated or reflects indirect actions, such as increased corticosterone secretion, remains to be determined.

386.6

ACUTE MDMA TREATMENT ALTERS CORTICOSTEROID RECEPTOR AND 5-HT_{2C} RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS. J.L.W. Yau* and J.R. Seckl. Dept. Med. Western Gen. Hosp. Edinburgh, U.K.

Serotonin and corticosteroids interact in the hippocampus, affecting electrophysiological, neuroendocrine and behavioural parameters. Chronic methylenedioxymethamphetamine (MDMA) administration causes selective neurodegeneration of 5-HT neurons. This decreases mineralocorticoid (MR) and glucocorticoid (GR) receptor and increases 5-HT_{2C} receptor gene expression in the hippocampus. Conversely antidepressant drugs, which potentiate monoamine action, increase hippocampal GR and MR gene expression. Acutely, MDMA releases 5-HT from nerve terminals, prior to damaging axons. In the present study, we examined the effects of acute MDMA on hippocampal MR, GR and 5-HT_{2C} receptor mRNA expression using *in situ* hybridisation. Rats were killed 16 h after MDMA (20mg/kg, s.c.). Hippocampal neuronal GR mRNA expression was decreased in the dentate gyrus(DG) (29% fall), CA2 (20%) and CA3 (24%; all $p<0.05$). In contrast, MR mRNA expression was increased in the DG (69% rise), CA1 (41%), CA3 (35%) and CA4 (52%; all $p<0.01$). 5-HT_{2C} receptor mRNA expression was decreased in CA3 of the dorsal (20%, $p<0.01$) and ventral (30%, $p<0.05$) hippocampus. Increased MR and decreased 5-HT_{2C} receptor gene expression in the hippocampus probably reflect acute MDMA-induced 5-HT release. Down-regulation of GR gene expression might be a consequence of the MR changes. These results support our previous findings suggesting 5-HT controls hippocampal corticosteroid receptor and $5HT_{2C}$ receptor gene expression.

386.7

Altered Expression of GLUT1 & GLUT3 Glucose Transporters in Neurohypophyses of Dehydrated/ Rehydrated Rats. Kang Li, Susan J. Vannucci, Ellen Koehler, Fran Maher and Ian A. Simpson* Bethesda, MD 20892 and Hershey, PA 17033.

The neurohypophysis (NH) is an extension of the central nervous system which contains axon terminals of vasopressin-and oxytocin-secreting neurons of the hypothalamus and glial-like pituicytes, but lacks a blood-brain barrier. Neuronal GLUT3 and 45 kDa GLUT1 are detected in the NH. Progressive dehydration due to water deprivation increases vasopressin (VP) secretion in rats; dehydration increases NH glucose utilization. This study examined the effects of progressive dehydration and subsequent rehydration on NH GLUT1 and GLUT3. Dehydration was achieved in adult (250g) male rats by water deprivation for 1, 2 &3d. At 3d of dehydration other rats had their water returned and were studied at 1, 2 & 3 days following rehydration. NH GLUT1 & 3 concentrations were determined in individual NH by Western blot analysis with specific anti-C-terminal antisera. With progressive dehydration, hematocrit increased and plasma VP levels, measured by RIA, increased 4-fold by 3 days with a corresponding depletion in NH VP. The concentration of GLUT1 was increased by 18% and 44% by 2 & 3 and GLUT3 was increased by 42% & 55%. With water repletion, physiological parameters, including weight gain and hematocrit returned to normal by two days. However, the levels of both GLUT1 and GLUT3 remained elevated, even at 3 days of rehydration. The results of this study suggest that NH glucose transporters are rapidly upregulated in response to the metabolic stress of dehydration but their turnover is apparently slower.

386.8

MODULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN BIRDS. L. Michael Romero* & John C. Wingfield Dept. of Zoology, Univ. of Washington, Seattle, WA 98195

Recent evidence suggests that some avian species can modulate corticosterone (B) release in response to a standard stressful paradigm (capture and serial blood sampling). Stess-induced release of B varies throughout the year and appears dependent upon the animal's physiological state. In one species (white-crowned sparrow, *Zonotrichia leucophrys*), B output is inhibited during molt. We tested whether this inhibition results from adrenal or pituitary nonresponsiveness by injecting various stimulating factors into molting white-crowned sparrows and comparing B release to similarly-injected nonmolting pigeons (*Columba livia*), a species not known to modulate their glucocorticoid response.

White-crowned sparrows from eastern WA were housed in outdoor aviaries until the onset of molt. Pigeons were captured in Seattle, injected, bled, and released. Intravenous ACTH stimulated B release in the sparrows, indicating that the adrenal can still respond to an ACTH signal during molt. CRF, on the other hand, failed to stimulate B release in the sparrows, but enhanced B release 300% in the pigeon compared to vehicle-injected controls. Similarly, arginine-vasotocin and mesotocin (AVT and MT - the avian equivalents of vasopressin and oxytocin, respectively) also failed to stimulate B release in sparrows whereas AVT caused a 200% increase in the pigeon. In neither species did the combination of CRF and AVT enhance B secretion than to either hormone alone. These data suggest that the inhibition of B release seen in white-crown sparrows during molt may be mediated by pituitary insensitivity to an ACTH secretagog signal but not to adrenal insensitivity to ACTH.

386.9

PERIPHERAL NGF ADMINISTRATION INCREASES HYPOTHALAMIC C-FOS EXPRESSION. F.Passarelli*,R.Nicolai# F.R.Buttarelli, D.Babiloni, S.Scaccianoce#, G.Cigliana#,and L. Angelucci#. Dept.of Neurosci.,Univ.of Rome "La Sapienza" and #Farmacologia, 2ª, Univ.of Rome "La Sapienza".

We have recently demonstrated in the rat the hypothalamic involvement in the adrenocortical activation induced by NGF (Scaccianoce S.,1993). The *c-fos* gene, expressed at low levels in most cells types, is induced rapidly in response to a variety of extracellular stimuli in the periphery and in the CNS. We have investigated whether NGF administration is followed by an induction of hypothalamic *c-fos* mRNA expression. Freely moving male Wistar rats were injected through a permanent jugular cannula with either 1or 5 nmol/kg of mouse βNGF (kindly donated by dr.J.R.Perez Polo,Univ. of Texas, Galvestone, Texas) or vehicle.The adrenal glands and the hypothalami were isolated 30 min after injection and total RNA was extracted by the guanidinium thiocyanate-cesium chloride method. The levels of *c-fos* and β-actin mRNAs were measured in each sample using a RT-PCR. Compared to vehicle in the hypothalamus a 2-fold *c-fos* mRNA increment was observed after 5 nmol/kg of NGF administration but not after 1 nmol/kg. The adrenal gland mRNA *c-fos* enhancement was detected after 1 and 5 nmol/kg NGF injection. These results confirm that the hypothalamus is involved in the adrenocortical activity induced by NGF and that the *c-fos* gene is a target of intracellular second messenger pathway. (Supported by CNR grant 93.00447.PF40 to Scaccianoce S.).

386.10

CATECHOLAMINERGIC AFFERENTS TO THE FETAL PARAVENTRICULAR NUCLEUS CONTAIN GLUCOCORTICOID RECEPTORS W.-W. Le, T.J. McDonald, P.W. Nathanielsz, and G.E. Hoffman*. Department of Neurobiology, Univ. Pittsburgh, Pittsburgh, PA, 15261 and Laboratory for Pregnancy and Newborn Research, Cornell Univ., Ithaca NY 14853.

In fetal sheep, implants of corticosteroids adjacent to the paraventricular nucleus of the hypothalamus suppress AVP and CRH expression. These effects of glucocorticosteroids are likely mediated by actions of glucocorticoid receptors within the paraventricular (PVN) neurons. Under physiological conditions, steroids could influence the function of the hypothalamus via actions to the PVN afferent neurons as well. To assess this possiblity, we determined whether the catecholaminergic afferents to the PVN in the ventrolateral medulla (A1/C1), and n. solitary tract (A2/C2) possessed immunoreactive type II glucocorticoid receptors. To accomplish this, fluorogold (FAu) was stereotaxically injected into the PVN of 5 fetal sheep (120 dGA). After 3 weeks, each fetus was removed by Caesarian section, administered an overdose of anesthesia and perfused. Immunocytochemistry for GRII (using anti BUGRII), tyrosine hydroxylase and FAu was performed in the brainstem. Staining of the hypothalamus for FAu, GRII, and CRH verified the accuracy of tracer injection and substantiated the presence of GR within the CRH neurons of the PVN. The dominant catecholamine innervation of the PVN arises from the A1/C1 and A2/C2 cell groups. In the A1/C1 cell group, 68% of the catecholamine neurons possessed GR; in the A2/C2 cell group 73% of the neurons were GRII+. Triple labling of tyrosine hydroxylase, GRII and FAu confirmed that approximately half of the GRII+ catecholamine neurons in both regions projected to the PVN. These data suggest that glucocorticoids can act on both the CRH neurons of the hypothalamus as well as on catecholamine afferents to the PVN. (*Supported by NIH HD 21350*)

386.11

GLUCOCORTICOID MODULATION OF C-TYPE NATRIURETIC PEPTIDE mRNA LEVELS IN PREOPTIC AREA AND LIMBIC STRUCTURES. M.C. Langub, Jr.*, Dreama Rucker and J.P. Herman Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

C-type natriuretic peptide (CNP) has been shown to exert inhibitory action on the HPA axis. The potential for CNP to act via central mechanisms is highlighted by recent data indicating localization to several stress relevant CNS nuclei, including the preoptic area and hippocampus. In light of the potential importance of CNP in regulation of HPA axis, we tested the hypothesis that CNP is glucocorticoid (GC) regulated by examining the effects of adrenalectomy and GC replacement on CNP mRNA. Groups of adrenalectomized (ADX) rats, ADX rats replaced with 30% corticosterone (CORT) pellets (yielding CORT levels of 40-70 ng/ml), and sham-ADX rats (CORT levels between 10-30 ng/ml) were sacrificed and brains processed for *in situ* hybridization histochemistry. Hybridization signal for CNP mRNA was present in the anteroventral periventricular area (AVPv), arcuate nucleus, hippocampal subfields CA1, CA2/3, dentate gyrus and cingulate cortex. The AVPv was the only region showing evidence of GC modulation. Semi-quantitative analysis revealed no changes between ADX and sham rats. ADX-CORT rats, which have significantly higher levels of circulating CORT than sham rats at the time of sacrifice, exhibited pronounced increases in CNP mRNA relative to ADX or sham rats. These results suggest the AVPv CNP-synthesizing neuronal population can respond directly or indirectly to high steroid levels, and therefore that these cells may modulate CNP actions on the stress axis. Supported by American Heart Association Kentucky Affiliate.

386.12

BEHAVIORAL, NEUROENDOCRINE AND NEUROIMMUNE ANALYSIS OF HIPPOCAMPAL STRESS INTEGRATION. J.P. Herman*, C.M. Dolgas and S.L. Carlson. Dept. of Anatomy and Neurobiology, Univ. Kentucky Medical Center, Lexington, KY 40536-0084.

Lesion and anatomical analyses suggest that the ventral subiculum (VSUB) plays a major role in hippocampal inhibition of the hypothalamo-pituitary-adrenocortical (HPA) axis. The present study was designed to assess the nature of VSUB involvement in behavioral, neuroendocrine and neuroimmune stress responses. Groups of rats received bilateral ibotenic acid lesions of the VSUB (IBO) (n=19) or injections of saline vehicle (SAL) (n=17). All animals underwent analysis of stress-related behaviors in an open field, and were subsequently divided into three groups for analysis of endocrine and immune responsivity to acute restraint (0, 60 and 120 m). IBO rats exhibited enhanced emotional responsivity to open field exposure, manifest as increases in freezing behavior and decreased ambulation. IBO lesion increased stress-induced ACTH secretion 60 m but not 120 m after stress induction; no changes were seen in baseline release. Restraint stress caused marked decreases in splenic lymphocyte proliferation following 60m of restraint, as measured by *in vitro* mitogen assay; however, no differences were noted between groups. The results suggest that VSUB damage affects both emotional responsivity and HPA activation following exposure to a stressful situation, yet do not impair either basal ACTH release or immune reactivity in a long-term fashion. The VSUB lesion appears to bias behavioral predispositions in a direction suggesting enhanced stress responsivity. Effects of VSUB lesion on HPA activation may therefore be mediated by alterations in salience of stressful stimuli, rather than modulation of glucocorticoid negative feedback. Supported by MH49698.

386.13

BILATERAL LESIONS OF THE CENTRAL AMYGDALOID NUCLEUS BLOCK THE EFFECTS OF CHRONIC STRESS ON THE HPA AXIS. C.M. MEYERS*, AND J.P. HERMAN. Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

Chronic stress elicits long-term hypersecretion of stress-hormones by the hypothalamic-pituitary-adrenal (HPA) axis. The central nucleus of the amygdala (ACE) has been shown to potentiate HPA activation; stress-induced ACTH secretion is increased by stimulation of the ACE and decreased following ACE lesion. The present studies address the hypothesis that the ACE is involved in chronic stress-induced HPA changes. The ACE was bilaterally lesioned by injection of ibotenic acid (IBO) (3.0 μg total in 0.3 μl) or sham-lesioned (0.3 μl saline/side). Groups of rats were chronically stressed using a variable stressor paradigm (bi-daily exposure to cold, warm water swim, cold water swim, crowding, vibration, isolation). Control rats were handled bi-daily. Rats were killed after 30 days of stress. Adrenal glands and thymus were removed and weighed; plasma stress hormones were determined by RIA; and CRH mRNA in the hypothalamic paraventricular nucleus (PVN) was analyzed by in situ hybridization histochemistry. Chronic stress decreases thymus and body weight, and increases adrenal weight and basal CORT level in sham-lesioned animals. However, in ACE lesioned rats, chronic stress did not decrease thymus or body weight and did not increase basal CORT level. Chronic stress upregulated CRH mRNA in the PVN of sham-lesioned animals, but no change was found in the IBO-lesioned rats. These results suggests that the ACE exerts an important action on maintenance of HPA axis activation following chronic stress. The ACE may therefore play a critical role in disease states associated with pathological hypersecretion of glucocorticoids. Supported by MH49698.

386.14

THE EFFECTS OF GLUCOCORTICOIDS ON THE ELECTRICAL PROPERTIES OF IDENTIFIED HYPOTHALAMIC NEURONS. S.S.Daftary*, K.Szabo and J.G.Tasker. Molecular & Cellular Biology Program and Dept of Cell & Molecular Biology, Tulane University, New Orleans, LA 70118.

Activation of the hypothalamic-pituitary-adrenal (HPA) axis causes an increase in the circulating levels of glucocorticoids (eg. during stress). Glucocorticoids feed back onto the brain to inhibit the secretion of corticotropin releasing factor (CRF) and suppress HPA activation. To determine whether glucocorticoids feed back directly onto neurosecretory neurons of the hypothalamus, we studied the effects of the glucocorticoid analog, dexamethasone, on the membrane electrical properties of identified neurons in the rat hypothalamic paraventricular nucleus (PVN). The effects of dexamethasone on specific PVN cell populations were studied using intracellular recordings, dye injection and immunohistochemistry in the hypothalamic slice preparation. Bath application (30 min) of dexamethasone (10^{-6} M) caused a 4-14mV hyperpolarization with an increase in input resistance in low-threshold spiking (LTS) cells, or putative parvocellular neurons, and a small depolarization (2-5mV) with a decrease in input resistance in non-LTS cells, or putative magnocellular neurons. The identity of recorded cells was verified anatomically with biocytin injection and subsequent immunohistochemical processing using antisera to CRF, oxytocin, vasopressin or neurophysin. These results suggest that glucocorticoids have a direct inhibitory effect on parvocellular neurons and a weak excitatory effect on magnocellular neurons of the hypothalamic PVN.

386.15

EVIDENCE FOR GLUCOCORTICOID REGULATION OF VASOPRESSIN V1a RECEPTOR GENE EXPRESSION

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The brain vasopressinergic system is intimately involved with the hypothalamo-pituitary-adrenal axis. We have isolated a genomic clone from a rat testis library encoding a putative vasopressin V1a receptor. Analysis of the 5' flanking region of this clone revealed the presence of several putative glucocorticoid response elements suggesting that glucocorticoids might play a role in transcriptional regulation of this gene. The rat mammary tumor cell line, WRK-1, expresses a vasopressin V1a receptor similar to that expressed in rat liver. Previous studies in our laboratory have shown that treatment of these cells for 24 hours with dexamethasone (DEX), leads to an increase in PI hydrolysis, receptor number and receptor binding. These effects were blocked by the glucocorticoid receptor (GR) antagonist RU486. To determine if these increases might be due to an enhancement of V1a gene transcription, we treated cells with DEX for 1, 3, 6, 12, and 24 hours and isolated poly A+ RNA . We observed a transient increase in mRNA by Northern blot analysis, that was maximal at 12 hours. This time period, in addition to blockade of this effect by a GR antagonist, suggests a genomic mechanism of glucocorticoid modulation of the V1a receptor. In vivo studies are currently in progress to assess the physiologic role of glucocorticoids in expression of this receptor in rat brain.
(Supported by NS20311, the VA and Pharmacological Sciences Training Grant 670489).

386.16

ADRENOCORTICAL EFFECTS ON TYPE I CALMODULIN-DEPENDENT ADENYLATE CYCLASE mRNA LEVELS IN RAT BRAIN.

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Manipulation of the hypothalamic-pitutiary-adrenal axis has pronounced effects on calmodulin-dependent (CaM) adenylate cyclase (AC) enzyme activity, measured in rat brain homogenates. In particular, hippocampal CaM AC activity shows diurnal variation, an effect abolished by ADX [J. Neuroendocrinol. 3:37-43, 1991]. The present study investigated whether, adrenocortical effects on AC enzyme activity were paralleled by changes in the expression of AC mRNA. Of the eight identified members of the AC gene family, only Type I ACmRNA is both present in high levels in brain and shows CaM-dependency. *In situ* hybridization analysis was conducted at the level of the dorsal hippocampus, using [35S]-oligonucleotide probes directed against rat Type I ACmRNA. Preliminary results indicate that ADX (14 days) attenuates Type I ACmRNA levels in rat dentate gyrus measured 2-4 hr into the dark cycle, but not 5-7 hr into the light cycle. Compared to ADX, corticosterone administration (40 mg/kg s.c. once daily) increased expression of Type I ACmRNA in the dentate gyrus at both time points. In a separate study, rats were housed on a 12:12 light:dark cycle, sham-operated or ADX (14 days), sacrificed at 4 hr intervals across the light:dark cycle and brain processed for *in situ* hybridization analysis. Both ADX and time of day had significant effects on Type I ACmRNA expression in the dentate gyrus. In contrast, 21 days stress, which attenuated cortical CaM AC enzyme activity [J. Neurochem. 55:276, 1990], had no significant effect on Type I ACmRNA expression in either cerebral cortex or hippocampus. These results suggest that part, but not all, of the effects of adrenocortical state on cAMP signal transduction in brain are mediated through effects on Type I ACmRNA expression.
Supported by grant MH41256.

386.17

MINERALOCORTICOID RECEPTOR mRNA VARIANTS IN THE DEVELOPING HIPPOCAMPUS: DISTRIBUTION AND CORTICOID REGULATION

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Our laboratory has previously reported an MR cDNA clone from rat hippocampus (HC) which shares a high degree of homology with the kidney MR rat clone. However, these two clones differ significantly at the 5' untranslated region (5' UT) and have been named alpha (α) (kidney clone) and beta (β) (HC clone). Most recently, a third HC 5'UT cDNA clone was identified which we termed gamma (γ) (Endo 133:5-2344). Studies have shown that these 5' UT regions are located on separate exons of a single gene and give rise to three mRNAs that encode the same protein. We were interested in investigating the presence of these distinct mRNA forms and their glucocorticoid regulation in the developing rat HC. Last year we presented the α and β HC distribution in animals 10, 18, 28 and 60 (adult) days old. We have expanded the *in situ* hybridization study to younger ages and included the γ 5'UT variant in our analysis. In general, we find that α and β expression increases slowly in all HC regions during the first 2 weeks of life. Whereas α levels remain constant thereafter, β expression decreases markedly in the pubertal animal. In contrast, γ is highly expressed in all regions early during development. In the dentate gyrus this high level of expression is retained until adulthood when low levels are detected. Adrenalectomy results in up-regulation of the α 5'UT form in all ages. Our results, show that the α and β 5' UT forms achieve an adult pattern of distribution in the HC after the Stress Hypo-Responsive Period. The γ 5'UT form maybe important for proliferation and differentiation of hippocampal neurons. Supported by NIMH MH09720 and MH422251.

386.18

ALPHA-TOCOPHEROL SUCCINATE, BUT NOT ALPHA-TOCOPHEROL OR OTHER VITAMIN E ANALOGS STIMULATES PROLACTIN RELEASE FROM RAT ANTERIOR PITUITARY CELLS in vitro.

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Green barley leaf extract, a dried extract of young green barley leaves, is widely used in Japan and other countries as a nutritional supplement. We have recently reported (Badamchian, et.al J. Nutr. Bioc. 5:145-150, 1994), the isolation of a vitamin E analog from green barley leaf extract that stimulates release of prolactin and growth hormone from rat anterior pituitary cells in vitro. This molecule was identified as α-Tocopherol succinate, an analog of α-Tocopherol, or vitamin E. In the present study we tested the commercially available forms of α-Tocopherol and also succinic acid. Treatment of normal anterior pituitary cells with different forms of tocopherol (100μg/ml) and succinic acid (50-100μg/ml) caused significant increase ($p<0.01$) in prolactin release in only α-Tocopherol succinate-treated cells. α-Tocopherol, δ-Tocopherol, α-Tocopherol acetate, α-Tocopherol nicotinate, succinic acid disodium salt, and succinic acid butenedoic acid did not effect the prolactin release in vitro. Supported by a gift from Hagiwara Institute of Health, Hyogo, Japan.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: GNRH RELEASE

387.1

GABA TURNOVER IN MICRODISSECTED BRAIN REGIONS DURING THE RAT ESTROUS CYCLE.

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GABAergic neurons terminating in the rostral and mediobasal hypothalamus are positively regulated by testosterone, an action that may play an important role in mediating the negative-feedback action of testosterone on hypothalamic GnRH release in the male rat. The present study determined whether endogenous changes in gonadal steroids during the estrous cycle were associated with changes in GABAergic neuronal activity measured in tissue punches from steroid-sensitive brain regions. Animals were decapitated at 0900 and 1700 h on diestrous day 1, and 0900, 1300 and 1700 h on proestrus, either without treatment, or 60 minutes after inhibition of the GABA degrading enzyme GABA-transaminase by amino-oxyacetic acid (AOAA, 100 mg/kg, ip). The rate of accumulation of GABA in the tissue after AOAA was taken as an index of GABA turnover. In brain regions examined so far, there was no change in GABA turnover between the morning and afternoon on diestrus, or between the morning of diestrus and the morning of proestrus. In the diagonal band of Broca at the level of the OVLT, GABA turnover was significantly reduced in the afternoon of proestrus compared with activity measured in the morning. By contrast, in the central portion of the MPN, no changes were apparent at any of the timepoints examined. Analysis is continuing in the median eminence, ventromedial nucleus, septal nuclei, medial amygdala, hippocampus CA1, suprachiasmatic nucleus and cortex. The results suggest that activity of specific subpopulations of GABAergic neurons in the rostral hypothalamus changes during the estrous cycle in the female rat. It is likely that decreased GABA turnover in these regions may be important for generation of the preovulatory GnRH surge. (Supported by NIH Grant HD21351)

387.2

EFFECT OF CHRONIC MORPHINE TREATMENT ON NORADRENERGIC MODULATION OF GT1-7 NEURONS.

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Stimulation of noradrenergic, serotonergic, and opiate receptors are known to regulate release of hypothalamic GnRH, but it is not known whether the receptors exist on GnRH neurons or on other cell types that contact GnRH neurons. GT1-7 neurons, which synthesize and secrete GnRH, were immortalized in culture from transgenic mice. The purpose of this study was to examine the possible interrelationship among opiate, noradrenergic, and serotonergic input to GT1-7 neurons. We measured GnRH by RIA, $[Ca^{2+}]_{cyt}$ with the fura-2 method, and cAMP accumulation as conversion of ^{3}H-ATP to ^{3}H-cAMP. Our results show that norepinephrine (NE) stimulated increases in cytosolic $[Ca^{2+}]$ and GnRH release. Serotonin (5HT) had no effect on $[Ca^{2+}]_{cyt}$, basal cAMP accumulation, or forskolin-stimulated cAMP accumulation. Exposure of these cells to morphine for 5 days resulted in enhanced NE stimulation of $[Ca^{2+}]_{cyt}$ but did not change the lack of responsiveness of the cells to 5HT. These data suggest that opiate receptors may modulate noradrenergic stimulation of GnRH neurons *in vivo* and also that 5HT regulation of GnRH neurons *in vivo* is not direct. Supported by USPHS grants DA06039, AA10112, and MH52369.

387.3

GONADOTROPIN-RELEASING HORMONE (GnRH) RELEASE IN OVARIECTOMIZED FEMALE MARMOSET MONKEYS. D.H. Abbott[1,2], W. Saltzman[1], N.J. Schultz-Darken[1] and E. Terasawa[1*]. [1]Wisconsin Regional Primate Research Center and [2]Dept. of Ob./Gyn., University of Wisconsin, Madison, WI 53715.

Socially subordinate female marmosets, *Callithrix jacchus*, fail to ovulate because of suppressed gonadotropin secretion, presumably caused by reduced GnRH release. To investigate the causes of this pronounced reproductive suppression, we developed a push-pull perfusion technique for characterizing GnRH release in conscious female marmosets. In order to validate this technique, 5 ovariectomized females were implanted with cranial pedestals using x-ray ventriculography and a stereotaxic apparatus. After at least 6 weeks, a micromanipulator was attached to the pedestal and was used to lower a push-pull cannula (outer cannula: 20 ga, inner cannula: 28 ga) into the pituitary stalk-median eminence. The female was then placed in a jacket/sling restraint beside its social group. Two days later, we perfused modified Kreb's Ringer phosphate buffer at 23µl/min and collected 10-min perfusate samples for 7h from fully conscious animals. GnRH in perfusate samples was measured by RIA. Following perfusion, the cannula and micromanipulator were removed and the animal was returned to its social group. We found that GnRH release in ovariectomized marmosets was pulsatile, with values ranging from 0.1-16.3 pg/ml. GnRH inter-pulse interval was 42.3±1.8 min (mean ± SEM) with a peak amplitude of 2.1±1.1 pg/ml. Perfusion sites were just ventral to the infundibular recess, within 1 mm of the midline. Repeated perfusion of individual animals indicated that multiple measurements of GnRH can be made from the same females without disturbing their social grouping. Such direct measurement of hypothalamic function now permits systematic investigation of the neuroendocrine control of female reproduction in the marmoset. [Supported by NIH grants RR00167, HD07678 and NSF grant IBN 92-21771.]

387.4

FOOD DEPRIVATION ENHANCES ESTRADIOL-INDUCED LH SURGE IN FEMALE SYRIAN HAMSTERS. R.A. Mangels, A.E. Jetton*, and G.N. Wade. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Reproductive function is sensitive to the availability of metabolic fuels. In Syrian hamsters, two days of food deprivation prevents ovulation, inhibits lordosis, and alters brain estrogen receptor immunoreactivity (ERIR). To further asses the role of changes in neural sensitivity to estradiol (E_2) during food deprivation, we examined the effect of food deprivation on the E_2-induced LH surge. Fifty three hamsters were individually housed in a 14:10 light-dark cycle (lights on 0700h). Two weeks after ovariectomy (OVX), animals were injected at 1300h with 5µg estradiol benzoate (EB) or oil, and food was removed from half of the animals in each group. Two days later, .4ml of blood was taken via cardiac puncture at each of three time points (1100, 1700, and 1800h). RIA revealed that EB reduced serum LH at 1100h and induced an LH surge at both 1700h and 1800h. There was no effect of food deprivation. In a second experiment, fifty nine animals were housed in identical conditions. Two weeks after OVX, food was removed at 1100h. Forty eight hours later, animals were injected with oil, 1µg EB, or 5µg EB, and food was returned. Two days after EB injection, animals were bled at 1100, 1600, or 1700h. Both doses of EB induced an LH surge at 1600 and 1700h, but only the 5µg dose inhibited LH at 1100h. Food deprivation enhanced the LH surge in hamsters treated with 1µg EB, but not in hamsters given 5µg EB. Food deprivation inhibited LH at 1100h in oil-treated animals. Thus, two days of food deprivation preceding a low dose injection of EB increases the magnitude of the LH surge. This effect could be related to the increase in mPOA ERIR following food deprivation. Supported by NS10873, MH00321, and HD07673

387.5

SUPPRESSION OF LUTEINIZING HORMONE SECRETION IN FOOD RESTRICTED LACTATING RATS: EFFECTS OF OVARIECTOMY AND BROMOCRYPTINE TREATMENT. C.-D. Walker*[#], J. B. Mitchell [+]and B. Woodside[$]. [#]Dept of Psychiatry, McGill University, [$]CSBN, Concordia University, Montreal, Que., [+]Psychology Dept. Boston College.

It has been shown that food restricting lactating rats for the first two weeks postpartum at a level of 60% of the ad-lib daily ration increases the length of lactational diestrus by about 7 days. The aims of the present studies were 1) to examine changes in LH, PRL, and ACTH secretion in food-restricted and ad libitum fed lactating rats at various stages of lactation, and 2) to determine whether the changes in hormonal parameters induced by ovariectomy or bromocryptine treatment differed between food-restricted and ad lib fed dams. Blood samples were obtained over a 2 hour period from freely moving lactating females on Days 4, 15 and 21 of lactation. GnRH receptor number was determined by autoradiography. Our results demonstrate that food restriction during the first two weeks of lactation did not affect PRL or ACTH secretion, but decreased plasma LH levels despite comparable GnRH receptor density between food-restricted and ad lib fed females. In addition, LH reduction in food-restricted lactating females was not affected by ovariectomy or bromocryptine treatment, although the latter treatment significantly increased GnRH receptor number. These data suggest that factors other than ovarian steroids, PRL or increased adrenocortical activity modulate LH secretion and the length of lactational diestrus in food-restricted lactating females.

387.6

BLOCKADE OF PREOVULATORY LH SURGES WITH A NEUROPEPTIDE Y RECEPTOR ANTAGONIST, PYX2. L.M. Besecke*, A.C. Bauer-Dantoin, D.K. Wong and J.E. Levine. Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Neuropeptide Y (NPY) plays an important role in the generation of preovulatory luteinizing hormone (LH) surges. NPY likely both stimulates LHRH release and facilitates LHRH-induced LH secretion as complementary components of an integrated LH-surge amplification process. It is not known, however, how NPY's actions are manifest at the level of the anterior pituitary, and if any such actions are requisite for the production of normal LH surges. The present experiments were undertaken to determine 1) if NPY receptor occupancy is required for the elaboration of spontaneous LH surges and 2) if pulsatile or continuous stimulation of the receptor is sufficient for expression of NPY's facilitatory effects. In experiment 1, either vehicle or the NPY receptor antagonist, PYX2 (0.5 mg/kg BW,iv), was administered at 1330, 1530, and 1730 h in proestrous rats. Blood samples obtained at 60 min intervals between 1100 and 2100 h were analyzed by LH RIA. Results revealed that the LH surges were completely blocked or significantly blunted ($p<0.05$) in all rats treated with the PYX2 regimen. In experiment 2, proestrous rats were anesthetized with pentobarbital (40 mg/kg BW) to block endogenous LHRH/LH surges, and then treated with pulses of LHRH (15 ng,iv) at 30 min intervals. Rats were cotreated with vehicle, continuous NPY (10 µg/h) or pulsatile NPY (1 µg/pulse/30 min). Both continuous and pulsatile NPY modes of treatment were effective in facilitating LHRH-induced LH surges. We conclude from these studies that occupation of a NPY receptor is required for the generation of LH surges in the rat. Our findings also demonstrate that the NPY stimulus pattern need not be pulsatile to affect its actions. Current work is directed at identifying the molecular characteristics of NPY receptors which may mediate these obligatory effects. (NIH HD20677, HD219121, HD00879 and HD28048)

387.7

ACUTE INHIBITION OF LUTEINIZING HORMONE SECRETION IN RESPONSE TO ETHYL β-CARBOLINE-3-CARBOXYLATE (β-CCE) AND DIAZEPAM TREATMENTS IN CASTRATED MALE RATS. C.A.Hodson, A.T.Davenport, G.T.Price and H.W.Burden*. Obstetrics and Gynecology, and Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858

Previous studies have shown that diazepam administration can inhibit LH secretion. The present study was conducted to determine if the inverse agonist β-CCE would affect LH secretion.

Male rats castrated for 7 days weighing 350-450g were used for the study. On day 7 at time 0 a single blood sample was taken from the retro-orbital sinus under ether anesthesia. Immediately after collection of the time 0 sample the rats were injected with vehicle (2:1 propylene-glycol:ethanol), 2mg β-CCE/kg bw, 2.5mg diazepam/kg bw or β-CCE and diazepam in combination. After treatment additional blood samples were collected at 15, 30 and 60 minutes from the retro-orbital sinus.

β-CCE reduced serum LH with respect to vehicle at 15 and 30 minutes post injection. Diazepam treatment caused a reduction in LH at 30 minutes post injection. The drugs did not have an additive effect. These results suggest that diazepam and β-CCE can acutely reduce LH.

387.8

SODIUM NITROPRUSSIDE-INDUCED RELEASE OF NITROGEN MONOXIDE (NO) FROM PROCESSES IN EWE MEDIAN EMINENCE (ME) RESULTS IN AN INCREASE OF IN VIVO ME-LHRH RELEASE. J.P. Advis*, D.K. Sarkar, J. Rabii and R.O. Kuljis. Depts of Animal Sciences, Rutgers University, New Brunswick, NJ 08903; VCAPP, Washington State University, Pullman, WA 99164; Biological Sciences, Rutgers University, Piscataway, NJ 08855; and Neurology, Univ of Iowa and VAMC, Iowa City IA 52242-1053.

Physiological evidence suggests a role for hypothalamic NO, since NO synthase has been reported to be present in the hypothalamus and since NO increases the release of LHRH in vitro. Thus, **1)** serial frozen sections of ewe hypothalamus and ME (n=5) were labeled histochemically for NADPH-diaphorase which is necessary for the synthesis of NO, and **2)** ME-LHRH release was determined in vivo by push-pull cannula (PPC) sampling, before and after infusion of sodium nitroprusside through a ME-PPC probe (NaNP, 500 µM, n=5). The mid-hypothalamic region exhibited a collection of labeled neurons and neurites in the ventro-medial nucleus (VMN) visible even at low magnification. Additional large labeled neurons were scattered around the VMN and throughout the hypothalamus. Virtually no labeled perikarya were visible at low magnification around the fundus of the third ventricle. However, a vast network of neurites is present at that level. The interface between ME and pituitaty stalk, near the palisade zone showed unequivocally labeled processes, indicating that NO-synthesizing neurites reach the ME. The precise pattern of distribution and source(s) of these neurites remain to be elucidated. Nevertheless, perfusion of NaNP increased in vivo pulsatile ME-LHRH release (10.8±1.3 vs 34.3±12.2 pg/100 µl PPC perfusate /2h, before vs after) predominantly by increases in pulse amplitude (2.32 ± 0.29 vs 4.79 ± 0.69). Therefore, it is possible that NO-containing processes might be involved in regulating LHRH release and other aspects of reproductive function and behavior (NJAES-Hatch 06108 and USDA 89-37240-4587 to JP Advis).

387.9

PARTIAL SURGES OF LUTEINIZING HORMONE (LH): A NEUROENDOCRINE MECHANISM FOR PROLONGED ESTROUS CYCLES. Susan E. Gans and Martha K. McClintock*, The University of Chicago, Chicago, IL 60637.

The LH surge that stimulates ovulation is triggered by a daily neural signal only when the brain has been adequately primed by ovarian steroids. This preovulatory surge is characterized by (1) a rise above diestrous LH level and (2) an accelerated rate of LH secretion. We hypothesized that these two characteristics are independent, and that rises in LH without acceleration would cause prolonged estrous cycles. We performed external jugular cannulations on 19 regularly cycling female rats. LH levels were assessed in plasma samples collected seven times a day, hourly, from each female on four proestrous days. Steroid levels were assessed in daily samples collected at lights out throughout two estrous cycles. In 57 full LH surges, the rise always preceded the acceleration in the rate of LH secretion. Nonetheless, the time of the rise did not predict the latency to the acceleration. These two independent characteristics of the LH surge correlated with different steroids. Moreover, in eight additional surges LH rose above diestrous LH levels but never achieved the acceleration typical of the rising limb of the LH surge. We termed these partial LH surges and hypothesized that they cause luteinization of the ovarian follicle without ovulation, thus initiating prolonged estrous cycles characteristic of estrous cycle distributions in our colony. The females whose full LH surges had a delayed acceleration were the ones most likely to have partial LH surges In addition, the steroid correlates of partial LH surges were the same as those of the delayed acceleration, not the rise.

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387.10

EFFECTS OF DIRECT INFUSION OF GLUTAMATE AND N-METHYL-D-ASPARTATE (NMDA) ON LHRH RELEASE IN FEMALE PREPUBERTAL AND PUBERTAL RHESUS MONKEYS. F. Marzban* and E. Terasawa, Wisconsin Regional Primate Research Center., University of Wisconsin, Madison, WI 53715.

It has been reported that systemic injection of both glutamate and NMDA stimulate LH and LHRH release in pubertal as well as prepubertal monkeys. In this study we examined the effects of direct infusion of glutamate and NMDA into the stalk-median eminence (S-ME) on LHRH release *in vivo* using a push-pull perfusion method. Glutamate (10^{-6},10^{-8} &10^{-10} M) or NMDA (10^{-6},10^{-8} & 10^{-10} M) was infused into the S-ME of prepubertal and pubertal monkeys for 10 min at 2 h intervals, while perfusates were continuously collected in 10-min fractions for over 10 h. Vehicle was infused as a control. LHRH in perfusates was measured by RIA. Comparison was made between the values 0-20 min before infusion and the first 20 min of the infusion. **Results:** Both glutamate and NMDA induced a small, but consistent, LHRH release in all age groups: The overall effects of glutamate and NMDA were significant (for both $p<0.05$). However, there were no significant differences between age groups or between different concentrations. Vehicle infusion did not induce any significant effects on LHRH release. **Conclusion:** The findings support the hypothesis that glutamatergic input stimulates LHRH release in prepubertal and pubertal monkeys. However, the effects of direct infusion of glutamate and NMDA into the S-ME (where LHRH neuroterminals are concentrated) on LHRH release *in vivo* were not as robust as those observed in an experiment with i.v. injection (*Biol. Reprod*: 40 suppl: 83, 1989), indicating that input to the LHRH soma, either directly on LHRH neurons or indirectly via interneurons, rather than input to LHRH neuroterminals, may be more important for the action of glutamate and/or the activation of NMDA receptors resulting LHRH release. (Supports: NIH grants HD11355, HD15433 & RR00167)

387.11

DOPAMINE AND THE INHIBITION OF LUTEINIZING HORMONE PULSES IN RATS: THE SIGNIFICANCE OF HYPOTHERMIA. P. H. Strutton and C. W. Coen* Division of Biomedical Sciences, King's College, London WC2R 2LS, U.K.

Previous work has indicated that a reduction in body temperature can be the primary factor in the suppression of luteinizing hormone (LH) in ovariectomized rats. Since it has been demonstrated in independent studies that centrally administered dopamine can induce hypothermia and inhibit LH pulses, we have examined the effects of the dopamine receptor agonist apomorphine on these two parameters concurrently; in some of the studies the agonist was administered after a dopaminergic antagonist and in others it was given at an ambient temperature (35°C) which precluded the induction of hypothermia. Ovariectomised rats were fitted with an intraperitoneal transmitter to monitor core temperature and with indwelling cannulae in the right atrium of the heart and either the lateral cerebral ventricle or the subcutaneous space. Every 5 minutes during the experiment core temperature was recorded automatically and a 25µl blood sample was obtained using an automated system. Drugs were administered without touching the animal after a control sampling period of 2.5 hours. Subcutaneous administration of apomorphine (1.5mg/kg) at an ambient temperature of 21°C resulted in hypothermia 30-120 minutes post-injection; during this period there was a reduction in mean LH concentration and pulse frequency but not pulse amplitude. When this experiment was carried out at 35°C there was no reduction in core temperature; nevertheless, a reduction in mean LH concentration and pulse frequency was observed. Intracerebroventricular (icv) administration of apomorphine (20µg) at 21°C produced hypothermia 15-45 minutes post-injection; during this period there was a reduction in mean LH concentration and pulse frequency. At 35°C the icv treatment did not significantly reduce core temperature; nevertheless a reduction in mean LH concentration and pulse frequency was observed. Subcutaneous or icv administration of vehicle at 21°C or 35°C had no effect on core temperature or LH release. Subcutaneous administration of the selective D_2 receptor antagonist sulpiride (50mg/kg) 90 minutes before subcutaneous administration of apomorphine prevented the apomorphine-induced hypothermia and suppression of the LH pulses. These results suggest that D_2 receptors are involved in the hypothermia and suppression of LH induced by apomorphine, and that this inhibitory effect on LH release is not secondary to the induced hypothermia.

387.12

PRESENCE OF FUNCTIONAL OPIOID BINDING SITES ON THE GT1-1 LHRH SECRETING CELLS.
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The secretion of the hypothalamic luteinizing hormone-releasing hormone (LHRH) is regulated by several neurotransmitter systems. Among these, endogenous opioids play a major role. However, it is not clear whether opioids exert a direct effect on LHRH neurons, or whether they interfere with other neuronal systems able to influence LHRH release. The neuronal LHRH-producing cell line GT1 provides a new model to evaluate which signals may directly modify LHRH release. The presence and the possible function of opioid binding sites were studied on a clone of the GT1 cells (GT1-1). A specific and saturable binding of labeled opioid antagonist diprenorphine (DIP) was detected both on crude membrane preparations and on intact GT1-1 cells. DIP apparently binds to a single high affinity site (Kd 0.2 nM), showing a Bmax of 125 fmol/mg protein (approximately 20.000 sites/cell). Displacement of DIP binding to GT1-1 cells with the selective opioid agonists DAGO, DPDPE and U50488, which bind respectively to mu, delta and kappa opioid receptors, showed that only the delta ligand DPDPE was able to inhibit the binding of DIP (K_I 5.5 nM). As expected, the inhibitory potency of DPDPE was significantly decreased by the presence of sodium ions in the binding buffer. DPDPE addition to GT1-1 cells brought about a significant and dose-dependent inhibition of the forskolin-stimulated LHRH release. The effect was blocked by the opioid antagonist naltrexone. These results show that functional opioid receptors of the delta type are present on GT1-1 cells. These receptors may mediate a direct opioid control of LHRH release. *(Supported by CNR through the Projects BTBS # 93.01103.PF70 ACRO # 93.0297.PF39, Aging #93.00438.PF40 and by MURST).*

387.13

NEURAL FOS-LIKE IMMUNOREACTIVITY IS NOT AUGMENTED FOR THE DURATION OF THE MATING-INDUCED LH SURGE IN THE FEMALE FERRET. **S.R. Wersinger* and M.J. Baum. Department of Biology, Boston University, Boston, MA 02215.**

Mating induces a prolonged (12-14 hr) preovulatory LH surge in the female ferret, most likely by stimulating the secretion of luteinizing hormone-releasing hormone (LHRH) into the pituitary portal vessels. Previous research in the ferret (Lambert et. al., Endocrinol 131: 1473, 1992) demonstrated that 1 h after mating there is an increase in the percentage of LHRH neurons colabeled with FOS-like immunoreactivity (FOS-IR), suggesting that these neurons are activated at the time LHRH is being released. Mating also induces FOS-IR in non-LHRH neurons in several forebrain and midbrain sites. We sought to determine whether FOS-IR was augmented in both LHRH and non-LHRH neurons throughout the unusually long preovulatory LH surge. Estrous females received one 5-minute intromission or remained alone in a testing cage for 60 minutes. Animals were perfused 0.5, 1.5, 3.0, 6.0, or 12.0 hours after the end of the intromission. The brains were processed for LHRH and FOS immunoreactivity. The percentage of forebrain LHRH neurons colabeled with FOS-IR and the number of FOS-IR non-LHRH neurons in the medial preoptic area, bed nucleus of the stria terminalis, medial amygdala, ventromedial nucleus of the hypothalamus and midbrain central tegmental field were significantly elevated 1.5 hours after mating in comparison with unpaired controls. FOS-IR was not significantly elevated in either LHRH or non-LHRH neurons at any other timepoint. However, plasma LH was significantly elevated at 12 h. These results suggest that FOS is not essential for the maintenance of the presumed preovulatory increase in LHRH secretion and may not be a suitable marker of prolonged neural activation in response to a physiological stimulus. (supported by HD210942 and MH00392).

387.14

IN VITRO EXPRESSION OF FOS-LIKE IMMUNOREACTIVITY IN IDENTIFIED ß-ENDORPHIN AND LHRH NEURONS. M. D. Loose* and M. L. Holcomb, Neuroscience Program, Oberlin College, Oberlin, OH 44074

To complement and extend in vivo studies we have developed an in vitro paradigm in which brain slices can be acutely exposed to neuromodulators and any effects on the expression of fos-like immunoreactivity (fos-l) can be determined in selected neuronal subtypes via dual label immunocytochemistry. Ovariectomized rats (60-90 days old) were injected with 10 µg estradiol benzoate 24 hrs before anesthesia and then perfused with ice cold, oxygenated Krebs buffer. Coronal brain slices (450 µm) were incubated in 100 ml buffer. Very few nuclear profiles had fos-l if slices were fixed either immediately after preparation or after incubation at 24° C. After 2-3 hrs at 34° C many cells had fos-l and 60 mM K^+ caused no subsequent increase. However, incubation near 31° C resulted in intermediate fos-l, and 60 mM K^+ induced additional fos-l profiles. Subsequent experiments were performed at 29-31° C. Cells containing both ß-endorphin and fos-l in control media were 8 ± 2.6% of the total ß-endorphin profiles (N=10 slices). Preliminary results (N=2 or 3) indicate that 30 mM K^+ caused a 2- to 5-fold increase in the incidence of double label (DL) cells whereas a phorbol ester (0.1 µM PMA) had little effect (87% of control). Incubation with 100 pM or 10 nM LHRH resulted in an apparent decreased incidence of DL (12% and 78% of control, respectively). In contrast, a majority of LHRH containing neurons (54 ± 6.9%, N=8 slices) expressed fos-l under basal conditions. No treatment tested (30 mM K^+, PMA, LHRH) has affected the percent of DL profiles in LHRH neurons.

387.15

CASTRATION DECREASES mRNA LEVELS ENCODING GLUTAMIC ACID DECARBOXYLASE WITHIN THAT REGION OF THE MEDIAL PREOPTIC AREA WHICH COINCIDES WITH THE SEXUALLY DIMORPHIC NUCLEUS (SDN-MPOA). C.A. Sagrillo* and M. Selmanoff, Center for Studies in Reproduction, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201-1559.

We have recently reported that castration decreases single cell message levels for glutamic acid decarboxylase (GAD), the rate-limiting enzyme for γ-aminobutyric acid (GABA) biosynthesis, within regions of the brain containing GnRH cell bodies. Closer examination has revealed that the majority of GAD-labelled cells in the MPOA are coextensive with the region previously described by Gorski and colleagues as the SDN-MPOA. The objective of the present study was to determine whether castration affects GAD mRNA expression specifically within this highly steroid-sensitive area of the brain. Brain sections from two or six day orchidectomized or sham-operated male rats were hybridized with an ^{35}S-labelled 48-base oligodeoxynucleotide (cDNA) probe complementary to feline GAD mRNA. Area of grains (μm^2) within the SDN-MPOA showed a reduction at 2 days (109,255 ± 13,267) and a further significant decrease at 6 days (84,848 ± 6,220) postcastration compared to intact controls (123,928 ± 10,311), while there was no change in the total area (μm^2) of the region. The data suggest that GABAergic neurons within the SDN-MPOA are important target sites for testosterone negative feedback in the male rat. GAD-labelled cells also are concentrated in the female SDN-MPOA, while the GABA concentration and turnover rate in microdissected tissue from this region are significantly lower in female compared with male rats. Current studies focus on whether a sex difference exists in the expression of the two isoforms of GAD, GAD_{65} and GAD_{67}, within this sexually dimorphic nucleus. (Supported by NIH Grant HD21351).

NEUROENDOCRINE REGULATION: GLUCOCORTICOIDS

388.1

Further Inquiry into the Effects of Corticosterone on CRH mRNA and CRH Content in the Paraventricular Nucleus of the Hypothalamus, Central Nucleus of the Amygdala and Lateral Bed Nucleus. Jay Schulkin*, Shinya Makino and Philip Gold, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD., 20892. In our earlier inquiry, we observed differential effects following chronic treatment with high doses of corticosterone (CORT) on CRH mRNA in the paraventricular nucleus of the hypothalamus (PVN) in contrast with that of the central nucleus of the amygdala (CEA). In the former region CORT reduced CRH mRNA expression, in the latter region it increased it. We have now extended these results by determining the effects of high levels of CORT on CRH cells in the lateral bed nucleus or extended amygdala. Rats were treated with either daily CORT sc injections or given a silastic implant sc to deliver CORT. Rats were sacrificed and their brains removed for analysis using in situ hybridization to determine CRH mRNA. We found that CRH mRNA levels in the lateral bed nucleus were elevated in the CORT treated groups when compared to controls. In a separate study we also measured CRH content in rats that were given the silastic capsule of CORT. Four brain regions were micro dissected (e.g. median eminence, PVN, CEA, BNST), and a radioimmunoassay for CRH content was performed on the tissue. The largest effect was the reduction of CRH content in the median eminence, there was no reduction in the PVN. CRH content was increased in the CEA, while there was no change in content in the BNST. Taken together these results suggest that while both CEA and BNST CRH mRNA can be raised when high levels of CORT are delivered, it is only the CEA that shows both elevated CRH mRNA and CRH content to CORT. Perhaps, the local activation of CEA CRH accounts for the behavioural expression of fear associated with central infusions of CRH and systemic CORT.

388.2

TRANSCRIPTIONAL CONTROL OF HIPPOCAMPAL GLIAL FIBRILLARY ACIDIC PROTEIN AND GLUTAMINE SYNTHETASE IN VIVO: OPPOSITE RESPONSES TO CORTICOSTERONE. Nicholas J. Laping*, Nancy R. Nichols, Jonathan R. Day, Steven A. Johnson, and Caleb E. Finch, Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, 3715 McClintock Ave., Los Angeles, CA 90089-0191

Transcriptional regulation of two astrocyte genes, glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS) by glucocorticoids was determined by nuclear run-on assay. Single stranded cRNA probes to the sense and antisense strands determined that transcription on the opposite strand of the GFAP and GS genes is undetectable. GFAP transcription in the hippocampus did not change 2 hours after 10 mg CORT injection in intact male rats (2-3 months old), but was reduced by 50% at 6 and 24 hours. In contrast, CORT increased GS transcription at 2 and 6 hours. GFAP, but not GS transcription, was increased seven days after adrenalectomy. Corticosterone (CORT) replacement (200 ug/ml in the drinking water) suppressed GFAP but did not increase GS transcription in adrenalectomized rats. Therefore, GFAP transcription is sensitive to low physiological levels of CORT whereas GS is not. Three non-overlapping probes for GFAP were used and detected the same degree of CORT-induced inhibition of transcription. This indicates that polymerase pausing is not a factor in CORT regulation of GFAP transcription. Because CORT regulation of GFAP is delayed compared with GS, we propose that additional transcription factors interact with glucocorticoid receptors to regulate GFAP transcription. (Supported by AG-07909 and J.D. and C.T. MacArthur Foundation to CEF and AG-05528 NRSA to NJL).

388.3

CORTICOSTERONE SELECTIVELY REGULATES $GABA_A$ RECEPTOR SUBUNIT EXPRESSION IN THE HIPPOCAMPUS. M. Orchinik*, N.G. Weiland and B.S. McEwen, Lab. of Neuroendocrinology, Rockefeller University, New York, NY 10021.

Adrenal corticosteroids (CORT) can modulate a variety of hippocampal functions. One mechanism of CORT action in the hippocampus may be via regulation of $GABA_A$ receptor subunit expression. We examined the effects of short-term adrenalectomy and low-level CORT replacement on the expression of 5 $GABA_A$ receptor subunits. *In situ* hybridization studies demonstrated that α1, α2, ß2, and γ2 subunits were altered by adrenalectomy in the hippocampus of ovariectomized female rats. Two days following adrenalectomy, levels of α1 and γ2 mRNA increased in CA3, α2 mRNA increased in the dentate gyrus, while ß2 mRNA decreased in the dentate gyrus and CA2 relative to sham-operated controls. These effects were reversed by replacement with non-stress levels of CORT (addition of 100 μg/ml CORT to the drinking water). Adrenalectomy had no effect on ß1 mRNA levels and no effect on the expression of any subunits examined in CA1 or the cingulate cortex. These data support the conclusion that corticosteroids can modulate hippocampal excitability through the site-specific regulation of the expression of specific $GABA_A$ receptor subunits. CORT-induced changes in subunit expression might modulate GABAergic synaptic inhibition by altering the density or pharmacological properties of $GABA_A$ receptors. (Supported by NRSA NS09129 to M.O., NIH grants NS30105 to N.G.W., MH41256 and NS07080 to B.S.M.)

388.4

ALLOPREGNANOLONE: A MEDIATOR OF PROGESTERONE'S SEDATIVE AND ANTIOXYTOCIC EFFECTS IN HUMAN PREGNANCY? Grace Erb, Robert Morrow, Robert H. Purdy*, and Neil J. MacLusky, Division of Reproductive Science, The Toronto Hospital Research Institute, Toronto, ON, Canada, M3C 3P5.

Numerous studies over the last five years have demonstrated that the naturally occurring 3α-hydroxy, 5α-pregnane metabolites of corticosteroids and progestins have pronounced sedative effects on the brain, mediated through benzodiazepine-like interactions of these compounds with the GABA-chloride channel complex. These effects may be involved in physiological modulation of GABAergic brain pathways, as well as in controlling the release of a number of pituitary hormones, but definitive information on circulating and tissue concentrations of progesterone (P) and corticosteroid metabolites under physiological conditions is lacking. To test the hypothesis that allopregnanolone (3α-OH-5α-pregnan-20-one, AP), the principal 3α-hydroxy, 5α-dihydro metabolite of P, may participate in P effects on the developing central nervous system during pregnancy, we have established high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA) systems to separate and measure P, AP, and other related P metabolites in biological tissue and fluid samples. Samples were extracted into diethyl ether, chromatographed on a Silica HPLC column in methylene chloride containing 0.2% ethanol. The separated P metabolite fractions were then assayed by RIA. Samples of human amniotic fluid and maternal serum were collected during cesarean section from third trimester patients in established labor, as well as from patients at the same stages of pregnancy who were not in labor at the time of surgery. For patients who were in labor and for those who were not, AP levels were approximately 10% of those of P. Levels of AP in amniotic fluid averaged 20-30% of those in the maternal circulation. These results suggest that AP is present in maternal serum and amniotic fluid at sufficiently high concentrations to potentiate GABAergic transmission, and that AP biosynthesis may contribute significantly to the actions of P on both the maternal and developing fetal central nervous systems during human pregnancy.

388.5

SYNAPTIC BURST ACTIVITY IN HYPOTHALAMIC NEURAL NETWORKS.
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Burst activity is important for the release of hormones from central neurons. In disinhibited (picrotoxin 20 μM) networks of hypothalamic neurons in culture, neurons exhibit 'rhythmic' burst activity that is blocked by the glutamate receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione). Using Ca^{2+}-imaging on Fura-2/AM loaded cultures and patch clamp techniques we found synchronous increases in $[Ca^{2+}]_i$ in a large majority of neurons (>85%) that corresponded to bursting activity recorded from a single neuron. In 20 μM CNQX, none of the neurons showed oscillations in $[Ca^{2+}]_i$.

Investigation of the intervals between bursts from single cell recording revealed a random distribution over a range of 400% from the minimum interval. Poincaré maps of burst intervals, i.e. graphs of all burst intervals I_n vs. their preceding burst interval I_{n-1}, revealed that a burst interval is unpredictable from its predecessor. When synaptic coupling was attenuated by low concentrations of CNQX (50 - 500 nM), the mean burst interval was considerably increased without a change in the random character of burst activity. 4-Aminopyridine (4-AP, 1mM), known to increase synaptic efficiency, reduced the mean burst interval. CNQX (200 nM) slowed the spread of activity during a population burst in the network and reduced resting $[Ca^{2+}]_i$. Increasing $[K^+]_o$ from 5 to 12.5 mM restored $[Ca^{2+}]_i$ but not mean burst frequency.

We conclude that burst activity in hypothalamic neural networks is generated primarily by a synaptically coupled network oscillator. Absence of a strong post-burst inhibition may be responsible for the wide distribution of burst intervals. Network oscillators are probably more reliable than single cell oscillators and may be controlled by synaptic modulation, a process that appears to be also important for the release of hormones and transmitters.

Supported by grant s from the BMFT to W.M. and from the DFG to D.S..

388.6

CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY IN THE BRAIN AND PITUITARY OF CHINOOK SALMON.
G.T. Hofeldt, S.P. Matz, and T.T. Takahashi*. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

In mammals corticotropin-releasing factor (CRF) regulates the pituitary release of adrenocorticotropin hormone (ACTH). In teleosts CRF is also thought to stimulate the release of ACTH, but in addition, may control thyrotropin-simulating hormone release from the pituitary (Moons et al., Gen. Comp. Endocrinol. 73:270-283, 1989). This is of particular interest since thyroxine has been implicated in the imprinting of salmon to their homestream odor.

We localized CRF-like immunoreactivity in the brain and pituitary of hatchery reared juvenile, 12 to 15 months, chinook salmon (*Oncorhynchus tshawytscha*). Fish were perfused with Bouin's fixative (10% Sublimate), brains frozen and sectioned at 30 microns, and then incubated with polyclonal CRF antisera for 48 h. Incubation for less than 48 h resulted in little or no staining. Preincubation of the antibody with CRF eliminated all observed staining.

Results show CRF-positive cell bodies in the parvocellular and magnocellular nuclei of the preoptic area (POA) and in the ventral zone of periventricular hypothalamus. These hypothalamic cell bodies also extend laterally into a region that may correspond to the nucleus lateralis hypothalamus. CRF-positive fibers, presumably from the cell bodies in the POA, reside along the rostral edge of the hypothalamus, in the pituitary stalk, and in the pituitary gland. CRF-positive fibers in the pituitary were clearly distinguished only in the neurohypophysis. However, intense CRF-immunoreactive material was also present in the rostral pars distalis and pars intermedia. These findings suggest that CRF might exert control on endocrine cells of the pituitary by neuroglandular and/or neurovascular interactions. The distribution of CRF-like immunoreactivity is consistent with the role of CRF as a pituitary releasing factor.

388.7

cFOS AND *cJUN* ARE EXPRESSED IN CULTURED HYPOTHALAMIC TRH NEURONS: STIMULATION BY GLUCOCORTICOIDS.
L-G. Luo and J.M.D. Jackson*. Division of Endocrinology Brown University, Rhode Island Hospital Providence, RI 02903.

There is evidence that glucocorticoids (GC) regulate the hypothalamic-pituitary-thyroid axis. Although GC have been reported to reduce proTRH mRNA levels in the thyrotrophic area of the hypothalamus *in vivo*, it has not previously been feasible to determine the direct effect on hypothalamic TRH neurons *in vitro*. Accordingly, we have studied the effect of GC on proTRH mRNA levels in hypothalamic cultures (Jackson IMD et al, 1994) and the possible involvement of the protooncogenes *cfos* and *cjun* in this process. A non-isotopic double label *in situ* hybridization histochemistry method (ISH)was chosen to explore the possibility of colocalization of TRH with *cfos* and *cjun* mRNAs. We demonstrated that the mRNA for TRH is coexpressed with both *cjun* and *cfos* in hypothalamic cells. Treatment with dexamethasone (Dex) enhanced the cell signal for both TRH and *cfos/cjun*, and also increased the number of cells coexpressing their mRNAs. Following the use of Image Analysis, Dex 10-8M enhanced mRNA of TRH 133% ($p< 0.01$ n=20), *cjun* 121% ($p< 0.01$ n=20) and *cfos* 148% ($p<0.01$ n=20). We also applied 32p-labeled RNA Blotting and Image Analysis to measure the steady state levels of *cfos/ciun* and TRH mRNA. The results show that Dex 10-8M enhanced expression of *cfos* 2.1 fold, *cjun* 4.2 fold and proTRH 3 fold ($n = 4$, $p< 0.01$). Nuclear run-on analysis of transcription was performed to measure the effect of Dex. The results showed that Dex 10-8M increased the transcription activity of *cfos* 3.4 fold, *cjun* 5 fold and proTRH 7.7 fold ($n = 3$, $P<0.01$). Conclusions: 1. Dex enhances the expression of proTRH mRNA as well as *cfos/cjun* mRNAs in hypothalamic neurons *in vitro*. 2. Dexamethasone increases the transcription activity of *cfos/cjun* and proTRH in hypothalamic cells *in vitro*. 3. The existence of *cfos/cjun* in TRH neurons and the increase of their transcription activity by Dex suggests that these protooncogenes may mediate the effect of glucocorticoids on TRH gene transcription.

NEURAL-IMMUNE INTERACTIONS: STRESS AND OTHER BEHAVIORAL MODELS

389.1

PRENATAL STRESS ALTERS IMMUNE FUNCTION OF JUVENILE AND ADULT RATS.
S.L. Klein* and D.R. Rager. Dept. of Psychology, Univ. of Georgia, Athens, GA 30602. We investigated the effects of prenatal stress on cellular and humoral immune responses in juvenile and adult male and female Long-Evans rats. Pregnant rats were either exposed to restraint under bright lights for 45 minutes three times daily ($n = 7$) or were left undisturbed ($n = 8$) during days 14-21 of gestation. The offspring of these rats were tested for cellular immune responses as measured by Concanavalin A-stimulated proliferation and Natural Killer (NK) cytotoxicity of splenocytes as juveniles (30 days of age) or adults (90 days of age), or were tested for specific humoral immune responses as measured by antibody levels in serum following *in vivo* challenge with the antigen Keyhole Limpet Hemocyanin (KLH) as adults (65 days of age). Results indicated that: (1) proliferative responses did not vary as a function of sex or prenatal treatment condition in either juvenile or adult offspring; (2) in juveniles, NK cytotoxicity was marginally lower in males as compared to females, and was also marginally reduced by prenatal stress in males but not females, whereas in adults, NK cytotoxicity was marginally enhanced by prenatal stress in both sexes; and (3) prenatally stressed offspring of both sexes had higher levels of anti-KLH IgM and IgG as compared to control offspring. In summary, prenatal stress administered during the third trimester of pregnancy altered immune function, and these effects varied as a function of the sex and age of the offspring. This study provides the first experimental evidence that prenatal stress can influence nonspecific cellular and specific humoral immune responses, and provides further support for the existence of neuroendocrine-immune interactions.

389.2

CHANGES IN NK CELL FUNCTION IN FEMALE MONKEYS IN REPSONSE TO ACUTE STRESS.
C. J. Rogers*, W. H. Chambers, C. S. Brissette-Storkus and J. L. Cameron. Depts. of Cell Biology & Physiology, Pathology, Pittsburgh Cancer Institute, Psychiatry, and Neuroscience. University of Pittsburgh, Pittsburgh, PA 15261.

Previous studies have shown acute stress-induced changes in both function and distribution of lymphocytes in peripheral blood in rats and humans, however the physiological mechanism underlying these stress-induced phenomena remain unclear. To begin to examine the effects of acute and chronic stress on lymphocyte function, in female cynomolgus monkeys, we measured both the number and the cytolytic activity of a specific subpopulation of lymphocytes, NK cells, at 0, 1, 2 and 3 hours after exposure to an acute stress paradigm involving the presentation of leather catch gloves (used in the colony to catch unanesthetized monkeys) twelve times at random intervals for 1 hour. Blood samples were collected before and 1, 2, and 3 hours after the stress via femoral venipuncture (n=4). The number of NK cells was measured using flow cytometry and the cytolytic function of NK cells assessed using a 4 hour chromium release assay against K562. NK cell activity was significantly decreased at 2 hours (37%) and 3 hours (49%) after the stress. To assess the effect of blood sampling alone as a stressor, the experiment was performed with and without glove presentation (n=14), and NK cell activity was quantified at two time points, 0 and 3 hours. Both blood sampling alone and glove presentation caused a significant reducton in NK cell cytolytic activity (39% and 48%, respectively) at 3 hours compared to time 0, with no significant difference between the two stress paradigms. There was no change in the percent of circulating lymphocytes identified as NK cells at any time point after stress exposure. These data indicate that the stressor resulted in reduced NK cell function on a per cell basis. We have thus chosen to use the acute stress of blood sampling as a probe to monitor changes in NK cytolytic activity in studies examining the effects of chronic stress (i.e. exercise training or chronic social stress) in adult and juvenile monkeys.

389.3

THE EFFECT OF COLD-WATER-STRESS ON MACROPHAGE FUNCTION AND SUBSTANCE P(SP) BINDING. C. CHANCELLOR-FREELAND*, G.F. Zhu, R. KAGE, D.I. BELLER, AND P.H. BLACK, Dept. of Microbiology, Boston Univ. Sch. of Med., Boston, MA 02118.

The macrophage, a critical component of the immune response, is profoundly affected by stress. We have previously reported stress-induced interleukin 1 (IL-1), prostaglandin (PGE_2), and Ia antigen alterations (Jiang, et al., 1990). We now report findings for increased interleukin 6 (IL-6) and tumor necrosis factor (TNFα) following exposure to stress ($p<0.05$, $p<0.01$, respectively). In addition, preliminary data show increased specific binding (B_{max}) and/or increased binding affinity (Ki) for substance P (SP) to the peritoneal macrophages from stressed mice. The stress paradigm for all work involved subjecting male C57BJ/6J mice to 5-minute swim tests in 10°C (+/-2°C) water twice daily for 4 days. Cytokine concentrations were determined by ELISA, and SP binding was assessed with fluorescein- and ^{125}I-labeled SP. Specificity and affinity were determined by competitive displacement with unlabeled SP. Results from this work, together with recent findings for stress-induced increases in immunoreactive SP in the peritoneal wash fluid, suggest that perhaps SP interacts with cell-surface receptors to mediate the various functional alterations in macrophages following stress.

389.4

CONDITIONED STRESS-INDUCED ALTERATIONS IN IMMUNE FUNCTION IN LACTATING RATS. N. Shanks*, M.A. Pezzone, G.E. Hoffman†, B.S. Rabin, Depts. of Clinical Immunopathology & Neurobiology†, University of Pittsburgh Med. Center, Pittsburgh, PA

Stress-induced CNS and endocrine alterations are attenuated in lactating females. We determined whether lactating rats were similarly resistant to stress-induced alterations in immune function. Sprague Dawley female rats were exposed to either 2 sessions of 16 footshocks (1.6 mA, 5 sec) where shock was paired with a tone (C+), or were left undisturbed in their homecages (HC). Half of the rats were bred and on day 10 of lactation half the C+ rats were re-exposed to 16 tones, but shock was witheld (C+C-), while the other half were left undisturbed (C+HC). Diestrous rats received identical stress treatments as did lactating rats (L) and served as nonlactating controls (NL). HCNLs, HCLs and C+HCNLs and C+HCLs exhibited similar resting levels of plasma corticosterone (6.8-8.3 μg/dl). Steroid levels were elevated in both C+C- groups immediately following re-exposure, but C+C-Ls exhibited lower levels of steroid (34.8 μg/dl) than C+C-NLs (50.1 μg/dl). Splenocyte aTCR stimulated proliferation was suppressed in C+C-NLs, but remained at nonstress levels in C+C-Ls. Whole blood mitogen responses (PHA, ConA) were suppressed following conditioned stress, but LPS stimulated proliferation was enhanced in both C+C-NLs and C+C-Ls. Mesenteric lymph node (MLN) lymphocyte proliferation stimulated by anti-T cell receptor antibody (aTCR) or ConA was enhanced in C+C-Ls, while this effect was less noticeable in C+C-NLs. These data suggest that lactating rats are resistant to stress-induced suppression of splenocyte proliferation, are as sensitive to alterations of blood lymphocyte immune function as are NL controls, and exhibit greater stress-related enhancement of MLN proliferation.

389.5

Peripheral Benzodiazepine receptor involvement in stress-induced suppression of *in vivo* antibody response. C. Cobb[1], M. Fleshner[1], B. Shaw[1], M. L. Laudenslager[2], L. R. Watkins[1], S. F. Maier[1], C. D. Anderson[3]*, R.C. Drugan[3] Dept Psych, UCO-Boulder, UCO HSC[2], Brown Univ, Providence, RI, 02912[3].

Exposure to inescapable tail shock (IS) results in decreases in anti-KLH Igs. The mechanism(s) responsible for this change are not well understood. The following studies examined the possible involvement of a putative endogeneous anxiogenic benzodiazepine ligand in modulating IS-induced suppression of anti-KLH Igs. Naturally occurring compounds (e.g. diazepam binding inhibitor, DBI) have been shown to increase during stressor exposure, bind to both the central-type (CBR), & the peripheral-type (PBR) benzodiazepine binding sites, & modulate the immune system. We examined the effect of CBR & PBR blockade during IS on the IS-induced reductions in anti-KLH Igs. Rats (12/grp) were immunized with KLH (200ug IP) & injected with either RO15-1788 (RO 10 mg/kg; CBR antagonist) or PK11 195 (PK 8 mg/kg; PBR antagonist) & exposed to 100 1.6 mA inescapable tail shocks (IS) or returned to their home cages (HC). Anti-KLH IgG levels were measured using ELISA. RO did not block the IS-induced decrease in anti-KLH Igs, whereas PK did. Since prior work demonstrated that glucocorticoid (GC) type II receptor antagonist administration could also block the IS-induced reduction in anti-KLH Igs, & PK is involved in steroidogenesis, we tested whether the effect of PK was due to PK's potential alteration of IS-induced GCs. Rats were injected with PK or vehicle & exposed to IS. Blood samples were taken after 50 & 100 shocks, & 90 min & 24 hrs after IS. PK had no effect on the IS-induced changes in serum GCs. Thus, PK blockade of IS-induced decreases in anti-KLH Igs suggest an involvement of the PBR/DBI system which is GC independent. NIH-MH45045

389.6

I. Mechanisms of stress-induced immunomodulation: T cells M. Fleshner*, J. Hermann, M. L. Laudenslager[1], L. R. Watkins, S. F. Maier Dept. of Psychology, Univ.of Colorado, Boulder, CO 80309, Univ. Colorado Health Sci. Cntr., Denver, CO[1].

Stressors can result in changes in immune function. Exposure to inescapable tail shock (IS) results in long-term decreases in anti-KLH Igs The mechanisms responsible for this change are not understood, but evidence suggests an involvement of altered T cell subpopulations. The following studies extended these earlier observations. Rats were immunized with KLH (200ug IP) and exposed to IS (n ~ 6) or returned in their home cages (n ~ 6). Lymphocytes from the mesenteric lymph node (MS), cervical lymph node (CN), and spleen (SPL) were removed 0, 48, 72, 96, or 168 hrs after IS termination. The percent of TCR+CD4+, TCR+CD8+, CD45RC+ CD4+ (Th1-like) and CD45RC-CD4+ (Th2-like) lymphocytes were assessed at each time point for each tissue using two color flow cytometry. The number of KLH specific splenic B cells were also measured 7 days (168 hrs) after KLH+IS using ELISPOT. IS results in a complex pattern of changes. The greatest shifts occurred immediately after IS + KLH and 96 hrs (4 days) after IS + KLH. IS resulted in an increase in CD4+ T cells and Th1-like cells per gram of wet tissue immediately after IS termination, but only in MS. Four days after IS + KLH, there was a decrease in Th1-like MS cells and an increase in Th2-like MS cells. Seven days after IS + KLH there was a reduction in the number of KLH specific splenic B cells. These changes in T cell phenotypes could be involved in causing the IS-produced reduction in the formation of KLH specific B cells and alterations in immunoglobulin levels and isotypes (IgG1 & IgG2a). NIH-MH45045

389.7

II. Mechanisms of Stress-induced immunomodulation: A role for the macrophage. J. L. Hermann*, M. Fleshner, K H Ray, L. L. Lockwood, L. Silbert, M. L. Laudenslager[1], L. R. Watkins, S. F. Maier. Dept. Psych, CU, Boulder, CO 80309, Univ. Colorado Health Sci. Cntr., Denver, CO[1].

Inescapable tail shock (IS) suppresses Ig response to KLH. Evidence suggests that alterations in early processing events such as antigen transport & processing by macrophages (MØs) may be involved. Stressors can alter MØ function & can make MØs suppressive. The following studies examined the role of MØs in stress-induced decreases in anti-KLH Igs. First, stress-induced changes in MØ numbers were investigated immediately after IS + KLH in the peritoneal cavity, the mesenteric lymph nodes (MS), cervical lymph nodes (CN), & the spleen (SPL). Cells were labeled with ED1 (MØ specific), anti-TCR, & anti-CD4 antibodies and were counted using two-color flow cytometry. IS decreased ED1+ peritoneal cell number & ED1+ splenic cell number immediately after IS + KLH. In contrast, the number of ED1+ cells in both MS & CN increased following IS + KLH. If the increase in MS ED1+ cells reflects an increase in a suppressive MØ population, then inhibition of these cells should result in a potentiated immune response when the antigen processing involves MS. Rats (12/gr) were injected intraperitoneally (IP) with either gandolinium chloride ($GdCl_3$; 7.5 mg/kg, a phagocyte inhibitor), or saline. Twenty-four or 72 hrs after $GdCl_3$, rats were immunized with KLH (200ug) either IP, which involves the draining nodes (MS), or intravenously (IV), which bypasses the draining nodes. The Ig response to KLH, measured using ELISA, was found to be enhanced in IP immunized rats both 24 hrs & 72 hrs after $GdCl_3$. In contrast, $GdCl_3$ had no effect in IV immunized rats 24 hrs after $GdCl_3$ & slightly suppressed the Ig response in these rats 72 hrs after $GdCl_3$. It appears, therefore, that MØs can have a suppressive effect on Ig production in response to IP KLH & that IS induced decreases in anti-KLH Ig production may be due to increased suppressive MØs in the MS. The importance of IS-induced decreases in peritoneal and SPL MØs is currently being investigated. NIH-MH45045.

389.8

III. Mechanims of stress-induced immunomodulation: RU 486 but not Naltrexone blocks the stress-induced decrease in anti-KLH Igs. L. R. Watkins*, M. Fleshner, F. X. Brennan, G. R. Dungan, L. L. Lockwood, M. L. Laudenslager[1],S. F. Maier. Dept. of Psychology, Univ.of Colorado, Boulder, CO 80309, Univ. Colorado Health Sci. Cntr., Denver, CO[1].

Stressors can result in changes in immune function. Exposure to inescapable tail shock (IS; 100 1.6 mA) results in decreases in anti-KLH Igs. The hormonal mechanisms responsible are not understood. Both glucocorticoids (GCs) as well as endogeneous opioids are potential candidates, as both are released during stress & are capable of altering immune function. Thus, the effect of RU 486 (RU; a glucocorticoid type II receptor antagonist) & Naltrexone (NAL; an opiate receptor antagonist) on the shock-induced reduction in anti-KLH Igs was examined. Rats (12/grp) were injected with NAL (5 mg/kg or 10 mg/kg), RU (5 mg/kg or 10 mg/kg), or the appropriate drug vehicle, then immunized with KLH (200ug IP) & exposed to either IS or returned to their home cage (HC). Anti-KLH IgM & IgG levels were measured using ELISA. Exposure to IS resulted in a reduction in anti-KLH IgM & anti-KLH IgG. The high dose of RU blocked the IS-induced reduction in anti-KLH Igs, whereas the low dose was without an effect. In contrast, the high dose of NAL did not block the effect of IS on anti-KLH Igs & suppressed the Ig response in HCs. The low dose of NAL also did not block the IS-induced reduction in anti-KLH Igs & had no effect in HC controls. These data suggest a potential role for GCs but not endogeneous opioids as hormonal mediators of the IS-induced reduction in anti-KLH Igs. NIH-MH45045.

389.9

IV. Mechanisms of stress-induced immunomodulation: High levels of steroids at the time of immunization are not necessary for IS-induced immunosuppression. T. L. Mayr*, M. Fleshner, M. L. Laudenslager[1], L. R. Watkins, S. F. Maier, Dept. of Psychology, Univ.of Colorado, Boulder, CO 80309, Univ. Colorado Health Sci. Cntr., Denver, CO[1].

Exposure to inescapable tail shock (IS; 100 1.6 mA) results in decreases in anti-KLH Igs. The mechanism(s) responsible for this change are not well understood. Previous work demonstrated that administration of the glucocorticoid (GC) type II receptor antagonist RU 486 before IS & KLH blocked the IS-induced reduction in anti-KLH IgM & anti-KLH IgG (see companion abst. III). These data suggested that GC released during IS could mediate the effects of IS on anti-KLH Igs. The timecourse of the changes in serum GCs during & after IS are well understood. GCs increase to ~ 40-50 ug/dl & return to basal levels 90-120 min after IS. The following studies examined whether rats must be immunized with KLH in the presence of high GCs for a reduction in anti-KLH Igs to occur. Rats (12/grp) were immunized with KLH immediately prior to, immediately after, or 3 hrs after exposure to IS. Anti-KLH IgM, IgG, IgG1, & IgG2a levels were measured using ELISA. Data to date reveal that IS resulted in a reliable reduction in anti-KLH IgM & IgG levels at all times of immunization. Importantly, rats immunized 3 hrs after IS termination had normal GC levels. IgG1 & IgG2a are not yet available. Subcutaneous injection of 100ug/kg of dexamethasone 2 hours prior to KLH also had no effect on anti-KLH Ig levels. Thus, high levels of GCs **at the time** of KLH are not necessary to produce IS-induced decreases in anti-KLH Igs. Dose response experiments involving short interval timing of KLH immunization & IS exposure as well as corticosterone & dexamethasone dose response studies are currently in progress. NIH-MH45045

389.10

FAILURE TO DETECT A DIRECT RELATIONSHIP BETWEEN STRESS-INDUCED CHANGES IN LYMPHOPROLIFERATION AND CIRCULATING CATECHOLAMINE CONCENTRATIONS L. Potla, S. Pouciau, C.A. Meseke, D.W. Horohov, S. Barker, M. Littlefield-Chabaud, J. Bu, S.G. Kamerling, P.A. Melrose*, School of Veterinary Medicine, Baton Rouge, LA 70803.

The present study was performed in order to determine whether stress-induced catecholamine release may be correlated with the inhibition of influenza virus (specific) and/or pokeweed mitogen (non-specific)-induced lymphoproliferation. Unconditioned Thoroughbred horses were subjected to sham, psychological or various intensities of exercise stress. Jugular blood samples were collected at 4 different time points, before and during each stress test. Triplicate cultures of $2x10^5$ peripheral blood mononuclear cells were incubated at 39° C with control media, high or low dose of influenza virus or pokeweed mitogen. Proliferation was measured based on radioactive thymidine incorporated on days 5 and 7. Plasma samples obtained at the aforementioned 4 time points were also assayed for catecholamines using high performance liquid chromatography. Non-specific lymphoproliferation was significantly decreased during slow speed exercise stress. Specific lymphoproliferation declined ($P<.05$) in all but the sham stress test and it was increased ($P<.05$) at the end of psychological stress. Circulating concentrations of norepinephrine and epinephrine were increased ($P<.05$) by all stress tests. Thus, stress-induced changes in lymphoproliferation were not related to increased catecholamine concentrations that resulted from psychological and physical stressors.

389.11

MONOAMINERGIC, NEUROENDOCRINE AND IMMUNE RESPONSES TO STRESS: THE INFLUENCE OF LATERALIZATION. C. Delrue, B. Deleplanque, K.-S.Li, S.Vitiello, J.-J. Bouyer* and P.J. Neveu.

Stress responses including modifications of immune reactivity, neuro-endocrine functions, and brain functioning were hypothesized to depend on lateralization. To answer this question, we studied the stress responses in mice selected as left- and right-handers in a paw preference test.

First, restraint stress, known to affect immune reactivity, induced modifications in the mesolimbic dopaminergic (DA) system which are asymmetrically expressed and are related to behavioral lateralization. Indeed, the asymmetry of DA metabolism in the nucleus accumbens observed in controls and correlated to paw preference scores, disappeared after stress. Conversely, an asymmetry in DA metabolism in the frontal cortex, correlated to paw preference, appeared in stressed animals.

Second, the injection of lipopolysaccharide (LPS) is usually followed by a decrease in T-lymphocyte proliferation, an increase in ACTH and corticosterone plasma levels and an augmentation in brain monoaminergic metabolism and thus may be considered as a stress inducer. The modifications of brain monoaminergic metabolism were asymmetrically expressed and depend upon behavioral lateralization. Plasma corticosterone was enhanced in both left- and right-handers, but plasma ACTH was increased only in right-handed animals. T-lymphocyte proliferation was depressed only in right-handers.

These results show that an immune challenge, which may be considered as a stressor, induces modifications in the activity of the central nervous system, the HPA axis and the immune system, that depend on brain and behavioral lateralization. Therefore, the bidirectional pathways between the brain and the immune system appear to be subjected to lateralization.

389.12

ALTERATIONS IN T-CELL AND MACROPHAGE FUNCTION INDUCED BY A METABOLIC STRESS, 2-DEOXY-D-GLUCOSE (2-DG): EFFECTS AND MECHANISMS. SH Chou, LD Kojic, & JE Cunnick*. Microbiology, Immunology & Preventive Medicine, Iowa State University, Ames, IA 50011; USA.

Administration of 2-deoxy-D-glucose (2-DG, 500 mg/kg) was used to characterize the effect of metabolic stress and its mediating mechanisms on immune function. Male Lewis rats were exposed to one or five injections (one every 48 h) of 2-DG. Control rats received saline injections. In blood and spleen, T-cell function was evaluated by mitogen proliferation and production of interleukin-2 (IL-2) and interferon-γ (IFN-γ), while macrophage function was evaluated by production of nitric oxide as measured by nitrite. 2-DG induced a suppression of mitogenic responsiveness and IFN-γ production in both whole blood and spleen lymphocytes. Additionally, IL-2 production was reduced in the blood, but not the spleen. Conversely, there was a significant increase in nitrite production in cultures of Con A and PHA stimulated splenocytes from 2-DG injected animals in comparison to saline injected controls. In blood cultures stimulated with Con A and PHA, the nitrite production of the group which received five injections of 2-DG was significantly higher than the group which received one injection of 2-DG or saline.

Administration of nadolol, a β-adrenergic receptor antagonist attenuated the 2-DG induced suppression of splenic T-cell responsiveness to mitogen and IFN-γ production, as well as attenuated the increased of nitrite production in splenic cell cultures. However, nadolol did not attenuate the 2-DG induced changes of immune parameters in blood cultures. These results demonstrate that the metabolic stressor 2-DG can alter cellular immune function via neuroendocrine pathways common to physical and psychological stressors. Additionally, the use of 2-DG in rats provides an important model to study metabolic stress.

389.13

ADVERSE EFFECTS OF CHRONIC NICOTINE ON IMMUNE ORGAN ATROPHY IN AN ANIMAL MODEL OF ANOREXIA NERVOSA. A. Chiou, D.M. Amos, H.A. Funk, T.S. Rieg* & P.F. Aravich. Virginia Governor's School for Science & Technology, Hampton, VA 23666; Eastern Virginia Medical School, Norfolk, VA 23501; Veterans Affairs Medical Center, Hampton, VA 23667.

Anorexia nervosa (AN) is an eating disorder associated with excessive activity, weight loss and immunosuppression. Nicotine increases activity, decreases body weight and affects immune function. This investigation determined if nicotine increases activity, promotes weight loss and worsens the thymus and spleen atrophy associated with a popular animal model of AN. The model was produced by giving moderately food-deprived rats (1.5 h/day food access) free access to running wheels (22.5 h/day). Three groups of rats (9-10 rats/group) were injected with either 0, 0.4 or 0.8 mg/kg/day of nicotine bitartrate (free base; s.c.) for 11 days prior to and throughout the syndrome. Contrary to predictions, nicotine did not affect wheel running, food intake and rate of weight loss in the model. However, nicotine worsened the thymus atrophy associated with the syndrome. This effect, which was specific to the thymus and did not occur with the spleen, was related to an exacerbation of the relative adrenal hypertrophy that occurs in the syndrome. It is proposed that nicotine stimulates glucocorticoid secretion, which adversely affects the immature T lymphocytes of the thymus but spares the mature lymphocytes of the spleen. These data raise the possibility that, while nicotine may not affect weight loss in AN, it may adversely affect immune function, which is already compromised in the disorder.

389.14

IMMUNOSUPPRESSIVE TREATMENT RESTORES EXPLORATORY BEHAVIOR IN AUTOIMMUNE MRL-lpr MICE. Boris Šakić*, Henry Szechtman and Judah A. Denburg. Depts. of Biomedical Sciences and Medicine, McMaster Univ., Hamilton, Canada L8N 3Z5.

Various behavioral deficits of unknown etiology accompany systemic autoimmune diseases in humans. Autoimmune MRL-lpr mice show a variety of behavioral deficits in comparison to undiseased age-matched MRL +/+ mice, suggesting an animal model of behavioral dysfunction in autoimmune disorder. We have recently observed impaired exploration of a novel object in the MRL-lpr group. Moreover, this behavioral deficit significantly correlated with high serum autoantibody titers (a reliable symptom of autoimmunity). In the present study we test the relationship between exploratory behavior and autoimmunity by treating young MRL-lpr and MRL +/+ mice for 6 weeks with immunosuppressive drug, cyclophosphamide (100 mg/kg/week, i.p). It was expected that such a treatment would prevent development of autoimmune symptoms and restore normal exploratory behavior in MRL-lpr mice. Indeed, chronic immunosuppressive treatment almost completely abolished the onset of autoimmune disease and increased the duration of novel object exploration in the MRL-lpr group. Thus, in MRL-lpr mice there may be a causal relationship between autoimmune factor(s) and impaired exploration of novel objects. (BS is a OMHF Postdoctorate Fellow; supported by funds from NSERC)

389.15

INTERLEUKIN-1 INHIBITS SEXUAL BEHAVIOR OF FEMALE BUT NOT MALE RATS. R. Yirmiya*, R. Avitsur, O. Donchin, and E. Cohen. Department of Psychology, The Hebrew University of Jerusalem, Mount Scopus, Jerusalem 91905, Israel.

In response to infection and injury, a variety of cells release the cytokine interleukin-1 (IL-1), which produces immunological, neuroendocrine and behavioral effects. The present study examined the hypothesis that the previously reported suppressive effects of IL-1 on reproductive hormones and goal-directed behaviors is also associated with inhibition of sexual behavior. The effects of IL-1 on sexual motivation and sexual receptivity in female rats were examined using the partner preference (PP) paradigm and the test of lordosis quotient. The effects of peripheral (i.p.) administration of IL-1β were examined during the estrus phase of intact cycling females. Administration of IL-1, at a dose of either 2 or 10 μg/kg, significantly decreased proceptive (soliciting) and receptive behaviors. The suppression of sexual behavior by IL-1 was further demonstrated in ovariectomized rats, following hormonal induction of estrus: IL-1β (2 μg/kg) injected 2 hr before testing significantly decreased PP scores as well as proceptive and receptive behaviors. Intracerebroventricular administration of IL-1β (10 ng/rat) suppressed PP scores and proceptive behavior in intact cycling females. The effects of IL-1β on male sexual motivation and performance were assessed using the PP paradigm and by counting the number of mounts, intromissions and ejaculations during a 15 min testing period. IL-1β had no effect on any component of male sexual behavior. The inhibition of female sexual behavior by IL-1 may be an adaptive mechanism, which prevents conception during an infection and therefore reduces the risk of prenatal infection.

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389.16

EMOTIONALITY AND LEARNING IN AUTOIMMUNE MICE L.M. Schrott* and L.S. Crnic. Depts. of Psychiatry and Pediatrics and the MRRC, Univ. of Colorado School of Medicine, Denver, CO 80262

Mice with lupus-like autoimmune disorders such as NZB x NZW F1 (B/W) hybrids display specific learning deficits in shock motivated active avoidance learning. Although this deficit is not due to altered sensitivity to shock, it may be due to alterations in responsiveness to emotional stimuli such as shock. To investigate this possibility, female B/W mice were tested in an elevated plus maze, a sensitive measure of anxiety, and a simple shock-motivated black-white discrimination task. B/W mice readily learn a swim version of this task. Mice were given 10 trials a day for 6 days. In comparison to female mice from the paternal progenitor strain NZW, female B/W mice displayed an anxiety profile in the plus maze. They had fewer percent entries and spent less percent time in the open arms of the maze. In the shock discrimination learning task, B/W females were faster and had a greater number of correct responses than NZWs. Both strains of mice initially used a positional strategy and showed a high degree of reponse lateralization. On days 4-6 both strains stopped using a positional strategy, and the B/W mice switched to the correct strategy of associating color with escape. In the swim version of the task, B/W mice learned more quickly and did not show the initial positional strategy. These findings, in combination with the plus maze data, suggest that shock motivation does not impede learning, but that alterations in responses to aversive stimuli (such as open arms in a plus maze or footshock) may influence performance. Neuroactive cytokines that are up-regulated in the autoimmune disease may be involved in mediating these responses.

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389.17

INTERFERON-ALPHA DETECTED BY *IN SITU* RT-PCR IN THE BRAINS OF AUTOIMMUNE MICE. L.S. Crnic* AND L.M. Schrott. Depts. of Pediatr. and Psychiat. and the MRRC, Univ. Colo. Sch. of Med., Denver, Co 80262

Interferons are known to be neuroactive and are up-regulated in lupus-like autoimmune diseases such as that seen in NZB x NZW F1 (B/W) hybrid mice. This study focused on the role of interferon-alpha (IFN-α) in the behavioral abnormalities seen in these mice by examining brains of female B/W mice for IFN-α mRNA. Using reverse transcription followed by polymerase chain reaction amplification (RT-PCR), IFN-α was detected in brains both before and after the onset of severe immunologic abnormalities (12 and 24 weeks). *In situ* RT-PCR was used to localize specific brain regions producing IFN-α. Following fixation and paraffin embedding, brains of 12 week old mice were sectioned in the sagittal plane. Sections were treated with trypsin to permeabilize membranes and DNAase to destroy genomic DNA. mRNA was converted to cDNA in an RT reaction and IFN-α cDNA was amplified by PCR. A digoxigenin-labelled nucleotide was incorporated into the amplified product and detected immunocytochemically. Negative and positive controls were run with each sample. Positive signal for IFN-α mRNA was found middle layers of the cerebral cortex, extending along most of the anterior-posterior axis. Signal was seen in membranes surrounding the brain and in occasional cells throughout the CNS. Dysmorphology of the cerebellum was also apparent. To determine the specificity of this pattern of signal, brains of mice injected with the IFN inducer Poly I:C were examined. These mice displayed IFN-α mRNA signal in neurons throughout the brain. Thus, it appears that the *in situ* RT-PCR technique is sensitive and specific. The production of IFN-α mRNA in the CNS may explain some of the behavioral alterations in autoimmune mice. Supported by MH49043, MH10643, MH15442 and HD04024 and by the Developmental Psychobiology Endowment fund of the Department of Psychiatry.

389.18

INTERLEUKIN-2 AND UNCONTROLLABLE STRESSORS INTERACT TO ALTER NEUROENDOCRINE AND IMMUNE ACTIVITY. S. Zalcman, J. M. Green-Johnson*, A-M. Brown, D. M. Nance and A. H. Greenberg. Manitoba Institute of Cell Biology and Univ. of Manitoba, Winnipeg, Mb. R3E 0V9.

Interleukin (IL)-2 potentiates a variety of immune responses and influences central nervous system activity. We reported that IL-2 induced alterations of central monoamine activity that were similar to those provoked by uncontrollable stressors and that IL-2-induced immunological effects were mediated by non-lymphoid mechanisms. In the present study, we determined whether IL-2 would interact with a stressor to alter neuroendocrine and immune activity. BALB/c mice received either Ringer's Solution or IL-2 (200 ng, ip) and were immediately exposed to a novel environment for 0, 30 or 60 minutes. Seven fold elevations of plasma corticosterone concentrations were evident in IL-2-treated mice exposed to the stressor for 30 or 60 minutes compared with mice sacrificed immediately after IL-2 injection. These elevations were significantly greater than those observed in vehicle-treated animals exposed to the stressor for comparable periods. Corticosterone levels did not differ among vehicle- and IL-2-treated mice that were returned to their home cages for 0, 30 or 60 minutes following injection. IL-2 also potentiated corticosterone elevations following exposure to restraint stress. Mice received vehicle or IL-2 and were either not stressed or were immediately restrained for 30 minutes. Corticosterone levels were determined 0, 30 or 60 miutes after stressor termination. In vehicle-treated mice, levels were significantly increased immediately following restraint but returned to control levels within 30 minutes of stressor termination. In IL-2-treated mice, corticosterone levels were markedly elevated 0 and 30 minutes after stressor termination and returned to non-stress levels within 60 minutes of stressor termination. Additionally, the enhancing effects of IL-2 on an antigen-specific IgM PFC response were not evident in mice exposed to a mild stressor immediately after IL-2 administration. This treatment also resulted in a suppression of splenic T cell proliferation and IL-2 production that was similar in magnitude and duration to that evident in mice exposed to restraint stress. It is suggested that neuroendocrine and immune consequences of a lymphokine (i.e., IL-2) are influenced by the stressor background on which it is superimposed. Supported by NIMH, MRC of Canada.

389.19

CLASSICAL CONDITIONING OF BRAIN C-FOS EXPRESSION AND IMMUNE CELL ACTIVITY. L. Wetmore*, W. Wan, J.G. Gartner and D. M. Nance. Depts. Physiol. and Path., Univ. Manitoba, Wpg, MB, R3E 0W3.

Stress is a complex phenomenon involving a highly coordinated activation of the endocrine, immune and central nervous systems . We have reported the central pattern of c-fos expression induced by intermittent footshock and the activation of the limbic system suggests that the psychological dimension of footshock may be an important component contributing to stressor effects. We have assessed the ability of conditioned fear to induce c-fos expression in the brain and to alter immune activity in male S/D rats. Rats were given three sessions of intermittent footshock (UCS) with a tone as the conditioning stimulus (CS). One week later, animals were either re-exposed to the CS (+CS) and immediately sacrificed or were sacrificed from the home cage (-CS). We found that footshock induced c-fos expression can be classically conditioned and that the +CS animals were immunoenhanced, relative to the -CS group. The CS induced c-fos protein expression occured in the same brain regions observed following the UCS, whereas the -CS group showed minimal brain c-fos expression. Conditioned immune effects were also found in that the +CS group had significantly enhanced splenic NK cell activity, relative to -CS animals. Spleen and blood cell lymphoproliferative responses to mitogen were also elevated in the +CS, relative to the -CS group, thus conditioned fear can induce c-fos expression in a distinct central circuit as well as produce an immunoenhancement. Presently, we are investigating the relationship between the strength of the conditioned response and the immune alterations in order to better characterize the significance of the concommitant effects of conditioned fear on central c-fos expression and immune cell activity. Supported by MRC of Canada.

389.20

TO THE MECHANISMS OF PRENATAL EPYGENETIC TRANSFER OF MATERNAL "ANTI-BRAIN" AUTOIMMUNITY. N.K.Yabi-shevich, A.B.Poletaev*. Chernobyl-Test Ctr., Moscow, Russia.

Transfer of the specific autoimmune reactions from a mother, but not from a father to a child (described earlier) is probably connected with the genesis of some forms of inborn defects of the nervous system. It is necessary to know the mechanisms that allow the maternal immune system to play a "matrix" role in regard to the immune peculiarities of a child. It can be supposed that the modulation of the AG-specific immune status of a child is dependent on:(1) the transplacentar transfer the own maternal AB; (2) the prenatal "mirror" activation of the certain clones of child lymphocytes (LC) by the maternal AB; (3) the "transplantation" of the immunocompetent LC, e.a. the maternal LC (including memory cells) transfer and their long (for years) persistence in a child. In experiments on rats and rabbits (passivelly or actively immunized by the S100 proteins during pregnancy) it was shown that the hypothesis (1) is groundless. In opposite real evidences for hypotheses (2) and (3) were recieved. Thus, the transfer phenomenon is probably based on the combination of the AB-dependent modulation of the child LC repertoires and a direct transfer of the maternal AG-specific LC with their subsequent persistence in a child.

390.1

INCREASED SUSCEPTIBILITY TO BREAST CANCER METASTASIS DURING PROESTRUS/ESTRUS PHASES IN F344 RATS: IMPLICATIONS FOR PROGNOSIS FOLLOWING THE REMOVAL OF A BREAST CANCER S. Ben-Eliyahu*, G.G. Page, J.C. Liebeskind & A.N. Taylor. Dept. of Psychology, Tel-Aviv University, Tel Aviv 69978, Israel.

Several studies report that women undergoing surgery for breast cancer during the first half of their menstrual cycle show a 3-fold increase in mortality rate over a ten-year period. We present evidence in rats in support of the hypothesis that this phenomenon is attributable to metastatic development that is induced by the surgical procedure itself and is facilitated by elevated estradiol/low progesterone levels at the time of surgery. Suppression of innate immunity is suggested to underlie this increased susceptibility to metastasis, rather than a direct effect of sex hormones on the tumor. Syngeneic mammary tumor cells (MADB106) were injected intravenously to inbred F344 rats during different phases of the estrous cycle. The metastatic efficiency of this tumor, which is confirmed here to be highly controlled by natural killer (NK) cell activity, was higher during estrous phases that are hormonally homologous to the high-risk periods in women. Estrogen treatment alone caused similar effects in ovariectomized rats and progesterone partly blocked these estradiol effects, whereas neither hormone affected the proliferation rate of the tumor in vitro. The tumoricidal activity per blood NK cell was diminished during the high-risk estrous phases. These findings introduce an animal model for further studying the clinical phenomenon and suggest hormonal and immunological mechnisms mediating it. Supported by NIH-HD07228, NIH/NS 07628, VA Medical Research Service, the UCLA Psychoneuroimmunology Task Force, and a research grant from Tel-Aviv University.

390.2

STRESS-INDUCED CHANGES IN LEUKOCYTE DISTRIBUTION -- HORMONE DEPENDENCY. F. S. Dhabhar*, A. H. Miller, B. S. McEwen, R. L. Spencer. Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

We have previously demonstrated significant and selective changes in numbers and percentages of peripheral blood leukocyte (PBL) subpopulations in the rat. These changes were rapidly induced by mild acute stress (2 h restraint), and consisted of a decrease in numbers and percentages of lymphocytes, and an increase in numbers and percentages of neutrophils. B cells, NK cells, and monocytes showed a greater stress-induced decrease than T cells. Helper T cell number showed the lowest magnitude of decrease with stress. These stress-induced changes were rapidly reversed upon the cessation of stress.

In the present series of studies we demonstrate that adrenalectomy significantly reduced the magnitude of the stress-induced changes. Furthermore, administration of cyanoketone, a corticosterone synthesis inhibitor, eliminated the stress-induced changes in PBL. However, catecholamine receptor antagonists (phentolamine (alpha antagonist) and propranolol (beta antagonist)), failed to inhibit the stress-induced changes in PBL. Acute administration of corticosterone to adrenalectomized animals resulted in a close replication of the stress-induced changes observed in adrenal intact animals. Furthermore, acute administration of glucocorticoid receptor agonists showed that the Type II receptor agonist (RU28362) replicated all the stress-induced changes in PBL (except the decrease in NK cell percentage). The Type I receptor agonist (aldosterone) had no effect. These results suggest that corticosterone is a major mediator of the stress-induced changes in leukocyte distribution. (MH 47674, MacArthur Foundation.)

390.3

SEXUALLY DIMORPHIC EFFECT OF PRENATAL DHEA TREATMENT ON T-CELL FUNCTION. S. G. Shelat, I. Halasz and E. Redei* Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

We have hypothesized that sexual differentiation of the immune function occurs prenatally and is driven by similar events as seen in the sexual differentiation of the brain. Therefore, pregnant dams were treated with different doses of dehydroepiandrostenedione (DHEA), a weak androgen, in the drinking water from day 8 of gestation until parturition when DHEA treatment was discontinued. Body weights of young, prepubertal (day 35 of age) male and female pups from the DHEA groups were significantly lower than those of controls, and showed a dose response effect even at this age (control males 133.6±2.62 g; DHEA males (250 ng/ml) 87.4±5.45 g; DHEA males (25 ng/ml) 98±3.2 g; DHEA males (2.5 ng/ml) 114±3.75 g; control females 118.2±1.66; DHEA female(25 ng/ml)82±2.26 g; DHEA female (2.5 ng/ml) 103.6±3.68 g). In contrast, both absolute thymic weights and thymic weights normalized to body weight were significantly higher in the offspring of the lowest DHEA treated dams (2.5ng/ml) compared to those of controls (control males and females: 3.74±0.23 and 3.59±0.2 mg/g BW; DHEA males and females 4.8±0.26 and 4.46±0.17 mg/g, respectively). Splenic lymphocyte proliferative response to Concanavalin A (Con A, 2.5 ug/ml) used spleens of individual animals representing different litters. There was no significant sex difference in the proliferative response to Con A in the control animals, although control males showed a tendency toward a higher response. Prenatal treatment with DHEA (2.5 ng/ml) significantly reduced proliferation in the male offspring (controls: 210,684±35,339 cpm; DHEA: 90,897±8,594 cpm) while DHEA females were not affected significantly and showed an increased rather than a diminished response (controls: 150,682±28,818 cpm; DHEA: 220,886±21,145 cpm). This data suggest that prenatal DHEA treatment permanently alters thymic development and T-cell dependent functions, particularly in the male offspring.

390.4

TYPE II GLUCOCORTICOID RECEPTOR (GR) GENE EXPRESSION PARTICIPATES IN THE DEVELOPMENT OF NEUROENDOCRINE-IMMUNOLOGICAL SEXUAL DIMORPHISM: EFFECT OF ABNORMAL GR FUNCTION IN TRANSGENIC ANIMALS BEARING TYPE II GR ANTISENSE RNA. B. Marchetti*, M.C. Morale and N. Barden. Dept. of Pharmacology, University of Catania, Italy, and Molecular Psychogenetics, CHUL, Québec, Canada.

Clinical and experimental evidence indicates that gonadal steroids can modulate immunological functions and that a sexual dimorphism exists in the immune response to different noxa. The molecular mechanism(s), however, involved in sexually-dimorphic immune responses are not completely understood. We have recently (Endocrine Journal 2: 181, 1993) demonstrated that type II GR gene expression within the thymus is markedly modulated by physiological alterations of the sex steroid hormone milieu. The aim of the present study was to determine the role of type II GR gene expression in the development of neuroendocrine-immunological sexual dimorphism using a transgenic animal model created by partially knocking out gene expression with type II GR antisense RNA. In intact mice, a sexual dimorphism in the post-natal development of type II GR gene expression paralleled the development of sexually dimorphic immune responses, initiating 9 days after birth, reaching a peack of activity at 25 days of age, and declining around puberty. The development of a sex-dependent apoptotic potential after thymocyte treatment in vitro with corticosterone followed a similar pattern. In transgenic animals, the abolishment of a sexual dimorphism of type II GR gene expression was accompanied by a lack of sexually dimorphic immune responses and marked inhibition of the specific suicide program within the thymus, suggesting a key role for type II GR gene expression in the molecular mechanisms underlying the development of sexual dimorphism of immune responses.

390.5

PERMISSIVE ROLE OF GLUCOCORTICOIDS ON INTERLEUKIN-1 ACTIVATION OF THE SEROTONERGIC SYSTEM. L. Gemma, A. De Luigi, M.T. Tacconi* and M. G. De Simoni. Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157, Milan - Italy.

Glucocorticoids (GCs) are widely used in therapy for their antiinflammatory and immunosuppressive properties but their use in cytokine-mediated pathologies needs a thorough understanding of their role in modulating cytokine actions. The present study was designed to clarify the effect of GCs on the action of IL-1β on the serotonergic system. Changes in the serotonin metabolite 5-hydroxyindolacetic acid (5HIAA) were recorded in freely moving rats by *in vivo* voltammetry using chronically implanted carbon fiber electrodes in the medial preoptic area. IL-1β (10 ng icv) induced a dual increase in 5HIAA levels: a rapid-short, term rise was followed by a lasting increase possibly due to newly synthesized IL-1. The synthetic GC dexamethasone (DEX, 3 mg/kg ip, 30 min before IL-1β), prevented the effect of IL-1β starting from 150 min, suggesting that it only inhibited the second increase. In adrenalectomized rats IL-1β had no effect but when these rats were given DEX (40 μg/kg a day for 3 days) the short-term increase was restored. The GC receptor antagonist RU38486 (25 mg/kg sc, 60 min before IL-1β) completely prevented IL-1β activation of the serotonergic system. The results indicate that although GC are effective inhibitors of IL-1 synthesis, their presence is necessary for IL-1β-induced activation of the serotonergic system. Thus GC may allow a number of serotonin-mediated actions triggered by IL-1 in the course of inflammation.

390.6

CORTICOTROPIN RELEASING FACTOR (CRF) SECRETION FROM THE HYPOTHALAMUS AND THYMUS GLAND IN THE RAT. C.P. Phelps, L.T. Chen, R.A. Menzies*, J. Oliver, E. Horvath, L. Poole. Dept. of Anatomy, U. South Florida, Tampa, FL 33612

The hypothalamus (HYP) and thymus gland (THY) both contain bio- and immunoreactive CRF and CRF mRNA (Redei, 1992; 1993). Hypothalamic secretion of CRF is a well-known final common pathway for direct and indirect immunomodulation, but the role of CRF in THY function is not well understood. We have compared the in vitro release of CRF during a 5 hr perfusion of preoptic area-mediobasal (POA-MBH) HYP and the THY. Individual POA-MBH and THY were removed from male SD rats (350-400g) and placed in parallel perfusion chambers containing Earles' balanced salt solution (pH 7.4) at 37°C. The POA-MBH was halved longitudinally and each lobe of the THY was quartered. After a 60 min preincubation period, fractions were (300μl/10 min) collected over 2 hr for basal release of CRF assayed by RIA (IgG Corp., Nashville) and after 56 mM KCl added to the medium during the third hr. At the end of the incubation each POA-MBH and THY was weighed and assayed for total CRF. As expected there was an approximate 10 fold higher concentration (per g wet wt) of CRF in the POA-MBH when compared to THY. However, an estimation of total secretion using a numerical integration of the release curve (trapezoidal approximation) revealed that total THY secretion of CRF was approximately 75% that of the POA-MBH. Experimental lesions of the HYP which resulted in THY involution and subsequent increased response to mitogen stimulus did not result in significant changes in CRF secretion by the thymus. Supported by NIH MH46808.

390.7

LOCALIZATION OF CRF IN THE PRIMARY AND SECONDARY LYMPHOID ORGANS OF THE RAT. S.M. Brouxhon, A.V. Prasad, S.A. Joseph, D.L. Bellinger, and D.L. Felten*. Dept. of Neurobiol. & Anat., Univ. of Rochester, NY 14642.

Numerous studies have reported the production of a variety of neuropeptides or peptide hormones in cells of the immune system, either constitutively or upon induction. Further, some cells of the immune system possess neuropeptide or hormone receptors that display ligand-receptor interactions similar to those described in the CNS. These findings suggest the capacity of some lymphoid cells to produce and respond to peptides heretofore thought to be principally neural.

Recently, the presence of CRF mRNA was detected in the rat thymus and spleen. We have performed a series of experiments to localize the CRF peptide and its mRNA in the rat spleen, thymus, and mesenteric lymph nodes employing immunocytochemistry (ICC) and *in situ* hybridization (ISH) techniques. Immunoreactive CRF was present in cells of the marginal zone and red pulp cord of the spleen, connective tissue septa and corticomedullary junction of the thymus, and the medullary cords and sinuses of the mesenteric lymph nodes. Dual ICC/ISH for CRF demonstrated CRF mRNA over CRF-immunoreactive cells suggesting CRF synthesis, not just uptake. Double-label ICC for CRF and markers for specific populations of immunocytes suggest that CRF positive cells in the spleen and the thymus are macrophages. CRF positive cells in primary and secondary lymphoid organs reside in compartments that are innervated by noradrenergic (NA) sympathetic nerves, raising the possibility that they may signal each other. The presence and production of CRF in cells in lymphoid organs suggest that CRF may play a direct or indirect role (eg induction of the POMC gene) in the immune system as part of a paracrine network that is interactive with neural fibers in these organs. Supported by R37MH42076, NS21323, R23MH47783, and a Markey Charitable Trust Award.

390.8

IMMUNE AND ENDOCRINE IMPLICATIONS OF LONG-TERM INTRACEREBROVENTRICULAR CORTICOTROPIN-RELEASING HORMONE ADMINISTRATION IN THE RAT. J.M.H.M. Reul*, M.S. Labeur, G.J. Wiegers, E. Arzt and F. Holsboer. Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich, FRG.

The brain, endocrine and immune system communicate as an integrative network, which is critical for maintaining homeostasis during disease and other stressful situations. To investigate the (patho)physiological consequences of chronic hypothalamic-pituitary-adrenocortical (HPA) axis hyperactivity, rats were intracerebroventricularly (icv) treated for 1 week with CRH via an osmotic minipump and HPA axis and immune system function were studied. As compared to the vehicle, CRH produced elevated a.m. plasma ACTH and corticosterone levels, increased anterior pituitary POMC mRNA expression, thymus involution and adrenal enlargement. Regarding immune function, CRH treatment had markedly differential effects on splenocyte proliferation and cytokine expression. Long-term CRH-treated rats displayed highly suppressed *in vitro* splenic T-cell and B-cell proliferative responses. Surprisingly, *in vitro* interleukin-2 (IL-2) production by stimulated T-cells of these rats was increased. Moreover, basal and *in vitro* LPS-stimulated IL-1ß mRNA expression in splenic macrophages of peptide-treated rats was elevated as well. Thus, chronically elevated HPA axis drive results in disparate changes in the immune system. Whether the observed changes in cytokine expression should be regarded as physiologically adaptive adjustments supporting immune function or as potentially pathological anomalies remains to be elucidated. (VW I/68 430)

390.9

SUPPRESSION OF MACROPHAGE FUNCTION BY ADRENOCORTICOTROPIC HORMONE. M.Ichinose*, M.Sawada and T.Maeno. Dept. of Physiology, Shimane Med. Univ. Izumo 693, Japan.

Macrophages play important functional roles in the host defense against neoplasia and microbial infection. Macrophages ingest and kill microorganisms, process antigens and present them to T cells. Phagocytosis is the most representative functions of the cells. Thus, phagocytosis of latex beads by peritoneal macrophages was examined by means of flow cytometry. This assay revealed that adrenocorticotropic hormone (ACTH) suppressed phagocytosis in a dose-dependent manner. ACTH(1-24) was more suppressive than ACTH(1-39). Control phagocytosis was partially suppressed in Ca^{2+}-free solution. Phagocytosis was suppressed by ACTH in Ca^{2+}-free solution to the same degree as in the normal solution. ACTH-induced suppression was reduced in the presence of IBMX, a phosphodiesterase inhibitor. These results suggest that ACTH suppresses extracellular Ca^{2+}-dependent and -independent phagocytosis, that the suppression is not mediated by cAMP and that the inhibition of macrophage phagocytosis by ACTH is one of mechanisms which modulate immune responses in stressful situations.

390.10

MODULATION OF PHAGOCYTOSIS BY SENSORY NEUROPEPTIDE. M.Sawada*, M.Ichinose and T.Maeno, Dept. Physiology,Shimane Med. Univ. Izumo 693, Japan.

Interactions between immunocompetent cells and neuropeptides such as substance P, somatostatin and vasoactive intestinal peptide (VIP) show modulation of local immunologic responses by the peripheral nervous system. In the present study, effect of VIP on phagocytosis in peritoneal macrophages was examined by means of flow cytometry. Present experiments clarified that VIP suppressed phagocytosis at a threshold concentration of 10^{-8} M and maximum concentration of 10^{-5} M examined. Control phagocytosis was partially suppressed in Ca^{2+}-free EGTA-containing solution. Phagocytosis in Ca^{2+}-free solution was reduced further in the presence of VIP. Phagocytosis was suppressed in the solution containing IBMX, a known phosphodiesterase inhibitor. Suppressed phagocytosis by VIP was the same degree irrespective of IBMX. These results suggest that VIP suppresses extracellular Ca^{2+}-dependent and -independent phagocytosis, that the suppression is mediated by cAMP and that the inhibition of macrophage phagocytosis by VIP is one of mechanisms which modulate immune responses by the nervous system.

390.11

SHIFT IN CD4 AND CD8 T CELLS IN ATHYMIC MICE GRAFTED WITH THYMUS OR THYMUS & PITUITARY. **G.O. Gaufo, T. Martin, R. Lin, and M.C. Diamond*.** Group in Endocrinology and Department of Integrative Biology, University of California, Berkeley, CA 94720.

Research on the developmental sequence of T cell maturation has been concentrated on paracrine interactions; few investigations have focussed on the role of the endocrine system in its regulation. Cell-to-cell communication is crucial, but the internal milieu created by classical endocrine hormones during the development of the organism may also influence T cell differentiation and maturation. To investigate the role of the pituitary on T cell development, a comparison was made of $CD4^+$ and $CD8^+$ T cells derived from the inguinal lymph nodes of 60-day-old female mice from immune-competent strains, BALB/c/BALB/c and nude/BALB/c, and from an immune-deficient strain, nude/nude, grafted at 30 days of age with a thymus or with the combination of a thymus + pituitary underneath the kidney capsule. Fluorescence-activated cell sorter (FACS) analysis using dual color direct immunofluorescence with fluorescein isothiocyanate (FITC)-labeled anti-CD3 combined with phycoerythrin (PE)-labeled anti-CD4 (L3/T4) or PE-labeled anti-CD8 (Ly-2) monoclonal antibodies revealed a $CD3^+4^+$ to $CD3^+8^+$ T cell ratio of 3.1 ± 0.2 for both immune-competent strains; whereas, the groups reconstituted with a thymus and thymus + pituitary showed significantly ($P<0.05$) higher ratios of 5.7 ± 0.9 and 9.1 ± 1.4, respectively. No significant levels of $CD3^+4^+$ or $CD3^+8^+$ T cells were observed in the non-operated, sham-operated, and pituitary-grafted nude/nude mice; therefore, T cell ratios were not calculated. Addition of the pituitary to the thymus resulted in the highest CD4/CD8 T cell ratio ($P<0.05$). The role of the pituitary seems to direct T cell development toward the $CD4^+$ T cell lineage. ***Aided by grants from the Fetzer Institute to M.C.D. and from Zenyaku Kogyo Company of Tokyo to H.A. Bern.***

390.12

ALPHA-MSH ACTIVATES THE SPLENIC NERVE AND IS DEPLETED FROM THE ARCUATE NUCLEUS FOLLOWING IL-1ß INJECTIONS. C.A. Vriend*, L. Janz, A. Jansen, L. Murray, A.H. Greenberg and D.M. Nance. Depts. of Psych. and Path. and Manitoba Inst. of Cell Biology University of Manitoba, Winnipeg, MB., R3E 0W3, Canada

The neuroimmune system utilizes messenger molecules such as cytokines and neuropeptides. Alpha-melanocyte-stimulating-hormone (α-MSH), is considered to be an endogenous antagonist of the central effects of interleukin-1ß (IL-1ß), a cytokine which mediates the acute phase response. Recent evidence indicates that the anti-inflammatory effects of central α-MSH are mediated by descending neural pathways which involve peripheral ß adrenergic receptors. To study the direct effects of α-MSH on the neural regulation of the spleen, we examined splenic nerve activity following ICV injections of α-MSH in the rat. α-MSH (5-20 ng) significantly increased splenic nerve activity (32-115%). Previously, we showed that ICV injections of IL-1ß increased norepinephrine (NE) turnover in the spleen. To examine further the functional role of α-MSH, we tested the effects of ICV α-MSH on splenic NE turnover following synthesis inhibition. ICV α-MSH (2.5 ng) significantly increased splenic NE turnover. To investigate the relationship between IL-1ß and α-MSH in the brain, we tested the effects of ICV (5 ng) and IP (100 ng) injection of IL-1ß on the immunocytochemical staining for α-MSH neurons in the arcuate nucleus. Two hrs post injection rats were perfused and brains processed for the presence of α-MSH. In the rats which received IL-1ß IP, the number of α-MSH possitive cells in the arcuate were reduced, relative to saline animals, and virtually eliminated in the animals receiving IL-1ß ICV. These results suggest that IL-1ß releases α-MSH from arcuate neurons and collectively, these data provide evidence that endogenous α-MSH may play an integral role in the sympathetically mediated downregulation of the immune response observed after IL-1ß injection. Supported by MRC and NIMH.

391.1

EFFECT OF BACTERIAL ENDOTOXIN ON HIPPOCAMPAL AND PREOPTIC NEUROTRANSMISSION, BODY TEMPERATURE, BEHAVIORAL ACTIVITY AND FREE CORTICOSTERONE LEVELS. A.C.E. Linthorst*, C. Flachskamm, F. Holsboer and J.M.H.M. Reul. Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich, FRG.

Evidence has accumulated for a bidirectional communication between the central nervous system and the immune system. However, on the level of the brain the neural circuitry involved in the processing of signals from the immune system largely remains to be resolved. We started a study on the effects of i.p. lipopolysaccharide (LPS, bacterial endotoxin) administration on hippocampal and preoptic serotonergic and noradrenergic neurotransmission using *in vivo* microdialysis in freely moving rats. In addition, we monitored hypothalamic-pituitary-adrenocortical (HPA) axis activity by measurement of free corticosterone levels in the dialysates. Behavioral activity was scored by measuring the time during which rats were active and body temperature was assessed via biotelemetry. I.p. LPS treatment produced a dose-dependent increase in the extracellular concentrations of serotonin in the hippocampus but not in the preoptic region. In addition, the endotoxin caused a dramatic increase in preoptic levels of noradrenaline, elevations in body temperature and HPA activity, and a decrease in behavioral activity. We conclude that signals from the immune system exert differential effects on brain neurotransmission. These distinct changes in neurotransmission may be involved in the regulation of specific neuroendocrine, autonomic and behavioral responses after an immune challenge. (VW grant I/68 430)

391.2

NEUROCHEMICAL SPECIFICITY OF ENDOTOXIN-INDUCED C-FOS-EXPRESSING NEURONS IN THE HYPOTHALAMUS. A.T.K. Jackson*, A.H. Greenberg and D.M. Nance, Depts. of Pathology and Physiology and Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3

It has been shown that injection of lipopolysaccaride (LPS), a powerful bacterial endotoxin, results in a dose- and time-dependent expression of the proto-oncogene c-fos in the brain (Wan et al, B.R.B. 32:581,1993). We have extended these studies and report here on the transmitter specificity of hypothalamic neurons that are activated following LPS injection. LPS (100 µg) was administered i.v. to male S-D rats. Two hrs later the rats were perfused and the brains processed for c-fos immunocytochemistry (rabbit anti-fos, Santa Cruz). Immunocytochemical and histochemical methods showed that many of the c-fos positive neurons were also positive for oxytocin, vasopressin or NADPH-diaphorase (NADPH-d). Double-labelled cells were predominately located in the paraventricular and supraoptic nuclei of the hypothalamus and analysis showed that NADPH-d > OXY ≈ AVP in numbers of c-fos co-localized cells. Raw cell counts showed that LPS decreased NADPH-d staining, increased OXY, and had no effect on AVP. The LPS-induced changes in NADPH-d suggests a functional role for nitric oxide (NO). Consistent with this finding, preliminary results indicate that inhibition of NO synthase by i.c.v. infusion of L-NAME potentiates the induction of c-fos protein by LPS. Supported by MRC and NIMH.

391.3

THE EXPRESSION AND SECRETION OF INTERLEUKIN-6 FOLLOWING CENTRAL ENDOTOXIN IS DIFFERENTLY REGULATED IN THE CENTRAL NERVOUS SYSTEM AND IN THE PERIPHERY. M. G. De Simoni*, R. Dal Bo, A. De Luigi, S. Symard and G. Forloni. Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea, 62, 20157, Milano - ITALIA.

Centrally administered IL-1 results in a large increase in serum IL-6 indicating the brain's role in the acute phase response. The mechanisms of this induction and the signal conveying the information from the brain to the periphery are not known yet. To help characterize the pathway of centrally-mediated IL-6 induction, IL-6 levels, measured as hybridoma growth factor on 7TD1 cell line, were evaluated in rat serum and cerebrospinal fluid (CSF) at different times after intracerebroventricular endotoxin (LPS, 2.5 µg/rat). In the same experiments, IL-6 mRNA expression, measured by Northern blot analysis, was evaluated in periphery (in adrenals and lymphonodes) and in the brain (in hypothalamus, hippocampus and striatum). In serum, the highest IL-6 levels were reached at t = 2 h after which they rapidly decreased. The same time-course was shown by IL-6 mRNA in adrenals and lymphonodes. A different pattern was present in the central nervous system: in the CSF, IL-6 was detectable starting from t = 2 h, reached a plateaux at t = 4-8 h and remained detectable until t = 16 h. IL-6 mRNA expression in the brain areas showed a similar time-course, reaching a maximum at t = 4 -8 h. The results indicate that IL-6 synthesis is differently regulated in the brain and in the periphery.

391.4

QUINOLINIC ACID IMMUNOREACTIVE CELLS IN THE RAT BRAIN AFTER INTRACEREBRAL INJECTION OF LIPOPOLYSACCHARIDE. M.G.Espey, J.R. Moffett, and M.A.Namboodiri*. Georgetown University, Biology Department, Washington, DC 20057-1028.

Quinolinic acid (QUIN) is a neurotoxic tryptophan metabolite. Antibodies to quinolinic acid were used to study its cellular localization in the brain 1 to 30 days after intra-hippocampal injection of lipopolysaccharide (LPS). Quinolinic acid immunoreactive (QUIN-IR) cells were observed in hippocampus, corpus callosum, neocortex, meninges and choroid plexus 24 hr after LPS application. The immunoreactive cells were larger than lymphocytes, ranged from round to rod-shaped to complex in morphology, and were often observed around the vasculature. The overall number of QUIN-IR cells increased at 48 hours, and peaked in number at 72 hr. In saline injected control animals, only rare QUIN-IR cells were observed at 72 hr surrounding the injection site. The number of QUIN-IR cells was reduced in the peri-injection region by 4 days after LPS injection, but did not return to control levels until 15 to 30 days later. Tissue destruction was observed in the cortex, hippocampus and corpus callosum, and progressed in severity between days one and four. No clear correlation with QUIN-IR was found with the monoclonal antibodies ED1, ED3, F4/80, OX17 or OX41. The tissue damage observed around the injection site correlated more closely with a central mass of infiltrating leukocytes than with the relatively scattered QUIN-IR cells observed in and around the damaged region. These results suggest that QUIN, derived from a select population of leukocytes, may contribute to some of the secondary neuronal death subsequent to CNS infections. We propose that QUIN, rather than being simply a neurotoxic byproduct of tryptophan metabolism, may be a cytokine or immune modulator involved in the initial reactions to pathogens.

391.5

PROHORMONE CONVERTASES PC2 AND PC3 IN RAT NEUTROPHILS AND MACROPHAGES. PARALLEL CHANGES WITH PROENKEPHALIN-DERIVED PEPTIDES INDUCED BY LPS *in vivo*. Q. Vindrola[a]*, A. M.S. Mayer[b], G. Citera[c], J. A. Spitzer[b] and L. R. Espinoza[c]. Dept. of Biochem. and Mol. Biol.[a], Dept. of Physiology[b] and Dept. of Med./Rheumatology[c]. LSU Medical Center, New Orleans, LA 70112.

Prohormone- or proneuropeptide-converting enzymes PC2 and PC3 have been observed exclusively in nervous and endocrine tissues. In this work the presence of these enzymes in cells of the immune system was demonstrated. PC2 was detected in peripheral and liver-infiltrated polymorphonuclear leukocytes (PMN) but not in alveolar macrophages (AM) or spleen mononuclear cells (SMC). PC2 proteins corresponded to 75, 71 and 56 kDa forms. PC3 appeared in AM and SMC but not in PMN, and a 66 kDa protein was the only PC3 form detected. Proenkephalin-derived peptides (PENKp) were observed in PMN and AM, showing peptides of 35, 28, 21, 18 and 14 kDa in the former cells and a doublet of 35 and 32 kDa in the latter. PC2 proteins and PENKp decreased in liver PMN and peripheral PMN 90 min after intravenous (i.v.) infusion of LPS, suggesting an increased release. However, *in vitro* assays showed that the chemotactic peptide FMLP but not LPS increased the basal secretion of PC2 proteins and PENKp in PMN. These results indicate that PC2 proteins are released from PMN, together with PENKp, and suggest that LPS *in vivo* may act through an indirect mechanism. Low levels of PC3 and PENK were detected in the AM of rats treated for 90 min with SAL or LPS. However, a significant increase of PC3 and PENKp appeared 30 h after LPS infusion. These results show for the first time that PC2 and PC3 are differentially expressed in PMN and AM, respectively, which were paralleled by the presence of different post-translational products of PENK. In addition, the *in vivo* effect of LPS on PC2, PC3 and PENKp levels in PMN and AM resembles the effect of LPS on prohormone levels in endocrine tissues, suggesting that similar mechanisms may control the turnover of PENK in neuroendocrine and in these immune cells.

391.6

A MATHEMATICAL MODEL OF THE NEUROENDOCRINE-IMMUNE (NEI) AXIS. M.W. Denker[1], M. Cavanagh[2], J-G. Liao[3], L.T. Chen[4], R.A. Menzies[4], J. Oliver[4], L. Poole[4], E. Horvath[4] and C.P. Phelps*[4]. Depts. [1]Psychiatry and Behav. Med., [2]Elec. Eng.[3], Epidem. and Biostat. and [4]Anat., University of South Florida, Tampa, FL 33612.

Prikrylova, Jilek and Waniewski (CRC Press, 1992) described a very comprehensive mathematical model of the immune response to antigen. This model consists of 12 simultaneous first order differential equations and 6 auxiliary equations ("switches"). Eight cell types and 4 molecular messengers are represented. Here we describe an extension of the Prikrylova model which includes a provision for NEI modulation. These equations are derived from our experimental investigation of the adrenal axis response to variable in doses of endotoxin (EN, E. Coli, 10, 50 and 1000 µg per 100g b.w.) administered to male SD rats (350-400g) through jugular catheters. Rats received either saline or EN at the indicated doses and blood was withdrawn at 0, 0.5, 1 and 4 hrs later. Plasma ACTH and corticosterone (B) was assayed by RIA. Relative to 0 hr., increasing doses of EN resulted in parallel increased release of B at all intervals. However, ACTH release after 10µg EN resulted in the greatest (20X) increment from time 0 when compared to other EN treatments. After 1000µg EN there was only a 3X ACTH increase, whereas 50µg resulted in a 10X increase. Inverse changes in ACTH after EN may be explained at the H-P level, whereas the B response may be a direct cytokine effect on the adrenal. Our proposed mathematical model of the NEI axis integrates these and other findings from our experiments to stimulate NEI regulation. Supported by NIH MH46808.

391.7

EFFECTS OF VIRAL INFECTION ON ADRENAL STEROID SECRETION AND ADRENAL STEROID RECEPTOR EXPRESSION. A.H. Miller*, C.A. Biron, R.L. Spencer, P. Tanapat, J. Leung, F. Dhabhar, B.S. McEwen. Mt. Sinai Sch. Med. and Rockefeller Univ., NY, NY 10021; Brown Univ., Providence RI, 02912.

It is generally believed that during a viral infection, cytokines stimulate the release of endogenous adrenal steroids which in turn provide feedback inhibition on evolving immune responses. Although ample data demonstrates that cytokines stimulate HPA axis activity, few studies have directly examined adrenal steroid secretion during a viral infection. Accordingly, we examined corticosterone secretion at multiple time points in the am and pm of mice infected with lymphocytic choriomeningitis virus (LCMV). Since our previous studies have indicated that adrenal steroid effects on target tissue function must be established at the receptor level, we also measured cytosolic type II adrenal steroid (glucocorticoid) receptor binding in immune tissues. LCMV-infected mice exhibited modest elevations in corticosterone secretion on Days 3 through 7 post infection. Pm corticosterone values were greater than am values in all cases. Cytosolic type II receptor binding in spleen and thymus of LCMV-infected animals was significantly decreased in the pm compared to non-infected animals, indicating that infection-induced increases in corticosterone in the pm were activating receptors in these tissues. Interestingly, however, significant decreases in cytosolic type II receptors were also observed in the am in the spleen of infected mice. These receptor decreases occurred in the absence of marked elevations in corticosterone and suggest that local factors (eg cytokines) elaborated during the immune response to LCMV may directly influence adrenal steroid receptor expression. Related studies on the interferon inducer, poly I:C, indicate that interferons may mediate these adrenal steroid-immune interactions in LCMV infection by augmenting corticosterone release and decreasing cytosolic receptor expression in the presence or absence of hormone. Supported by MH47674 and MacArthur Foundation

391.8

IMMUNE CELLS MEDIATE CHROMAFFIN AND SYMPATHETIC GANGLION CELL CATECHOLAMINE SECRETION. S. Jones, H. Lujan, J. Pinxteren, J. Roberts, J. Walter* and W. DePotter. Loyola Univ. Med. Center and Hines VA hospital, Maywood IL. Univ. Health Sci./The Chicago Med School (Mt. Sinai Hospital) USA and Neuropharmacology, Univ Antwerp, Belgium.

Epinephrine (E) secretion from bovine chromaffin cells in response to mononuclear cell conditioned media (Life Sci,53:PL447-451) suggests that immune cells may mediate stress hormone secretion. In the present study, porcine chromaffin (C) and ganglion (G) cells were used to test the possibility that porcine and bovine immune cells could stimulate secretion. Blood, spleen, adrenal and superior cervical ganglion were obtained from local slaughter houses. Mononuclear cells isolated from blood or spleen were incubated (37C) overnight in RPMI media without serum; cells were removed and conditioned media (CM) frozen and kept at -20C. Porcine C and G cells were isolated using collagenase and maintained in culture at 37C. Secretion experiments were conducted after 5-7 days for C (cultured in DMEM/F12, 10% FBS) and at 4 days for G (cultured in F12, 5% Horse serum). For secretion experiments, growth media was replaced with CM or control RPMI for 90 min. at 37 C. For C, Epinephrine released into media was measured by HPLC while G secretion involved assessment of preloaded ^{3}HNE. Secretion in response to CM is expressed as % of total cell content.

	Basal	Bovine CM	Porcine CM
C Cells	1.4±.3*	16.7±1.8	12.1±1.7
G Cells	29.3±.5	55.1±.9	41.4±1.0

*Mean ±SEM with at least 3 different cell preparations. Results suggest that immune cell products can mediate secretion from the adrenal medulla and sympathetic ganglia. Thus, peripheral immune cells may be an important effector in the sympathetic - immune axis. (Supported by The Falk Research Trust)

391.9

IMPLICATION OF HYPOTHALAMIC INTERLEUKIN-6 CONCENTRATIONS AND RESPONSIVENESS OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN PRIMARY IMMUNIZATION. E. Khan Shaghaghi[1], M. Pallardy[2] H. Lebrec[2], J.C. Duché[3], C. Jacquot*[1] and A.M. Gardier[1]. [1]Dept. Pharmacol., [2]Dept. Toxicol., Fac. Pharmacie, Chatenay-Malabry F-92296, Dept. Pharmacol. CHIC, F-94010 Créteil, France.

Recently we demonstrated that primary immunization with sheep red blood cell (SRBC) decreases hypothalamic and cortical serotonin (5-HT) levels in F344 rats while increasing extracellular 5-HT levels (Gardier et al., *Brain Res.*, 1994). Pretreatment with an immunosuppressive drug, cyclosporine A prevented these effects suggesting that a T-lymphocyte product, but not a macrophage one, may be involved in the central serotoninergic activation induced by SRBC. Thus, interleukin-6 (IL-6) might be a good candidate for being a link between the immune system and the CNS. To characterize further this hypothesis, we have now investigated the effects of keyhole limpet hemocyanin (KLH, 200 µg i.p.) on both hypothalamic IL-6 levels and the hypothalamic-pituitary-adrenal axis (HPA) response (plasma ACTH and corticosterone concentrations). The presence of specific antibodies for KLH (IgM) in the serum was also assessed by using an ELISA-method at 3, 4 and 5 days following KLH injection. In the hypothalamus, 5-HT level significantly decreased by - 26% ($p<0.01$), 5-HIAA levels increased by +43% ($p<0.01$) while Il-6 levels increased as compared with controls 4 days following the primary immune response to KLH. In the cortex and hippocampus, no changes were observed in the serotoninergic markers studied. Antibodies directed to KLH were present since Day 3 following KLH injection. Plasma ACTH levels in KLH-treated rats were higher than responses in controls at all points, while corticosterone levels peaked at Day 4 post-immunization. These data confirm our previous results showing that long-lasting changes occur specifically in the hypothalamus as well as in the HPA axis when the production of specific antibodies is maximal following primary immunization.

391.10

N-(2-HYDROXYETHYL)HEXADECANAMIDE IMPROVES NEUROLOGICAL DEFICITS ACCOMPANYING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN B10/PL MICE. A. Leon*, R. Canella, and S. Mazzari. Research*life* S.c.p.A., Centro di Ricerca Biomedica, Ospedale Civile, 31033 Castelfranco Veneto, Italy.

Mast cells (MCs) present in the normal CNS have been proposed to regulate interactions between the immune and nervous systems. In addition, it has been suggested that MCs are involved in the pathogenesis of experimental allergic encephalomyelitis (EAE). Recently, the endogenous N-Acylamide, N-(2-hydroxyethyl)hexadecanamide, has been proposed to behave as a local autacoid capable of negatively modulating MC activation. In agreement with this concept, termed autacoid local inflammation antagonism (ALIA) orally given N-(2-hydroxyethyl)hexadecanamide (LG 2110/1) prevents MC-dependent edema formation and substance P-induced MC activation in the mouse ear pinna (Mazzari et al., this meeting). Here we have evaluated the effect of MC modulation by LG 2110/1 in the development/progression of EAE in B10/PL mice after immunization with 1-9 Nac, the NH_2-terminal-acetylated peptide of mouse myelin basic protein (Jiang et al., Science 256: 1213, 1992). Oral treatment with LG 2110/1 (10 mg/kg) beginning on the day of immunization delayed the onset (6 day post-immunization) as well as the frequency of animals displaying the neurological syndrome. In addition, when treatment was begun on day 4 post-immunization (P.I.), LG 2110/1 significantly reduced the severity of EAE. The exogenous N-acylamide ameliorated the clinical scores at day 8 P.I. up to day 31 P.I. (χ^2 test, $p < 0.05$ versus vehicle, n = 19-20). These findings not only support a physiological role for N-(2-hydroxyethyl)hexadecanamide, but also suggest that this type of molecule may represent a novel therapeutic approach to the management of inflammatory conditions of the nervous system modulated by MCs, including autoimmune CNS diseases.

391.11

THE CD4 CORECEPTOR MEDIATES EFFICACY OF T CELL ANTIGEN RECEPTOR INTERACTIONS WITH AUTOLOGOUS MYELIN BASIC PROTEIN M.D. Mannie* and G. White. Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville NC 27858-4354.

Guinea pig (GP) myelin basic protein (MBP) is approximately 100-fold more active than rat (R) MBP as measured by induction of experimental autoimmune encephalomyelitis (EAE) in Lewis rats. A Ser for Thr substitution at position 80 comprises the distinguishing structural difference between the two 72-86 encephalitogenic regions of GPMBP and RMBP, respectively. To test whether the CD4 co-receptor has a differential role in T cell responses to these two proteins, we measured the inhibitory effects of the anti-CD4 monoclonal antibody W3/25 on in vitro proliferative responses of MBP-specific T cell lines. W3/25 only partially inhibited proliferative reponses to GPMBP but fully inhibited the response to RMBP. This differential susceptibility to the inhibitory effects of W3/25 was observed across of broad concentration range of antibody and antigen as well as across a broad range of T cell densities. T cell subclones exhibiting functional uncoupling or downregulation of CD4 exhibited responses to GPMBP but did not respond to RMBP. These results suggest that the response to autologous RMBP, unlike GPMBP, is completely dependent upon CD4. In addition, T cell proliferative responses to GPMBP that were obtained in the presence of W3/25 were fully antagonized in a concentration dependent manner by RMBP. These results indicate that RMBP is a specific antagonist when CD4 is neutralized by an anti-CD4 monoclonal antibody. This work was supported by a grant from the National Multiple Sclerosis Society.

391.12

LOCALIZATION OF TUMOR NECROSIS FACTOR RECEPTOR mRNA IN NORMAL AND HERPES SIMPLEX VIRUS INFECTED TRIGEMINAL GANGLIA IN THE MOUSE. E.T. Cunningham, Jr.*[1] P.P. Sanna,[2] F.E. Bloom [2]and T.P. Margolis[1]. Francis I. Proctor Foundation and Dept. of Ophthalmology, UCSF, San Francisco, CA, 94143,[1] and Dept. of Neuropharmacology, Scripps Research Foundation, La Jolla, CA, 92037.[2]

In situ hybridization of ^{35}S-labeled cRNA probes was used to investigate the distribution of cells expressing mRNA encoding the p60 and p80 subtypes of the tumor necrosis factor receptor (TNF-R) in normal and herpes simplex virus (HSV) infected trigeminal ganglia in the mouse. Riboprobes were derived from previously characterized murine full-length cDNA species provided by Immunex Corporation. Trigeminal ganglia were infected *via* corneal innoculation using a McKrae strain of HSV three days prior to analysis. *In situ* hybridization for both TNF-R subtypes produced a moderately intense autoradiographic signal over subgroups of neurons and neuroglial cells in both normal and HSV infected ganglia. In general, the signal intensity and the number of neurons and neuroglial cells labeled in trigeminal ganglia did not change in response to acute HSV infection. However, infection was accompanied by an intense white blood cell infiltrate, and many of these non-resident cells did display signal for both TNF-R subtypes. Signal over control sections hybridized with sense p60 and p80 TNF-R cRNA was comparable to background. The presence of TNF-R mRNA over neurons and neuroglial cells in normal and HSV infected trigeminal ganglia suggests a role for TNF in normal trigeminal functioning, and supports studies implicating TNF in the pathogenesis of immune-mediated peripheral neuropathies.

Supported by a grant from Research to Prevent Blindness (T.P.M.) and grant # MH47680 (F.E.B.).

391.13

ISOLATION OF C1q PROTEIN FROM RAT BRAIN FOLLOWING INJURY.
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There is increasing evidence for a role of complement (C) components in neurodegeneration during Alzheimer disease (AD). To date there has been immunohistochemical detection of activated C products in AD brain associated with senile plaques and neurofibrillary depostis (e.g. Eikelenboom et al., 1992). Work in this laboratory demonstrated a 2 to 3-fold increase in C mRNA in AD brain (Johnson et al., 1992; Lampert-Etchells et al., 1993). Animal studies showed an increase in C mRNA after systemic kainic acid (KA) or knife lesions (Pasinetti et al., 1993; Johnson et al., 1992). Although C1q mRNA is increased, translation of protein in brain is not certain. Present studies utilized protein chromatography and Western blots to identify C1q protein increases in brain after injury. Animals were treated in one of two paradigms. 1.Systemic injections of KA, KA + pentobarbitol, pentobarbitol, or saline. 2. Unilateral neocortical lesions or sham surgery. After 3 or 10 days respectively, blood was collected via cardiac puncture then rats were perfused with saline. Whole brains (excluding cerebellum) were processed for the KA group. Ipsi- and contra-lateral striata were assayed separately from cortially lesioned rats. Tissues were processed by a batch chromotography procedure modified from the methods of Tenner et. al., (1981). SDS-PAGE was perfomed as described by Sambrook et al., (1989). Western blots were run using a goat anti-human antibody against C1q (Sigma C3900) which identified three bands of expected molecular weight (aprox. 26KDa) in both human and rat sera. C1q protein (three bands aprox 26 KDa) was increased in the KA rat brains compared to controls in which the protein was not apparent. C1q was also increased in brain ipsilateral to the lesions as compared to the unlesioned side. (Supported by AG 07909 and AG 00093.)

391.14

NERVE GROWTH FACTOR IMMUNOREACTIVE MAST CELLS IN HUMAN PERIPHERAL NERVE.
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Mast cells purified from the rat peritoneum express NGF mRNA. In addition, these mast cells store and release biologically active NGF protein (Leon et al., PNAS, in press). Mast cell-derived NGF may thus control adaptive-reactive responses of the nervous and immune systems towards noxious tissue perturbations. The fact that mast cells are found in the peripheral nerve trunk, especially in the endoneurium, raises the possibility that mast cells could contribute a readily available source of NGF for the regenerative process. Utilizing a goat anti-mouse β NGF IgG and standard immunohistochemical techniques, specimens of adult peripheral nerve from patients undergoing reconstructive surgery for traumatic nerve injury were examined for the presence of NGF-immunoreactive mast cells. NGF immunopositive mast cells were observed not only in the epineurium, but also within the endoneurium. Particularly striking was the intensity of the staining, which was totally absent in the surrounding tissue. The anti-NGF antibodies used here block as well the activity of NGF released from peritoneal mast cells. Endoneurial mast cells may be activated by mediators liberated from the damaged nerve fiber or from mechanical stress to the nerve, possibly providing a rapid supply of NGF to counteract early neurodegenerative events. In extreme cases, release of supraphysiological quantities of NGF could, conceivably, lead to undesirable neurological consequences, including hyperalgesia, chronic pain, and formation of neuromas.

391.15

RECRUITMENT OF MAST CELLS INTO THE CNS DURING DEVELOPMENT: IS IT TIME AND TISSUE SPECIFIC
X. Zhuang[1]*, A.-J. Silverman[2], and R. Silver[1,3], [1]Dept. Psychology, [2]Dept. Anat. & Cell Biol., P&S, and [3]Barnard College, Columbia Univ., New York, NY.

In isolated adult ring doves mast cells are relatively rare in the CNS but enter the medial habenula (MH) in large numbers at the initiation of courtship (Silver et al., J. Neuroendocrinol., 1992). During normal development mast cells are first observed in the most dorsal aspect of MH on day 21 post-hatch and gradually increase in number, until by 4 months they are distributed throughout MH. At puberty (6-7months) mast cells reach a maximum and then decline to adult levels. At both 3 and 6.5 months, filamentous processes enclosing extracellular space are characteristic of cells in MH, suggesting migration through the tissue. Throughout this period mast cells are identified by their metachromatic properties, by their staining with alcian blue and GnRH-like immunoreactivity. Ultrastructurally, secretory granules are immature. To begin to understand what quality of MH might contribute to its being populated by this cell type, we implanted embryonic (E15) MH and, as a control, embryonic optic lobe, into the lateral ventricles of 4 months old hosts. Each host received both types of tissues and was sacrificed 2-3 months later. Both the host and transplanted MH had numerous mast cells though densities appeared to vary. The transplanted optic lobe did not contain mast cells. These data suggested that MH can 'attract' mast cells regardless of location or connectivity. Furthermore, access to the CSF is not the determining factor in mast cell recruitment into CNS tissue. Some factor(s) intrinsic to the MH may play a role in attracting, sustaining, or in the maturation of mast cells. Supported by NIMH grant 29380 (to RS).

391.16

MIGRATION OF MAST CELLS INTO THE MEDIAL HABENULA (MH) OF ADULT RING DOVES.
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In adult ring doves kept in visual isolation from conspecifics mast cells can be identified using several criteria: metachromasia to aniline dyes such as acidic toludine blue, alcian blue staining in the presence of safranin, histamine and abundant secretory granules with complex sub-granular patterns (Silverman et al, PNAS in press). In this species brain mast cells are also contain a GnRH-like peptide (Silver et al., J Neuroendo 4:207, 92). Mast cells are present in small numbers in the MH (n~400) and increase dramatically (n~1200) when birds are allowed to court for 2 hrs (Zhuang et al., Horm&Behav 27:283,'93). The question remains whether these mature mast cells enter the CNS *de novo* or rapidly produce the GnRH-like epitope used in our initial study for identification. Independent lines of evidence support the migration hypothesis. First, the same increase in mast cell number is observed using GnRH-like ir or the cytochemical markers. Second, immature mast cells which might have been resident in the tissue have very few secretory granules compared to those seen in the MH during courtship. Finally, ultrastructural images show mast cells inserted between the ependymal cells, with numerous filamentous surface processes oriented toward the grey matter. One to two cell diameters into the MH, mast cells these processes enclose considerable extracellular space (ECS) suggesting an alteration in the local environment to create room for movement. Only deep within the MH does the ECS around the mast cell disappear, although blunt processes are still present. These two sets of observations are consistent with the transmigration of mast cells from the ventricular surface into the brain parenchyma. NIMH29380 (RS) & HD10665 (AJS).

391.17

STAPHYLOCOCCAL ENTEROTOXIN CAUSES ACUTE BLOOD-BRAIN BARRIER (BBB) PERMEABILITY IN THE RAT.
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Staphylococcal enterotoxins (SE) act as superantigens that can activate then deplete specific T-cell clones. In the rat, SE D and SE E have been reported to inhibit the clinical expression of experimental autoimmune encephalomyelitis, while SE B in the mouse can inhibit or exacerbate EAE depending upon the timing of treatment to the induction of EAE. While the effects of the SEs may be related to T-cell activation, the impact of the SEs upon BBB integrity has never been examined.

We tested the effects of SE B and SE D upon BBB permeability in Sprague-Dawley rats, 24 hours after intraperitoneal SE using 14C-aminoisobutyric acid autoradiography. Rats were treated with 75 ug of SE B or 50 ug of SE D, based upon pilot studies. SE D inoculated rats showed an increase in cortical BBB permeability to 7.36 +\- 2.17 (mls/gm/min x 10^3) (n=3) which was significantly increased compared with rats treated with SE B, 2.50 +\- 1.10 (n=3), and untreated controls, 2.87 +\- 1.50 (n=6). A similar increase in BBB permeability occurred in the basal ganglia, but this did not approach significance. No cellular infiltrates were detected in the CNS.

These findings show that a specific SE can alter BBB integrity acutely. This alteration can affect signaling between the CNS and the systemic immune system, and may partially explain the SE D inhibition of EAE in the rat.

391.18

LYMPHOCYTE AND MACROPHAGE TRAFFICKING IN THE NORMAL CHOROID PLEXUS.
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Activated T lymphocytes exit the blood-brain barrier and enter the normal CNS, thereby permitting immune surveillance of the brain. Because the choroid plexus(CPx) lacks a BBB, CNS exposure to circulating immune cells may be maximized in this structure. Therefore, we blindly evaluated post-mortem CPx from 22 patients for the presence of T and B lymphocytes and monocytes, using immunohistochemistry and monoclonal antibodies for cell identification (CD45Ro, CD45 and CD68 respectively, Dako Corp). The intensity of infiltrate was graded as none, mild, moderate or severe. There was one 3 m infant and 21 adults whose age averaged 47 yr. (range 22-84 yr); 15 were male. None had known infections, immunosuppression or HIV risk factors and infections were absent in the 8 with complete autopsies. T lymphocytes were present in all cases; the infiltrate was mild in 55%, moderate in 36% and severe in 9%. In contrast, lymphocyte infiltration in the brain parenchyma was rare. The CPx contained rare B lymphocytes in 9% of cases. CPx monocytes were present in 86% and the infiltration was usually mild. There was no apparent correlation between immune cell infiltration and age, sex, race or clinical disease. This prominent T cell trafficking into CPx in normal brain may be important in initiating CNS immune-related diseases or infections and may explain the periventricular localization of multiple sclerosis, certain infections, and brain lymphomas. (supported by NIH, RO1-27416)

391.19

ACTIVATION OF THE BLOOD COMPLEMENT SYSTEM IN THE HUMAN SUBARACHNOID SPACE P.J.Lindsberg*, J.Öhman, T.Lehto, M.Kaste, S.Meri. Departments of Neurology, Neurosurgery, and Bacteriology and Immunology, University of Helsinki, Helsinki FIN-00014, Finland.

The blood-brain barrier largely secludes the mammalian CNS from circulating immunological factors such as complement (C), a main mediator of humoral immunity and killing of foreign cells. C activation in plasma is under stringent regulation to prevent its attack on host cells. Since a number of CNS disease states induce plasma extravasation, which may carry C proteins to escape the regulation effective in plasma, we examined whether spontaneous C activation occurs in CSF in vitro and in vivo during subarachnoid hemorrhage (SAH). CSF specimens from healthy individuals were incubated at 37°C for 30 min with varying concentrations of serum before SC5b-9 concentrations were assayed with ELISA. In CSF:serum admixtures of 1:1 and 4:1, the assembly of SC5b-9 was up to five-fold enhanced by the addition of human CSF (n=5). CSF and plasma from SAH patients were studied on day 1 and 8 after SAH (n=15) and compared to controls with no CNS disease (n=8) and patients with ischemic stroke on day 1 (n=7). The CSF SC5b-9 concentration during SAH on day 1; 210±61 ng/ml, was higher than that in plasma; 63±17 ng/ml (p=0.01), while no SC5b-9 was detected in the CSF of controls or patients with stroke (p<0.001). The level of CSF SC5b-9 decreased during 8 days after SAH (24±10 ng/ml). We conclude that CSF lacks C regulatory capacity, thereby permitting intrathecal C activation during SAH. C activation may promote chemotaxis, vasoactive perturbations and membrane lysis, but its potential pathophysiologic role in SAH needs further studies.

391.20

BRAIN INFILTRATION BY AUTOREACTIVE ANTIBODIES INDUCED BY CENTRAL INTERLEUKIN-2 (IL-2) INFUSION. U.-K. Hanisch[1,2]*, J. Neuhaus[1], R. Quirion[2] and H. Kettenmann[1]. Max [1]Delbrück Center of Molecular Medicine, Cellular Neurobiology, Berlin-Buch, 13122, Germany and [2]Douglas Hospital Research Centre, McGill University, Montreal, Quebec, H4H 1R3, Canada.

IL-2 regulates activities of immune and neural cells and has clinical application as an immunotherapeutic agent to treat peripheral and intracranial tumors. In this study, infusion of IL-2 (i.c.v., 5 U/hr for 14 days) in rats is shown to cause neuronal loss and myelin damage arround the infusion site, along with a pronounced activation of glial cells. Within the ventricle, masses of non-neural cells intermingled with collagen and fibronectin formed a vascularized pseudotissue clot that dislodged adjacent brain regions. By means of specific antibodies, immunocytology revealed not only extravasating T cells, but B cells scattered throughout the clot as well as peri-ventricular tissue. At the ultrastructural level, the B cells were identified as plasma cells endowed in protein synthesis. Accordingly, a massive inundation by genuine immunoglobulin (Ig) was detected in widespread brain areas. Fractions of the rat Ig apparently recognized populations of neuronal cells and astroglia. The results may thus be relevant both to CNS disorders involving an autoimmune component and to the adverse neurologic and neuropsychiatric symptoms induced by the application of IL-2 in the treatment of cancer. Supported by the BMFT (Germany).

NEURAL-IMMUNE INTERACTIONS: CYTOKINE EFFECTS ON THE NERVOUS SYSTEM

392.1

DOSE-DEPENDENT RESPONSE OF N1E-115 NEUROBLASTOMA CELLS TO MURINE AND HUMAN TNF-α. K. Sipe, K. Kelley, R. Dantzer, A. Wonderlick, D. Srisawasdi and J. Weyhenmeyer*, Department of Cell and Structural Biology, Department of Animal Sciences, University of Illinois, Urbana, IL 61801 and INRA-INSERM, Unité 394, Bordeaux, France

Tumor necrosis factor- α (TNF) has been reported to have a variety of effects on neurons and neuroblastomas. It promotes axonal projection beyond the point of injury in severed optic nerve (Schwartz et al., *Brain Res*, 1991, **545**, 334), induces differentiation of neuroblastomas in conjunction with interferon-gamma (Ponzoni et al., *Canc Res*, 1992, **52**, 3194), and protects neurons against metabolic-excitotoxic insult (Cheng et al., *Neuron*, 1994, **12**, 139). Using the MTT viability assay, we have observed cytotoxicity in differentiated and undifferentiated N1E-115 neuroblastoma cells (cytotoxicity indices [CI's] of 54±15% and 70±11 for differentiated and undifferentiated cells, respectively) treated with 250 U/ml rMuTNF-α. Treatment of undifferentiated N1E's with rHuTNF-α, an agonist specific for the murine 55 kD TNF receptor, resulted in a CI of 79±2% at 400 U/ml TNF, indicating the likelihood that cytotoxicity in N1E's is mediated through the 55 kD TNF receptor. Low TNF concentrations produce little or no cytotoxicity in N1E's. The CI's for differentiated and undifferentiated cells treated with 0.025 U/ml rMuTNF-α are -11±4% and 18±17%, respectively. In undifferentiated N1E's treated with recombinant human TNF-α, the CI is -10±14% at 0.004 U/ml TNF. It is not clear whether the negative cytotoxicity index observed in differentiated N1E's at low TNF concentrations is due to an increase in cell proliferation or an increase in cell survival. The former seems unlikely, as the cells are treated with actinomycin D to inhibit proliferation during the course of TNF treatment. However, experiments measuring ^{3}H-thymidine uptake are being performed to verify that proliferation is not occurring. It has also been reported that TNF is capable of promoting cell adhesion (Mackay et al., *J Exp Med* 1993 **177**, 1277), so it is conceivable that TNF may promote the survival of differentiated N1E's by increasing their adhesion, either to substrate or to one another.

392.2

LOCUS COERULEUS NEURONS RESPOND DIFFERENTLY TO INTERLEUKIN-1β AND CORTICOTROPIN-RELEASING HORMONE. M.K. Borsody and J.M. Weiss*. Neuroscience Program and Department of Psychiatry, Emory University School of Medicine, Atlanta GA, 30322.

We administered i.c.v. recombinant human interleukin-1β (IL-1β; 0.1-2.5ng) while recording extracellular activity of single LC neurons in Sprague-Dawley rats anesthetized with chloral hydrate or halothane. Our findings show that, in chloral hydrate-anesthetized rats, IL-1β dose-dependently suppresses LC firing rates. LC sensory-evoked responses, measured as the response to 1-second paw compression, were decreased by IL-1β proportionately with the baseline firing rate. In contrast to the effects seen in animals anesthetized with chloral hydrate, firing rates of LC units in halothane anesthetized rats did not change in response to any dose of IL-1β. Since corticotropin-releasing hormone (CRH) is an intermediary of IL-1 in many of the cytokine's physiological actions, we compared the IL-1β responses of LC neurons to i.c.v CRH (1-3μg) under halothane and chloral hydrate anesthetics. CRH did not change LC firing rates in rats anesthetized with chloral hydrate. As reported elsewhere (Valentino et al., Brain Res. 270: 363, 1983), LC units in halothane anesthetized rats were excited by CRH at both 1μg and 3μg doses. The magnitude of sensory-evoked responses under halothane was not affected by CRH, but since baseline firing rates increased, the relative size of the sensory-evoked response was decreased. Supported by NIMH grant MH50420.

392.3

NEUROIMMUNOMODULATORY EFFECTS OF INTERFERON. R.A. Menzies[1], C.P. Phelps[1], L.T. Chen[1], N.R.S. Hall[2], J. Oliver[1], L. Poole[1], E. Horvath[1], M. Wiranowska*[3]. Depts of Anatomy[1], Psychiatry and Behavioral Medicine[2] and Neurology[3], U. South Florida, Tampa, FL, 33612.

Our laboratory has been studying the effects of interferon (IFN) on the hypothalamic pituitary adrenal axis (HPA). Last year we reported (Soc. Neuroscience Abs.209.5,1993) that natural rat IFNα/β (RIFNα/β)(Lee BIOMOLECULAR Research Labs) stimulated the HPA as determined by the RIA measurement of ACTH, β-endorphin and corticosterone (B) in plasma whereas human recombinant IFNα (rHuIFNα)(Hoffman LaRoche) had a minimal effect. In this report we extend those observations using the same animal preparation, e.g., 350-450 g Sprague-Dawley freely behaving male rats with indwelling jugular catheters. When either rHuIFNα or RIFNα/β is administered i.v. at doses of either 300 or 600 International units (IU) per g body weight, secretion of both ACTH and (B) is stimulated. Due to large variances, the differences at individual time points between control (either saline, serum albumen or heat inactivated RIFNα/β) and IFN injected animals were not always significant although mean differences were 2 to 4 fold. Total secretion of both ACTH and B is significantly stimulated by both IFNs although the response to RIFNα/β was greater and with less variability as compared to rHuIFNα, Total secretion was estimated by numerically integrating the release curve (trapezoidal approximation). The time course of hormone release was similar for both IFNs with the effects of RIFNα/β and rHuIFNα peaking at 30 and 60 minutes respectively and approaching baseline by 120 minutes. Both IFNs exhibit an inverse dose effect with the mean secretion of ACTH after 600 IU/g being about half that of 300 IU/g whereas there is no apparent reduction in B secretion. Supported in part by MH46808, DA 05723 and DA07976.

392.4

EFFECTS OF INTRACERERAL AND SYSTEMIC IL-1α ON NE TURNOVER IN THE RAT HYPOTHALAMUS USING MICRODIALYSIS. D. Kaur, G. Chen, H. K. Manji* and W.Z. Potter Section on Clinical Pharmacology, NIMH, Bethesda, MD 20892

IL-1 and other cytokines are known to be an integral part of the acute phase response and the hypothalamus has been postulated to be,site of interaction of cytokines, particularly with respect to regulating NE release in the brain. In previous experiments we had shown that cytokines including IL-1 probably do not act directly on the hypothalamus. In our current experiments we used microdialysis in an attempt to show that IL-1 does not act centrally but does act peripherally, using NE as index of measurement. We used microdialysis for our experiments and a specially constructed CMA-12 / Cannula Combo . Animals used were Sprague Dawley rats weighing between 230-300 gms (Tacocnic Farms). Anesthesia used was Chloral Hydrate. Basal values of NE were established 60 min post insertion of probe. At 140 min IL-1 was then infused through the cannula at a concentration of 100 ng / ml (total delivered was 5 ul over a 20 min period. Analysis of dialysate was done by HPLC with amperomatic detection and samples were analyzed for norepinephrine. Averaged NE values in fmol/20 min collection were: Basal 358.8, Post Hypothamic Rx: 417.2; Post i.p. Rx: 568.8 . Infusion of IL-1 produced a very modest nonsignificant 11% increase in hypothamic NE (in fact 3 of the 5 animals actually showed a decrease). By contrast, the i.p. treated animals produced a robust increase in hypthalamic NE in all 5 animals (48%). These data suggest that i.p. IL-1 induces a cascade of events which eventually result in an elevation of hypothalamic NE. Further experiments to delineate these mechanisms are underway.

392.5

Effect of cytokines on vasopressin and corticotropin releasing factor release from the hypothalamus and amygdala in vitro: possible involvement of nitric oxide mediated signalling. J.Raber*, G.F.Koob, and F.E.Bloom, Department of Neuropharmacology, The Scripps Research Institute, 10666 North Torrey Pines Rd., La Jolla, CA 92037.

Much attention has focussed on the role of selected cytokines as stimulatory factors of the HPA axis and the involvement of hypothalamic release of corticotropin-releasing factor (CRF) and arginine vasopressine (AVP) in this response. The amygdala, involved in stress related reactions and the regulation of the HPA axis, includes CRF and AVP containing neurons. An in vitro paradigm was used to investigate the influence of selected cytokines on the release mechanisms of CRF and AVP in the hypothalamus and amygdala, and the possible involvement of nitric oxide in this release. CRF and AVP are released, in a calcium-dependent manner from both sites, and this release is responsive to acetylcholine, norepinephrine, or high KCl (60 mM). The norepinephrine-induced AVP release is antagonized by phentolamine, but not by propanolol, while the norepinephrine-induced CRF release is antagonized by both adrenergic antagonists, suggesting an α-adrenergic receptor mediated AVP response and an α- and β-adrenergic receptor mediated CRF release. The acetylcholine-induced release is antagonized by atropine or mecamylamine, indicating that both muscarinic and nicotinic receptors can mediate the cholinergic response in the hypothalamus and amygdala. IL-2 stimulated AVP and CRF release in both regions, in a calcium- and dose dependent manner. Nitroprusside, which generates nitric oxide (NO), also induced AVP and CRF release. The IL-2- and acetylcholine-induced release is antagonized by Nᴳ-methyl-L-arginine, a potent inhibitor of nitric oxide formation, indicating a role for NO in this release. Nᴳ-methyl-L-arginine does not effect the norepinephrine-induced release. Finally, IFN-α stimulated CRF and AVP release from both the hypothalamus and amygdala. These data suggest that in addition to the hypothalamus, the amygdala may also play an important role in the bi-directional communication between neuroendocrine and immune systems (supported by grant MH 47680).

392.6

TUMOR NECROSIS FACTOR DECREASES THE LEVEL OF VASOPRESSIN BUT NOT OXYTOCIN IN RAT PITUITARY. G. V. SHURIN, D. A. PRENOVITZ, M. D. FITZSIMMONS, A. G. ROBINSON.*Dept. of Endocrinology , Univ. of Pittsburgh., PA 15261.

Cytokines derived from the immune system can modulate the activity of the hypothalamic-pituitary-adrenal (HPA) axis and thereby regulate the involvement of the neuroendocrine system in the inflamatory process. The role of major cytokines such as IL-1, IL-2, IL-6 in release of ACTH, TSH, GH has been well documented, but little is known about the effect of cytokines on arginine-vasopressin (AVP) and oxytocin (OT) secretion. In the rat many stressors stimulate OT release greater than AVP release. Yet, excess secretion of AVP is a common accompaniment of certain illness, causing excess water retention and hyponatremia. The stimulus for such elevated secretion of AVP has not been determined. We postulated that it might be mediated, at least in part, by cytokines. The present study was designed to test whether Tumor Necrosis Factor-alpha (TNF), derived from the immune system, can stimulate AVP and OT neuronal pathways. TNF (1ug) was injected (iv) into conscious, freely moving rats and the levels of pituitary AVP and OT were measured by radioimmunoassay 2 hours after cytokine injection. Pituitary AVP levels were significantly decreased after injection of TNF: baseline values (n=8) were 811±41 ng/pituitary for control animals and 671±27ng/pituitary (p=0.012) for animals (n=8) received TNF. However, pituitary OT levels were not changed after adminuistration of TNF (p>0.5). These data suggest that the immunmodulatory system (TNF) can specificaly regulate AVP depletion in the posterior pituitary. The lack of effect on oxytocin indicates this is not a direct vascular (necrotic) effect on the pituitary. As "stress" stimulates OT in the rats, neither is the increased AVP a non-specific response to stress. Rather, there might be a specific neuro-immuno interaction between cytokines and vasopressin neurons.

392.7

MODULATION OF NMDA CURRENTS BY INTERFERON-α (IFN-α) IN THE PREOPTIC ANTERIOR HYPOTHALAMUS (POA). S.Take*, T.Katafuchi, and T.Hori. Dept. Physiol., Kyushu Univ. Fac. Med., Fukuoka 812, Japan

IFN-α is produced within the brain and induces a variety of central symptoms. We have reported that microinjection of IFN-α into the POA enhances the electrical activity of the splenic sympathetic nerve and reduces the cytotoxic activity of splenic natural killer cells. We have also observed that microinjection of glutamate into the POA inhibits the activity of the splenic spmpathetic nerve. NMDA type of glutamate receptors in the POA is reported to mediate the thermal information from the skin and non-NMDA type of receptors is reported to play a major role in the control of neuroendocrine neurons in the hypothalamus. Taken these things together, we have speculated that IFN-α could suppress the glutamate responses in the POA which might be involved in the regulation of the immunity as well as body temperature. In the present study, we recorded whole cell currents from the POA of male WKA rats of 10-20 days old and investigated the effects of recombinant human IFN-α on glutamate and NMDA induced inward currents with slice patch method.

Glutamate(100μM)-evoked inward currents was markedly attenuated by the concurrent application of D-AP5 (200μM). Evoked inward currents by both glutamate and NMDA (200μM) were suppressed by perfusion with IFNα (100U/ml, 2 min) for more than one hour. IFN-α-induced suppression of NMDA currents was partially abolished by simultaneous application of naloxone (10μM), sodium salicylate (10μM) and Nw-nitro-Larginin (100μM). The results suggest that the POA IFNα-induced suppression of NMDA currents is mediated by opioid receptors, prostaglandins and the nitric oxid.

392.8

CHRONIC EFFECTS OF IL-1: PATHOLOGIC AND ELECTROPHYSIOLOGIC CHANGES IN THE RABBIT. M. Litwak*, S.Seto, J. Martinez, J. Arezzo, C. Brosnan. Dept. of Neuroscience, Neurology and Pathology, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

Interleukin 1 (IL-1) is a protein secreted predominately by activated macrophages that has direct acute effects on neural conduction. The present study examined the chronic effects of IL-1 using the rabbit eye model. 5 New Zealand White rabbits were treated with 300 units of IL-1 in the Rt eye, 2 controls were treated with heat inactivated IL-1 in the Rt eye and all 7 had saline in the Lt eye. Visual Evoked Potentials (VEPs) were recorded at 24 hr, 48 hr, 1wk, 2wk, 3wk, and 4wk timepoints. A parallel 5wk serial sacrifice study was conducted to determine pathological correlates of 300 units of IL-1. At 24 hrs the VEPs in the treated eye were prolonged by an average of 7.5% (1.1msec, $p=0.004$) of the baseline value; at 2wks the VEPs were increased by 11.9% (1.8 msec, $p=0.000$) of the baseline value. The latencies for the saline control eyes were unchanged; animals injected with heat inactivated IL-1 had no delay in the VEPs. Pathology demonstrated intravascular and extravascular accumulation of inflammatory mononuclear (MN) cells and evidence of swollen and reactive astrocytes. The increase in MN cells persisted through 3wks, while altered permeability of epiretinal vessels was detected through the 2wk timepoint. These changes were not detected in control eyes (saline and HI). These results support the conclusion that a single intraocular injection of IL-1 induces inflammatory effects that persist for up to 4wks. These alterations in function could contribute to the progressive and chronic inflammatory responses observed in diseases such as multiple sclerosis and HIV encephalopathy, where IL-1 may be released at sites of CNS lesions. Supported by NS 11920.

392.9

INTERLEUKIN-1 AND ENDOTOXIN INCREASE RELEASE OF NOREPINEPHRINE IN HYPOTHALAMUS.
Adrian J. Dunn*, Gennady N. Smagin and Jan Lavicky. Department of Pharmacology, Louisiana State Univ. Med Ctr, Shreveport, LA 71130.

Previous studies have indicated that administration of interleukin-1 (IL-1) and endotoxin (lipopolysaccharide, LPS) increased the cerebral metabolism of norepinephrine (NE), with the greatest response in the hypothalamus. To extend these observations, we have now performed *in vivo* microdialysis to determine extracellular concentrations of NE in the hypothalamus and cortex. Intraperitoneal injection of 5 μg LPS into freely moving rats increased microdialysate concentrations of NE and dopamine (DA) and their metabolites from the medial prefrontal cortex (PFM) and medial hypothalamus of rats. 5-HIAA was also increased. Peak responses in all cases occurred around 2 hours, and returned to baseline by about 4 hours. The response in PFM was much smaller than in the hypothalamus, but 100 μg of LPS produced an effect in PFM comparable to that of 5 μg in the hypothalamus. These responses were blocked by pretreatment with indomethacin.

IL-1β (1 μg) injected either ip or iv increased microdialysate concentrations of NE in the hypothalamus. Both injection routes elicited similar responses with dialysate concentrations increasing slowly to reach a peak after 2-3 hours. This temporal pattern differs from the elevations of plasma ACTH and corticosterone reported for IL-1; iv IL-1 elicits a rapid response with a peak around 20-30 min, whereas ip injections elicit a slower response with a peak around 2 hours after injection. This suggests that different mechanisms may be involved in the activation of cerebral NE systems and the HPA axis.

Supported by a grant from NIMH (MH 46261).

392.10

SYSTEMIC INTERLEUKIN-1ß AND MILD STRESS RAPIDLY & ADDITIVELY INCREASE INTERSTITIAL LEVELS OF 5-HIAA & HVA AT THE NUCLEUS ACCUMBENS.
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It has been proposed that the CNS interprets immunologic challenge as a stressor and that cytokines, such as IL-1, may serve as mediators between the immune system and CNS. The present investigation assessed the effect of systemic administration of hrIL-1ß and/or mild (air puff) stress on interstitial neurotransmitter and metabolite levels in the nucleus accumbens (nAcb) using microdialysis. Following steady baseline condition, male Sprague-Dawley rats were injected with IL-1ß (1 or 2 μg/rat; i.p.) or saline (0.5 ml; i.p.) and 5 (20 min) samples collected, following which some animals were exposed to an air puff stressor (1 puff/min for 5 min) and additional 5 samples collected. Analysis of our data revealed a slight transient increase in 5-HIAA levels in control animals following saline injection stress and a second slightly larger increase following exposure to the air puff. In comparison to controls, rats injected with IL-1ß showed a significantly larger and more sustained post-injection increase in 5-HIAA. A still larger increase following air puff was seen in the IL-1ß treated animals. The HVA profile was very similar to that of 5-HIAA. However, neither DA nor DOPAC levels were affected by IL-1ß or the air puff stress. Our results support the contention that IL-1ß may be perceived by the CNS as a stressor and may cumulatively interact with other stressors to affect changes in CNS neurotransmission, particularly that of 5-HT.

392.11

INTERLEUKIN-1α INDUCES CORTICOTROPIN-RELEASING FACTOR SECRETION AND SYNTHESIS FROM NPLC-KC CELLS THROUGH VARIOUS SECOND MESSENGER PATHWAYS J. W. Kasckow*, J. J. Mulcahey, J. Han, M.J. Owens, M.D. Stipetic, D. Breitman and C.B. Nemeroff. Dept. of Psychiat. Behav. Sci., Emory Univ. Sch. of Med., Atlanta, GA 30322

It is well documented that stress exerts effects on both endocrine and immune functions. The cytokine, interleukin-1 (IL1) is known to activate the hypothalamic-pituitary-adrenal axis by stimulating release of corticotropin-releasing factor (CRF) and to also play a preeminent role in immune function. CRF secretion and synthesis in the NPLC-KC human hepatoma clonal cell line has previously been shown to be increased by IL1. The purpose of this study was to elucidate which second messenger pathways mediate this effect. NPLC-KC cells were grown in 6 well Costar plates and treated for 12 or 24 hours with 500 pM IL1α in the presence of the protein kinase C (PKC) inhibitor, H-7 (50 μM), the protein kinase A inhibitor, IP20 (5 μM), or the cyclooxygenase pathway inhibitor, indomethacin (IND, 300 nM). Both cell extracts and secretion media were assayed for CRF-like immunoreactivity in our radioimmunoassay. All 3 inhibitors reduced the IL1 effect on CRF secretion. This effect was statistically significant ($P < 0.05$) at 12 hours; although all three inhibitors reduced CRF secretion at 24 hours, only the H-7 effect was statistically significant. Only the PKC inhibitor, H-7 reduced the IL1-induced increase in CRF synthesis, an effect that was statistically significant at 12 and 24 hours ($P < 0.05$). Thus, all three signal transduction pathways are implicated in IL1-induced CRF secretion while only the PKC pathway is implicated in IL1-induced CRF synthesis. Supported by NIMH MH 42088. The work was also completed while Dr. Kasckow was a Pfizer awardee.

392.12

BIOTINYLATED INTERLEUKIN 1 RECEPTOR ANTAGONIST (IL1RA) LABELS PARAGANGLIA IN THE RAT LIVER HILUS AND HEPATIC VAGUS. L.E. Goehler*, J. Relton, S.F. Maier, & L.R. Watkins. Dept.C&S Biology, UCHSC, Denver, CO, Synergen, Boulder, CO, & Dept. Psych., U. Colo., Boulder, CO, 80309.

Hyperalgesia resulting from immune activation by bacterial endotoxin is blocked by both IL1ra and by section of the hepatic branch of the vagus nerve (Watkins et al.,1994). Thus, some information about immune activation is conveyed to the central nervous system via peripheral nerves presumably sensitive to circulating cytokines. To determine the site of action of the IL1ra, we incubated cryostat sections of rat liver and hepatic vagus with biotinylated IL1ra. The IL1ra labeled vascular and connective tissue, tissues known to express IL1 receptors. The biotinylated IL1ra also labeled a subpopulation of cells in the paraganglia imbedded in the hepatic branch of the vagus and in the connective tissue of the liver hilus. These cells appear to be chemoreceptive, and are innervated by the vagus nerve (Prechtl & Powley, 1985). These preliminary results suggest that paraganglia are sensitive to circulating cytokines associated with immune activation, and convey this information to the brain via the vagus nerve. Supported by Synergen and NIH NS31569, MH14617.

392.13

I^{125}INTERFERON-*α*2A BINDING IN RAT BRAIN. A.C. Andorn* and D. Saphier, Depts. of Psychiat. and Human Behav., St. Louis Univ. Schl. of Med., St. Louis VAMC, 63125 and Depts. of Pharmacol. and Psychiat., LSU Schl. of Med., Shreveport, LA., 63125.

The mechanism by which inteferon-*α*2A (IFN*α*2A) contributes to neuropsychiatric sequelae in patients treated with this medication is unknown. If IFN*α*2A had direct central nervous system (CNS) effects then IFN*α*2A would be likely to have specific binding sites in brain. We had IFN*α*2A custom radiolabeld (ICN) with I^{125}. Using a centrifugation assay and nuclear and microvessel enriched particulate membrane fragment fractions of rat frontal cortex in the presence of 2 mM $MgSO_4$ we observed that at 4°C and 60 min incubation, and 0.1 nM I^{125}IFN*α*2A, specific binding represented 40-60% of total binding when non-specific binding was determined in the presence of 10^{-8}M unlabeled IFN*α*2A. I^{125}IFN*α*2A (0.1 nM) rapidly associated to the particulate membrane fragments reaching an apparent steady state within 30 min. with K_{obs} from computer assisted analyses of 3-10 and a binding maximum of .6-1 pM (N=2). I^{125}IFN*α*2A binding can be dissociated by a 20-fold dilution from its putative binding sites with $t_{½}$ of 20-40 min (N=2). Using these $t_{½}$ and the average of the K_{obs} an approximate K_D of 1.0 pM is derived. Saturation studies (5-400 pM I^{125}IFN*α*2A) had K_A of 5E10 ± 2E10M^{-1} (K_D = 19E-12M) and B_{max} of 0.7 ± 0.2 pM (N=2). The computer assisted analyses of these specific binding data showed that a 1-site fit was preferred to a 2-site fit. These very preliminary findings suggest that IFN*α*2A may have specific binding sites in brain, that these binding sites are of high affinity, of limited capacity, and that binding at these sites is reversible. These findings further suggest that IFN*α*2A may have direct CNS effects.

RESPIRATORY REGULATION: CHEMORECEPTIVE MECHANISMS

393.1

ATP-SENSITIVE POTASSIUM CHANNELS ARE FUNCTIONAL IN NORMOXIA IN THE RESPIRATORY NETWORK OF ADULT CAT. O. Pierrefiche and D.W. Richter*. II. Physiol. Inst. Univ. Göttingen, Humboldtallee 23, 37073 Göttingen, Germany.

K^+ channels sensitive to intracellular levels of ATP participate in the cellular response induced by oxygen deprivation. These channels are activated when intracellular levels of ATP are reduced and closed when ATP levels are normal. In some central nervous structures, a role for these channels in the hyperpolarization of cells induced by hypoxia has been described (Mourre et al. 1989; Brain Res. 496, 159). However, a functional role for such channels has not been described in respiratory neurons of adult mammals. Therefore we attempted to identify the presence and functional role of the ATP-sensitive K^+ channels in the respiratory network of adult cats.

Experiments were performed in anesthetized, paralyzed, vagotomized and artificially ventilated cats. PO_2 and PCO_2 were monitored continuously and kept within physiological ranges to insure normoxia. Expiratory neurons were recorded intracellularly with glass micro-electrodes, filled with a solution of ATP (5mM) in $K\text{-}CH_3SO_4$ (1.5M) in order to study the effect of ATP injection. A specific agonist (diazoxide, 2-20mM) and 2 specific antagonists (tolbutamide, 2-20mM; glibenclamide, 5mM) were applied topically over the expiratory neuronal pool.

Fourteen cells tested for intracellular ATP injection showed a membrane depolarization of 4.1±2.4mV. Activation of the channels with diazoxide produced a hyperpolarization of 3.1±0.95mV in 12 out of the 16 cells tested with a decrease in input resistance by 18±12% (n= 4). Blockade of ATP-K^+ channels by either tolbutamide or glibenclamide evoked a depolarization of 3.4±1.02mV in 10 out of 14 cells tested with an increase in input resistance of 29.3±8.6% (n=8).

We conclude that 1) ATP-sensitive K^+ channels are present in respiratory neurons of adult cats, 2) some of the channels are activated in normoxia and 3) opening of the remaining channels might contribute to the respiratory response during hypoxia.

This work was supported by DFG (Ri 279/7-11 and Ri 279/11-1).

393.2

EFFECTS OF HYPERCAPNIA ON NEURONS IN THE DORSAL MEDULLA DURING PERFORATED-PATCH RECORDINGS IN THIN SLICES. R.-Q. Huang* and J.B. Dean, Department of Physiology & Biophysics, Wright State University School of Medicine, Dayton, OH 45435.

Our previous thick slice (≈400μm) studies in adult rat have shown that CO_2-induced excitation of neurons in the solitary complex (SC), hypothesized to function in central chemoreception, is disrupted by washout during conventional whole-cell recording. The present study employed the perforated-patch technique (Amphotericin B) in thin slices (≈120 μm, 1-12 day old rat) to prevent washout of the CO_2 sensing mechanisms during patch-clamp recording. Chemosensitivity was tested by increasing the percent CO_2 in the perfusion medium from 5% CO_2 (pH≈7.4) to 10-11% CO_2 (pH≈7.1) for 3-12 minutes. Stable recordings were maintained for ≤3 hours at 25°C (R_a ≤10 MΩ) from cell bodies ranging 10-40 μm in diameter. Approximately 20% of the cells tested were depolarized (ΔV_m ≤10 mV) by CO_2, often accompanied by an increased input resistance (R_{in}). Excitatory responses to high CO_2 were usually maintained during high Mg^{++}/low Ca^{++} synaptic blockade, but were attenuated by the carbonic anhydrase II (CA II) inhibitor acetazolamide (10^{-4} M). Approximately 30% the neurons tested were hyperpolarized (ΔV_m ≤7 mV) by increased CO_2, sometimes accompanied by a decreased R_{in}. In some cases, increased IPSP activity or decreased EPSP activity occurred during CO_2-induced hyperpolarization. The remainder of neurons tested (≈50%) were unaffected by hypercapnia. Our findings show that when whole-cell washout is prevented, SC neurons have similar types of responses to hypercapnia whether studied in thick slices (adult) or thin slices (neonate). Moreover, our results suggest that CA II plays an important role in CO_2-chemoreception in SC neurons (supported by NIH grant HL 46308).

393.3

DOES HYPOXIA-INDUCED INHIBITION OF GLOMUS CELL OUTWARD CURRENT CAUSE INCREASED CHEMORECEPTOR NERVE ACTIVITY?
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One major theory of chemo-transduction postulates that hypoxia inhibits a glomus cell K^+ current, resulting in depolarization, calcium influx, secretion, and, thus, increased nerve activity. To test this theory, a preparation was developed which allowed for simultaneous afferent nerve recording from the intact organ and patch-clamp recording of glomus cells within the organ. Experiments were conducted using rat carotid bodies, *in vitro*, superfused with HEPES-buffered saline at 30°C. Purported glomus cell were identified based on their I/V profile: small inward currents and large, voltage-dependent outward current (760±88 pA @+40mV, n=104). Repeated measures of membrane currents and nerve activity demonstrated that hypoxia (30-60s duration, PO_2=0 Torr, at nadir) caused a large increase in nerve activity (15.4 x baseline, $p<0.001$), but no significant change in holding current ($p=0.1$) or Ro ($p=0.11$). Outward current significantly decreased during hypoxia ($p<0.01$), but the magnitude was small (-36±17 pA) and did not recover following reoxygenation despite the return of afferent nerve activity. Administration of the K^+ channel blocker TEA (20mM) caused a large reduction in outward current to 54% of control ($p<0.02$), but caused no significant change in afferent nerve activity ($p=0.55$). These results demonstrate an independence between changes in glomus cell outward currents and afferent nerve activity and, thus, suggests that hypoxia-transduction is not primarily mediated by modulation of glomus cell K^+ conductance.

393.4

IMMUNOHISTOLOGICAL DIFFERENTIATION OF CULTURED CAT CAROTID BODY CELLS.
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Adult cats have been widely used to study the respiratory and circulatory responses to hypoxia as well as the increase in neural activity from the carotid body (CB) responsible for these reflex responses. To understand mechanisms of hypoxic chemotransduction in the CB the availability of functioning chemoreceptor cells from adult cats is extremely important. Recently we have developed methods for culturing cat CB cells which respond to hypoxia for up to one month in culture. However, a critical problem with using cats in cellular studies is identification of type I cells, because (1) the ratio of type I to type II cells is only about 2:1, and (2) both types of cells in culture are similar in size and shape. The purpose of this study was to find surface markers for identification of live type I cells. Three types of cells were clearly distinguished with three antibodies against tyrosine hydroxylase (TH), glial fibrillary acid protein (GFAP) and galactocerebroside (Gal-C). Type I cells were positive only for TH. Type II cells were positive for GFAP, but not for Gal-C or TH, suggesting that type II cells are similar to astrocytes. A third type of cell expressed only Gal-C. Both type I and type II cells were stained with tetanus toxin fragment. Since astrocytes in the central nervous system expressed immunocompetent cell markers such as CD21 and MHC-class II, we tested these markers as well. Neither type I or II cells expressed CD21, but type II cells expressed MHC-class II molecules. These results suggest that live type I cells in culture can be identified by immunohistological methods. Supported by HL 47044 and HL 50712.

393.5

FICTIVE VENTILATORY RESPONSE TO pH CHANGE IN AN *IN VITRO* BRAINSTEM OF *RANA CATESBEIANA*.
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We have developed an *in vitro* brainstem preparation from the bullfrog, *Rana catesbeiana* for investigation of the cellular basis of central respiratory pH/CO_2 sensitivity and transmission of related signals within the CNS. In this study we assessed whether extracellular pH changes within a physiologic range result in an appropriate response in the multi-unit activity of the Vth cranial nerve, regarded as an index of the fictive respiratory motor output. Trials consisted of alternating two CO_2/bicarbonate buffered mock CSF solutions with pH of 8.10 and 7.60 ±0.05 pH units, respectively, made three times for each of the six preparations studied. For fictive lung ventilation cycles, the periods and area under the bursts were measured. All of the preparations demonstrated an appropriate fictive ventilatory response, defined as an increase in the product of instantaneous respiratory rate and burst area, to the pH reduction. Changes in either the average burst area or the period alone were less consistent. Small, high frequency "buccal" oscillations, when present, demonstrated a decrease in average amplitude with pH reduction, but no change in period. Thus an *in vitro* brainstem preparation of *R. catesbeiana* has pH sensitivity and offers an attractive model for elucidation of the basic neuronal mechanisms of central chemical control of ventilation. (Supported by NIH Anesthesia Research Training Grant: GM 07612)

393.6

NITRIC OXIDE MODULATES CO_2 CHEMORECEPTION IN THE PULMONATE SNAIL.
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Winlow *et al.* (Soc. Neurosci. 19(2):A1172, 1993) have implicated the interneuronal messenger NO in the feeding and respiratory programs of the aquatic mollusc, *L. stagnalis*. We studied the role of nitric oxide (NO) in CO_2 chemoreception in the terrestrial, pulmonate snail, *H. aspersa*. Using an isolated brain-pneumostome preparation, we compared the effects of the reversible NO-synthase inhibitor, L-N^G-nitroarginine methyl ester (L-NAME) and NO donors, sodium nitroprusside (SNP), L-arginine (L-arg) and hydroxylamine (HX), on pneumostomal function during normocapnia and hypercapnia (6% CO_2). Previously, we have shown that focal exposure of a discrete area between the visceral and right parietal ganglia to 6% CO_2 causes large increases in the diameter of the pneumostome (Pn), the breathing orifice in these animals. The addition of L-NAME (10 mM) to brain perfusate decreased Pn area during normocapnia and focal hypercapnic stimulation compared to control saline, but did not affect CO_2 responsiveness *per se*. The decreased Pn area resulting from L-NAME treatment could be reversed by adding L-arg (10 mM). The inactive enantiomer, D-NAME (10 mM), had little effect on Pn area during normocapnia and hypercapnia. The NO donors, SNP (400 µM) and HX (1 mM), added to the brain perfusate increased Pn area during normocapnia and focal hypercapnic stimulation compared to control saline, but also had no effect on the responsiveness to CO_2. We conclude that NO modulates respiratory activity in the snail, but does not appear to be involved in the CO_2 chemotransduction process. (Supported by grants HL-01998, HL-19827 and HL-07449).

393.7

ANATOMY OF CO_2/H^+-CHEMOSENSITIVE NEURONS IN THE SOLITARY COMPLEX.
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Neurons in the solitary complex (SC; *i.e.*, nucleus tractus solitarii, NTS; dorsal motor nucleus vagus, DMNX) depolarized by CO_2/H^+ are hypothesized to function in central chemoreception of the cardiorespiratory systems. The present study was undertaken to determine morphological and neurochemical properties of chemo-sensitive SC neurons. Intracellular and perforated-patch recordings (amphotericin B) were made in transverse slices (120-400 µm) prepared from adult or neonate rat. Neurons were stained with lucifer yellow or biocytin during intracellular recording or after rupturing the perforated patch. Chemosensitivity was tested by increasing the $\%CO_2$ saturating the perfusion medium (pH ~7.4 → ~7.1). Chemosensitive NTS neurons had somata measuring 17-28 µm in diameter, and 3-4 primary dendrites. Two-3 dendrites coursed dorsally or dorsomedially, producing secondary and tertiary branches, before exiting the brain slice, or terminating close to the dorsal surface of the medulla, central canal, or fourth ventricle. One dendrite usually coursed ventrally before branching and running along the dorsal border of, or extending into, the DMNX. The dendritic tree of a chemosensitive DMNX neuron extended dorsally into the NTS. The axon of chemosensitive SC neurons usually originated from the proximal part of the ventral dendrite, exited the SC ventrolaterally and projected into the medullary reticular field/nucleus before fading or exiting the slice. To date, lucifer yellow- and biocytin-labeled neurons were localized adjacent to immunopositive neurons (tyrosine hydroxylase, CGRP); however, none of the recorded neurons were immunopositive for these markers. Immunohistochemical experiments are continuing to test other neurochemicals known to be located in SC neurons. Our findings show that CO_2/H^+-chemosensitive neurons in the SC have 1) dendritic trees that radiate towards the surfaces of the dorsal medulla, and 2) dendrites that interconnect the NTS and DMNX (supported by NIH grants HL46308 and NS25547).

394.1

EFFECT OF HYPOXIA ON RESPIRATORY NEURONS IN THE VENTROLATERAL PONS OF ADULT RATS.

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Bilateral chemical lesioning of the ventrolateral pons in the A5 region ablates the post-hypoxic ventilatory depression (Coles and Dick, ATS Abstract, 1994). Data from subsequent experiments using extracellular recording techniques indicate that neurons in the A5 region display highly-modulated respiratory activity (Dick et al., Neurosci. Abs., 1994). We hypothesized that if respiratory neurons in the ventrolateral pons mediate post-hypoxic ventilatory depression, these cells would be excited by hypoxia. The purpose of the present study was to characterize discharge patterns of these neurons before, during, and after exposure to acute periods of hypoxia.

Adult, male Sprague-Dawley rats (n=7) were anesthetized with Equithesin, vagotomized, paralyzed, and ventilated with 100% O_2. Glass micropipettes (R=25 MΩ) filled with a mixture of L-glutamate and 2% Fast Green were used for recording pontine neural activity extracellularly and for marking recording sites. Glutamate was iontophoresed to distinguish cell bodies from axons of passage. Respiratory activity before, during, and after hypoxia (8% O_2) was recorded from neurons located in the dorsolateral (n=3), lateral (n=13), and ventrolateral (n=10) pons.

Discharge frequency increased clearly in three expiratory cells during and following hypoxia. Surprisingly, activity was remodelled, *i.e.*, not only did the onset and offset of neuronal discharge shift (n=5), but also cells (n=5) became active in additional phases of the cycle. Changes in activity were not evident in all cells; in 13 cells discharge frequency and activity remained stable.

These data suggest that post-hypoxic ventilatory depression is mediated potentially through respiratory neurons in the ventrolateral pons, and that the process may include "remodelling" neural activity in response to hypoxia.

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394.2

PHRENIC AND HYPOGLOSSAL NERVE RESPONSES TO SUSTAINED HYPOXIA IN DECEREBRATE CATS.

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A biphasic ventilatory response is observed in adults and newborns of many species during sustained hypoxia; an initial increase in ventilation is followed by a "roll off" of the response to a level greater than or equal to the control value, but less than the peak response. While the mechanism responsible for hypoxic rolloff is uncertain, previous reports suggest that it is both centrally mediated and dependent on peripheral chemoreceptor stimulation. Furthermore, the depressive effects of hypoxia differs between muscles of the upper airway and the diaphragm in piglets [Martin et al., JAP 68(2) 672-677]. In this study, we questioned 1) whether hypoxic rolloff, in the absence of changes in baroreceptor input and end tidal CO_2, is demonstrable in the adult decerebrate cat, and if so 2) whether hypoxic depression is expressed equally in hypoglossal and phrenic nerves. We measured hypoglossal and phrenic whole nerve recordings in 16 paralyzed vagotomized mechanically-ventilated decerebrate cats while arterial blood pressure and end tidal CO_2 were held constant. Following ventilation with 100% O_2, inspired O_2 was lowered to 12, 14 or 16% for 10-20 minutes. Nerve responses at all levels of inspired O_2 were biphasic. Overall, phrenic activity increased to a maximum of 17% above control before returning to the pre-hypoxic level after 7 minutes of hypoxia. Phasic hypoglossal nerve activity increased to a maximum of 40% above control and remained 25% above control after 7 minutes of hypoxia. There was no correlation between peak nerve activity and the decay constant for rolloff in either nerve. We conclude that hypoxic rolloff can be elicited in adults cats in the absence of centers rostral to the pons. Moreover, the hypoglossal nerve is more resistant to the depressive effects of hypoxia compared to the phrenic nerve in cats. (Supported by grants HL-01998, HL-19827 and HL-07449).

394.3

CHANGES IN AUTOREGRESSIVE SPECTRA OF THE PHRENIC NEUROGRAM DURING SEVERE HYPOXIA AND SUBSEQUENT RECOVERY.

M. Akay, J.E. Melton*, W. Welkowitz, N.H. Edelman and J.A. Neubauer. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

Progressive hypoxemia in anesthetized, peripherally chemodenervated cats results in initial depression of the phrenic neurogram (PN) culminating in phrenic silence and, eventually, gasping. These changes reverse during reoxygenation. To determine if changes in the PN power spectrum correspond to changes in temporal patterning, we examined autoregressive (AR) spectra of 100 msec bins of PN in 4 anesthetized, glomectomized, vagotomized cats during isocapnic hypoxia and reoxygenation. During normoxic eupnea, the AR spectra had peaks in the 30-60 and 60-120 Hz ranges. Hypoxia resulted in power loss at all frequencies and a shift of the 30-60 Hz peak to frequencies <30 Hz. Gasping resulted in increased power in all peaks but peaks were seen only in the 0-30 and 60-120 Hz ranges. During isocapnic recovery, the PN reverted from gasping to eupnea. Phrenic amplitude, initially 200-400% of pre-hypoxic values, returned to control values after 30 min. Power was initially increased in all peaks and returned to control levels over the same time period. The AR spectra of the PN during reoxygenation was initially gasp-like with significant power only in the 0-30 and 60-120 Hz ranges. During return to eupnea, low frequency power shifted from 0-30 to 30-60 Hz ranges. The AR spectra during intermediate recovery had characteristics of both eupnea and gasping, i.e., significant power in both the 0-30 and 30-60 Hz ranges. These results suggest that hypoxia results in a reversible reconfiguration of the central respiratory pattern generator. (Support: HL44678, HL16022, AHA/NJ)

394.4

IMAGING OF VENTRAL MEDULLARY SURFACE CHANGES DURING HYPOXIC CHALLENGES IN WAKING GOATS.

H.V. Forster, D. Gozal, D. M. Rector, P. J. Ohtake, L. G. Pan, T. F. Lowry, J. Marsh* and R.M. Harper. Dept. Physiol., Medical Coll. of Wisconsin and Zablocki VA Hospital, Milwaukee, WI 53226; Dept. of Anatomy and Cell Biology, UCLA, Childrens Hosp., USC, Los Angeles, CA 90024.

We examined activity changes on the rostral ventral medullary surface (VMS) of 5 non-anesthetized, unrestrained goats during poikylocapnic hypoxia (10% O_2 in N_2) using optical imaging procedures. Under sterile surgery, a coherent fiber probe with a charged couple device camera was placed over a rostral VMS region which elicited ventilatory and blood pressure depression during local cooling. Tracheostomy and vascular cannulation were performed for assessment of cardiorespiratory measures. Onset of 10% hypoxia was associated with a substantial increase in activation on the VMS, which was sustained for a period of time, and then gradually declined to a lower, stable level of activity as hypoxia was maintained. The temporal trends in neural activity observed are suggestive of the reported ventilatory "roll-off" phenomenon commonly associated with sustained hypoxic challenge.

(Supported by HL-22418, NIDR DE-07212, USPHS-25739 and Parker B. Francis Foundation.)

394.5

VENTRAL MEDULLARY SURFACE RESPONSES TO TRANSIENT HYPOXIA AND HYPEROXIA IN THE CAT: EFFECT OF CAROTID SINUS DENERVATION.

G. Aljadeff, D. Gozal, J.L. Carroll, D.M. Rector, O. Omidvar, R.K. Harper* and R.M. Harper. Dept. of Anatomy & Cell Biology, UCLA, Childrens Hosp., USC, Los Angeles, CA 90024; Johns Hopkins School of Medicine, Baltimore, MD 21287.

Carotid body afferents mediate components of the response to mild hypoxia within the intermediate area of the ventral medullary surface (IVMS). Changes in IVMS activity following transient peripheral chemoreceptor stimulation are unclear. IVMS neural activity in 6 spontaneously breathing, pentobarbital-anesthetized cats was measured as changes in 660 nm scattered light from the surgically-exposed IVMS, using a 3.2 mm coherent optical probe attached to a charge-coupled device. Two tidal breaths of 100% N_2 or O_2 were randomly administered before, and after carotid sinus denervation (CSD). Activity significantly increased (10.1±2.4 %) occurring within 3.1±0.7 sec following N_2 onset while decreases in activity (4.8±1.8%) were observed with O_2. CSD significantly prolonged the latency of the N_2 responses to 7.4±1.1 sec ($p<0.01$), and increased the magnitude of the response (14.5±1.8%; $p<0.01$). Similarly, CSD enhanced the effect induced by O2 (7.2±1.3%; $p<0.05$) as well as prolonging the latency (8.8±1.8; $p<0.01$). We conclude that CSD abolishes the early IVMS responses, although deafferentation allows for development of a late IVMS activity response, possibly of central origin. *(Supported by HL-22418 & Parker B. Francis Foundation.)*

394.6

INFLUENCE OF HYPERCAPNIA ON THE EXPIRATORY VOLUME-TIMING RELATIONSHIPS DURING EXPIRATORY RESISTIVE LOADING IN THE CAT.

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Previously, we have shown that the inspiratory volume-timing relationship during inspiratory resistive loading is linear, and that increasing levels of hypercapnia significantly increase the slope of this relationship. The specific aims of this study were to describe the expiratory volume-timing relationship during expiratory resistive loading and to determine whether this relationship is modulated by elevated CO_2 levels. Six chloralose anesthetized cats were presented with three graded levels of expiratory resistive loads (13-540 cm H_2O/l/sec) and tracheal occlusion at three levels of inspired CO_2 (room air, 7%, and 9%). The relationship between tidal volume (V_T) and expiratory duration (T_E) can be described by the following equation:

$$V_T = a + b \cdot T_E + c \cdot T_E^2.$$

The influence of different CO_2 levels on this relationship is shown in the following table:

	a	b	c	r^2
Room air	-264.63	3.53	-0.0084	.94
7% CO_2	-140.89	1.92	-0.0030	.85
9% CO_2	-167.02	2.23	-0.0034	.89

Following vagotomy, T_E was no longer modulated in response to expiratory resistive loading. We conclude that the relationship between V_T and T_E during expiratory resistive loading can be modulated by hypercapnia and that the modulation of T_E in response to both mechanical and chemical loading is dependant upon intact vagi. (Supported by NIH #PO1 40369)

394.7

ACETAZOLAMIDE INJECTED INTO THE MEDULLARY RAPHE OF RATS INCREASES PHRENIC NERVE ACTIVITY. D.G. Bernard*, A. Li and E.E. Nattie. Dept. of Physiology, Dartmouth Medical School, Lebanon, NH 03756

The medullary raphé was examined for chemoreceptor responses (increased phrenic nerve amplitude (PNA)) to localized tissue acidosis produced by microinjection of acetazolamide (AZ). Adult male Sprague-Dawley rats were anesthetized, vagotomized, paralyzed, and artificially ventilated. The ventral medullary brainstem was exposed and 1nl injections of mock cerebrospinal fluid (mCSF), AZ ($5x10^{-6}$M) or an inactive AZ-analogue ($5x10^{-6}$M) were made in the raphé while recording integrated PNA, femoral arterial blood pressure and end-tidal CO_2. The injection locations were demonstrated by post-mortem anatomical evaluation. PNA was expressed as percent maximum defined by 9% CO_2 stimulation. The mean change in PNA evaluated at 10, 20 and 30 min following 27 AZ injections was greater ($p < 0.001$; ANOVA) than after 16 control injections. Of the 27 AZ injections, 11 had responses greater than any control response. It appears that the traditional ventral medullary chemosensitive regions may not be the exclusive sites for sensing decreased pH by increasing PNA. Other sites, namely the locus coeruleus and the nucleus tractus solitarii have recently been described and the medullary raphé may be another such chemoreceptor site. (Supported by HL 28066 & HL 26091)

SOMATIC AND VISCERAL AFFERENTS: VISCERAL AFFERENTS

395.1

PRENATAL DEVELOPMENT OF SPINAL VISCERAL AFFERENTS IN THE RAT. **Y.F. McKee*, K.J. Townley, and K.G. Ruit. Department of Anatomy and Cell Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202.**

We have previously described the characteristic patterns of dendritic outgrowth by sympathetic pregnalionic neurons (SPNs) in the thoracic and lumbar spinal cord. In an effort to relate the temporal development of the dendritic arborizations of SPNs with the temporal development of afferent systems to SPNs, we have undertaken to describe the prenatal ingrowth of visceral afferents carried into the spinal cord via dorsal roots.

Visceral afferents were labelled by placing small crystals of DiI within celiac ganglia of aldehyde-fixed rat embryos beginning on embryonic day 15 (E15) and continuing through the day of birth (E21). Central projections of visceral afferent neurons were visualized in lower thoracic and lumbar spinal cord by fluorescence microscopy, photoconverted using diaminobenzidine, and drawn by camera lucida.

On E15, visceral afferents begin to penetrate the gray matter of the dorsal horn, entering medially. Later in prenatal development these afferents ramify primarily within ventral laminae of the dorsal horn and medial regions of the gray matter adjacent to the central canal. This medial region of the gray matter contains SPN cell bodies of the central autonomic area and also contains the medially-extending dendrites of SPNs located within the intermediolateral cell column. These neurons and their processes are clearly present in this central location before the arrival of the ingrowing visceral afferents. The appropriate development of SPNs may be important in regulating the development of spinal visceral afferents necessary for visceral reflex function.

395.2

SPINAL VAGAL AFFERENT PROJECTIONS TO THE CERVICAL SPINAL CORD. S. Kapadia, C.C. LaMotte, C. Shapiro*, K. Arsenault, and M. Wolfe. Section of Neurological Surgery, Yale University Med. New Haven, CT. 06510.

Injection of the rat cervical vagus nerve with WGA-HRP results in labeled axons and terminals at the light and EM level not only in the brainstem but also in the upper cervical spinal cord. The projection to the cervical cord consisted of a small compact bundle of nonmyelinated axons which was located in the reticular zone immediately lateral to the narrow neck of lamina V and a second, less distinctly organized, set of axons at the medial border of the dorsal horn between lamina I and the dorsal columns. Axons and terminal fields extended from each of these small bundles into lateral lamina V-VII and medial lamina I, respectively. The lamina I terminal fields were compact, lying as small "islands" in the gray matter. All axons identified at the EM level were nonmyelinated. This correlates with calculations of the diameter of vagal spinal afferents suggested by the physiological studies of the cervical respiratory neurons and dorsal horn neurons shown to be affected by vagal stimulation (Dawkins et al., '92; Fu et al., '92). Both simple and glomerular terminals were labeled, with clear vesicles and few dense core vesicles. These results extend the previous evidence (Panneton, '91; McNeill et al.,'91 ; Robertson et al., '92.; Kalia and Sullivan,' 82) for the presence of afferents from branches of the vagus to the high cervical cord. (NIH grant 13335)

395.3

PEROXIDE-SENSITIVE MESENTERIC AFFERENTS: AN IN VITRO STUDY. D.W. Adelson, J.Y. Wei, and L. Kruger*. Scripps Institution of Oceanography, UCSD 92093, the Departments of Medicine and Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Receptive fields of single unit afferent C-fibers (conduction velocity < 2.5 m/sec) were carefully delimited and characterized on the basis of impulse activity evoked by focal mechanical and heat stimuli in an in vitro rat splanchnic nerve-mesentery preparation. Units were then tested for responsiveness to 1 µl of 3% hydrogen peroxide (H_2O_2) applied to their receptive field. H_2O_2-responsive units displayed a characteristic discharge pattern comprising windup in discharge rate, decay back to background levels, followed by bursting discharge lasting several minutes. This two-phase response and the latencies involved suggest possible participation of a second cell type. Some units initially unresponsive to H_2O_2 exhibited the characteristic response pattern upon subsequent H_2O_2 stimulation, implying that units responding to initial applications of peroxide may previously have been exposed to sensitizing factors. H_2O_2 stimulation was observed to sensitize unit discharge in response to subsequent mechanical stimulation. Since a number of immunocompetent cells (e.g. neutrophils, mast cells, macrophages) generate reactive oxygen species as a result of activation, the observed sensitivity to peroxide may mediate an interaction between the sensory nerve terminal and activated immunocompetent cells.

Supported by NIH grants NS-5685 and NS-28433.

395.4

QUADRUPLE COLOCALIZATION OF CALRETININ, CALCITONIN GENE-RELATED PEPTIDE, VASOACTIVE INTESTINAL PEPTIDE AND SUBSTANCE P IN SENSORY FIBERS WITHIN THE VILLI OF THE RAT INTESTINE. **K.R. Isaacs*, L. Winsky, K.I. Strauss and D.M. Jacobowitz. NIMH, LCS, Bethesda, MD 20892**

The distribution of calretinin (CR), a calcium binding protein, was compared with several neuropeptides in fixed frozen cross-sections of rat intestine (20 µm) as well as dorsal root ganglia (DRG) using double-labelling immunofluorescence. CR was found to coexist with calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and substance P (SP) but not calbindin D-28K and galanin in the fibers innervating the lamina propria of the rat intestinal villi. An acetylcholinesterase (AChE) histochemical stain revealed that the majority of CR cells in the myenteric ganglia were cholinergic and about one half of the submucosal CR cells contained AChE but very few fibers in the villus contained both substances. *In situ* hybridization studies confirmed the presence of CR mRNA in the DRG and enteric ganglia and a ribonuclease protection assay verified the presence of CR message in the intestine. A small proportion of the CGRP immunoreactive cells in the DRG were also immunoreactive for CR and prior studies have revealed that CGRP coexists with SP and VIP in the DRG. These findings combined with reports that the primary source of CGRP in the rat intestine is the DRG (Sternini and Anderson, Somatosensory and Motor Research, 9:45-59, 1992), suggest the source of the quadruple colocalization is the DRG. While the function of CR within these nerves is unknown, the three potent vasodilatory neuropeptides may influence the uptake of metabolized food products within the vasculature of the villi.

395.5

EFFECTS OF ANTIOXIDANTS ON CISPLATIN-INDUCED EMESIS AND RELEASE OF SEROTONIN IN *SUNCUS MURINUS*. N. Matsuki*, Y. Torii, Y. Chen and H. Saito. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan.

Nausea and emesis are frequent side effects during cancer chemotherapy. Antitumor drugs, such as cisplatin, are considered to release serotonin from the enterochromaffin cells that stimulates serotonergic 5-HT3 receptors located on the vagus afferents. However, little is known how cisplatin releases serotonin from the enterochromaffin cells. We have proposed the involvement of free radicals in the process (Y. Torii et al. Eur. J. Pharmacol. 248, 131, 1993). To assess the hypothesis, effects of various antioxidants and radical scavenging drugs on cisplatin-induced emesis and the release of serotonin from the intestine were studied in *Suncus murinus*, a house musk shrew. Cisplatin (20 mg/kg) was injected intraperitoneally, and the antioxidant was injected subcutaneously either one or 24 hours before the cisplatin. Butylated hydroxyanisole, propyl gallate, ascorbic acid and acetyl-cysteine attenuated the number of vomiting animals and episodes per animal. Acetyl-cysteine also decreased cisplatin-induced release of serotonin measured *in vitro*. These results further support the hypothesis that free radical-mediated oxidation is involved in cisplatin-induced release of serotonin.

395.6

FUNCTIONAL ASSESSMENT OF CHOLECYSTOKININ (CCK) PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT (NST) TO THE PARABRACHIAL NUCLEUS (PBN) IN THE RAT. R.D. HOFBAUER, P.L. FARIS*, B.K. HARTMAN. Dept. of Psychiatry, Div. of Neuroscience Research, University of Minnesota, Minneapolis, MN. 55455

Research in our laboratory has been focused on the anatomy and function of CCK-ergic neural projections from the NTS to the PBN. It has been hypothesized that this is the first central nervous system component of a polysynaptic neuronal relay carrying satiety information from vagal afferents to the hypothalamus. Our laboratory began by anatomically detailing both the location of CCK-ergic neurons in the NTS and localizing those CCK-ergic parakarya which project to the PBN. To accomplish this the retrograde tracer flourogold and immunohistochemical methods localizing CCK were used. Next, it was determined through the use of a computer aided data acquisition program (Bioquant), that approximately 98% of the CCK containing neurons in the medial subdivision of the NTS project to the PBN. Additionally, it was determined that those CCK neurons which project to the PBN and those which do not are of disperate morphology. To assess the functionality of the circuit CCK-8 was injected intraperitonealy. C-Fos immunohistochemistry was then performed on areas of the NTS receiving vagal afferents. Regions of the NTS known to contain CCK-ergic neurons which project to the PBN were found to contain a high number of C-Fos containing cells when compared to controls. The results of the aforementioned experiments indicate that those CCK cells which project to the PBN may indeed be a subset of those neurons excited by IP injections of CCK-8. Future studies will be conducted to answer this question.

395.7

INHIBITION OF AXOPLASMIC TRANSPORT IN THE VAGUS NERVE ALTERS THE NUMBERS OF NEUROPEPTIDE AND TYROSINE HYDROXYLASE mRNA-CONTAINING AND IMMUNOREACTIVE VISCERAL SENSORY NEURONS IN THE RAT NODOSE GANGLION. H. Zhuo*, A.C. Lewin, E.T. Phillips, C. Sinclair and C.J. Helke. Deparment of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Previous work showed that axotomy-induced deafferentation altered the numbers of neuropeptide and tyrosine hydroxylase (TH) mRNA containing and immunoreactive neurons in the nodose ganglion. The present study was designed to selectively evaluate the loss of axonal transport on the numbers of vasoactive intestinal polypeptide (VIP), TH, and calcitonin gene-related peptide (CGRP) mRNA-containing and immunoreactive (ir) neurons of the nodose ganglion. Vinblastine (VB, 0.15mM) was administered to the cervical vagus nerve to block axonal transport. In situ hybridization and immunocytochemistry were used to label nodose ganglion neurons at 1, 3, 7, and 14 days after VB treatment. VB treatment of the vagus nerve markedly increased the numbers of VIP mRNA-containing and VIP-ir neurons, and slightly increased the numbers of CGRP mRNA-containing and CGRP-ir neurons in the nodose ganglion. The average labeling density of VIP mRNA-containing neurons was increased following VB treatment. VB treatment led to the appearance of low-labeling density CGRP mRNA-containing neurons which reduced the average labeling density of the CGRP mRNA-containing neurons. Application of VB to the cervical vagus nerve, decreased the number of TH mRNA-containing and TH-ir neurons in the nodose ganglion. The efficacy of VB to inhibit axonal transport and the absence of VB-induced neuronal damage were verified. These data suggest the presence of an axonally-transported influence regulating neuropeptide and neurotransmitter enzyme synthesis in mature placode-derived visceral sensory neurons of the nodose ganglion. (Supported by NIH grant R01 NS20991)

395.8

SPINAL AND SUPRA-SPINAL C-FOS EXPRESSION IN RESPONSE TO PERITONITIS-INDUCED DIGESTIVE ILEUS IN RATS. B.Bonaz*, P.J.M.Rivière, X.Pascaud, J.L.Junien and C.Feuerstein. Lab. of Neurophysiology, CHU grenoble, 38043 Grenoble cedex 09 and Institut de Recherche Jouveinal, 94265 Fresnes cedex, France.

Peritonitis induced by i.p. injection of acetic acid is responsible for an inhibition of gastric emptying and intestinal transit in rats(1). Purpose: to map spinal and supraspinal pathways that are activated by peritonitis-induced ileus in rats. Methods: male fasted SD rats were injected i.p. either with vehicle (NaCl 0.9%) or with 0.6% wt/vol acetic acid (10ml/kg). Rats pretreated with systemic capsaicin (125mg/kg subcutaneously) also received acetic acid. Sixty min after injections rats were perfused with 4% PFA. Frozen sections (30 µm) of the brain and spinal cord were processed for Fos immunoreactivity (Fos-IR)(2). Results: no or a few Fos-IR was observed after i.p. injection of the vehicle. Acetic acid induced Fos-IR in the spinal cord, nucleus tractus solitarius (NTS) and area postrema, parabrachial nucleus, supraoptic and paraventricular nucleus (PVN) of the hypothalamus. A dramatic decrease of Fos-IR was observed in capsaicin pretreated rats. Conclusions: peritonitis induces c-fos expression in spinal cord and selective brain nuclei (NTS, PVN) involved in the control of digestive motility in rats. Such expression is vehiculated through capsaicin-sensitive afferent fibers. (1) Rivière P.J.M. et al., Gastroenterology 104: 724-731, 1993. (2) Bonaz B. et al., Brain Res. 600: 353-357, 1993.

395.9

C-FOS EXPRESSION IN SPECIFIC AREAS OF THE CENTRAL NERVOUS SYSTEM INDUCED BY PROXIMAL COLON DISTENSION IN CONSCIOUS RATS. V. Martínez, L. Wang and Y. Taché*. CURE, VA Med. Cter., Brain Res. Inst. and Dept. Medicine, UCLA. Los Angeles, CA 90073.

Colorectal distension evokes *c-Fos* expression in the central nervous system (CNS) of the anesthetized rat. The aim of this study was to characterize the response of neurons in the CNS to proximal colon distension in conscious rats. A flexible balloon (5 cm) was inserted, through a medial laparotomy, into the proximal colon in ketamine anesthetized rats. 48 h later mechanical stimulation of the colon was performed for 10 min (30 s on, 30 s off), by inflating the balloon with 10 ml of air in conscious rats. In the control group the balloon was inserted into the colon but the distension was not performed. 60 min later the animals were deeply anesthetized and perfused; cryostat sections from brain and spinal cord (lumbar and sacral areas) were processed for Fos-like (Fos-LI) immunohistochemistry. After the proximal colon distension, the number of Fos-LI neurons was increased in the paraventricular nucleus of the hypothalamus (4159 ± 756 vs 393 ± 71 cells in the control group; mean ± SEM, $p<.01$), the locus coeruleus (357 ± 37 vs 116 ± 27 cells in the control group, $p<.01$) and the nucleus tractus solitarius (1947 ± 236 vs 508 ± 151 cells in the control group, $p<.01$). In the sacral part of the spinal cord, the number of Fos-LI cells was higher in the treated rats both in the laminae I-X (59 ± 2 vs 40 ± 2, $p<.05$) and the intermediolateral nuclei (12 ± 0.6 vs 4 ± 0.4, $p<.01$); while no differences were observed in the lumbar part. These data show that visceroceptive inputs activate selective spinal cord and brain areas involved in the integration of autonomic functions, and provide anatomical substrata for the CNS pathways involved in the regulation of colonic function and pain perception. *V. Martínez personal support: Ramón Areces Foundation (Spain).*

395.10

WHOLE-CELL PATCH CLAMP RECORDINGS OF CULTURED DORSAL ROOT GANGLION NEURONS THAT INNERVATE THE KIDNEY OF THE ADULT RAT. M.A. Vizzard* and W.C. de Groat. University of Pittsburgh, School of Medicine, Department of Pharmacology, Pittsburgh, PA 15261.

Whole-cell patch-clamp recordings in combination with axonal tracing techniques were used to examine the electrophysiological properties of afferent neurons innervating the kidneys of the adult rat. A fluorescent axonal tracer (Fast Blue, FB or Fluorogold, FG) was injected into each kidney 7-14 days prior to the removal of the T8-L2 dorsal root ganglia (DRG) bilaterally. DRGs were dissociated with enzymatic-mechanical methods and individual FB or FG-labeled cells were identified with a fluorescent microscope. Renal afferent cells were cultured for 12 hours to 2 days prior to whole-cell recordings. Renal afferent cells were small (major axis mean; 26.5 ± 1.7 µm; range 11.9 - 44 µm) and had an average resting membrane potential of -50.5 ± -1.7 mV. Action potentials of these cells were of two types: 1) tetrodotoxin (TTX)- resistant, long duration (12.6 ± 1.2 ms) with an inflection on the repolarization phase, 2) TTX-sensitive, shorter duration (7.4 ± 0.9 ms). 64% of renal afferent neurons exhibited low threshold (-40 to -45 mV) TTX-sensitive (1 µM) action potentials and Na^+ currents. Cells that exhibited TTX-resistant action potentials had high threshold Na^+ currents (-20 to -30 mV). Some cells exhibited both TTX-sensitive and TTX-resistant Na^+ currents. In contrast to bladder afferent neurons in which TTX-resistant and -sensitive Na^+ currents were present in small and large cells, respectively, TTX-resistant and -sensitive Na^+ currents in renal afferents were present in similar size neurons (26.8 ± 3.7 µm and 26.5 ± 2.4 µm, respectively). These results demonstrate that axonal tracing with fluorescent dyes is useful for identifying specific populations of visceral neurons for patch-clamp studies. The results provide the control data for future studies of changes in renal afferent cells following pathology in the kidney. [Supported by NIH grants DK 37241, DK 422369 and NRSA 1 F32 DK 08916-01].

395.11

MUSCULAR HYPERALGESIA FROM URETERAL CALCULOSIS IN RATS: RESPONSES OF SPINAL CORD NEURONS TO OBLIQUE MUSCLE STIMULATION. M.A. Giamberardino,* A. Dalal, R. Valente and L. Vecchiet, Institute of Medical Pathophysiology, University of Chieti, 66100 Chieti, Italy.

Previous studies have shown that rats implanted with an artifical stone in one ureter develop referred hyperalgesia of the ipsilateral oblique musculature which lasts many days. The present study examined input to the spinal cord dorsal horn from the hyperalgesic muscle in 15 stone-implanted rats and from the corresponding normal muscle in 15 controls, using electrophysiological techniques. Recordings were made from 113 single dorsal horn neurons in calculosis rats and 137 units in controls in the T10-T12 segments. Testing was as follows: gentle brushing of the dorso-caudal body surface, noxious skin pinch, graded pressure over muscle, noxious muscle pinch. In calculosis rats, 25.66% of the recorded neurons received input from the oblique musculature, with additional input from the skin in 21% of the cases. Of the neurons with muscular input, whether musculo-cutaneous or deep only, 79.31% displayed background activity and 17.24% were exclusively activated by noxious stimuli. In normal rats, 13.13% of the neurons received input from the oblique musculature, with additional input from the skin in 50% of the cases. Of the neurons with muscular input, 50% displayed background activity and 11.11% were exclusively driven by noxious stimuli. The statistical analysis showed that in calculosis rats with respect to controls a significantly higher number of cells with muscular input was recorded ($p<0.001$) and, of these cells, a significantly higher number displayed background activity ($p<0.001$).The results seem to indicate a condition of central sensitization of dorsal horn neurons in rats with referred muscle hyperalgesia from viscera.

395.12

EVIDENCE THAT THE VAGUS NERVE MEDIATES A RESPONSE TO VAGINOCERVICAL STIMULATION AFTER SPINAL CORD TRANSECTION IN THE RAT. R. Bianca*, G. Sansone, R. Cueva-Rolon, L. Gomez, M. Ganduglia-Pirovano, C. Beyer, B. Whipple, and B.R. Komisaruk, Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

Vaginocervical stimulation (VS)-induced increases in pupil dilatation (PD) and analgesia [as measured by vocalization threshold (VOCT) to tailshock] are reduced, but not abolished, by combined bilateral pudendal, pelvic, and hypogastric neurectomy (NX). In the present experiment, rats underwent aspiration transection of the spinal cord at levels L5 or T7 to determine if such procedures would replicate the effects of NX, and to determine which of the residual responses, if any, are affected by vagotomy (VX). A control group received a sham operation. VS significantly increased both PD, and VOCT to front paw shock, in the SH, L5, and T7 groups. The increases in PD and VOCT in the SH group were significantly greater than those in either the L5 or T7 groups. The differences in VS-induced increases in PD and VOCT between the L5 and T7 groups were not statistically significant. VX abolished the VS-induced increase in PD in the L5 and T7 groups. The effects of VX on the VS-induced increase in VOCT could not be adequately determined after VX due to low survival rate at the time of assessment. In summary, VS-induced responses of PD and analgesia persist after spinal transection at L5 and T7, and subsequent VX abolishes VS-induced PD. These findings provide evidence that a physiological response (PD) to VS is mediated, in part, by the vagus nerve.
Support: NIH-1-RO3-TW00394-01A1, 3-S06-GM08223-10, Busch Fndtn.

395.13

EVIDENCE THAT THE VAGUS NERVE MEDIATES SOME EFFECTS OF VAGINOCERVICAL STIMULATION AFTER GENITAL DEAFFERENTATION IN THE RAT. R. Cueva-Rolón[1], G. Sansone[1], R. Bianca[1], L. E. Gómez[1], C. Beyer[2], B. Whipple[1], E. J. Muñoz-Martínez[2]*, and B. R. Komisaruk[1]. [1]Institute of Animal Behavior, Rutgers University, Newark NJ 07102 and [2]CINVESTAV IPN, Apdo. Postal 14-740, México, 07000, D. F. MEXICO.

In order to ascertain whether any effects of vaginocervical stimulation (VS) are mediated by the vagus nerve, we first transected all obvious afferent nerves from the reproductive tract and then tested for residual responses to VS. After combined bilateral transection of the pelvic, hypogastric and pudendal nerves (NX), the following responses to VS were greatly reduced or abolished: lordosis to flank-perineum palpation, leg extension, immobilization and blockage of both tail withdrawal to radiant heat and leg withdrawal to foot pinch. However, after these nerve cuts, the following persisted as significant residual responses to VS: 1) Pupil Dilatation (PD): subsequent bilateral subdiaphragmatic vagotomy significantly reduced the magnitude of this residual response; 2) Analgesia: (measured as an increase in vocalization threshold to tail shock); the subsequent vagotomy abolished the analgesia; 3) Heart rate (HR): the subsequent vagotomy produced no significant effect on the HR increase to VS. The present findings provide evidence that the vagus nerve conveys vaginocervical afferent activity that produces pupil dilatation and analgesia. Support: NIH-1-RO3-TW00394-01A1; 3-S06-GM08223-10, Busch Foundation.

395.14

COMPLETE SPINAL CORD INJURY DOES NOT BLOCK PERCEPTUAL RESPONSES TO VAGINAL OR CERVICAL SELF-STIMULATION IN WOMEN. B.R. Komisaruk* and B. Whipple. IAB and College of Nursing, Rutgers - The State Univ. NJ, Newark, NJ 07102

In 8 women diagnosed as having complete spinal cord injury (SCI) [4 women @ T6-10, 4 women @ T11-L2], and a non-injured control group [5 women], vaginal or cervical self-stimulation (VCSS) significantly increased group mean pain thresholds in the SCI groups by up to 78% and in the control group by up to 67%. There were no significant differences among groups in magnitude of this effect. Seven of the 8 women with SCI reported that they experience menstrual cramps. The threshold to perceive cervical self-stimulation *per se* was significantly higher in the 4/8 women with SCI who perceived this stimulus than in the control group. The perceptual responses to VCSS in the women with SCI @ T11-L2 may utilize hypogastric nerve afferents that enter the spinal cord as far rostral as T10; however, the perceptual responses to VCSS in the T6-10 group were unexpected. Possible afferent pathways include: 1) intraspinal visceral afferent pathways that remain intact after SCI, are not tested for, and are not part of the criteria for defining "complete" SCI (i.e. absence of voluntary movement, cutaneous pain and touch below the level of SCI); 2) a vagal afferent pathway from uterine cervix and/or adjacent regions that could bypass the spinal cord and enter the medulla oblongata directly. The latter is supported by recent findings in rats.
Support: NIH: R01 HD 30156.

395.15

PUTATIVE C-FIBER SOMATIC AFFERENTS FROM THE HINDLIMB DO NOT CONVEY THERMAL SENSORY INFORMATION TO NUCLEUS TRACTUS SOLITARIUS (NTS) IN RATS G.M. Toney* and S.W. Mifflin, Department of Pharmacology, University of Texas HSC, San Antonio, TX 78284

Activation of hindlimb somatic afferents elicits NTS neuronal discharge, but the sensory modalities mediating this excitation are unknown. Experiments were conducted in anesthetized rats to determine if hindlimb somatic afferent inputs to NTS convey thermal sensory information. The left hindlimb sciatic nerve was electrically stimulated (1 Hz frequency, 0.5 ms duration) and extracellular neuronal discharge evoked in the right NTS was recorded. Units were located medial to the tractus, near obex, at depths from 450 to 1100 μm. To categorize unit responses as being a-fiber or c-fiber evoked, compound action potentials were recorded from the sciatic nerve and the averaged response to 30 stimuli compared at different stimulus intensities (n=3). Short latency excitation, presumably a-fiber mediated, was first recorded at an intensity of ~10 μA and became maximal near 100 μA. Longer latency excitation, presumably c-fiber mediated, appeared near 50 μA and became maximal at intensities of ~400 μA. NTS unit discharge was evoked at intensities averaging ≥217.0 ± 36.9 μA (n=8), suggesting activation by c-fibers. Unit activity was further categorized as c-fiber-evoked by injecting capsaicin (10 ng) into the hindlimb arterial supply. In 7 of 8 units, discharge significantly increased from 3.1 ± 1.2 Hz to 8.9 ± 1.5 Hz ($p<0.05$), while 1 unit failed to respond. Units were then tested for responses to thermal stimuli. Over a 10 second period, non-noxious (40 °C) and noxious (47 °C) heat was applied to the hindlimb. In no instance did heating affect NTS neuronal discharge frequency. These data indicate that NTS units activated by hindlimb somatic nerve stimulation appear to receive c-fiber inputs, but these inputs do not transmit thermal sensory information. (supported by HL 36080)

396.1

A FLUORESCENCE-BASED METHOD FOR ASSESSING NEUROGENIC DURAL INFLAMMATION AS INDICATED BY DURAL PROTEIN EXTRAVASATION INDUCED BY TRIGEMINAL GANGLION STIMULATION. K.W. Johnson and L. A. Phebus*. Central Nervous System Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Neurogenic dural inflammation has been proposed as a source of pain during migraine. This inflammation is produced by the release of inflammatory neuropeptides from sensory afferents in dural tissue. A method for artificially inducing dural inflammation was reported utilizing unilateral electrical stimulation of the trigeminal ganglion. This "artificial trigger" caused the unilateral release of inflammatory neuropeptides, resulting in ipsilateral dural protein extravasation from post-capillary venules and increased dural blood flow. Most groups using this animal model of migraine have quantified the amount of protein extravasation using exogenous radiolabeled albumin.

We modified the technique to measure the amount of endogenous albumin leaking into the dural tissue by exploiting the complexation reaction of albumin with the fluorescent dye, Evans Blue. The dye was injected intravenously in guinea pigs, prior to stimulation of the trigeminal ganglion to label circulating albumin. Following unilateral ganglion stimulation, samples of dura overlying both hemispheres were removed, rinsed, and mounted on a microscope slide. The amount of Evans Blue trapped in dural tissue was measured using a Zeiss fluorescence microscope equipped with a spectrophotometer. This computerized system automatically performed readings on 25 different sites on the tissue and calculated the associated statistical values. Values were expressed as the ratio of average fluorescence in ipsilateral vs. contralateral dura for each animal. This method utilized multiple measurements on each sample which resulted in very precise measurements with a small number of animals per point (n = 3). The ability of several compounds to prevent dural extravasation was evaluated. This model was successful in discriminating the clinically effective and ineffective compounds.

396.2

EFFECTS OF CONTINUOUS, CONSTANT-RATE SPINAL INFUSION OF SNX-111 (SYNTHETIC ω-CONOPEPTIDE MVIIA) ON FORMALIN-EVOKED CUTANEOUS PAIN IN THE RAT: DOSE-RESPONSE RELATIONSHIP. S. Bowersox, T. Singh, K. Lau, K. Valentino*, and R. Luther Pharmacology Department, Neurex Corporation, Menlo Park, California, 94025.

Male, Sprague-Dawley rats were used to evaluate the analgesic properties of SNX-111 (synthetic ω-conopeptide MVIIA) administered by continuous, constant-rate spinal (intrathecal) infusion. We and others previously reported (Gohil et al. Neurosci Abstr. 19:235, 1993) that spinal bolus injections of SNX-111 significantly attenuate tonic nociceptive responses (ED_{50} = 0.1 μg) in the rat hindpaw formalin test. In the current study, animals with indwelling spinal catheters were given infusions of either sterile saline (0.9%) or SNX-111 (0.001, 0.003, 0.01, 0.03, 0.1 μg/μl/hr) for 72 hours before analgesia testing. Nociceptive behavior was elicited by injecting 50 μL of 5% buffered formalin subcutaneously into the dorsal hindpaw. Nociceptive responses were quantified by counting paw flexions at regular intervals across a 90-minute observation period beginning immediately after formalin injection. The number of paw flinches during acute (Phase 1:0-9 minutes post-formalin) and tonic (Phase 2: 10-90 minutes post-formalin) response phases were summed for animals of each group. Group values were compared by ANOVA and *post hoc* t-tests. SNX-111 dose-dependently blocked formalin-induced nociceptive behavior in both the acute and tonic response phases. Half-maximal blockade of Phase 1 and Phase 2 responses (ED_{50}) was produced by doses of 14 ng/hr (Phase 1) and 0.82 ng/hr (Phase 2). Behavioral signs observed included stiff tails (low dose groups) and mild tremor in the 0.1 μg/hr group. We conclude that sub-tremorgenic doses of SNX-111 administered by continuous intrathecal infusion have analgesic efficacy in both the acute and tonic phases of the rat paw formalin test.

396.3

STIMULUS-STRENGTH DEPENDENT INHIBITION OR FACILITATION OF A WITHDRAWAL REFLEX BY A 5-HT_{1A}-RECEPTOR AGONIST IN THE RABBIT A.K. Houghton, S.E. Edwards & R.W. Clarke (SPON: Brain Research Association) Dept. PES, University of Nottingham, Sutton Bonington Campus, LE12 5RD, U.K.

Selective blockade of spinal 5-HT_{1A}-receptors strongly potentiates spinal withdrawal reflexes in the decerebrated rabbit, a result which implicates these receptors in tonic descending inhibition (R.W. Clarke & A.K. Houghton, *J.Physiol.*, **473**, 123P, 1993). In the present study we have investigated the effects of a selective 5-HT receptor agonist, (±)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), on withdrawal reflexes in spinalized rabbits. Twelve animals were decerebrated and spinalized under halothane/N_2O anaesthesia. The left sural nerve was stimulated electrically to excite either Aß axons only or Aß + Aδ fibres. Reflexes were recorded from the ipsilateral gastrocnemius medialis (GM) muscle nerve, averaged, and integrated. 8-OH-DPAT was given i.v. in doses of 1, 9 and 90μg/kg, followed by the selective 5-HT_{1A}-antagonist (+)-WAY-100135 (a gift of Wyeth Research (U.K.) Ltd.), also given i.v. at 1mg/kg. When high stimulus strengths were used, 8-OH-DPAT always increased reflexes at doses up to 10μg/kg, after which the reflex was a median of 214% (n=5, range 152-362%) of pre-drug controls. Increasing the dose of 8-OH-DPAT could increase reflexes further (2 rabbits) or decrease responses (3 animals). WAY-100135 always had the opposite effect to the highest dose of the agonist. In contrast, reflexes evoked by low intensity stimuli were usually (6 of 7 animals) suppressed after 8-OH-DPAT. In these experiments, the median reflex after the 10μg/kg dose was 46% of controls (n=7, range 10-101%). The reflex increased after WAY-100135 to a median of 166% of the pre-drug values (range 44-431%). Thus, 5-HT_{1A}-receptor activation can facilitate *or* suppress polysynaptic reflexes, presumably through actions at multiple sites in the reflex pathway. *AKH is an SERC scholar.*

396.4

IONTOPHORETIC APPLICATION OF SEROTONERGIC AGONISTS PRODUCES BOTH EXCITATION AND INHIBITION OF PHYSIOLOGICALLY CHARACTERIZED PUTATIVE PAIN MODULATING NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA OF LIGHTLY ANESTHETIZED RATS. S.M.Roychowdhury and M.M.Heinricher*. Dept. Neurol., Univ. Calif., San Francisco, CA 94143.

Two classes of presumed pain modulating neurons have been identified in the rostral ventromedial medulla (RVM) of the lightly anesthetized rat: "on-cells" display a burst of activity, and "off-cells" become quiescent prior to a noxious heat-evoked tail flick reflex (TF). A third class of cells, "neutral cells," show no TF-related change in firing.

The role of serotonin within the RVM is not well understood. The aim of the present study was to examine the effect of iontophoretically applied 5HT upon the activity of on-, off- and neutral cells. Fifty-three of 59 cells (89%) responded to 5HT. The predominant effect on all cell classes was a facilitation of spontaneous (34/59) and glutamate-evoked (5/7) activity, an effect blocked by the 5-$HT_{2/1C}$ antagonist ketanserin. 5HT also enhanced the TF-related on-cell burst. Ketanserin by itself had no effect on cell discharge.

The $5HT_{1a}$ agonist 8-OHDPAT and the nonselective $5HT_1$ agonist 5-CT inhibited the majority of cells tested. Neurons inhibited by 8-OHDPAT were on many occasions excited by iontophoretic application of 5HT itself. Fourteen neurons were inhibited by 5HT.

These results suggest that individual neurons within the RVM can be both excited and inhibited by serotonergic agonists acting at different receptor subtypes. The results further indicate that the actions of 5HT within the RVM cannot be attributed to an influence on a particular cell class, suggesting that 5HT plays a more general role in modulating the circuitry of the region.

Supported by grants from NIDA (DA05608) and the National Headache Foundation.

396.5

ACTIVATION OF 5-HT_2 RECEPTORS POTENTIATES PAIN PRODUCED BY INFLAMMATORY MEDIATORS. Y. Hong*, F.V. Abbott and P. Blier. Dept. Psychiatry, McGill Univ, Montreal, PQ H3A 1A1.

Serotonin (5-HT) strongly potentiates the spontaneous pain response produced by intraplantar injection of inflammatory mediators (Hong & Abbott, Neurosci., in press). The present study explored the receptor subtype(s) mediating this synergistic effect of 5-HT in algogenesis, and the effects of 5-HT antagonists on formalin-induced pain.

5-HT agonists were injected, alone or with noradrenaline (NA) or prostaglandin E_2 (PGE_2), into the plantar surface of the paws of rats. The behavioral response was recorded using the rating scale developed for quantifying formalin-induced pain. 8-OH-DPAT and 2-methyl-5-HT produced only transient responses by themselves and did not interact with PGE_2 or NA. The 5-HT_2 agonists DOI, mCPP and α-methyl-5-HT produced little response alone, but induced lifting and licking lasting more than 30 min when combined with PGE_2 or NA. The algogenic effect of α-methyl-5-HT plus PGE_2 was dose-dependently blocked by intraplantar pretreatment with the 5-$HT_{2A/2C}$ antagonists ketanserin (0.5-5 μg) and ritanserin (5-50 μg) and the 5-HT_{2A} antagonist spiperone (0.03-0.3 μg), but not by α- or ß-adrenergic antagonists. Similar doses of these 5-HT_2 antagonists attenuated the second phase of the pain response to intraplantar injection of 1.0% formalin. These data imply that 5-HT_{2A} antagonists may be effective peripherally acting analgesics or analgesic adjuncts in pain states associated with 5-HT release from platelets, such as acute injury and, perhaps, some chronic pain states.

396.6

EFFECTS OF MICRODIALYSIS APPLICATION OF 5-HT_{1A} AND 5-HT_3 AGONISTS ON HEAT HYPERALGESIA AND JOINT INFLAMMATION IN RATS. D.M. Rosenstein and K.N. Westlund*. Marine Biomedical Institute and Dept. of Anatomy and Neurosciences, Univ. of Texas Medical Branch, Galveston, TX 77555-0843.

It is known that systemic application of non-selective 5-HT agonists can block nociceptive transmission. We attempted to determine the antinociceptive and anti-inflammatory effects of selective 5-HT_{1A} and 5-HT_3 agonists (8-OH-DPAT and 1-phenylbiguanide (PBG), respectively) applied directly to the lumbar spinal cord in arthritic rats. It was found that treatment with both 8-OH-DPAT and PBG five hours after induction of arthritis (by intraarticular injection of the knee joint with 3% kaolin/3% carrageenan) significantly reversed the behavioral responses to noxious thermal stimuli seen in rats at four hours post-induction. However, joint circumference was not significantly reduced by post-treatment of these drugs. Application of these drugs prior to the induction of arthritis failed to significantly inhibit arthritic changes in behavior in response to noxious thermal stimuli at four hours post-induction. After eight hours, however, behavioral responses did show some return toward baseline levels. Pre-treatment with both of these drugs failed to block changes in joint circumference associated with acute arthritis. These findings suggest that while 5-HT plays a role in the processing of thermal nociception, it is not involved in the process of peripheral inflammation. (Supported by NS01445, NS28064, NS11255)

396.7

MDL 100,687A IS A 5HT-RECEPTOR AGONIST WITH UNUSUAL POTENCY VERSUS TRIGEMINO-VASCULAR COMPONENTS OF MIGRAINE. B.M. Baron*[1], J. Sprouse[1], M. Petty[2], M. Johnson[1], M. Linnik[1], J. Kehne[1], M.M. Racke[1], B.W. Siegel[1], A.L. Slone[1], R. Bernotas[1], M. Hibert[2], E. Hamel[3], W.S. Lee[4], and M.A. Moskowitz[4]. [1]Marion Merrell Dow Res. Institute, Cincinnati OH 45215, [2]Strasbourg, France 67080; [3]McGill Univ., Montreal, Canada H3A 2B4; [4]Mass. General Hosp., Boston, MA 02114.

The clinical success of 5HT-receptor agonists has indicated a role for a $5HT_1$-like receptor in migraine. Two models have been proposed which postulate either a vascular or neural location for the 5HT receptor. MDL 100,687A and sumatriptan were characterized *in vitro* and then evaluated *in vivo* for their ability, following i.v. administration, to alter blood flow in the carotid circulation (closure of arteriovenous anastomoses in cats) or transmitter release (attenuation of plasma extravasation following trigeminal nerve stimulation in guinea pigs). MDL 100,687A was similar in potency and efficacy to sumatriptan in affecting blood flow (ED_{50} values were 438 and 726 nmol/kg, respectively). In contrast, respective ED_{50} values vs. plasma extravasation were 2-4 orders of magnitude lower (0.035 and 5.5 nmol/kg). In addition, the minimally effective dose of MDL 100,687A was 1,000-fold lower than that of sumatriptan in the neurogenic model. Thus, in general, the $5HT_1$ agonists were more potent against the neural vs. the vascular compo-nents of migraine and specifically, MDL 100,687A was exceedingly potent (compared to sumatriptan) against its neural components. These data suggest that the two responses might have differing pharmacological mechanisms.

396.8

SIMULTANEOUS ASSESSMENT OF PRE- AND POSTSYNAPTIC EFFECTS OF SEROTONIN IN THE RAT DORSAL HORN *IN VITRO*. J.A. Lopez-Garcia and A.E. King. Department of Physiology, University of Leeds, Leeds LS2 9NQ, UK. (SPON: Brain Research Association).

In an attempt to study cellular mechanisms of serotonin (5-HT) in the dorsal horn (DH), simultaneous recordings were obtained from primary afferents and DH neurons during continuous monitoring of synaptic activity in the hemisected spinal cord of young rats (10-14 day old). Two suction electrodes were placed in contiguous lumbar dorsal roots and used for stimulation and recording of primary afferent depolarization (PAD) respectively. Conventional sharp electrodes were lowered in the DH. After impalement of a neuron a dorsal root stimulation protocol was initiated and 5-HT superfused at different concentrations (0.01-100 μM).

Preliminary results obtained from 22 preparations show that 5-HT had effects on both pre- and postsynaptic elements. 5-HT induced a long lasting (8-15 min) PAD of small amplitude (230 μV) in all preparations tested. The dose response curve for this response was bell shaped with a peak at 5 μM. Direct effects of 5-HT on the membrane potential of the postsynaptic neuron were found in only 4 out of 16 neurons. A depolarization was observed in 3/16 neurons and hyperpolarization in 1 neuron. These responses developed slowly, were of small amplitude (3-10 mV) and displayed dose-dependency. The EPSP elicited by dorsal root stimulation was transiently depressed by 5-HT in 13/16 cases, potentiated in 2/16 and unchanged in the remaining case. 5-HT doses equal or greater than 1 μM were required to produce changes in both the EPSP and the membrane potential of the postsynaptic neuron.

These data suggest a role for 5-HT in the modulation of transmission from primary afferents to DH neurons via complex mechanisms which may include activation of low and high affinity 5-HT receptors at pre- and postsynaptic sites.

Supported by The Wellcome Trust.

396.9

ANTAGONISM OF NERVE GROWTH FACTOR (NGF)-INDUCED HYPERALGESIA BY THE SUBSTANCE P (SP) N-TERMINAL METABOLITE, SP(1-7). A.A. Larson* and K.F. Kitto. Department of Veterinary PathoBiology, University of Minnesota, St. Paul, MN 55108, U.S.A.

NGF has been shown to produce hyperalgesia 24 hr after injection in rats. We tested the effect of 0.1 μg of NGF injected intrathecally in mice. The withdrawal latency of the tail from a hot water bath maintained at 49°C was significantly decreased at 24 and 48 hrs after injection of NGF. In contrast, the abdominal stretch response (writhing) to an intraperitoneal injection of acetic acid was not altered by NGF pretreatment at these times. Parallel studies indicate that SP(1-7), the predominant N-terminal metabolite of SP, was antinociceptive in the abdominal stretch assay at 24 and 48 hr. However, a dose of 100 nmoles of SP(1-7), injected 24 hr before testing, elicited no change in the hot water tail-flick response. In spite of its lack of efficacy in the tail-flick assay, when 1 to 100 nmoles of SP(1-7) was administered together with 0.1 μg of NGF, this N-terminal fragment of SP inhibited the hyperalgesic effect of NGF in a dose-related fashion. D-SP(1-7), a D-amino acid substituted isomer of SP(1-7) that antagonizes SP N-terminal binding and activity, reversed the inhibitory effect of SP(1-7) on NGF-induced hyperalgesia. Together these data suggest that N-terminal metabolic fragments that are presumed to accumulate in the spinal cord during pain transmission serve as long-term modulators of pain by antagonizing the action of NGF. *(Supported by NIDA 04090)*

396.10

DELTA-OPIOID RECEPTOR MEDIATED CONTROL OF SUBSTANCE P RELEASE IN THE DORSAL HORN OF THE RAT FOLLOWING MECHANICAL OR THERMAL NOXIOUS PERIPHERAL STIMULATION. V. Zachariou* and B.D. Goldstein. Dept. of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

The superficial laminae of the dorsal horn of the spinal cord (laminae I and II) are areas rich in the undecapeptide Substance P (SP). These are the areas where the small lightly myelinated and unmyelinated Aδ- and C-fibers terminate. Activation of these fibers elicits an increase in the release of SP in the dorsal horn of the spinal cord. The opioid peptide met-enkephalin, is also found in high concentrations in the dorsal horn and is a selective δ-opioid receptor agonist. The aim of this study was to determine whether δ-opioid receptors are involved in the control of the release of immunoreactive SP in the dorsal horn when noxious mechanical or thermal stimuli are applied to the ipsilateral hindpaw of the rat.

A push-pull canula was introduced into the dorsal horn at the lumbar enlargement in decerebrate/spinal transected rats. The spinal cord was perfused with artificial CSF and immunoreactive SP (SPLI) was measured using RIA, before and after the application of a noxious mechanical or thermal stimulus. All drugs were applied to the dorsal horn through the perfusion apparatus.

ME reduced the tonic release of SPLI. It also blocked the increase in SPLI following either the mechanical or thermal stimuli. The effect of ME was blocked by naltrindole (NTD), a selective δ-opioid receptor antagonist. NTD alone, elicited an increase in the tonic release of SPLI. These results suggest that there is a δ-opioid receptor mediated control of the tonic as well as, the evoked release of SPLI.

396.11

RPR 100893: INHIBITION OF MEMBRANE CURRENT ACTIVATED BY SUBSTANCE P IN CULTURED CELLS OF THE HUMAN ASTROCYTOMA LINE, U 373 MG. J.C.R. Randle* and M. Laville, Rhône-Poulenc Rorer S.A., Central Research, Biology Department, 94403 Vitry-sur-Seine, France

RPR 100893 is a potent and selective antagonist of human neurokinin type 1 (NK_1) receptors. We used whole-cell patch clamp techniques to evaluate the antagonist activity of RPR 100893 on the peak outward (potassium) current recorded in the human astrocytoma cell line, U 373 MG, following NK_1 receptor activation by substance P (SP; 10 nM for 5 secs once every 5 mins). At least two control responses to SP were recorded prior to a 10 minute continuous application of antagonist. RPR 100893 (10^{-12}-10^{-7} M) inhibited SP-induced current in a concentration-dependent manner with an IC_{50} value of 0.44±0.21 nM. The three enantiomers of RPR 100893 (RPR 102530, RPR 103023 and RPR 103253) were ~1000-fold less potent, with IC_{50} values of 300-1000 nM. Under these experimental conditions, the inhibitory effect of RPR 100893 developed gradually over the 10 minute period of application but did not appear to be use-dependent. Little reversal of the inhibition was observed during washout of 10 nM and 100 nM RPR 100893. In contrast, two other NK_1 antagonists, RP 67580 (100 and 300 nM) and (±)CP 96345 (10 nM), inhibited SP responses rapidly and reversibly. These results confirm that RPR 100893 is a potent and stereoselective antagonist of human NK receptors, and indicate that it may have much slower association and dissociation kinetics than previously-described NK_1 receptor antagonists.

396.12

THE SUBSTANCE P N-TERMINAL HEPTAPEPTIDE, INJECTED 24 HRS BEFORE THE FORMALIN ASSAY, POTENTIATES THE ACUTE AND INHIBITS THE TONIC PHASE. V.M. Goettl* and A.A. Larson. Graduate Program in Neuroscience, Univ. of Minnesota, St. Paul, MN 55108, U.S.A.

There is much evidence that substance P (SP) is released in the spinal cord in response to a nociceptive stimulus. Since conditions such as hyperalgesia can occur days or months after nociceptive stimulation, we investigated whether SP produces long-term effects. The SP N-terminus and SP N-terminal metabolites, such as SP(1-7), produce antinociception in the abdominal stretch assay when injected intrathecally (i.t.) 30 min before testing. However, in the formalin assay, in which mice are injected s.c. with 20 μl of 5% formalin into the dorsum of the rear foot and the amount of time spent licking or biting the foot is counted, SP(1-7) was without effect in the acute phase (0-5 min) or the tonic phase (20-30 min) of the behavioral response when SP(1-7) was injected i.t. 5 or 30 min before formalin injection. However, when administered i.t. twenty-four hrs before testing, SP(1-7) produced a U-shaped dose-response curve in both phases. The acute phase was potentiated to 127% and 135% of control at doses of 1 and 10 nmol, respectively. In contrast, the tonic phase was inhibited with decreases to 64% percent of control at 0.1 nmol, 52% at 1 nmol and 59% at 10 nmol. Ten nmol of SP(5-11), a C-terminal SP metabolite active at neurokinin receptors, was without effect. These data implicate an accumulation of SP N-terminal metabolites in the long-term and differential modulation of pain, perhaps contributing to the development of hyperalgesia or analgesia chronically, depending on the type of nociception measured. (Supported by NIDA T32 DA07234 and DA04090.)

396.13

EFFECT OF L-TYPE CALCIUM CHANNEL ANTAGONISTS ON THE RESPONSES OF DORSAL HORN NEURONS TO PERIPHERAL CUTANEOUS STIMULI AND TO SUBSTANCE P IN CATS. V. Radhakrishnan and J.L. Henry*, Departments of Physiology and Psychiatry, McGill University, Montreal, Quebec

Substance P, a mediator of nociceptive transmission at the level of the primary afferent synapse, is known to elevate intracellular levels of calcium in dorsal horn neurons. Substance P (NK-1) receptor antagonists, such as CP-96,345 and CP-99,994, have been shown to interact with L-type calcium channels, thereby raising doubts about their NK-1 receptor selectivity in blocking nociceptive responses. Therefore, in the present study we have tested the effects of L-type calcium channel blockers, verapamil and diltiazem, on the responses of spinal dorsal horn neurons to peripheral cutaneous stimulation and to iontophoretic application of substance P. Extracellular recordings were obtained using multibarrel electrodes from L_4-L_7 segments of the spinal cord in cats anesthetized with α-chloralose and spinalectomized at the L_1 level. Substance P, verapamil and diltiazem were applied to the neurons by iontophoresis. Verapamil and diltiazem depressed spontaneous activity by 20-40% in 8/10 neurons tested. The response to hair stimulation was depressed (20-50%) in 7/10 neurons. Responses to noxious mechanical and noxious thermal stimuli were depressed (40-60%) in 6/6 neurons. The response to substance P was also blocked by verapamil and diltiazem in 5/5 neurons. Thus, in contrast to NK-1 receptor antagonists which did not affect the spontaneous firing or the response to hair stimulation at doses that blocked the response to substance P, L-type calcium channel antagonists showed a suppression of all types of response, irrespective of the modality. In addition, it is likely that the antagonism of nociceptive responses by CP-96,345 and CP-99,994 is unrelated to their interaction with calcium channels. *(Supported by MRC of Canada and Pfizer Central Research)*

396.14

EFFECTS OF THE CALCIUM CHANNEL ANTAGONISTS, SNX-111 (MVIIA) AND SNX-230 (MVIIC), ON THE RELEASE OF GLUTAMATE AND CGRP FROM SPINAL CORD SLICES. S. Gaur, J. Ramachandran and G. Miljanich*. NEUREX Corporation, Menlo Park, CA 94025

During nociception, several neuropeptides (e.g., substance P, CGRP) and excitatory amino acids (e.g., glutamate) are released at spinal cord synapses associated with the nociceptive pathway. The identities of the voltage-sensitive calcium channel (VSCC) subtypes regulating this release are not yet clear. The N-type calcium channel antagonist, SNX-111 (synthetic ω-conopeptide MVIIA), has been shown to be potently anti-nociceptive in pain models, including the rat formalin test (ED_{50}=0.01ug intrathecally), whereas the P/Q-type antagonist, SNX-230 (synthetic ω-conopeptide MVIIC), is not anti-nociceptive in this model (up to 1ug intrathecally; Malmberg and Yaksh, *J. Neurosci.*, in press). We have studied the effects of SNX-111 and SNX-230 on K^+-evoked, Ca^{++}-dependent release of exogenously loaded radiolabelled d-aspartate (*d-Asp, a non-metabolizable glutamate congener) and endogenous calcitonin gene related peptide (CGRP) in transverse rat spinal cord sections. The N-type VSCC blocker, SNX-111, has no detectable effect on K^+-evoked CGRP or *d-Asp release. In contrast, the P/Q-type ligand, SNX-230, blocks approximately 100% of both *d-Asp (IC_{50}=10nM) and CGRP release. These findings indicate that the bulk of glutamate and CGRP release from spinal cord nerve terminals is not mediated by N-type VSCC, but is mediated by P/Q-type VSCC. Whether the antinociceptive effects of SNX-111 are due to inhibition of a minor but critical fraction of CGRP and/or glutamate release in the spinal cord or result from another unrelated mechanism remains to be determined.

396.15

HYPERALGESIA INDUCED BY SPINALLY ADMINISTERED CELL PERMEABLE ANALOGS OF CYCLIC 3':5' GUANOSINE MONOPHOSPHATE (CGMP) Ethan Abraham§, Mary G. Garry†, Kenneth M. Hargreaves† and Lin Aanonsen§*. §Dept. of Biology, Macalester College, St. Paul, MN 55105; † Depts. of Restorative Sciences and Pharmacology, University of Minnesota, Minneapolis, MN.

Recent studies have shown that spinal levels of cyclic 3':5' guanosine monophosphate (cGMP) may be involved in the development of hyperalgesia. The purpose of the present study was to investigate whether direct, spinal application of cell permeable cGMP analogs would result in a hyperalgesic response as measured in the hot-plate assay. Male, ND4 Swiss mice (10/group) were injected intrathecally (i.t.) with 8-bromoguanosine 3':5'-cyclic monophosphate (8-bromo-cGMP; 10nmol/5µl) or saline in order to determine 1) if there was a hyperalgesic effect (a decreased latency to lick the hind-paw) and 2) the time course of the effect. 8-bromo-cGMP produced a significant hyperalgesic response when compared to a saline control from 3 to 30 minutes post-injection. Maximal effects were observed at 10 min., thus, this time was used in subsequent studies. 8-bromo-cGMP and N^2, 2'-O-dibutyryl-guanosine 3':5'-cyclic monophosphate (db-cGMP) produced dose-related hyperalgesia with ED_{50}s of 0.69±1.59 nmol/5µl and 0.79±1.11 nmol/5µl, respectively. The selectivity of this response was determined by observing the response to i.t. injection of other, cell impermeable guanosine compounds and cAMP. The other guanosine compounds did not have an effect, while cAMP significantly increased hot-plate latency. These results suggest that spinal cGMP may be involved in the facilitation of thermal hyperalgesia in mice.

396.16

ACTIVATION OF PROTEIN KINASE C INCREASES RELEASE OF NEUROPEPTIDES FROM RAT SENSORY NEURONS. L. A. Barber* and M. R. Vasko. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202.

Although activation of protein kinase C (PKC) has been shown to enhance excitability of sensory neurons, it is not known whether this activation alters the release of neurotransmitters from sensory neurons. Thus, we examined the effects of altering PKC activity on the resting and potassium-evoked release of substance P (SP) and calcitonin gene-related peptide (CGRP) from rat sensory neurons in culture.

Sensory neurons were dissociated from fetal rat dorsal root ganglia (E15-17) and grown in culture for 9-12 days. Release experiments were performed by exposing neuronal cultures for 10 min intervals to HEPES buffer containing 3.5 or 30 mM KCl, in the absence or presence of two activators of PKC, phorbol 12,13 dibutyrate (PDBu) and 1-oleoyl-2-acetyl-sn-glycerol (OAG), and a PKC inhibitor, staurosporine. SP and CGRP in the perfusate were measured by radioimmunoassay.

Exposing neurons to 10, 50, or 100 nM PDBu significantly increases SP and CGRP release at least 10-fold above resting levels in a concentration-dependent manner. One nM PDBu or 25 µM OAG does not alter resting release, but augments K^+-stimulated release of both peptides 2-fold. This sensitizing action of PDBu on peptide release is attenuated by 10 nM staurosporine. Peptide release is not enhanced by 100 nM 4-alpha phorbol ester, a phorbol that does not activate PKC .

These results suggest that activation of PKC is involved in the augmentation of release of SP and CGRP from sensory neurons, and also is important in sensitizing these neurons to release evoked by a depolarizing stimulus. Supported by PHS AR20582.

396.17

N-(2-HYDROXYETHYL)HEXADECANAMIDE GIVEN ORALLY REDUCES HYPERALGESIA BY DOWN-MODULATING MAST CELL ACTIVATION. S. Mazzari*, R. Canella, G. Marcolongo and A. Leon. Research*life* S.c.p.A., Centro di Ricerca Biomedica, Ospedale Civile, 31033 Castelfranco Veneto, Italy.

Inflammatory mechanical hyperalgesia induced by tissue injury (e.g. exposure to carrageenan) is due to activation/sensitization of sensory nerve terminals by mediators which are also released by mast cells. Tissue damage is also reportedly associated with increased generation of the N-acylamide N-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, termed autacoid local inflammation antagonism (ALIA), the effectiveness of N-(2-hydroxyethyl)hexadecanamide (LG 2110/1) in decreasing functional correlates of mast cell activation in vivo was examined. Oral administration of LG 2110/1 (1-10 mg/kg) to adult rats decreased carrageenan-induced hyperalgesia and hindpaw edema. Similar anti-edema effects were observed against dextran and formalin, known to also cause mast cell activation. Locally given LG 2110/1 was likewise effective in minimizing dextran-induced hind paw edema. LG 2110/1 diminished plasma extravasation induced by injection of substance P (SP) in the mouse ear pinna but not that induced by the SP fragment (peptide 6-11), the latter known to be inactive on mast cells. The pharmacological effect after SP was associated with a reduced frequency of degranulated mast cells. Thus, orally administered N-(2-hydroxyethyl) hexadecanamide both reduces inflammatory hyperalgesia and down-modulates mast cell activation. This not only supports the proposed physiological role of the N-(2-hydroxyethyl)hexadecanamide but also suggests that this type of molecule may represent a novel therapeutic approach for the management of inflammatory conditions modulated by mast cell activity, including nerve inflammation and autoimmune diseases (Leon et al., this meeting).

396.18

SUPERSENSITIVITY TO MORPHINE ANALGESIA INDUCED BY INESCAPABLE TAILSHOCK: TIMECOURSE OF EFFECT. **S.E. Lea*, L. Sutton, R.E. Grahn, L.R. Watkins & S.F. Maier. Dept. Psychology, Univ. of Colorado, Boulder, CO 80309[2].**

This lab has previously reported that inescapable tailshock produces marked enhancement of morphine analgesia, tested 24 hrs after shock. The present experiments replicate & extend this initial report by examining the timecourse of morphine supersensitivity.

Adult male albino rats were exposed either to 100 inescapable tailshocks (5 s, 1 min variable ITI, 1 mA) delivered by fixed fuse-clip electrodes or to an equivalent period of restraint (control). Rats were then tested for pain responsivity (tailflick [TF] test) in a novel environment either 1, 24, 48, or 72 hrs later. At these times, rats were first assessed for baseline pain responsivity (3 TF), followed by either 1 mg/kg s.c. morphine or equivolume (1 ml/kg) s.c. sterile saline. Beginning 15 min after injection, 3 TF latencies were assessed each 10 min for 60 min. Analgesia was not observed in any saline-injected groups. Likewise, morphine failed to produce analgesia in restraint controls at any time tested after shock. In contrast, profound morphine analgesia was observed in rats exposed to tailshock 24 or 48 hrs previously. This effect was strikingly time-dependent since no morphine analgesia was observed 1 hr after shock, & only modest analgesia occurred 72 hrs after shock.

These data demonstrate striking time-dependent supersensitivity to the analgesic effects of morphine following inescapable tailshock. This timecourse is in perfect agreement with other previously reported learned helplessness phenomena. NIH MH 50479

396.19

SUPERSENSITIVITY TO MORPHINE ANALGESIA INDUCED BY INESCAPABLE TAILSHOCK: NEUROPHARMACOLOGY. L. Sutton*, R.E. Grahn, M.J. Will, S.F. Maier & L.R. Watkins. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

Inescapable tailshock produces supersensitivity to morphine analgesia (see companion abstract). The timecourse of supersensitivity is in agreement with other previously reported learned helplessness effects. Since several learned helplessness effects can be prevented by opiate antagonists or benzodiazepines prior to inescapable shock, these drugs were tested to determine if they would also prevent supersensitivity to morphine analgesia.

Rats were injected ip with 5 mg/kg diazepam, 10 mg/kg naltrexone, or vehicle. Rats then received either 100 tailshocks (5 s, 1 min variable ITI, 1 mA) or an equivalent period of restraint (control). Rats were then tested for pain responsivity (tailflick [TF] test) in a novel environment 24 hrs later. Rats were first assessed for baseline pain responsivity (3 TF), followed by either 1 mg/kg s.c. morphine or 1 ml/kg s.c. sterile saline. Beginning 15 min later, 3 TF latencies were assessed each 10 min for 60 min. Regardless of drug received prior to shock, no analgesia was observed in any group injected with s.c. saline at time of pain testing. Likewise, no analgesia was observed in any morphine-injected restraint control group. In contrast, profound morphine analgesia was observed in vehicle-injected rats exposed to tailshock 24 hrs previously. This supersensitivity to morphine analgesia was prevented in rats which received either naltrexone or diazepam at the time of inescapable tailshock.

These data replicate the striking morphine supersensitivity induced by inescapable tailshock. Prevention of these effects by opiate antagonists & benzodiazepines at time of shock is in perfect agreement with other previously reported learned helplessness phenomena. NIH MH50479

RETINA AND PHOTORECEPTORS V

397.1

GROWTH FACTOR STIMULATION OF OPSIN IMMUNOREACTIVITY IN CULTURED PHOTORECEPTORS FROM *rd* MICE. J. C. Blanks*, E. Barrón, S. Y. Schmidt, Y. Courtois and D. Hicks. Doheny Eye Institute, Los Angeles, CA 90033; INSERM, Paris, France.

Retinal cell cultures from postnatal day 1 (P1) control and *rd* mice were used to assess the effects of various growth factors on neuronal differentiation. In *rd* retinas, the photoreceptor cells degenerate during early development due to a deficiency in cGMP PDE. Our objective is to develop an *in vitro* system in order to enhance photoreceptor cell survival and/or differentiation. Retinal suspensions from P1 control and *rd* mice were grown on coverslips at 5 X 10^5 cells/coverslip under basal conditions or in the presence of bFGF (10 ng/ml), NGF (50 ng/ml), EGF (20 ng/ml) or bFGF and NGF combined. Photoreceptor cell viability in cultures of control and *rd* mice were evaluated by counting the numbers of opsin-positive cells (opsin$^+$) throughout the entire coverslip after 4 days in culture. The numbers of opsin$^+$ cells were similar in control and *rd* cultures, and in both there was a two- to three-fold increase relative to baseline in cultures with bFGF and NGF. EGF had no effect on photoreceptor numbers. There did not appear to be a synergistic effect when bFGF and NGF were combined. Cultured cells survived for a minimum of 9 days. Double-label studies are in progress with opsin and neuron-specific enolase (NSE) antibodies to determine if bFGF and NGF have a specific effect on photoreceptor cells or if their effects extend to other neuronal cell types in the retina labeled by NSE. The finding that opsin$^+$ photoreceptor cell numbers were increased by growth factors in *rd* as in control retinal cultures raises the possibility that bFGF and NGF may also help the survival of *rd* photoreceptors.

397.2

SURVIVAL OF SPECIFIC CELL TYPES IN THE RETINA OF TRANSGENIC MICE THAT LACK ROD PHOTORECEPTORS. M.A. McCall*, K. R. Merriman & L.R. Stanford. The Waisman Center, and the Department of Comparative Biosciences. University of Wisconsin, Madison, WI 53706

We reported previously that the expression of a transgene, the rhodopsin promoter linked to an attenuated diphtheria toxin gene, eliminates rod photoreceptors in the retinae of transgenic mice, leaving what appear morphologically to be cone somata in the outer nuclear layer (ONL). In our present studies, we provide further evidence that the cells that remain in the ONL of our transgenic mouse retinae are cones, and, therefore, that the direct effect of this genetic ablation is limited to the rods. In one experiment, we used reverse transcription PCR to quantify the levels of rod and cone opsins. In our "rodless" mice, rhodopsin expression is lower at 14 days postnatally than in age-matched controls, and rhodopsin cannot be demonstrated after 21d. Blue cone opsin expression in the "rodless" retinae, while lower than in controls after 35d, can still be demonstrated in "rodless" adults. Similar experiments are now being performed to quantify the levels of red/green opsin remaining after rod ablation. We also used peanut agglutinin to selectively label cone and cone outer segments in our "rodless" mice. The proportion of labelled somata in the ONL increases with age, and only labelled somata remain at 35d. In addition, we crossed our "rodless" mice to transgenic mice in which cones express the beta-galactosidase (β-gal) gene. In the retinae of the progeny, all somata that remain in the ONL at 28d express β-gal. We are extending these observation to determine if the cones are maintained in "rodless" adults. We also examined the inner retina in the progeny of our "rodless" mice crossed to transgenic mice in which bipolar and ganglion cells express the β-gal gene. The number of bipolar cells in these retinae appears normal. Their location within the inner nuclear layer (INL) appears altered, however. Labelled bipolar cells are no longer found only near the outer margin of the INL, but are distributed throughout this layer. The selective loss of the rod photoreceptors also does not appear to affect the number of retinal ganglion cells. Supported by NIH EY10003 & EY04977.

397.3

IMMUNOCYTOCHEMICAL IDENTIFICATION OF COMPONENTS OF A PHOSPHOINOSITIDE PATHWAY IN ROD OUTER SEGMENTS. Y.-W. Peng[1], S.G. Rhee[2], T. Schoen[3] G.J. Chader[3] and K.-W. Yau[1]*. [1]Dept Neurosci., Johns Hopkins Sch. Med.,[2]Lab. Biochem., NHLBI/NIH and [3]Lab. Retinal Cell Mol. Biol., NEI/NIH.

Phototransduction in retinal rods involves a signaling cascade that leads to cGMP hydrolysis and the closure of cGMP-gated cation channels open in darkness, producing a membrane hyperpolarization as the light response. For many years there has also been some evidence for the presence of a phosphoinositide pathway in the rod outer segment, though its function and the molecular identities of its components are still unclear. We have studied this problem with immunocytochemistry using antibodies against phosphoinositide-specific phospholipase C (PLC) isozymes and also alpha subunits of the G_q family of G proteins. Among the PLC isozymes we have examined (β1-4, γ1-2 and δ1-2), only PLCβ4-like immunoreactivity is found in bovine and rat rod outer segments. At the same time, we have localized $G_{\alpha 11}$, a member of the $G_{\alpha q}$ family, also in the rod outer segments of the same species. Immunoblottings of total retinal proteins with anti-PLCβ4 and anti-$G_{\alpha 11}$ antibodies also gave a single protein band of correct molecular weight in each case, suggesting specific labelings. The co-localization of PLCβ4 and $G_{\alpha 11}$ in the rod outer segment is consistent with the recent biochemical finding that PLCβ4 is activated by the G_q family of G proteins (S.G. Rhee, submitted; Jiang et al., J. Biol. Chem. 269, 7593, 1994). We conclude that $G_{\alpha 11}$ and PLCβ4 are likely to be part of the phosphoinositide signaling pathway in the rod outer segment. Biochemical studies are in progress.

397.4

DOCOSAHEXAENOIC ACID UPTAKE IN THE CONE-DOMINANT LIZARD RETINA. W.C. Gordon, H.E.P. Bazan* and N.G. Bazan. LSU Neuroscience Center, LSU Medical Center, New Orleans, LA 70112.

The retina effects the high-affinity uptake of docosahexaenoic acid (DHA). Photoreceptors actively accumulate DHA for synaptic and photosensitive membranes. Light- and electron microscope-level autoradiography of [^{3}H]DHA has shown that from 65% (primates) to 92% (amphibia) of total retinal label occurs within photoreeptors. In the frog, rod photoreceptors incorporate much more DHA than cone photoreceptors, but [^{3}H]DHA labeling of rods and cones is similar in monkey and rabbit retinas. To determine if some cone photoreceptors are more similar to rods, we used the American chameleon (*Anolis carolinensis)*. which has a cone-dominant retina with a pronounced foveal region consisting of morphologically distinct, long, slender cone cells, and a peripheral region of shorter, wider cones. As controls, we used an all rod retina, from the mediterranean gecko (*Hemidactylus turcicus)*, and the frog *(Rana pipiens)*. Retinas were incubated 4 h in 2 μCi/ml [^{3}H]DHA (SA 23 Ci/mmol). Half of each retina was prepared for lipid analysis, the other half for autoradiography. Analysis of retinas revealed typical profiles in the frog, and good, consistant labeling of the gecko rod cells. However, the density of chameleon foveal cone inner segments was only 46% that of peripheral cone inner segments. Retinal DHA mole percent content was similar in frog and gecko (32%) but only half in chameleon (18%). von Scantz et al. (Invest. Ophthalmol. Vis. Sci 32:2558-2566, 1994) recently described rod-cone similarities in the cone-dominant squirrel retina at a molecular level. Based on these differences among the cell types of the animals studied, we suggest that some cone photoreceptors may possess pathways of phospholipid metabolism that are closer to those found in rods. (Supported by NIH NEI EY05121)

397.5

IDEAL OBSERVERS OF NEURAL SIGNALS IN PHOTO-TRANSDUCTION AND THE NOISE CHARACTERISTICS OF A PHOTOTRANSDUCTION MODEL. P. N. Steinmetz and R. L. Winslow*, Dept. of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The rod phototransduction model of Forti *et al.* was linearized about operating points corresponding to various background light levels. The linearized model was used to predict low frequency quantal noise in the outer segment current. The just noticeable difference (JND) in intensity (in units of photoisomerizations/s) based on observation of outer segment current in response to 200 msec light flashes was determined at different background light levels using ideal observer theory. Performance was compared to that predicted based on optimal processing of photon counts.

Results show that the linearized Forti model of phototransduction reproduces features of low frequency outer segment current noise measured experimentally, such as: a) the magnitude of the noise is a non-monotonic function of the background light level, with the maximum occuring at a dim illumination level; and b) trapping BAPTA (a calcium chelator) in the rod increases noise level.

Ideal observer analyses indicate that intensity JNDs based on observation of outer segment current in response to light flashes are 5-10 times higher than JNDs based on photon counts. The phototransduction process therefore results in an information loss at the earliest stages of neural processing within the retina.

397.6

D_4 DOPAMINE RECEPTORS, NEGATIVELY COUPLED TO ADENYLATE CYCLASE, BLOCK THE HYPERPOLARIZATION ACTIVATED CURRENT (I_h) IN MAMMALIAN ROD PHOTORECEPTORS. B. Longoni*, G.C. Demontis, S. Bisti, P.L. Marchiafava, and L. Cervetto. Ist. Policattedra and Dipartimento Fisiologia e Biochimica, Univ. di Pisa, Ist. Neurofisiologia , CNR, Pisa, ITALY.

The voltage response of retinal rods reflects both the suppression of the dark current entering at the outer segment and the activation of voltage dependent conductances of the inner segment. We have measured the voltage dependent currents at the inner segment of mammalian rods by using the whole-cell voltage clamp technique. We found that the main component is an inward current (I_h) activated by stepping the membrane potential to voltages between -70 and -140, from an holding of -30 mV. Application of 200 μM 8-Br-cAMP shifts the half-activation voltage from -90 to -70 mV. Dopamine 1-10 μM reversibly shifts the half-activation voltage of I_h from -90 to -105 mV, an effect opposite to cAMP application. The results suggest that dopamine receptors, located on mammalian rod inner segments, inhibits the adenylate cyclase. The properties of dopamine receptors were further investigated on membrane homogenates obtained from bovine rods, after isolation by mechanical dissociation and purification on Ficoll gradients. By using the [^{3}H]-spiperone binding assay we found the specific binding to be saturable and of high affinity with a K_d of 0.05 nM. The K_i obtained from antagonist displacement curves are consistent with the known pharmacology of D_2-like receptors; moreover the K_i for clozapine (40 nM) is typical for D_4 receptors. Biphasic displacement curves for agonists suggest that dopamine receptors are coupled to an endogenous G-protein; accordingly GTP-γ-S converts the receptor from an high affinity state for agonists to a low affinity one. We conclude that dopamine affects the membrane properties of mammalian rods by activating D_4 receptors which are negatively coupled to adenylate cyclase.

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397.7

Rp-cAMPS: A COMPETITIVE ANTAGONIST OF CYCLIC NUCLEOTIDE-GATED CHANNELS. Richard H. Kramer*[1] and Gareth Tibbs[2] [1]Dept. of Molec. & Cell. Pharmacol., Univ. of Miami School of Med, Miami, FL 33101, and [2]Ctr. for Neuro. & Behav., Columbia Univ., NY, NY 10032

Cyclic nucleotide-gated (CNG) channels generate the primary electrical signal in photoreceptors and olfactory neurons during sensory responses. Cyclic nucleotides bind to a site on CNG channels that is homologous to the cyclic nucleotide-binding domain of cAMP- and cGMP-dependent protein kinases. Therefore we investigated the effect on CNG channels of thioate derivatives of cAMP and cGMP (Rp-cAMPS and Rp-cGMPS); competitive antagonists of cAMP- and cGMP-dependent kinases, respectively. In CNG channels from catfish olfactory neurons (OLF channels), Rp-cAMPS is a partial agonist. Applied alone it weakly activates OLF channels, but strongly antagonizes activation by co-applied cAMP or cGMP. The inhibition is competitive, with a large effect at low cAMP levels and no effect at saturating cAMP. Single channel analysis shows that Rp-cAMPS reduces open probability without affecting single channel conductance. In contrast, Sp-cAMPS, the diastereoisomer of Rp-cAMPS, is a full agonist of OLF channels. One might expect Rp-cGMPS to antagonize activation of rod photoreceptor CNG channels (RET channels), since these channels are selectively activated by cGMP. However, Rp-cGMPS is an agonist, activating up to 80% of the current elicited by saturating cGMP. Suprisingly, Rp-cAMPS is a pure antagonist of RET channels, exhibiting a K_i of 200-500 μM. Rp-PCPT-cAMPS is an even higher affinity antagonist that is also membrane-permeant. Extracellular application of this analog may be useful for investigating the functional role of CNG channels in these and other cell types. Support: NIH grant NS30695.

397.8

ROD PHOTORECEPTOR M-CURRENT (I_{Kx}) IS STABILIZED BY CALCIUM-DEPENDENT PROTEIN KINASE. D.E. Kurenny and S. Barnes*, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

When recorded with conventional ruptured-patch whole cell recording in bright light, kinetic parameters of the non-inactivating voltage-sensitive rod M-current (I_{Kx}) change gradually ("run down") in the following manner: the maximum current amplitude and time constant of current activation decrease, while the activation curve shifts to the left. In contrast, I_{Kx} was stable during ruptured-patch recording in complete darkness or when using the perforated patch technique. However, the stability of I_{Kx} could be maintained in ruptured-patch recordings in bright light when the intracellular calcium concentration was increased by using either Ca-BAPTA buffer ($[Ca]_i$ = 1 μM), or caffeine (1 mM). IP_3, a second messenger that releases calcium from inracellular stores, stabilized I_{Kx} when included in the pipette solution at 10 μM, but not at 1 μM. Including the phorbol ester PMA in the pipette solution (20-100 nM) also prevented rundown of I_{Kx} but in only 50% of cells. The protein kinase C inhibitor, H-7, added to a pipette solution containing 1 μM free calcium (Ca-BAPTA buffer) restored I_{Kx} run-down. 1,2-dioctanoyl-rac-glycerol (50 μM), KF/$AlCl_3$ (10 mM/100 μM), cAMP or cGMP (200 μM), ATPγS or GTPγS (up to 1mM), and arachidonic acid (50-200 μM) failed to stabilize I_{Kx}. None of the drugs caused any effect on I_{Kx} when applied extracellularly. We conclude that a calcium-dependent substance inhibited by H-7, probably protein kinase C, is present in photoreceptors and is necessary for I_{Kx} stability.

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397.9

MULTIPLE CALCIUM CHANNEL SUBTYPES IN TIGER SALAMANDER CONE PHOTORECEPTORS. M.F. Wilkinson* and S. Barnes, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Numerous studies confirm that photoreceptor Ca channels, activating near -40 mV and showing little if any inactivation, are of the high-voltage-activated subtype. In this study, nystatin-permeabilized patch recordings of cone photoreceptors bathed in 5 mM Ba^{2+} revealed HVA Ca channel subtypes. The majority of Ca channel current was L-type as it was blocked in a voltage dependent manner by, for example nifedipine (0.1 - 100 μM), but a component of Ca channel current was resistant to even the highest concentration of dihydropyridine. Kinetic properties of the current component remaining in 10 μM nifedipine differed from the L-current in that activation occured faster and more inactivation was seen. Most of this component was reversibly blocked by *w*-conotoxin GVIA (1 μM), indicating that these Ca channels are of the N-type. The voltage range of activation was indistinguishable for L- and N-type. Bay K 8644 enhanced the Ca channel current markedly and increased the time constant of tail current deactivation from 1 ms to 6 ms. Application of *w*-conotoxin GVIA (1 μM) to Bay K treated cells blocked a smaller proportion of current during the depolarizing test step and had no effect on the slowed tail current, suggesting that the conotoxin was not blocking L-channels. The P-channel blocker *w*-agatoxin IVA (200 nM) had no statistically significant blocking effect. A small current component that could be blocked by Cd^{2+}, persisted in the presence of all three channel type blockers.

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397.10

Extra-Cellular pH and Cl^- Concentration modulate independently Ca^{2+} Currents in Tiger-Salamanders' Photoreceptors. R. Nitzan* and R.F. Miller. Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

Ca^{2+} currents and intracellular pH were studied in dissociated photoreceptors from tiger salamander retinas. Cells were loaded with either Fura-2 (Ca^{2+} indicator) or BCECF (pH indicator) and imaged using a dual wave length ratioing technique. The fluorescence was collected using a cooled CCD camera (Photometrics). Ca^{2+} currents were evoked by introducing high potassium concentration in the bathing solution for short durations (<30sec).

High potassium evoked Ca^{2+} currents were mainly localized in the photoreceptors soma and pedicle. Elevation of $[Ca^{2+}]_i$ was also observed in the inner segment and to a much lower extent in the outer segment. The Ca^{2+} current was slowly suppressed following introduction of Cl^- free medium (both $SO4^{2-}$ or $CH3SO4^-$ were used as Cl^- replacers). At the same time no change in intracellular pH was observed. On the other hand, introducing rapid (not sufficiently long to induce intra-cellular pH changes) extra-cellular pH acidification (pH of 8.0 to 6.0) along with the high potassium stimulus was sufficient to suppress the Ca^{2+} currents, suggesting an extra-cellular mode of action of suppression of Ca^{2+} currents.

These results point to parallel mechanisms for the effects of pH and extra-cellular Cl^- on Ca^{2+} currents in photoreceptors.

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397.11

INNER SEGMENTS OF SUNFISH SINGLE CONE PHOTORECEPTORS ARE GRADED INDEX WAVEGUIDES. M. P. Rowe, N. Engheta, S. S. Easter, Jr., J. M. Corless and E. N. Pugh, Jr.* Inst. of Neurol. Sci., Univ. of PA, Phila., PA 19104; Dept. Electr. Eng., Univ. of PA, Phila., PA 19104; Dept. Bio., Univ. of Mich., Ann Arbor MI 48109; Depts. Cell Bio. Neurobio. & Ophth., Duke Univ. Med. Center, Durham, NC 27710, Dept. Psych., Univ. of PA, Phila., PA 19104

Cone cells isolated from the retina of the green sunfish (*Lepomis cyanellus*) were examined with a double beam scanning interferometer and also with transmission electron microscopy. With the interferometer, optical path lengths (OPLs) were measured perpendicular to the natural direction of propagation of light in the living fish eye. Measurements gave a resolution of 220 nm in diameter by 6 nm in path length for 632.8 nm (free space) incident radiation. By assuming cylindrical symmetry for the inner segment region of the cone cells, variations in refractive index can be inferred from these OPL measurements. Our analysis indicates that the average refractive index increases monotonically from around 1.39 at the base of the inner segment to 1.43 near its junction with the outer segment. This increase is consistent with general predictions based on an analysis of electron micrographs previously presented (Rowe et al, *J. Optical Soc. A*, **11**:55-70).

397.12

SPECTRAL SENSITIVITY OF RETINAL CONES IN *DANIO MALABARICUS* (giant danio): A. G. Palacios* R. Srivastava & T. H. Goldsmith. Department of Biology, Yale University, New Haven, CT, USA.

The spectral sensitivity of individual cones of a fresh water fish (*Danio malabaricus,* Cyprinidae) was measured between 280 and 700 nm by recording membrane photocurrents with suction electrodes in response to brief flashes of monochromatic light of known quantal flux. These measurements extend further into the UV than have previously been obtained by this technique. We found four classes of cones with maximal sensitivity at 360 (n=5), 406 (n=7) , 480 (n=10) and 561 (n=4) nm. With the exception of the UV-sensitive cone, all spectral sensitivity curves were well fit by an iodopsin template.

The identity of the retinoid chromophore was established by HPLC. Retinal oximes were recovered from eyecups by extracting with a 1:1 mixture of methanol and 1M hydroxylamine (pH 6.5). The oximes were separated on a normal-phase silica column developed with 9% dioxane in hexane. Individual eyes contained an average of 580 pmol of retinal and 10 pmol of 3-dehydroretinal.

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397.13

HUMAN CONE ELECTRORETINOGRAM CAUGHT BY SECOND FLASH. M. Saeki, P. Gouras*, W. Li, +P. Lachapelle. Department Ophthalmology, Columbia University, NYC 10032 and +McGill University, Department Ophthalmology, Montreal, Canada

The b-wave of the cone electroretinogram (ERG) of monkeys and man to a flash can be caught and reduced in amplitude by a second flash if it occurs within 20 msecs after the first flash. The reduction is maximum at 10-15 msec after the first flash. It occurs at different states of retinal adaptation, flash intensities and rates of double flash presentation. We suggest that this interaction between double flashes may reflect timing differences in the cone to on- and off-bipolar synapses (Ashmore & Falk, 1980; Copenhagen et al., 1983) that lead to the generation of opposing ionic currents (Sieving et al., 1994) which underly this retinal response.

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397.14

THE VERTICAL PATHWAY IN THE RETINA IS MODULATED BY D_2 DOPAMINE RECEPTOR D.Križaj*[1], R.Gabriel[2], J.Zhang[3] and P.Witkovsky[1,3]. Depts Physiol[1] and Ophthalmol[3], NYU Med.Ctr, New York ;Dep.Biology[2], Janus Pannonius Univ.,Pecs, Hungary

Introduction: Rod and cone signals in amphibian retina may interact at the level of the photoreceptors themselves and/or on second order cells. We have examined the role of D_2 dopamine receptors in modulation of both rod-cone coupling and transmission between *Xenopus* photoreceptors and L-horizontal /bipolar cells. **Results**: a null test, based on equal rod absorbance for red and green flicker indicated a significant presence of cone signal in mesopic and photopic rods. EM showed small gap junctions between rods and cones and injection of neurobiotin into rods labeled both adjacent rods and cones (ratio 10:1). The magnitude of cone input to rods could be manipulated with D_2 agonists/antagonists. The flicker fusion frequency ν_c of dark adapted rods (4- -5Hz) was increased by the D_2-agonist, quinpirole (1-10 μM) to 12-20 Hz. This effect was observed with red, but not green flicker, its magnitude depended on the intensity and it was not affected by SKF 38393, CNQX or kynurenate. Spiperone, a D_2 antagonist, reduced ν_c to 2-3Hz. In the dark adapted retina the cone signal was absent from the HC's, but was expressed strongly in bipolar cells, as evidenced by intracellular recording and ERG. Cone input to bipolar cells was blocked by the D_2 antagonist and increased by the D_2 agonist. **Conclusion**: Transmission of cone signals to rods and to bipolar cells is modulated by a D_2 dopamine mechanism. In the dark adapted state, cone signal continues to be expressed in the vertical (bipolar cells) pathway as a result of D_2-dependent modulation, but is suppressed in the horizontal pathway. **Acknowledgement:** supported by EY 03570 to P.W.

AUDITORY, VESTIBULAR, AND LATERAL LINE: HAIR CELLS

398.1

ELECTRICAL RESONANCE OF FROG SACCULAR HAIR CELLS MEASURED IN THE PERFORATED-PATCH AND WHOLE-CELL RECORDING CONFIGURATIONS. D. Lenzi*, and W.M. Roberts. Institute of Neuroscience, University of Oregon, Eugene, Oregon, 97403

Frequency discrimination in the inner ears of birds, reptiles and amphibians is accomplished in part by an electrical resonance innate to mechanoreceptive hair cells. Saccular hair cells of the frog (*Rana pipiens*) express a damped membrane potential (Vm) oscillation caused in part by the interactions of voltage-gated Ca channels with Ca-gated K channels colocalized at active zones. Since an abundant, mobile Ca buffer appears to shape the Ca landscape at active zones, we asked whether its presence (in perforated-patch recordings, PP) or absence (in whole cell recordings, WC, where the native buffer was replaced by 1 mM EGTA) influenced Vm oscillations recorded in current clamp. Oscillations during and after current steps were fitted with a damped sine wave by a least-squared-error algorithm to measure frequency (f), damping time constant, and quality factor (Q) of oscillations in 7 cells in each recording configuration. Zero-current potentials were -53 ±8 mV (mean ±S.D.) in PP (series resistance, Rs: 4.8 ±3.3 MΩ), and -60 ±3 mV in WC (Rs: 3.2 ±1.0 MΩ) measured at least 90 s after break-in. The relationship between Q and Vm was bell shaped; peak Q (where the resonance was most robust) was 10.1 ±6.7 in PP, and 8.9 ±10.1 in WC. the potential at peak Q was 2 ±3 mV positive to the zero-current potential in PP, and +6 ±2 mV in WC. The f measured at peak Q was 198.1 ±21.2 Hz in PP, and 213.0 ±26.8 Hz in WC. Comparisons between peak Q, f at peak Q, and zero-current potentials in PP and WC were not significantly different using the Mann-Whitney test. These results suggest that the mobile Ca buffer does not greatly affect the resonant properties of frog saccular hair cells. Supported by NIH grant NS27142 and a McKnight Scholars Award to WMR.

398.2

TWO TYPES OF INWARD RECTIFIERS IN HAIR CELLS OF THE MOUSE UTRICLE. A. Rüsch* and R.A. Eatock; Dept. of Otolaryngology, Baylor College of Medicine, Houston, TX 77030

Whole-cell currents were recorded with the patch-clamp technique from hair cells in utricles excised from 2 to 17 d old mice. Recordings were obtained from both type I and II hair cells, the two morphological classes of hair cells in mammalian vestibular organs. Two kinds of inwardly rectifying conductances were found.

One type, resembling the inward rectifier I_{K1}, activated negative to -50 mV with fast monoexponential kinetics. Its mean chord conductance at -124 mV was 3.3±1.1 nS (mean±SD; n=8 cells). It was blocked by 1 mM external Ba^{2+} or Cs^+. The second type activated more slowly and with a sigmoidal time course at potentials negative to -60 mV. Its activation curve could be fit by a first-order Boltzmann relation with a $V_{1/2}$ = -101±5 mV. The reversal potential was -43±1 mV (n=4cells), and the chord conductance at -124 mV was 1.7±0.3 nS (n=8 cells). This current persisted in 1 mM external Ba^{2+} or 4-AP or intracellular Cs^+, but was blocked by 1 mM external Cs^+. This current is similar to I_h of photoreceptors and cardiac cells.

I_h was found in 24 out of 48 cells, including both type I and II. I_{K1} occurred in most type II cells, including cells with I_h. We have not yet determined whether I_{K1} occurs in type I hair cells.

(Supported by NIH grant DC02290.)

398.3

K CURRENTS IN TYPE I AND TYPE II HAIR CELLS OF THE RAT UTRICLE. M. Saeki* and R.A. Eatock, Physiology Dept., Univ. of Rochester; Otolaryngology Dept., Baylor Coll. of Med., Houston, TX 77030.

Whole-cell currents in rat utricular hair cells were studied with the ruptured- or perforated-patch technique. Cells with constricted necks were identified as type I; cylindrical cells were identified as type II. A low-voltage-activated K current ($I_{K,L}$) was correlated with type I cells: 95 and 2% of type I and type II cells, respectively, had $I_{K,L}$. $I_{K,L}$ activated above -95 mV and saturated by -30 mV. Boltzmann fits to the activation curve gave a slope (s) of 5±0.2 mV (SEM, n=6) and a half-maximal voltage (V_{half}) that varied between -55 and -73 mV. Because $I_{K,L}$ was substantially on at the zero-current potential (V_Z; -72±1 mV, n=37), type I cells had lower input resistances and smaller, faster responses to input currents than did type II cells (V_Z: -68±2 mV, n=22). The distributions of input resistances at -64 mV of cells with and without $I_{K,L}$ had modes of 21 MΩ (n=24) and 3.6 GΩ (n=40), respectively. $I_{K,L}$ activation was slow and sigmoidal. It was fit by an equation for a 3 state model (2 closed, 1 open), giving τ's of ~150 and 30 ms at -64 mV. Type II cells had outwardly rectifying K currents that activated above -60 mV and had variable activation and inactivation kinetics. Monoexponential fits to inactivating currents at +40 mV produced τ's that varied from 20 ms to 700 ms. The data are consistent with the cells having, in different proportions, two outwardly rectifying conductances with distinct kinetics. The cells with the slowest currents may have just one current; their activation curves were fit with a Boltzmann function with a V_{half} of -17±1 mV and an s value of 8±1 mV (n=14). Ca^{2+}-dependent K currents in type II cells were negligible, even with the perforated-patch method. (Supported by NIH grant DC02290.)

398.4

POTASSIUM CURRENTS IN HAIR CELLS ISOLATED FROM THE TURTLE COCHLEA. M. B. Goodman* and I. I. Art. Committtee on Neurobiology, University of Chicago, Chicago, IL 60637.

The frequency of electrical resonance in turtle auditory hair cells in the range 5 to 300 Hz is correlated with the kinetics of the cell's K current. Using whole-cell voltage-clamp recordings, we have examined the extent to which these kinetics are determined by the relative contributions of distinct conductances with different pharmacology, single-channel conductance, calcium- and voltage-sensitivity. In high frequency cells (>200 Hz), superfusion with TEA and 4-AP block the K current with affinities of ~200 μM and ~10 mM, respectively. The current is also blocked by reduction of extracellular calcium, $[Ca]_O$= 1 μM, or internal Ca-chelation with 5 mM BAPTA. It is highly voltage-dependent, and noise analysis of the whole cell current suggests a large-amplitude unitary conductance of the calcium-activated, BK type. In low frequency cells (< 50 Hz), the K current is less sensitive to TEA (K_d ≈ 50 mM) and more sensitive to 4-AP (K_d ≈ 25 μM). This current is resistant to lowering $[Ca]_O$ or intracellular perfusion with 30 mM BAPTA. It activates less steeply with voltage, and the current noise is consistent with a smaller unitary conductance. A K-selective inwardly rectifying current is also present. Cells tuned to intermediate frequencies exhibit a mixture of both 4-AP- and TEA-sensitive K currents. Across the frequency spectrum, an additional, small, voltage-independent K current is present to a variable degree, and it is most easily observed under conditions that elevate $[Ca]_i$. We propose that ionic currents underlying resonant behavior vary systematically in the auditory epithelium and solitary cells that resonate at the lowest frequencies employ an ensemble of K currents distinct from those characterized previously for resonant frequencies greater than 50 Hz.
Support: HHMI Predoctoral Fellowship (MBG) & NIH grant DC00454 (JJA).

398.5

CALCIUM-ACTIVATED POTASSIUM CHANNELS OF TURTLE HAIR CELLS. I. I. Art*, Y-C. Wu and R. Fettiplace. Department of Neurophysiology, University of Wisconsin, Madison, WI 53706 and Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

A major factor determining the electrical resonant frequency of turtle cochlear hair cells in the range 50-500 Hz is the time course of the Ca-activated K current. We have examined the notion that this time course is dictated by the K channel kinetics by recording Ca-activated K channels in inside-out patches from isolated cells. A cell's resonant frequency was estimated from its known correlation with the dimensions of the hair bundle. All cells possess BK channels with a similar unit conductance (300 pS in symmetrical 150 mM K) but with different mean open times of 0.25-12 ms. The time constant of relaxation of the average single channel current (-50 mV, 4 μM Ca) varied between cells from 0.4 to 13 ms and was well-correlated with the hair bundle height. The magnitude and voltage-dependence of the time constant agree with the expected behavior of the macroscopic K current, whose speed is thus limited by the K channel kinetics. All BK channels had similar Ca sensitivities with a mean half-activation at 5 μM (0 mV); we estimate that under physiological conditions these channels may be completely activated by a local rise in Ca to 50 μM. Membrane patches also contained 30 pS SK channels which were about ten times more Ca-sensitive than BK channels at -50 mV. Charybdotoxin (0.1 μM) blocked both types of K channel. The SK channels may underlie the hair cell's efferent ipsp.
Supported by: NIH grants DC00454 (J.J.A) & DC01362 (R.F.)

398.6

CALCIUM REGULATION IN TURTLE COCHLEAR HAIR CELLS. T. Tucker* and R. Fettiplace. Department of Neurophysiology and Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

The large size (up to 0.1 nA/pF) of the sustained Ca current in turtle hair cells imposes a considerable burden on the cell's Ca regulation about which little is known. Whole-cell recording of Ca currents from isolated hair cells using cesium-filled electrodes revealed a slow inward component of the tail current which declined over 0.2 to 2 seconds. The size of the tail current increased with the duration of the preceding voltage-step, exhibited a dependence on membrane potential that paralleled the Ca current and, like the Ca current, was blocked by 20 μM nifedipine. The tail current was abolished by removal of external K but not by removal of Na or Cl. These properties argue that the current is carried through Ca-activated K channels, its time course reflecting the decline in Ca concentration at the membrane. Thapsigargin (0.2 μM), a specific blocker of the endoplasmic reticulum Ca ATPase, and intracellular vanadate (1 mM) caused a progressive increase in the tail current on consecutive voltage pulses culminating in a permanent activation of an inward K current, which suggests the Ca load was no longer being cleared. We conclude that a Ca ATPase, probably located in an intracellular compartment, plays a major role in the Ca regulation in turtle hair cells and speculate that Ca may eventually be extruded by fusion of the stores with the plasma membrane.

398.7

MOLECULAR CHARACTERIZATION OF A VOLTAGE–ACTIVATED CALCIUM CHANNEL FROM THE CHICKEN'S INNER EAR. R. Kollmar* and A. J. Hudspeth. Howard Hughes Medical Institute and Center for Basic Neuroscience Research, University of Texas Southwestern Medical Center, Dallas, TX 75235.

The influx of Ca^{2+} into hair cells of the inner ear is necessary both for frequency tuning by electrical resonance and for synaptic transmission to auditory neurons. Unlike most other voltage-activated, L-type Ca^{2+} channels, those of hair cells open at a relatively negative membrane potential and are not inactivated by Ca^{2+} influx. To understand the molecular basis of their unique characteristics, we have begun to determine the structure of Ca^{2+} channels from the chicken's inner ear.

Using RNA from the sensory epithelium of the basilar papilla and polymerase chain reactions with degenerate primer pairs, we obtained 5 kilobases of cDNA, lacking only the 5′ end, for the α_1 subunit of a voltage-activated Ca^{2+} channel. The predicted protein sequence of the four homologous repeats is more than 90% identical to those of mammalian L-type channels of class D; however, several short stretches diverge markedly from any published sequence. We observed additional diversity at the 3′ end: two classes of isolated cDNAs encode alternative cytoplasmic domains at the carboxy termini, neither of which resembles published sequences. We are now testing whether these divergent sequences are expressed in hair cells and whether they account for the unusual electrophysiological properties of the voltage-activated Ca^{2+} channels there.

This work was supported by NIH grant DC00317.

398.8

MOLECULAR IDENTITY OF A POSSIBLE HAIR-CELL MYOSIN I. A. B. Metcalf and A. J. Hudspeth*. Howard Hughes Medical Institute and Center for Basic Neuroscience Research, University of Texas Southwestern Medical Center, Dallas, TX 75235-9117.

The hair cell's mechanosensitive organelle is the hair bundle, an organ-pipe array of stereocilia. A tip link connects each stereocilium to the tallest neighboring process; at or near this connection lie transduction channels that are gated by bundle displacements. During a hair cell's adaptation to protracted stimulation, the current flowing through transduction channels returns toward its resting level. Adaptation is thought to involve adjustment of the tension within tip links by an ATP-requiring molecular motor, possibly a myosin isozyme. Consistent with this hypothesis, monoclonal antibodies raised against a bovine myosin I, MMIβ, recognize a 120-kDa protein from hair bundles of the frog's sacculus. Immunofluorescence microscopy reveals that this protein is concentrated at the bundles' top edges, where the tip links and transduction channels are located.

To determine the molecular identity of the myosin isozyme in hair bundles, we have investigated expression in the frog of a gene similar to that encoding MMIβ. Frog brain, which contains a 120-kDa protein recognized by the same antibodies, expresses mRNA whose sequence closely resembles that of MMIβ message. The saccular macula contains mRNA with a similar sequence. When expressed in bacteria, a portion of the sequence specifies a fusion protein that is recognized by the antibodies raised against MMIβ. We are now attempting to determine the full-length sequence of the myosin message from frog brain.

This work was supported by NIH grant DC00241.

398.9

ELECTROPHYSIOLOGICAL EVIDENCE THAT 'GLUTAMATE' IS A HAIR CELL TRANSMITTER IN THE TURTLE. S.L. Cochran* and M.J. Correia. Dept. Otolaryngology, Univ. Texas Med. Branch, Rt. J63, Galveston, TX 77555-1063.

An acidic amino acid, such as glutamate, has been implicated as the hair cell transmitter in anamniotes (e.g. frogs), which lack Type I hair cells. Since there is scant electrophysiological evidence of the nature of the hair cell transmitter(s) in amniotes, we have recorded from canal afferents of the turtle inner ear (in vitro), while bath-applying transmitter agonists and antagonists in order to relate their activity with the endogenous hair cell transmitter-mediated activity. During extracellular recordings from individual afferents, glutamate (5 mM) reversibly induced over a ten-fold increase in the firing rate of the afferents (N=29). Aspartate (5 mM) mimicked this effect (N=5). Kynurenic acid (1 mM) reversibly eliminated the resting firing frequency of the afferents (N=8) and reduced the excitatory action of both of these agonists (N=6). NMDA (0.5 and 5 mM) also resulted in afferent excitation (N=5). GABA (5 mM) had no consistent effect upon afferent firing (N=10), while carbachol (0.5 mM) resulted in an increase in firing, weaker than that induced by glutamate and which to some extent desensitized during application (N=6). These findings are consistent with the hypothesis that glutamate or a related compound is a hair cell transmitter in amniotes as well as anamniotes. The major difference between the turtle and the frog appears to be the sensitivity in the turtle to NMDA, not found in the frog. Otherwise the turtle afferent responses resemble those found from afferents innervated by Type II hair cells in the frog. It is as yet unclear as to whether or not axons innervated by Type I hair cells are also included in the current sample.

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398.10

EXPRESSION OF mRNA ENCODING RAT KIDNEY WATER CHANNEL IN RAT ENDOLYMPHATIC SAC. K. Doi*[1], S. Iwakura[1], K. Fukazawa[1], M. Sakagami[1], M. Toyama[2], T. Kubo[1], K. Fushimi[3], S. Sasaki[3]; 1. Dept. of Otolaryngology, Osaka Univ. Med. Sch.; 2. Dept. of Anatomy II, Osaka Univ. Med. Sch.; 3. 2nd Dept. of Internal Medicine, Tokyo Med. Dent. Univ., JAPAN.

The cloning of cDNAs encoding two water channels from rat kidney has been recently completed. One is CHIP28 which is responsible for the high water permeability in the proximal tubule and the descending thin limb of the loop of Henle. Another one is WCH-CD which is specifically expressed in the collecting duct where water reabsorption is regulated by the antidiuretic hormone arginine vasopressin (AVP). In the present study, the expression and cellular localization of water channel in rat endolymphatic sac was investigated, since this end-organ has been believed to be highly water permeable and play the critical role in regulation and reabsorption of endolymph in the inner ear.

Approximately 0.4 μg total RNA was extracted from each endolymphatic sac with GTC-CsTFA method. First strand cDNAs were synthesized from 3 μg total RNA with reverse transcriptase. Two degenerated oligonucleotide primers were designed from the sequence of water channel (WCH-CD) and the homologous cDNAs were amplified by using polymerase chain reaction (PCR). The amplified cDNAs were separated on 1% agarose gel and subcloned into pGEM7zf(+) vector for sequence analysis by using Sanger's dideoxy-termination method. Cryosections of dissected endolymphatic sac were immunostained with anti-WCH-CD/C, a polyclonal antibody against the peptide corresponding to the C-terminal amino acids of WCH-CD.

A cDNA band with the expected size of 369 base pairs was detected on agarose gel after PCR amplification. The sequence analysis identified the expression of mRNA encoding WCH-CD in rat endolymphatic sac. Immunofluorescence staining was detected in the cells lining the lumen of the endolymphatic sac. The results strongly suggest that water channel (WCH-CD) is the molecule responsible for the AVP-mediated reabsorption of endolymph in rat endolymphatic sac.

398.11

LOCALIZATION OF NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DIAPHORASE IN THE RAT INNER EAR. MJ Lyon[1], D Godin[1] and B Mayer[2]. 1) Dept. Otolaryngology, SUNY Health Sci. Ctr., Syracuse, NY; 2) Inst. Pharmakologie Toxikologie Univ. Graz, Graz Austria.

Nitric oxide (NO) has received considerable attention and appears to play several roles in brain function. There is evidence showing that NO can function as a neurotransmitter (Bredt et al., Nature 351:714, 1991); is involved in ischemic injury (Beckman, Nature 345:27, 1990); plays a role in regulation of cerebral blood flow (Iadecola, Proc. Natl. Acad. Sci. USA 88:4651, 1992). To determine if NO may be involved in inner ear function, a combination of histochemical and immunohistochemical techniques were used (Mayer et al., FEBS Lett 277:215, 1990) to localize nitric oxide synthase immunoreactivity (NOS) within the adult rat temporal bone. Preliminary results show similar patterns of labeling for both techniques. Spiral and Scarpa's ganglion cells are positive as are their nerve projections. Label is also observed within the neuroepithelium of all the vestibular end organs with dense label near base of some hair cells bases. The stria vascularis contained NADPH-diaphorase label but little NOS immunoreactivity. These results indicated that NO may play a role in inner ear function and that a different or previously undescribed type of NOS is located in stria vascularis.

398.12

THE EXPRESSION OF GROWTH FACTOR RECEPTOR GENES IN MAMMALIAN HAIR CELL EPITHELIA ASSESSED BY RT-PCR. L.D. Saffer and J.T. Corwin*. Depts. of Otolaryngol. and Neurosci., Univ. of Virginia, Charlottesville, VA 22908.

Hair cell loss in the vestibular organs of mammals has been shown to result in the proliferation of supporting cells, which are the progenitors of regenerated hair cells. In normal cell proliferation, mitogenic growth factors must bind to specific receptors before cell division can occur. Since we are interested in controlling hair cell regeneration, we need to identify the receptors that are expressed in these cells. RT-PCR provides a sensitive method to assay for the presence of low levels of messenger RNA for such receptors. Hair cell epithelia were isolated from undamaged utricles of rats, trimmed of all edges and extrasensory epithelial cells, and frozen in liquid nitrogen. Total RNA was isolated from these tissues and reverse transcribed into cDNA. Specific oligonucleotide primers were selected from published sequences for growth factor receptors and these were used in hot-start PCR to amplify unique fragments of receptor cDNAs. Positive and negative controls were run with each reaction. PCR products were resolved by gel electrophoresis. These experiments have demonstrated the presence of mRNA for the insulin receptor, but as yet have not shown detectable levels of mRNA for the PDGF receptor, the IGF-1 receptor, or the EGF receptor in central regions of undamaged hair cell epithelia from mammalian utricles. Damaged utricles will be assayed for increased expression of those messages.

398.13

THE PROLIFERATION OF HAIR CELL PROGENITORS IS DEPENDENT ON CELL-SUBSTRATE ADHESION. L.L. Cunningham, J.E. Finley, M.E. Warchol* and J.T. Corwin, Depts. of Otolaryngol. and Neurosci., Univ. of Virginia, Charlottesville, VA 22908.

We conducted experiments to determine whether adhesion to a substrate is required for the proliferation of supporting cells from the chick ear. Basilar papillae and utricles were dissected from young (7-14 days) White Leghorn chicks, and sensory epithelia were cultured for 5-7 days in Medium 199 supplemented by 20% fetal bovine serum. After 1-2 days in culture, hair cells extruded from the epithelium leaving sheets of supporting cells. A subset of the supporting cell sheets attached to the substrate and began proliferating. Other sheets continued to float in the culture medium during the same period. Bromodeoxyuridine (BrdU) was added to each culture for 4 hours to measure the proliferation rates of substrate-adherent supporting cell sheets and free-floating sheets. Eighteen adherent cultures had a mean of 29.2 BrdU labelled cells per 100,000 μm^2 (range: 8-59). Whereas nine free-floating cultures had a mean of 4.66 BrdU labelled cells per 100,000 μm^2 (range: 0-24), a significantly ($p > 0.005$) lower proliferation rate. The supporting cells were in the presence of the many mitogenic growth factors in serum, but only showed a significant proliferation after substrate adhesion, demonstrating that cell-substratum adherence is required for the proliferation of auditory and vestibular supporting cells. Preliminary histochemical studies reveal the presence of integrin receptors on the supporting cells which may be responsible for the adhesion-dependence of their proliferation.

398.14

CYCLIC AMP IN AN INNER-EAR HAIR CELL SHEET PREPARATION. M. J. Drescher[1,2*], D. I. Margolis[1,2] and D. G. Drescher[1,2,3]. [1]Lab. of Bio-otol., Depts. of [2]Otolaryn. and [3]Biochem., Wayne State Univ., Detroit, MI 48201.

Previously, we localized adenylyl cyclase activity to specific sites within the sensory epithelium of the trout saccular macula, utilizing a modification of Mees' histocytochemical procedure (Kern et al., Soc. Neurosci. Abstr. 17: 29, 1991). Reaction product was concentrated in the stereocilia and apical surface of the mechanosensory hair cell. Precipitate was also found in two groups of nerve terminals in contact with hair cells, one characterized by efferent synaptic specializations and the second by efferent/afferent synaptic specializations at what appeared to be reciprocal synapses.

With an enzyme immunoassay for cAMP (Amersham, RPN 225), we have now measured cAMP levels in a hair cell sheet preparation for which the hair cell is the only intact cell type. Perchloric acid extracts of the hair cell sheet and associated saccular nerve fraction contained 5.2 ± 1.1 (5) and 15.3 ± 2.7 (5) fmol cAMP/μg cell protein, respectively [mean ± SEM (n)]. Depolarization of the hair cell sheet by a 10.5 mM elevation in extracellular [K^+] in superfusion experiments induced an increase in efflux of cAMP of approximately 0.6 fmol/70,000 hair cells, compared to 11 pmol for glutamate, the latter presumed to be involved in receptoneural transmission. Extracellular application of ATP at 25 μM (5 min) was observed to elicit a 30% increase in intracellular levels of cAMP in the hair cell sheet relative to the paired control, significant by paired-variate analysis ($p < 0.05$, two-tailed t test).

The low level of cAMP in the hair cell sheet appears consistent with its limited and site-specific presence in the receptor cell by histocytochemical determination. The modulation of intracellular cAMP in the hair cell sheet by extracellular ATP, if not elucidated with regard to mechanism, represents preliminary evidence of the existence of a cAMP-mediated second messenger pathway in hair cells.

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398.15

A MODEL FOR VESTIBULAR PRIMARY AFFERENT INTEGRATION OF HAIR CELL MORPHOLOGICAL POLARIZATION VECTORS.
T.C. Chimento*, B.R. Parnas, M.D. Ross. Biocomputation Center, NASA, Ames Research Center, Moffett Field CA 94035-1000.

The functional polarization vectors derived from otolith primary afferents are commonly fit to a cosine. The morphological polarization vectors (MPV) of hair cells contribute to the afferent's response characteristics. Transmission electron microscopy of rat utricular stereocilia demonstrates that adjacent hair cells often have MPVs that vary significantly. Physiological studies of individual hair cells have demonstrated that the functional polarization vector is not described by a cosine. Instead, the differential gains for the positive and negative half cycles are not equal resulting in a clipped response waveform. We believe that this nonlinearity accounts for the deviation from the cosine relationship found by Fernández, et al. (J. Neurophys. 35, 1972) in their studies of otolith afferent responses. We have simulated the effects of hair cell MPVs on the resultant afferent polarization vectors using a simple representation for the nonlinear hair cell input/output characteristics. Early simulation results using this model exhibit the same type of nonlinearity found in otolith afferent fibers. These findings are unchanged when the contributing hair cells have widely varying MPVs. A comparison of the simulation results with otolith response characteristics suggests that the response properties of otolith afferent fibers is consistent with relatively simple integration of input from contributing hair cells. The attendant nonlinearity in the responses results from the input/output characteristics of the hair cells.

This work was supported by NASA and the National Academy of Sciences.

398.16

TOLUENE EFFECTS ON *Gallus domesticus*' VESTIBULAR SENSORY EPITHELIA IN THE EMBRYOGENESIS.
A. Illescas-Landgrave* and M. Lorenzana-Jimenez. Departamento de Anatomía y Departamento de Farmacología. UNAM, México, D. F. 04510.

All biologically active substances interfere, in one way or another, with the chemical system that governs the homeostatic mechanism of living organism. Many drugs create temporary or permanent alterations in auditory or vestibular function.

The purpose of this study is to show that toluene induces cellular alterations on *Gallus domesticus* internal ear vestibular sensory epithelia during its morphogenesis. To accomplish this purpose 42 fertile stage 6 (23-25 hr), eggs were used arranged on 7 groups. The applied toluene doses were 0.60, 1.12, 2.5, 5.0 and 10 µL for the first 5 groups; 10.0 µL of normal saline solution for the 6th group and the last one was taken as a control group. After 14 days of hatching the embryons were removed from their egg-shells. Immediately were beheaded and their internal ear were prepared to be analysed through electronic transmition microscopy. Toluene provoked loss of some hair cells in all cristae and otolithic organs, in other cells we found various stages of degeneration: loss of cilia, cytoplasmic degeneration and vacuolization of membranes. In the supporting cells the common damage observed was edema. These data suggest that toluene injured avian vestibular receptors during the embryogenesis.

398.17

AUTORADIOGRAPHIC LOCALIZATION OF MINERALOCORTICOID (TYPE I) BINDING SITES IN THE SENSORY EPITHELIUM OF THE CRISTA AMPULLARIS.
P.G. Reddy[1], R.C. Kern[2] and D.Z. Pitovski[1]*. Depts. of Otolaryngology - Head and Neck Surgery, [1]Wayne State University, Detroit, MI, 48201 and [2]Northwestern University, Chicago, IL 60201.

Aldosterone, a mineralocorticoid, enhances Na retention and K excretion in various ion transporting epithelia. Previous studies have demonstrated that this mineralocorticoid regulates Na, K-ATPase in the inner ear. Na, K-ATPase has been implicated in the maintenance of high K and low Na in endolymph, ionic properties which support transduction by inner ear hair cells. Other studies have revealed a similar pattern of distribution for mineralocorticoid (type I) binding sites and Na, K-ATPase. These results indicate that mineralocorticoids may regulate endolymphatic ion content and thus modulate hair cell transduction. The purpose of our present study was to determine if mineralocorticoid binding sites are associated with sensory hair cells of the crista ampullaris.

The ampullae of the posterior, lateral and superior semicircular canals were dissected from male Hartley guinea pigs and incubated in media containing [^{3}H]-aldosterone (60 nM) and a 1000-fold excess (60 μM) of RU 28362, a glucocorticoid agonist, to reduce low affinity binding of tritiated ligand to glucocorticoid receptors. Competitive controls were incubated with an additional 2,000-fold molar excess (120 μM) of unlabelled aldosterone. Specimens were then processed for autoradiographic analysis.

Examination of autoradiographs revealed silver grains in the sensory epithelium of the ampullae. Specifically, binding was localized in the nucleus, cytoplasm, and stereocilia of both type I and type II hair cells. Localization of mineralocorticoid binding sites to these cellular components suggests that aldosterone may participate in both genomic and non-genomic modulation of hair cell transduction.

(Work supported by NIH CIDA DC00046 to D.Z.P. and NIH T32 DC00026)

398.18

RAPID DENDRITIC GROWTH IN VESTIBULAR NEURONS OF PERINATAL CHICKS.
K.D. Peusner * and C. Giaume. Dept. Anatomy and Neuroscience Program, George Washington Univ., Washington, D.C. 20037.

The objective of this study was to determine whether perinatal remodeling of primary vestibular synapses in the medulla is accompanied by developmental changes in their target vestibular sensory neurons, the principal cells (PC) of the tangential nucleus. PCs are vestibuloocular collic neurons which receive large axosomatic "spoon" endings from primary vestibular fibers. Spoon synaptogenesis follows an unusual pattern: first in embryos (E7) forming chemical synapses, which then associate with gap junctions (GJs) to form mixed synapses (E15), and finally mainly GJs in hatchlings (H) (Peusner, J. Comp. Neurol. 230:386-392, 1984).

This is the first report describing PC dendritic morphology and comparing them at two ages, E15-16 and H1-2. From preliminary observations made on PCs intracellularly injected with biocytin, we observed that the characteristic oval somata do not change appreciably in size (28±5 SD x 18±3 μm; n=12 vs. 28±4 x 17±5 μm; n=9). Also, in our small sample there is no apparent change in the average number of primary dendrites (3.7 dendrites/neuron) nor in the dendritic branching pattern. However, the number of dendritic branch points per neuron increases by 32% (12.7 vs. 16.8) as well as the dendritic spread in both the mediolateral (98%; 168±87 μm vs. 333±76 μm and the dorsoventral axes (127%; 103±59 μm vs. 234±71 μm). Finally, a major change of 147% was observed in total dendritic length per neuron (605±426 μm vs. 1495±358 μm).

These observations suggest that parallel to the important changes occurring at spoon ending terrminals at hatching, their postsynaptic target cells rapidly and extensively generate new dendrites. (This work was supported by NIH grant RO1- DC00970).

398.19

MACROMECHANICAL ENDOLYMPH PRESSURE AND FLOW DESCRIBE RESPONSES TO STEP MECHANICAL INDENTATION OF THE FISH VESTIBULAR LABYRINTH.
R.D. Rabbitt[1]*, R. Boyle[2], S.M. Highstein[3], and E.R. Damiano[1]. Univ. Utah, Salt Lake City, UT 84112[1]; Oregon Health Sci.. Univ., Portland, OR 97201[2]; and Washington Univ., St. Louis, MO 63110[3].

A piezoelectric micro-actuator was used to generate mechanical indentations of the long-and-slender region of the horizontal canal (HC) and the utricle (U) in the toadfish, *Opsanus tau*, while recording extracellular afferent responses. In the toadfish, sinusoidal ±1µm HC indentation commutates linearly with ∓15µm U indentation and ±4 deg/sec head rotation. Having established this correspondence, step mechanical indentation was applied to simulate a step increase in head velocity (Dickman and Corriea, *J. Neurophysiol.* 62, 1989). Afferent responses show a jump increase (τ <0.02 sec) in firing rate followed by a fast transient decay ($\tau \approx 1$ sec) and a slow exponential decay ($\tau \approx 30$ sec) approaching the resting rate. The slow exponential is relatively uniform across units and is consistent with models describing the mechanical recovery of the cupula to its original resting position. The jump increase and fast transient decay show significant inter-unit variability, from being absent in some units to exceeding the peak magnitude of the slow response in others. These fast components cannot be described by models of macromechanical cupular displacement or by established processing associated with the hair-cell-afferent complexes. The range of afferent responses to head rotation can by described by a model in which individual units exhibit varying sensitivities to macromechanical endolymph flow and pressure (Rabbitt et al., submitted *J. Neurophysiol.*). We apply the same model here to describe the afferent responses to the step stimuli. Results show that the fast components can be described by unit-specific sensitivity to macromechanical pressure and the slow decay by the sensitivity to macromechanical displacement of the endolymph. It is presumed that gross endolymph flow and pressure combine within the ampulla to elicit deflections of stereocilia, although the micromechanical and/or biophysical mechanisms responsible for the apparent unit-specific sensitivities to these components remain yet unknown. *[Supported by NIH]*

398.20

UNIFORM RESPONSES OF RAT SEMICIRCULAR CANAL AND OTOLITH AFFERENT NEURONS TO TRAPEZOIDAL AND SINEWAVE POLARIZATION OF THE LABYRINTH.
J. Kleine and O.-J. Grüsser, Department of Physiology, Freie Universität, 14195 Berlin, Germany. **(SPON: European Neuroscience Association)**

Action potentials of primary afferent vestibular neurons were recorded with tungsten microelectrodes from the *Ggl. Scarpae* in pigmented anaesthetized Norwegian rats. Sinewave mechanical stimulation in yaw, pitch and roll (0.5-1 Hz) and steady inclination at different angles around different axes identified the origin of the neurons (semicircular canal or otolith afferents). Galvanic stimulation was applied through electrodes in the ear bars. Most, but not all, of the regularly discharging units (rdu) exhibited sensitivities well below the range observed in irregularly discharging units (idu). When sinewave stimulus frequency was varied between 0.1 and 100 Hz, most rdu and idu followed up to stimulus frequencies of 60-70 Hz at least; some discharged action potentials in a stimulus-dependent pattern (1:1 or 1:2) up to 100 Hz. Depending on sensitivity and spontaneous discharge rate, non-linear response components appeared at varying stimulus amplitudes and frequencies and consisted mainly of a discharge pause during the positive phase of ipsilateral labyrinth polarization. The "galvanic" frequency response properties of afferent neurons from the vertical and horizontal semicircular canals and the otoliths did not differ essentially. At low galvanic sinewave stimulus frequencies (1-2 Hz) the response threshold was about 5 uA. No responses to galvanic stimulation were obtained in afferent auditory nerve fibers (Supported in part by a BMFT-grant).

399.1

TEMPERATURE DEPENDENCE OF TWO-TONE RATE SUPPRESSION IN THE NORTHERN LEOPARD FROG, *Rana pipiens pipiens.* P.M. Narins*, J.H. Benedix Jr, Marisa Pedemonte and Ricardo Velluti. Univ. of California, Los Angeles, CA 90024.

Stiebler and Narins (1990) reported that increasing body temperature causes low-frequency auditory nerve fibers in the frog to increase their CFs and decrease their thresholds. If two-tone rate suppression (TTRS) and excitatory tuning in the frog were to be codependent on the same processes, then excitation and suppression might be expected to exhibit similar temperature-induced shifts.

The goal of the present study was to define the TTRS existence region in the northern leopard frog and to determine its temperature dependence. Ectotherms such as anuran amphibians may experience daily temperature changes of more than 20°C in their natural habitat; thus it is of biological interest to characterize the temperature-dependence of auditory function in these animals.

Core temperature shifts were induced experimentally and the resulting changes in TTRS were quantified. The existence region of TTRS in the frog was expanded to include a suppressive region below a fiber's characteristic frequency. In response to a 3°C temperature rise, the area and the best suppressive frequency (BSF) of the low-side suppressive region increased significantly. Increasing core temperature of the frog by 6°C resulted in significant changes in the high-side suppressive region: its area decreased, and its BSF and best suppressive threshold (BST) increased. Moreover, constant-temperature control trials were run in which the probe tone was shifted within the tuning curve to partially mimic the temperature-induced changes in neural tuning properties. We found that lowering the probe tone by 0.5 oct had no effect on the low-side suppressive region, but significantly increased the area and lowered the BSF and BST of the high-side suppressive region. Temperature shifts in the frog appear to have a differential effect on the low- and high-side suppressive areas of auditory nerve fibers. Excitation and suppression also respond differentially to 6°C temperature shifts.
(Supported by NIDCD grant DC-00222 to PMN and Fulbright Awards to MP and RV)

399.2

SIZE, DISTRIBUTION, AND THEORETICAL CONDUCTION VELOCITIES OF AUDITORY NERVE FIBERS IN THE FROG INNER EAR. B. Pesaran, D.D. Simmons, P.M. Narins, S. Parham, C. Bertolotto, and A. Newman*. Dept. of Biology and Brain Research Institute, UCLA, and the House Ear Institute, Los Angeles, CA 90024.

The leopard frog (*Rana pipiens pipiens*) has two specialized auditory organs: the amphibian papilla (AP) and the basilar papilla (BP). Nerve fibers to the AP have characteristic frequencies (CFs) from 100-1250 Hz. Nerve fibers to the BP have CFs ranging between 1200 Hz and 3000 Hz. Previous studies suggest that click latency is a function of CF i.e., low CF fibers have maximum response latencies. The purpose of this study was to estimate theoretical conduction velocities (CVs) and compare with known CFs and response latencies.

Frog inner ears were embedded in plastic and 2 μm sections were cut through the AP and BP nerve branchlets. An automated computer algorithm was used to compute number, diameter, and area of nerve fibers. AP and BP nerves were also prepared for electron microscopy.

The AP nerve had an average of 717 fibers with areas ranging from 2-6 μm^2 and diameters between 2-4 μm. Nerve fibers terminating in caudal (high frequency) regions had smaller diameters than fibers terminating in rostral (lower frequency) regions. Virtually all of the fibers were myelinated (average myelin thickness of 0.13±0.05 μm). The BP nerve had 200-300 myelinated fibers with areas between 2-3 μm^2, and diameters between 1-2 μm. Larger diameter fibers were more heavily myelinated and were situated centrally within the nerve bundle.

CVs were estimated for both AP and BP nerve fibers. Rostral terminating AP fibers have the highest estimated CV, while caudal terminating AP fibers and BP fibers have similar estimated CVs. Variables involved in determining response latency are discussed.
(Supported by grants from the NSF and the UCLA Academic Senate)

399.3

β1 CLASS INTEGRIN IMMUNOREACTIVITY IS DEPRESSED IN THE RAT COCHLEA FOLLOWING NOISE INDUCED TTS. N.K. Woolf*, F.J. Koehrn, C. Jackson-Friedman, D.V. Jaquish, V.L. Woods, and A.F. Ryan. Depts. of Otolaryngology and Medicine, UCSD and the VA Medical Center, La Jolla, CA 92093.

Previously we demonstrated that fibronectin-like immunostaining in Deiter's cells is transiently depressed following noise induced temporary threshold shift (TTS). The current study investigated temporal changes in β1 class integrin immunolocalization in the cochlea during TTS and subsequent recovery. Under quiet control conditions, β1 class integrins are strongly expressed within the cochlea in stria vascularis and spiral ganglion cells. Following exposure to a two-octave band of noise (5656-22,624 Hz) at 115 dB (peSPL), a 30 dB TTS was induced, which resolved within 24 hours. No changes in the intensity of β1 class integrin immunostaining were observed in the inner ear at 1-24 hours post-exposure. At 7 days post-exposure, a significant decrease in β1 immunostaining was observed in spiral ganglion cells, but not in stria vascularis. These long term changes following TTS may be explained by either downregulation of β1 integrin expression or a conformational change in the protein preventing antibody binding.

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399.4

2f1-f2 *vs* 2f2-f1 DISTORTION-PRODUCT OTOACOUSTIC EMISSION GENERATORS. B.L. Lonsbury-Martin*, G.K. Martin, M.L. Whitehead and B.B. Stagner. Univ. of Miami Ear Inst., Miami, FL 33101.

Early results imply that: 2f1-f2 DPOAEs are produced in frequency regions between the primaries; 2f2-f1 DPOAEs are generated basal to the f2 place; and 2 sources of DPOAE generation exist [one source is relatively invulnerable to cochlear insult and dominates at high primary levels and small f2/f1 ratios, whereas the other operates at low stimulus levels (<65 dB SPL), dominates at large f2/f1 ratios, and is vulnerable to cochlear trauma]. Other data suggest that the high-level generator located basal to f2 at high stimulus levels is the source of 2f2-f1 DPOAEs. The present study examined these notions by measuring detailed suppression areas and the latency of both DPOAEs at 3 primary levels (45,60,75 dB SPL). Suppressor tones swept in 250-Hz steps from 0.25-17.25 kHz were varied in 1-dB steps over a 40-50 dB range, and onset latencies were measured directly from DPOAE-time waveforms. Suppression contours clearly indicated that 2f1-f2 DPOAEs were generated between the primaries, with tuning-curve tips being slightly below f2, whereas 2f2-f1 DPOAEs were tuned slightly above f2. Related onset latencies revealed slightly shorter latencies for 2f2-f1s than for 2f1-f2s, consistent with evidence indicating that the 2f2-f1 is generated basal to the 2f1-f2 place. 2f1-f2 DPOAEs were measured at all primary-tone levels, whereas 2f2-f1 DPOAEs were reliably elicited by 60- or 75-dB SPL primaries, consistent with the notion that 2f2-f1 DPOAEs are generated by the high-level distortion mechanism that operates basally to the generator of low-level, physiologically vulnerable 2f1-f2 DPOAEs. (Supported by the PHS: DC00613, DC01668, ES03500.)

399.5

EXPRESSION OF mRNA ENCODING RAT KIDNEY WATER CHANNEL IN RAT COCHLEA. S. Iwakura[1], K. Doi[1], K. Umemoto[1], M. Toyama[2], T. Kubo[1], K. Fushimi[3], S. Sasaki[3], F. Kimura*[4], 1. Dept. of Otolaryngology, Osaka Univ. Med. Sch.; 2. Dept. of Anatomy II, Osaka Univ. Med. Sch.; 3. 2nd Dept. of Internal Medicine, Tokyo Med. Dent. Univ.; 4. Dept. of Neurophysiology, Osaka Univ. Med. Sch., JAPAN.

The cloning of cDNAs encoding two water channels from rat kidney has been recently completed. One is CHIP28 which is responsible for the high water permeability in the proximal tubule and the descending thin limb of the loop of Henle. Another one is WCH-CD which is specifically expressed in the collecting duct where water reabsorption is regulated by the antidiuretic hormone arginine vasopressin (AVP). In the present study, the expression and cellular localization of water channel in rat cochlea was investigated, since the stria vascularis in the cochlea has been believed to play the critical role in regulation of production/reabsorption of endolymph in the inner ear.

Approximately 0.4 μg total RNA was extracted from each endolymphatic sac with GTC-CsTFA method. First strand cDNAs were synthesized from 3 μg total RNA with reverse transcriptase. Two degenerated oligonucleotide primers were designed from the sequence of water channel (WCH-CD) and the homologous cDNAs were amplified by using polymerase chain reaction (PCR). The amplified cDNAs were separated on 1% agarose gel and subcloned into pGEM7zf(+) vector for sequence analysis by using Sanger's dideoxy-termination method. Cryosections of dissected cochlea were immunostained with anti-WCH-CD/C, a polyclonal antibody against the peptide corresponding to the C-terminal amino acids of WCH-CD.

A cDNA band with the expected size of 369 base pairs was detected on agarose gel after PCR amplification. The sequence analysis identified the expression of mRNA encoding WCH-CD in rat cochlea. Immunofluorescence staining was detected in the marginal cells of the stria vascularis. The results strongly suggest that water channel (WCH-CD) is the molecule responsible for the AVP-mediated reabsorption of endolymph in rat cochlea.

399.6

INNER HAIR CELLS IN THE COCHLEA OF THE FETAL BRONX WALTZER MOUSE. D.S.Whitlon*, C.Gabel and X. Zhang. Waisman Center, University of Wisconsin, Madison, WI 53706.

In the adult Bronx waltzer cochlea, more than 75% of the normal number of inner hair cells is missing or deformed. The specific loss of inner hair cells makes the mutant a potentially valuable tool to study the effects of neuron-target interactions on development and neuronal plasticity. Theories differ on whether normal inner hair cells ever form in this mutant, but all observations have been based on postnatal animals. We directly analyzed cochleas from fetal Bronx waltzer mice (E16-E18), using light and electron microscopy. Almost the complete complement of inner hair cells exists in the E17 cochlea. The youngest inner hair cells have a normal, elongated shape; stereocilia are present. Beginning in the base and progressing toward the apex, the cells protrude through the reticular lamina and eventually become rounded. The cells begin to degenerate in the basal turn at about E17-E18, and the degeneration progresses toward the apex by P2. The adhesion molecules NCAM, on nerve fiber and hair cells, and L1, on nerve fibers, show a normal distribution. However, in contrast to normal inner hair cells, inner hair cells in the mutant accumulate the extracellular matrix protein tenascin. In summary: In the Bronx waltzer cochlea, inner hair cells are formed successfully, but shortly thereafter, they express abnormal amounts of at least one protein, tenascin, and most of them degenerate. The sequence of the degeneration suggests that it coincides with the timing of base-to-apex afferent innervation. (Supported by NIDCD grant #R29DC00653).

399.7

DOPAMINE RECEPTOR SUBTYPE MESSAGES IN THE MOUSE COCHLEA. D.G. Drescher[1,2*], J.M. Lasak[1], J. Chomchai[1], M.J. Drescher[1] and K.M. Khan[3]. Lab. Bio-otology, Depts. [1]Otolaryng. and [2]Biochem., Wayne State Univ. Sch. of Med., Detroit, MI 48201; and [3]Aga Khan Univ., Karachi, Pakistan.

Published evidence indicates that dopamine is present in the auditory inner ear. Presynaptic dense-core vesicles have been observed in olivocochlear fibers by electron microscopy, and dopamine has been detected at femtomole levels in the rat cochlea by HPLC. Immunocytochemical studies of olivocochlear fibers in the cochlea and neurons in the lateral superior olivary complex have suggested the presence of tyrosine hydroxylase and absence of dopamine ß-hydroxylase and phenylethanolamine N-methyltransferase.

We have utilized polymerase chain reaction (PCR) analysis to identify messages for dopamine receptor subtypes in the mouse cochlea. Specific D1A and D1B receptor primers were designed based on published rat brain sequences, and D2, D3, and D4 subtypes formulated from the corresponding mouse brain sequences. Poly A RNA was isolated from total RNA in guanidine thiocyanate extracts of cochleas of 30 CBA_J mice (16 days old) and reverse-transcribed with oligo dT primer. PCR-amplified products were separated by agarose gel electrophoresis. Results were compared to those obtained without reverse transcription (negative control) and to those using mouse brain RNA with reverse transcription (positive control).

PCR primers for all of the dopamine receptor subtypes yielded bands of expected molecular mass with cDNA from mouse brain samples, indicating that the primers were functioning. The primer designed to detect the D2 long form produced a PCR amplification product of the predicted size from mouse cochlear cDNA. D1B, D3, and D4 primers yielded faint bands after PCR amplification. The D1A amplification product was not observed. These results suggest that dopamine has a neurotransmitter role in the cochlea.

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399.8

THE ROLE OF MINERALOCORTICOIDS IN THE REGULATION OF COCHLEAR HOMEOSTASIS. H.H. Salem[1], W. S. Quirk[1], J. A. Kaltenbach[2*] and D.Z. Pitovski[1]. Dept. of Otolaryngology-Head and Neck Surgery[1] and Dept. of Audiology[2], Wayne State University, Detroit, MI 48201.

Although many studies have localized receptors postulated to control cochlear blood flow, there is still a dilemma about the exact mechanisms by which these receptors modulate inner ear function. A previous autoradiographic study conducted in our laboratory localized mineralocorticoid (type I) receptors in the walls of cochlear vasculature (Sinha et al., Soc. Neurosci. Abstr. 580.2: 1418, 1993). Furthermore, the possible physiological significance of these receptors in the regulation of cochlear blood flow (CoBF) was assessed by use of laser Doppler flowmetry (Sinha et al., Assoc. Res. Otolaryngol. Abstr. 13: 303, 1990). In both studies, we postulated that mineralocorticoids participate in regulation of CoBF.

In order to elucidate the mineralocorticoid participation in cochlear homeostasis, male Hartley guinea pigs were infused with aldosterone (10 $\mu g/kg$) for 10 min. Continuous measurements of lateral wall capillary blood flow velocity were assessed with intravital microscopy. Blood samples, withdrawn 2 and 60 mins following completion of infusion, were analyzed for serum aldosterone and electrolytes values. Preliminary results show that serum aldosterone levels fell significantly after 60 min post-infusion (731 ng/dl) compared to its 2 min post-infusion levels (5200 ng/dl). Systemic blood pressure, serum electrolyte values (Na^+, K^+ and Cl^-), and capillary blood velocity were unchanged during the experimental protocol. The stability of capillary velocity supports the presence of autoregulatory mechanism for CoBF which preserves constant capillary blood perfusion. Future studies, especially the functional effects of such steroids on arteries (and arterioles) supplying the cochlea, are needed to further delineate the association between mineralocorticoid hormones and cochlear vasculature.

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AUDITORY SYSTEMS: CENTRAL ANATOMY I

400.1

REEVALUATION OF FUNCTIONAL COMPONENTS OF THE CRANIAL NERVES. K.M. Khan*. Dept. of Anatomy, The Aga Khan University, Karachi 74800, Pakistan.

The four functional components of the cranial nerves are classified as general somatic and visceral afferents and efferents. Three additional components associated with special senses are also recognized to be present in certain cranial nerves. These fibers are classified as special visceral afferents (olfactory and gustatory), special visceral efferents (striated visceral muscles), and special somatic afferents (visual, auditory, and vestibular). The sensory cells of the vertebrate inner ear are innervated by both vesiculated and nonvesiculated endings: the vesiculated endings are regarded to be efferent in nature. Anatomical studies in all classes of vertebrates have invariably demonstrated that these efferent fibers originate from specific brain stem nuclei. The vesiculated endings form axosomatic synapses with the sensory cells as well as axodendritic synapses with the peripheral processes of the bipolar sensory neurons of the 8th cranial nerve. Physiological data indicate that efferent innervation of the sensory cells of the vertebrate inner ear is either facilitatory or inhibitory in function. Establishment of efferent innervation of the vertebrate inner ear merits a reevaluation of functional components of the cranial nerves. This report is suggesting that these efferent fibers be classified as "special somatic efferent" component of the cranial nerves.

400.2

COMPARISON OF AVIAN AND MAMMALIAN COCHLEAR EFFERENT NEURONS. R.A. Code*. Dept. of Zoology, Univ. of Maryland, College Park, MD 20742-4415.

Neurons of the mammalian olivocochlear (OC) system can be divided into two groups based on their location in the superior olivary complex and on their morphology. Neurons in the lateral OC group (LOC) are usually ovoid or fusiform in shape and project primarily to the ipsilateral cochlea, while those in the medial OC (MOC) group are typically stellate or radiate and project predominantly to the contralateral cochlea. The LOC and MOC can also be distinguished neurochemically: some cells of the LOC that are immunoreactive for choline acetyl transferase (ChAT), the biosynthetic enzyme for acetylcholine, are also immunoreactive for the neuropeptide, enkephalin (ENK) and calcitonin gene-related peptide (CGRP). Although cells in the MOC stain for ChAT, they do not appear to colocalize ENK or CGRP (see Eyalin, '93 for review). In order to determine whether the chick OC system can be divided into two neurochemically distinct groups similar to those in mammals, we studied cochlear efferent neurons in the brainstem of the chick (*Gallus domesticus*) using antisera to ChAT, to leu- and met-ENK, and to CGRP. Similar to mammals, ChAT-immunoreactive (ChAT-I) OC neurons in the chick brainstem are found in two groups. The morphologies of chick OC neurons, however, appear to be more varied than those of mammals and do not appear to be spatially segregated within these two groups as are those in the LOC/MOC. Some CGRP-I neurons in the chick are in locations similar to ChAT-I OC cells. Unlike mammals, chick OC neurons are not ENK-I; instead, they appear to receive ENK-I terminals. Thus, there are several striking differences in the organization of the avian and mammalian OC systems.

The ChAT antiserum was kindly provided by Dr. Miles Epstein, U. Wisconsin, Madison; the CGRP antibody was donated by Dr. Catia Sternini, UCLA. This work was supported by NIDCD grant DC01867 to RAC.

400.3

POSTNATAL IMMUNOREACTIVITY AND RETROGRADE LABELING OF OLIVOCOCHLEAR NEURONS IN THE BRAINSTEM. D.D. Simmons* J. Raji-Kubba, and J.H. Kim. Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1606.

The olivocochlear (OC) system is the major efferent pathway to the cochlea and is responsible for modulating activity of the cochlear nerve. Acetylcholine and calcitonin gene-related peptide (CGRP) both serve as neurochemical markers for the OC system. Very little is known about the maturation of this brainstem system. Standard immunohistochemical and an *in vitro* brainstem technique were used. OC neurons were defined by injections into the crossed OC bundle (COCB) at the floor of the IV ventricle.

During the first postnatal week, all COCB injections resulted in labeled terminals under IHCs and labeled cell bodies in the VPO region of the superior olive. Both ChAT and CGRP immunoreactive cell bodies were either non-existent or weakly labeled during within the VPO and LSO. During the second and third postnatal weeks, injections resulted in labeled terminals underneath IHCs and OHCs. The majority (>70%) of retrogradely labeled cell bodies were in the VPO region. Retrogradely labeled and ChAT positive cells had similar VPO distributions. A smaller (< 15%) population of LSO capsular cells also were retrogradely labeled and double labeled with ChAT antibody. The ChAT positive VPO cells were distinct from ChAT positive LSO cells. ChAT positive LSO cells had similar distributions to CGRP positive LSO cells. The majority of double labeled ChAT and CGRP cells were found either in the medial limb of the LSO or in the capsular regions of the LSO.

Thus, the majority of COCB cell bodies are ChAT positive, CGRP negative, and found within VPO regions. Our results are consistent with the hypothesis that periolivary OC neurons initially terminate on IHCs and then on OHCs before their neurotransmitter expression is mature.

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400.4

THE CYTOARCHITECTURAL ORGANIZATION OF THE MEDIAL OCTAVOLATERALIS NUCLEUS OF THE GOLDFISH. J.G. New[1], S. Coombs[1*], C.A. McCormick[2], and P. Oshel[1]. [1]Parmly Hearing Institute and Biology Department, Loyola University of Chicago, Il. 60626 and [2]Oberlin College, Oberlin, Oh. 44074.

The cytoarchitecture of the medial octavolateralis nucleus (MON), the principal first-order nucleus of lateral line mechanoreceptors, is not well known in any fish. Using Golgi, immunocytochemical and other histological techniques, we recognize at least three layers associated with the MON. The molecular layer (cerebellar crest) consists of axons from granule cells in the eminentia granularis and axons with GABAergic terminals from cells in the metencephalic nucleus praeeminentialis. Apical dendrites from underlying crest cells extend into the molecular layer, where small, intrinsic neurons are also found. The principal cell layer is composed of large multipolar (crest) cells known to project to the torus semicircularis. Some crest cells have a single, thick basilar dendrite that extends into deeper nuclear regions, whereas others have 2 or more thin basilar dendrites that ramify near the cell body. The deep layer consists of ventral dendrites of overlying crest cells, small intrinsic neurons, terminals from primary afferent fibers and commissural GABAergic neurons. The goldfish MON shares a number of organizational features with first-order nuclei of other octavolateralis systems and thus, its function may be shaped by similar neural circuits.

400.5

EVIDENCE FOR NEUROMODULATORS IN THE COCHLEAR NUCLEI OF THE NEWLY HATCHED CHICK. E.G. Dobbins* & G. Laurent, Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125.

Glutamate mediates rapid transmission of auditory information between the VIIIth cranial nerve afferents and the cochlear nuclei. The presence of glutamate (Raman & Trussell, Neuron, 9('92):173) and GABA (Code & Churchill, Hearing Res. 54('91): 281) receptors in chick, and of GABAergic terminals in owl (Carr, Fujita, & Konishi, J Comp. Neurol. 286('89):190) has been demonstrated. The potential contribution of neuromodulators to transmission across these primary synapses is unknown. The existence, and possible co-localization of neuromodulators with GABA and glutamate, in the avian cochlear nuclei has not been investigated. Immunohistochemistry was used to locate somata and terminals containing acetylcholine, catecholamines, and neuropeptides in the cochlear nuclei of the newly hatched chick. Preliminary results suggest that serotonin and acetylcholine are not present. Somatostatin is found in terminals in nucleus angularis (NA) but not magnocellularis (NM) or laminaris (NL), and in no somata. Substance P is found in terminals in NA and caudolateral NM, and in no somata. Enkephalin (5-L-methionine) is found in somata and terminals of NA. Cholecystokinin was found in somata: NM>NA>NL, and in terminals in NA. Supported by NIMH and the McKnight Foundation.

400.6

A GOLGI STUDY OF THE COCHLEAR NUCLEUS ANGULARIS IN THE BARN OWL J.R. Carpenter and C.E. Carr.* Dept. of Zoology, Univ. of Maryland, College Park, MD 20742-4415.

Sound localization in vertebrates is achieved largely by analysis of available binaural disparities. Previous work has demonstrated that two major localization cues are interaural time differences (ITDs) and interaural intensity differences (IIDs). Moreover, such functional specialization may be reflected in structural characteristics of individual auditory cell types.

The barn owl (Tyto alba) is a nocturnal predator that relies on the accurate processing of binaural information to locate prey. The incoming auditory nerve fibers bifurcate upon entering the brainstem, innervating the cochlear nucleus magnocellularis, the first step in a time-coding pathway, and the cochlear nucleus angularis (NA), which encodes sound-intensity information.

Rapid-Golgi impregnated material from existing collections was used for the present study. Selected sections of NA from 3-to-30-day posthatch owls were examined by light microscopy and camera-lucida drawings were made of various cells. Observations include:

1. NA resembles an inverted U-shape oriented dorsomedial to ventrolateral on the dorsolateral edge of the brainstem, with a hilus extending ventromedially.
2. NA contains at least nine specific cell types, as defined by morphology, location and relation to afferent fibers.
3. Several cell types bear considerable morphological similarity to cell types previously defined in the mammalian posteroventral and dorsal cochlear nuclei.
4. A small cell cap containing granule and fusiform-like cells is present on the dorsolateral aspect of NA throughout its rostrocaudal extent.

The results suggest structural parallels in the cellular organization of the avian and mammalian primary auditory nuclei, possibly due to common principles of information coding.

(Supported by NIH Grant DCD00436 to CEC)

400.7

Retrograde labeling of cochlear ganglion cells with injection of biotinylated dextran (BD) into granule-cell (GC) and small-cell-cap (SCC) regions of the cat anteroventral cochlear nucleus (AVCN). **D.O. Kim*, K. Parham, H. Zhao and S. Ghoshal. Div. Otolaryn., Surg. Res. Ctr., Ctr. Neurol. Sci., Univ. Conn. Health Ctr., Farmington, CT 06030-1110**

The SCC (also called juxtagranular) region of the cat AVCN receives preferential input from low spontaneous rate (SR) auditory nerve fibers (Liberman, 1991). Low-SR cochlear ganglion cells are preferentially located on the scala vestibuli (SV) side (Kawase and Liberman, 1992). From these two, we hypothesize that a focal injection of a tracer restricted in the SCC region should label ganglion cells preferentially located on the SV side. The goal of this study is to evaluate this hypothesis. After a 3-10 day survival period following BD injection, the cats were perfused. The label yielded Golgi-like filling of ganglion cell somata and their axons. In cases where the injection was centered in the deep AVCN or deep DCN, the labeled cells tended to be evenly distributed on the SV and scala tympani (ST) sides in general. In cases where the injection target was the GC/SCC region, by contrast, labeled cells in certain ganglion regions were preferentially on the SV side. This supports the hypothesis. A complicating factor was that there were additional ganglion regions in the latter cases with labeled cells either on the ST side or evenly distributed. Also in these cases, label was found in type II ganglion cells and olivocochlear bundle (OCB) fibers in the intraganglionic spiral bundle. This confirms previous findings that the GC/SCC region receives input from type II cells and OCB collaterals. [Supported by NIDCD-NIH]

400.8

REDUCED AUDITORY FUNCTIONAL ACTIVITY REVERSES SPONGIFORM DEGENERATION OF THE GERBIL COCHLEAR NUCLEUS. B.T. Faddis* and M.D. McGinn. Dept. Otolaryngology, Univ. of Calif. Sch. of Med., Davis, CA 95616

Spongiform degeneration of the gerbil cochlear nucleus has been shown to be dependent on auditory functional activity. The degenerative lesions primarily affect dendrites while sparing the neuronal cell body.

Ligation of the external auditory canal (EAC) in Mongolian gerbils for one week resulted in >30 dB elevation in ABR thresholds at frequencies from 1-32 kHz. This manipulation reduced the area density of a naturally occurring spongiform degeneration in the ipsilateral cochlear nucleus to 30% of that found on the contralateral (unligated) side.

Ultrastructural studies showed that some lesions "fill in" with a tubulovesicular material. Aggregations of mitochondria and filaments are also found. The tubulovesicular material is immunoreactive with antibodies to glucose regulated protein-78 (grp78), an ER-resident protein involved in the proper assembly and folding of newly synthesized proteins.

400.9

SYNAPTOPHYSIN IMMUNOREACTIVITY IN THE COCHLEAR NUCLEUS AFTER COCHLEAR ABLATION. C.G. Benson*, J. S. Gross, S. K. Suneja and S. J. Potashner. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06030.

A monoclonal antibody to synaptophysin, an integral membrane protein found in presynaptic vesicles, was used to immunohistochemically quantify changes in nerve terminal density in the guinea pig cochlear nucleus (CN) 2-161 days after complete unilateral cochlear ablation. In the ipsilateral ventral CN a significant reduction in the density of immunoreactive synaptic profiles was apparent 4-7 days after ablation, presumably corresponding to the loss of cochlear afferents. In the anterior division of AVCN immunoreactive density decreased to 35% of that contralaterally, and in the anterior part of PVCN to 62%. No reduction in immunoreactive density was seen in the deep layer of DCN. After 161 days there was an apparent complete restoration of immunoreactive density in the ventral CN. In PVCN this increased density could be attributed to progressive tissue shrinkage, presumably resulting from loss of cochlear nerve fibers, but in the AVCN tissue shrinkage only partially accounted for the increased density. These results suggest that the initial loss of synaptic terminal density in the AVCN after cochlear ablation may be followed by a partial resurgence at longer survival times. (Supported by DC00199 from NIDCD).

400.10

COCHLEAR ABLATION: LONG-TERM EFFECTS ON UPTAKE AND RELEASE OF D-ASPARTATE, GLYCINE AND GABA IN BRAIN STEM AUDITORY NUCLEI. S.K. Suneja, C.G. Benson and S.J. Potashner* Department of Anatomy, University of Connecticut Health Center, Farmington, CT, 06030.

To determine if unilateral cochlear ablation leads to plastic changes in the auditory nuclei, the freshly dissected guinea pig brain stem was cut transversely into 500 μm slices and samples of the LSO, MSO, MNTB, VNLL, and ICc were micropunched to measure uptake and release activities. Five days after cochlear ablation, ^{3}H-D-aspartate uptake and release were decreased slightly in the LSO, MSO and VNLL bilaterally, but these activities returned toward control levels at 59 and 145 days. ^{3}H-D-Aspartate uptake and release were increased slightly at 59 and 145 days in the MNTB and ICc bilaterally. ^{14}C-Glycine or ^{14}C-GABA release, unchanged after 5 days except for a bilateral increase in ^{14}C-GABA release in the ICc, increased slightly at 59 and 145 days in the MSO, MNTB, and ICc bilaterally. ^{14}C-Glycine uptake decreased after 5 days, but increased at 59 and 145 days in the LSO and MSO bilaterally. Although modest, these changes suggest that unilateral cochlear ablation may lead to plastic compensatory responses in auditory brain stem nuclei.

(This work was supported by grant DC00199 from NIDCD)

400.11

EXPRESSION OF C-FOS IN THE AUDITORY BRAIN STEM FOLLOWING ELECTRICAL STIMULATION OF THE COCHLEA. S. Nagase, H.H. Lim, J.M. Miller* and R.A. Altschuler. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109-0506.

C-Fos expression (as a marker for activation of neurons) was examined in the brain stem of deafened and undeafened rats after electrical stimulation with cochlear implants. Rats were either normal hearing or deafened by inner ear injection of neomycin. After assessment of auditory brain stem response (ABR), cochleae were implanted with electrodes. Either a single ball electrode was inserted in scala tympani of the basal turn of the cochlea or bipolar electrodes were inserted into basal and apical turns. Three days later electrically evoked ABRs were taken; after five days animals were electrically stimulated at three times the EABR threshold for 90 minutes. Thirty minutes after the cessation of stimulation animals were anesthetized and perfused with 4% paraformaldehyde. Vibratome sections were cut through the auditory brain stem and sections were immunoreacted with antiserum to C-FOS (Oncogene Sciences) using ABC Vector Stain immunoperoxidase methods.

In both hearing and deafened animals, many C-Fos immunoreactive (IR) neurons were observed ipsi and contralateral to the stimulated side in the dorsal cochlear nucleus, nuclei of the lateral lemniscus and the inferior colliculus. The C-Fos IR cells were arranged in band-like patterns in the IC and the contralateral DCN. The number of IR neurons increased with bipolar stimulation. C-Fos IR neurons were occasionally seen in the superior olivary complex in periolivary nuclei and the lateral superior olive. One deafened animal demonstrated many C-Fos IR cells in the ventral cochlear nucleus but this was atypical. These results suggest that C-Fos upregulation may provide a method for assessing central auditory activation with cochlear implants and may be useful for comparing different stimulation paradigms as well as assessing their influence on deafness-related changes.

400.12

CHARACTERIZATION OF MOSSY FIBER PROJECTIONS FROM THE CUNEATE NUCLEUS TO THE COCHLEAR NUCLEUS. D. D. Wright*, T. Pongstaporn, and D. K. Ryugo. Center for Hearing Sciences, Depts. of Neuroscience and Otolaryngology, Johns Hopkins University Sch. of Med., Baltimore, MD 21205

The cochlear nucleus (CN) is a brainstem auditory nucleus which receives direct input from the auditory nerve. Some non-auditory inputs to the CN have also been described, including a somatosensory projection from the cuneate nucleus (Weinberg and Rustioni, 1987, *Neurosci.*, 20:209-219). We have recently shown that the cuneate projections form mossy fiber terminals in the granule cell domains of the dorsal CN (Wright and Ryugo ARO Abst., 1994). Further studies using the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) combined with immunofluorescence have demonstrated that cuneate projections give rise to mossy fiber terminals in granule cell domains throughout the CN. The trajectory of the cuneate fibers is demonstrated in coronal sections of the brainstem where fibers are seen traversing the descending tract of V before entering the dorsal acoustic stria. Labeled fibers course along the stria for varying distances before entering the nucleus at different dorso-ventral levels, where they give rise to many *en passant* and terminal boutons in granule cell regions.

Cuneate mossy fiber terminals contain round synaptic vesicles and form asymmetric synapses with their targets, indicating that they are excitatory in nature. Using immunocytochemical techniques, we have begun to investigate which neurotransmitter is used by the cuneate mossy fibers. Preliminary experiments indicate that anterogradely labeled cuneate fibers are not double labeled with an antibody against choline acetyltransferase (Boehringer Mannheim), an enzyme used in the synthesis of acetylcholine. However, in adjacent sections, PHA-L labeled cuneate fibers coincide with glutamate immunoreactive fibers. We will test the hypothesis that the cuneate mossy fibers are glutamatergic using double labeling techniques for PHA-L and glutamate at light and electron microscopic levels.

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400.13

MUSCARINIC ACETYLCHOLINE RECEPTORS IN RAT COCHLEAR NUCLEUS: AUTORADIOGRAPHIC DISTRIBUTION OF [^{3}H]SCOPOLAMINE BINDING. W. Yao* and D.A. Godfrey. Dept. of Otolaryng., Med. Coll. of Ohio, Toledo, OH 43699.

There is evidence that cholinergic effects in the cochlear nucleus (CN) may be mediated primarily by muscarinic acetylcholine receptors (mAChR). We used [^{3}H] scopolamine to study mAChR distributions in the rat CN by receptor binding autoradiography. Rat brain sections 15 μm thick were incubated with 10 nM [^{3}H]scopolamine at 20 °C for 30 min, followed by two 5-min rinses with cold PBS-EDTA and a brief cold H_2O rinse. The sections were exposed to ^{3}H sensitive film for 2 weeks, followed by exposure to NTB2 emulsion for 2 weeks. The scopolamine binding densities in various CN subregions were estimated by measurements of optical density (using NIH Image 1.44, courtesy of Dr. E. Tietz). The specificity of scopolamine binding was confirmed by blocking with 1 μM atropine. To determine which mAChR subtypes participate in the binding, we included unlabeled subtype-preferential ligands in some preparations to block [^{3}H]scopolamine binding. Overall CN binding was measured on isolated CN portions of sections incubated with [^{3}H]scopolamine with and without unlabeled ligands, followed by scintillation counting. Our preliminary results suggest that scopolamine binding is highest in granular regions, followed in order by dorsal CN (DCN) deep and fusiform soma layers, DCN molecular layer, posteroventral CN, anteroventral CN, and auditory nerve root (which was similar to background). Ligands which preferentially block some mAChR subtypes competed more effectively than others with scopolamine binding. (Supported by NIH grant DC 00172)

400.14

MORPHOLOGICAL AND MORPHOFUNCTIONAL CHANGES IN THE ADULT GUINEA PIG AND RAT BRAINSTEM AUDITORY NUCLEI AFTER DESTRUCTION OF THE INNER EAR. J. Dupont, J.M. Bonneau, K. Sato, S. Bledsoe*, J.M Aran and R. Altschuler. Kresge Hearing Research Institute, Ann Arbor, MI 48109.

In this study, changes in cell size, GABA and glycine immunoreactivities, glycine receptor subunit expression and neurotransmitter release have been analysed in auditory brainstem nuclei after unilateral and/or bilateral deafferentation. Evaluations of these changes were performed by morphometrical and image analysis of the cells, of GABA and glycine immunocytochemistry, by in situ hybridization of glycine receptor subunits and the implantation of microdialysis probes in brainstem auditory nuclei. Most of the neurons within the lateral superior olive (LSO) are able to shrink and then recover to their normal size after uni- or bilateral destruction. This morphological plasticity parallels the changes in the density of GABA+ cell bodies in the LSO and in the central nucleus of the inferior colliculus. The fact that this plasticity of the auditory neurons is also observed in bilateral deafferentation suggests that the central auditory system (CAS) of adult mammals has the property to recover even after total auditory deprivation. We also observe an ipsilateral decrease of glycine positive terminals in the medial nucleus of the trapezoid body (MNTB) and the dorso medial peri-olivary nucleus with a simultaneous bilateral decrease following unilateral deafferentation. Modifications of glycine immunoreactivity in LSO and MNTB appear to parallel some interesting changes in the expression of the different glycine receptor subunits. We are trying now to correlate these morphofunctional changes of the neurotransmitters with our results of microdialysis in the same experimental model. Even if the mechanisms behind these changes are still unknown, our results indicate that the CAS of mature mammals has a remarkable property of plasticity in response to partial or total auditory deprivation. *Supported by MRT Grant # 88C0558 and NIDCD Grant # DC 00383*

400.15

LOCALIZATION OF GLYCINE RECEPTOR IN THE RAT COCHLEAR NUCLEUS AND SUPERIOR OLIVARY COMPLEX. K. Sato, H. Kuriyama, J. Dupont, J. Bonneau and R.A. Altschuler.* Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, MI 48109-0506.

Glycine is a major inhibitory transmitter that is especially prominent in the cochlear nucleus and superior olivary complex. The glycine receptor is composed of α subunits, a β subunit and a 93 K anchoring protein (AP), also termed gephyrin. Different subunits can combine to form complexes with different physiological properties. We used radioactive and non-radioactive *in situ* hybridization methods to localize neurons expressing glycine receptor subunits in the cochlear nucleus and superior olivary complex. This expression was then compared to immunolocalization of the 93 K AP. In the lateral superior olive (LSO) and medial nucleus of the trapezoid body (MNTB) the amount of expression of different subunits was compared to number of glycine immunoreactive terminals received as well as the amount of 93 K AP. Expression of β, α1 and α3 subunits were seen over principal cells of all major CN and SOC nuclei, while the expression of the α2 subunit was considerably lower. All cell types that showed expression of these subunits also showed immunoreactive labeling for the 93 K AP. While there was good correlation between the number of 93K AP immunoreactivity and the amount of glycine immunoreactive innervation a cell received, there was not always a correlation between the number of silver grains over cells expressing receptor subunits and the glycinergic innervation. LSO principal neurons receive many more glycinergic terminals than MNTB principal neurons. There was a comparable increase in expression of the α3 subunit but not of the β subunit. We are continuing this quantitative assessment and comparison.

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400.16

SYNAPTIC ORGANIZATION WITHIN THE MEDIAL SUPERIOR OLIVE OF UNILATERALLY DEAFENED GERBILS. F. A. Russell and D.R. Moore*. University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Neurons of the medial superior olive (MSO) are thought to contribute to sound localization by signalling the temporal correlation between excitatory input from the left and right anteroventral cochlear nuclei (AVCN). Following unilateral cochlear ablation in infancy (P0-P5), neurons in the ipsilateral AVCN die and axons from AVCN neurons on the unoperated side innervate the MSO in regions vacated by terminals from the operated side. Cochlear ablation after P10 does not produce these effects. We have used electron microscopy to examine sagitally sectioned tissue from the ipsilateral MSO of normal, P5 and P18 ablated gerbils. Synapses were classified on the basis of synaptic morphology as round (R), pleomorphic (P) or flat (F). Samples were collected at 15μm intervals from 60μm either side of the nucleus center. All types of synapses were found on both sides of the MSO in all animals. R synapses were found throughout the medio-lateral extent of the nucleus. P and F synapses were more abundant around the soma. Ablation at P5 did not reduce the density of R terminals on the operated side of the nucleus, but vesicle numbers per synapse were reduced. Ablation at P18 also resulted in reduced vesicle numbers and in a reduction in the number of dendrite profiles and synaptic active sites on the ablated side of the nucleus.

400.17

LARGE SYNAPTIC TERMINALS IN THE LATERAL NUCLEUS OF THE TRAPEZOID BODY OF THE CAT ARE PEP-19 IMMUNOREACTIVE. G.A. Spirou* and A.S. Berrebi. Dept. of Otolaryngology, West Virginia Univ. Sch. of Med. Morgantown, WV 26506-9200.

The lateral nucleus of the trapezoid body (LNTB) is a prominent periolivary cell group that contributes a large feedback projection to the cochlear nucleus. This cell group is known to receive ascending activation via collaterals of globular bushy cell axons, although existing descriptions of these terminals are sketchy. We have determined that PEP-19 antiserum labels substantial numbers of fibers and terminals in the LNTB, including globular bushy cell axons (Berrebi and Spirou, this volume). We studied the immunolabeled terminals using both light (LM) and electron microscopy (EM) to gain a clearer picture of globular bushy cell inputs to the LNTB.

Based on LM terminal labeling patterns, two zones within the LNTB can be identified. The first zone, located medially and ventrally and concentrated caudally, contains large PEP-19-IR puncta and fibers. The puncta in many cases form rings which define the perimeters of immunonegative cell bodies, and are typically on the order of 6-8μm^2. The second and larger zone is distinguished by having the highest density of immunolabeled puncta and fibers. This zone contains some large punctate profiles, although on average puncta are smaller than in the first zone and do not form pericellular rings. Pre-embedding EM immunocytochemistry reveals PEP-19-IR presynaptic boutons apposed to unlabeled cell bodies and dendrites. Their internal morphology is consistent with their being excitatory terminals.

These results support the notion that LNTB neurons are rapidly and securely activated by bushy cells, and may exert rapid feedback effects on cochlear nucleus neurons. Supported by NIH grant DC01387

400.18

PEP-19 IMMUNOLABELING OF VCoN BUSHY CELLS AND THEIR TERMINALS IN THE SUPERIOR OLIVARY COMPLEX OF THE CAT. A. S. Berrebi* and G. A. Spirou., Dept. of Otolaryngology, West Virginia University School of Medicine, Morgantown, WV 26506-9200.

Bushy cells are specialized to preserve temporal features of auditory nerve spike patterns, and are considered to play important roles in sound localization. In general, studies of bushy cell connections within the superior olivary complex (SOC) have employed techniques that label only sub-populations of neurons. Such questions as the distribution and diversity of terminals on particular SOC cell types can only be effectively addressed by bulk labeling of all bushy cells and their axonal processes.

We demonstrate here that antiserum to PEP-19 (generously provided by Dr. J. I. Morgan), a putative calcium-binding polypeptide, is an excellent marker for the entire population of VCoN bushy cells in cats. Spherical cell axons located dorsally and thicker globular cell fibers located ventrally in the trapezoid body are well labeled. Large calyceal endings in the MNTB are distinctly immunoreactive as are small punctate profiles that outline neuronal cell bodies and dendrites in the MSO and mainly dendrites in the LSO. In the LNTB, differences in the pattern of punctate immunolabeling provides the basis for parcelling the subregions of the nucleus (Spirou and Berrebi, this volume).

Within the entire SOC, only the posterior and dorsolateral periolivary nuclei contain significant numbers of PEP-19 immunoreactive neurons, but these regions are not thought to provide significant input to principal SOC nuclei. Thus, although expression of PEP-19 is not exclusive to bushy cells, antiserum to this protein should prove useful in quantitative light and electron microscopic investigations of bushy cell synapses.

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400.19

ORIGINS AND TARGETS OF THE COMMISSURAL CONNECTIONS BETWEEN THE COCHLEAR NUCLEI IN GUINEA PIGS.
B.R. Schofield*, Dept. Neurobiology, Duke Univ., Durham, NC 27710.

Projections from the ventral cochlear nucleus (CN) to the opposite CN and to the inferior colliculi (IC) arise primarily from multipolar cells. In our first experiment, we injected different fluorescent tracers into one CN and into each IC to determine whether individual cells project to more than one of these targets. Each of the 3 tracers labeled many cells in the uninjected CN; however, no individual cell in the ventral CN contained more than one tracer. Thus, commissural projections and collicular projections arise from different populations of cells.

In a second experiment, we injected PHAL into one CN and examined the labeled axons in the other CN. In the ventral CN, labeled boutons appeared to contact primarily multipolar cell somas but were also seen apposed to spherical and globular cell somas. Many labeled terminals were also found in the granule cell regions of the CN and in the deep layer and fusiform layer of the dorsal CN.

The commissural projections arise from cells that do not project to the IC. It appears that CN cells that *do* project directly to the IC, including a subset of multipolar cells in the ventral CN as well as cells in the dorsal CN, are themselves targets of the commissural projections. Other cell types, namely globular and spherical cells, are also targets of commissural projections. These cell types project to the IC indirectly, via relays in the superior olivary complex. The commissural projections thus provide an opportunity for binaural interactions at early stages in a variety of ascending auditory pathways.

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400.20

VENTRAL NUCLEUS OF THE TRAPEZOID BODY: DENDRITIC MORPHOLOGY BY INTRACELLULAR LABELING. N. Kuwabara.* *Dept. of Biol. Sci. and OUCOM, Ohio University, Athens, OH 45701.*

The ventral nucleus of the trapezoid body (VNTB) is a periolivary cell group at the floor of the mammalian superior olivary complex (SOC). This nucleus is strategically positioned in the trapezoid body just below the medial nucleus of the trapezoid body (MNTB) and the medial superior olive (MSO). The VNTB contains a mixed population of elongate and multipolar cells and is known to receive extensive ascending projections from the ventral cochlear nucleus (VCN; Warr, '72). Some cells of the VNTB are thought to contribute to the medial olivocochlear bundle system (Warr, '75). The VNTB also has projections to the inferior colliculus ipsilaterally (Nordeen, '83) and the cochlear nucleus bilaterally (Brown et al., '88; Winter et al., '89). However, little is known about the organization of the VNTB, its relation to the rest of the SOC, or the relationship between its afferent and efferent connections. We have begun our studies of the VNTB by examining its dendritic morphology as revealed by intracellular labeling in a gerbil brainstem tissue slice preparation.

Intracellular labeling with Neurobiotin suggests that the main dendrites of most elongate VNTB cells are oriented dorsoventrally. Dorsally oriented dendrites often invaded and arborized within the MNTB. Some small dendritic branches were traced dorsolaterally into the columnar region of the MSO. Ventral dendrites often extended across the entire width of the ventral trapezoid body. Previously, we have observed collateral axons from MNTB principal cells projecting to the VNTB (Kuwabara et al., '89). The dendritic pattern of the VNTB suggests that this nucleus may sample ascending information in the neural plexuses of both the MNTB and the MSO as well as direct projections from the VCN. (Supported by DC01303, DC00038 and OUCOM)

AUDITORY SYSTEMS: CENTRAL ANATOMY II

401.1

SUPERIOR PARAOLIVARY NUCLEUS (SPON): CONNECTIVITY REVEALED BY TRANSPORT OF BIOTINYLATED DEXTRAN. E. Saldaña* D. R. Lough and A. S. Berrebi. Univ. of Salamanca Med. Sch., 37007-Spain, and Dept. of Otolaryngology, West Virginia Univ., Morgantown, WV 26506.

The SPON is a prominent periolivary nucleus in rodents. In order to clarify its synaptic targets and sources of inputs, we injected biotinylated dextran (BD) into the inferior colliculus (IC) or SPON of rats. This tracer provides both retrograde and anterograde labeling of cell bodies and processes.

The projection from SPON to IC is extensive, primarily ipsilateral, and originates from large multipolar cells with flattened, parasagittally oriented dendritic trees. Medially situated SPON neurons project to ventromedial (high frequency) regions of the IC, and those situated laterally project to dorsolateral (low frequency) regions. Thus, the SPON projection to the IC appears to be tonotopically organized.

After injections restricted to the SPON, an extremely dense fiber plexus, but very few cell bodies, are labeled in the IC. Numerous retrogradely labeled neurons are found in the ipsilateral medial nucleus of the trapezoid body (MNTB) and contralateral posteroventral cochlear nucleus (PVCoN). The predominant neuronal type labeled in the PVCoN corresponds to octopus cells, although occasional multipolar and scattered small stellate and bushy cells are also found.

These data suggest that SPON activity is modulated by the balance of its excitatory inputs arising from octopus cells and glycinergic synapses originating in the MNTB. Since the SPON contains primarily inhibitory neurons, it appears that complex inhibitory/disinhibitory phenomena at the level of the IC may represent an important aspect of its functional role.

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401.2

EFFECTS OF STIMULUS INTENSITY ON c-*fos* mRNA EXPRESSION IN THE AUDITORY BRAINSTEM OF THE RAT. R. L. Saint Marie[1]*, L. Luo[1], and A. F. Ryan[2]. [1]Neuroanatomy Dept., House Ear Institute, Los Angeles, CA 90057 and [2]Div. Otolaryngology - Head and Neck Surgery, Univ. California, San Diego, CA 92093.

Expression of the c-Fos protein has been shown to be up regulated in some auditory brainstem neurons in response to tonal stimulation. In the present study we examined the expression of c-*fos* mRNA following 1 hr of free-field stimulation with a low-frequency, narrow-band noise (1.41-5.65 kHz) at intensities ranging from 80-120 dB SPL. At 80 dB elevated expression was found in subsets of neurons in the low frequency areas of the dorsal (DCN), posteroventral, and anteroventral cochlear nuclei, the medial nucleus of the trapezoid body (MNTB), the ventral and dorsal nuclei of the lateral lemniscus, and the inferior colliculus (IC). The areas containing labeled neurons in these structures increased in size with 90 and 100 dB stimulation, but the signal began to diminish at 110 dB. In some auditory nuclei, label in low-frequency areas was nearly absent at 120 dB, which may reflect damage to the low-frequency part of the cochlea. At 90 dB additional neuronal labeling was found in the highest frequency regions of some of these structures, particularly the DCN, MNTB, and IC. These areas of label also increased in size and intensity with increasing stimulus intensity, which may reflect the greater sensitivity of the high-frequency part of the cochlea to high intensity sounds of all frequencies. Alternatively, some central auditory neurons, i.e., those in high frequency regions of DCN, MNTB, and IC, may have more sensitive stimulus/transcription coupling mechanisms than others. At the highest stimulus intensities the middle frequency regions of the auditory nuclei became heavily labeled, presumably as the tail of the tuning sensitivity for these neurons was activated. C-*fos* mRNA expression appears to be related to a biochemical response driven by, but not necessarily synonomous with, neuronal activity. Its expression may be useful as an indicator of neuronal activity in many, though perhaps not all, neurons of the auditory brainstem.

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401.3

EXPRESSION OF c-*fos* mRNA IN THE RAT AUDITORY BRAINSTEM FOLLOWING UNILATERAL OR BILATERAL COCHLEAR ABLATION.

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Transcription of the c-*fos* gene was examined from 30 min to 60 days after surgical ablation of one or both cochleae in adult rats using *in situ* mRNA hybridization. After unilateral ablations, basal expression in the ipsilateral ventral cochlear nucleus (VCN) and contralateral inferior colliculus (IC) began to decline 1-2 hrs post injury, was maximally depressed by 2-4 hrs, and remained suppressed at least 60 days post injury. In the cochlear nucleus contralateral to the ablation there was a rapid (30 min - 1 hr post injury) increase in neuronal expression in the deep layers of the dorsal cochlear nucleus (DCN) and some areas of VCN which remained elevated at least 60 days post injury. This suggests a release from inhibition normally driven by the contralateral ear. An intense glial expression in the acoustic and vestibular nerves of the ablated side, reflecting degeneration of the afferent axons, was first evident at 30 min and gradually involved the nerve roots, octopus cell area, and spinal tract of the trigeminal. Elsewhere there were delayed increases in neuronal expression bilaterally in periolivary nuclei, dorsal nuclei of the lateral lemniscus, paralemnisci, and commissural nuclei of the IC and in the ipsilateral ventral and intermediate nuclei of the lateral lemniscus and central nucleus of the IC. Onsets ranged from 2 hrs to 2 wks and persisted for at least 60 days after ablation. The delayed onset of these responses suggests transynaptic effects and possibly reorganization of the central pathways in response to deafferentation in the adult. No neuronal expression was evident in any auditory nuclei 4 hrs or more after bilateral ablations, suggesting that all observed expression was acoustically driven. A single exception was in the superficial layers of the DCN, where there was an initial rapid increase in expression in medium-sized neurons that reached a maximum at 2 hrs and disappeared by 1 day. This may reflect a release from acoustically driven inhibition of high spontaneous rate neurons with subsequent adaptation or delayed depression of higher order excitatory input(s).

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401.4

C-FOS EXPRESSION ELICITED BY SOUND STIMULATION IN THE AUDITORY NEURONS OF THE BIG BROWN BAT, *EPTESICUS FUSCUS*

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Because proto-oncogene c-fos can be expressed in neurons following sensory stimulation, c-fos immunocytochemistry has been used as a rapid and sensitive marking technique to identify stimulus activated neurons. This technique has the advantage of examining c-fos expressed neurons in several brain nuclei thus providing an overall profile of the stimulus-related activity within a specific sensory system. In this study, we examined sound elicited c-fos expressed neurons in the auditory cortex, the cerebellum and subcortical nuclei of the big brown bat, *Eptesicus fuscus* under natural and monaurally plugged conditions. When stimulated with 30 and 50 kHz sounds at 79 dB SPL under natural condition, c-fos sensitive neurons were symmetrically and bilaterally observed in the ventral and dorsal cochlear nuclei (VCN, DCN), the superior olivary complex (SOC), the nucleus of lateral lemniscus (NLL), the nucleus of trapezoidal body (NTB), the inferior and superior colliculi (IC, SC), the pontine nuclei (PN), the medial geniculate body (MGB), the auditory cortex, the cerebellar fastigial and dental nuclei. However, c-fos sensitive neurons were greatly reduced and were observed in a more restricted area of all auditory nuclei when stimulated with a 20 dB SPL sound. When stimulated under monaurally plugged condition, c-fos sensitive neurons were predominantly observed in the contralateral DCN, VCN, SOC, NLL, NTB and the ipsilateral IC, SC, MGB, PN and the auditory cortex. These observations are consistent with the known neural circuits of the mammalian ascending auditory pathway. In the cerebellum, however, c-fos sensitive neurons were symmetrically observed in the rostral portion of both deep nuclei. but were predominantly observed in the caudal portion of the ipsilateral deep nuclei. (work supported by HFSP and NIH DC 247)

401.5

STRUCTURE AND INNERVATION OF NEURONS IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS THAT PROJECT TO THE INFERIOR COLLICULUS.

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A multiplicity of pathways transmit auditory information from the cochlear nucleus to the inferior colliculus. While some of these are binaural, others may convey monaural information. One potentially monaural pathway leads to the inferior colliculus via the ventral nucleus of the lateral lemniscus (VNLL). The VNLL is a major source of input to the inferior colliculus, yet neither its inputs nor the morphology of its neurons have been extensively studied.

Ascending input to the VNLL was examined by injecting biotinylated dextran in the cochlear nucleus of rats. Dextran-labeled axons that ran in the lateral margin of the lateral lemniscus periodically gave off dense arborizations that entered the VNLL. These horizontal arbors were roughly perpendicular to the lemniscus, and resembled the rungs of a ladder.

In a second experiment, neurons in the VNLL that project to the inferior colliculus were labeled with retrogradely transported fluorescent microspheres, and then intracellularly injected with a mixture of Lucifer Yellow and biotinylated Lucifer Yellow in slices of fixed tissue. The somata of stained neurons were spherical or elongate in shape. Dendrites were typically filled up to 50-150 µm from the soma but rarely branched more than three times. Some neurons had fusiform dendritic fields oriented perpendicular to the lemniscus. Others had multipolar dendritic fields that were less oriented, but which were more parallel to the lemniscus.

Neurons with their dendrites perpendicular to the lemniscus would likely receive input from one arbor, or "rung" of fibers, from the cochlear nucleus, whereas those with dendrites parallel to the lemniscus may integrate inputs over a larger region. The relationships of dendritic orientation in the VNLL to the input from the cochlear nucleus suggests that the VNLL may play more than one role in auditory processing.

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401.6

ORIGIN OF ASCENDING PROJECTIONS TO THE NUCLEI OF THE LATERAL LEMNISCUS IN THE BIG BROWN BAT, *EPTESICUS FUSCUS*.

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The nuclei of the lateral lemniscus (NLL) in the echolocating bat, *Eptesicus fuscus*, are unusually large and highly differentiated. Electrophysiological studies demonstrate marked differences in the response properties of neurons in the different divisions of NLL; thus, each nucleus presumably sends a unique transformation of auditory input to the inferior colliculus. To determine whether the dissimilar response patterns of the NLL divisions are due in part to differential patterns of ascending input, we placed small deposits of HRP or WGA-HRP in the NLL of 18 bats.

The retrograde transport from these injections shows that, as in other mammals, the dorsal NLL (DNLL) of *Eptesicus* receives the bulk of its input from binaural structures. These include the lateral and medial superior olives (LSO and MSO) and contralateral DNLL. Both LSO and MSO project bilaterally; the bilateral MSO projection is unusual in that it is found in only a few mammalian species. DNLL also receives a minor projection from the anteroventral cochlear nucleus (AVCN).

The projections to the intermediate NLL (INLL) and the two divisions of the ventral NLL (VNLL) are almost exclusively monaural. Major sources of input are the AVCN, posteroventral cochlear nucleus (PVCN) and the medial nucleus of the trapezoid body (MNTB); lesser inputs originate in the lateral nucleus of the trapezoid body (LNTB) and ventral periolivary area (VPO). Although INLL and the two divisions of VNLL all receive input from AVCN, PVCN, MNTB, LNTB and VPO, they differ in the relative proportions of connectivity with these brain stem nuclei. For example, in terms of the numbers of cells labeled, the pattern of labeling for VNLL (AVCN>>PVCN>MNTB) is quite different from that for INLL (MNTB>>PVCN>AVCN). These data suggest that the diverse neural response properties in INLL and VNLL are not the result of projections from disparate sets of brain stem nuclei, but rather that they depend upon differing magnitudes of the constituent projections. [Supported by NIH grant DC00607 and NRSA DC00094]

401.7

MORPHOLOGY OF COMMISSURAL AXONS IN THE INFERIOR COLLICULUS OF THE GERBIL.

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We made small injections of biocytin into the central nucleus of the inferior colliculus and reconstructed the axons and terminals which arise from this area and project into the contralateral colliculus. All subdivisions of the inferior colliculus receive commissural connections; the patterns of branching are different in each one. Most commissural axons entering the central nucleus form planar terminal fields extending from the rostral to caudal and from the dorsomedial to ventrolateral aspects of the central nucleus; they appear to be parallel to the fibrodendritic laminae that characterize the central nucleus. Less often, labelled axons enter the central nucleus from its laterodorsal aspect almost at right angles to the fibrodendritic laminae and travel in the ventromedial direction, giving rise to simple branches.

The internal part of the external nucleus is innervated by axons which enter it by passing through the central nucleus, where some of them give rise to branches. The terminal field of each axon is confined to a limited strip along the dorsal to ventral axis of the external nucleus.The superficial part of the external nucleus receives the sparsest intercollicular connections. Most of the terminals there arise from branches of axons passing into the brachium. In the dorsal cortex, terminals of commissural axons are found most often in the middle half of the deep layer. Most of them arise from branches of axons that enter the central nucleus or the brachium of the inferior colliculus.

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401.8

GABAergic PROJECTIONS FROM THE INFERIOR COLLICULUS TO THE MEDIAL GENICULATE BODY IN THE CAT.

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The mammalian auditory system is unique among sensory modalities in that many of its brain stem projections are GABAergic or glycinergic and probably inhibitory. These include ascending (feed-forward) and commissural (interaural) projections. Until recently, no such feed-forward projections had been identified in the tectothalamic system (K.A. Hutson et al., *Proc. Soc. Neurosci.*, 1993, **19**:1203). We injected horseradish peroxidase (HRP), apo-HRP gold, and wheat germ apo-HRP gold in subdivisions of the auditory thalamus, and studied GABA-immunoreactivity in Vibratomed tissue or in postembedded, semi-thin preparations from the inferior colliculus (IC).

We found that (1) GABAergic, retrogradely labeled neurons were present throughout the IC, including the central nucleus, dorsal cortex, and lateral nucleus; (2) such cells were diverse morphologically, ranging from the smallest to the largest GABAergic IC cells (D.L. Oliver et al., *J. Comp. Neurol.*, 1994, **340**:27-42); (3) GABAergic projection cells were distributed bilaterally, though most were ipsilateral to the injection, and (4) the brachium of the IC contained many myelinated GABAergic preterminal axons.

There is an ascending, feedforward inhibitory pathway from the IC to the MGB that is parallel to the much larger, probably excitatory, midbrain input. This is one of three GABAergic inputs to the MGB, besides those from the thalamic reticular nucleus and that of Golgi type II cells with local axons. This implies a convergence of inhibitory influences arising from both auditory and non-auditory sources, and whose physiological role(s) remain obscure.

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401.9

Distribution of NADPH diaphorase and cytochrome oxidase in relationship to functional organization of the inferior colliculus in guinea pig. J. Syka, R. Druga, J. Astl and J. Popelář, **(SPON: European Neuroscience Association)** Inst. Exp. Medicine, Czech. Acad. Sci., 142 20 Prague, Czech Republic.

The inferior colliculus (IC) in mammals consists of three subdivisions: central nucleus (CN), external cortex (EC) and dorsal cortex (DC). We analysed the distribution of NADPH - diaphorase (NADPH-d) and cytochrome oxidase (CyOx) in the IC subdivisions and compared it with functional organization of the IC. Animals anaesthetized with pentobarbital were perfused with aldehyde fixation, sections through the hemispheres and brainstem were cut on a freezing microtome and processed for NADPH-d according to Scherer-Singler *et al.* (J. Neurosci. Meth., 9: 229, 1983) and for CyOx according to Wong-Riley (Brain Res. 171:11,1979). In another group of anaesthetized guinea pigs, the IC was penetrated with a microelectrode and parameters of neuronal responses to acoustical stimuli, such as the characteristic frequency, Q_{10} ,latency and binaural interaction, were evaluated. The inferior colliculus in guinea pig contains, as in the rat (Druga and Syka, Neuroreport 4:999, 1993), large amounts of NADPH-d positive neurons and neuropil. Positive neurons are present mainly in the dorsal and external cortices of the IC whereas the CN contains few positive perikarya. NADPH-d positive neurons and fibers were also found in the intercollicular commisura. CyOx in the IC was localized mainly to the neuropil. In contrast to NADPH-d, CyOx was present predominantly in the CN of the IC, mainly in its basal part. CyOx positivity was particularly weak in the DC. Isofrequency layers reconstructed from microelectrode penetrations declined in frontal sections from the dorsomedial to the ventrolateral and they mostly continued from the CN to the EC. However, the EC neurons were characterized by broader tuning curves as well as by longer latencies. Investigation of distribution of histochemical markers together with the mapping of functional properties of individual neurons enables better delineation of the subdivisions of the IC and further understanding of their role in the processing of acoustical information.

401.10

CALCIUM-BINDING PROTEIN EXPRESSION DELINEATES SUBDIVISIONS OF RABBIT MEDIAL GENICULATE COMPLEX. N.T. McMullen* R.K. de Venecia, C.B. Smelser and S.D. Lossmann. Dept. of Anatomy, Univ. of Arizona College of Medicine, Tucson, AZ 85724

Previous cytoarchitectonic studies of the medial geniculate body have recognized three major divisions: ventral, dorsal and medial. Although additional parcelling of the MGB is warranted based on Golgi and connectional studies, further subdivisions are frequently difficult to discern in routine Nissl studies. We report that immunocytochemistry for the calcium-binding proteins parvalbumin (PV) and calbindin (CB) clearly delineate subdivisions of the medial geniculate complex. Furthermore, these MGB subdivisions appear to correspond to those recently defined by AChE and NADH-diaphorase activity in the rabbit (Caballero-Bleda et al, J. Chem. Neuroanat., 4, 1991). NZW rabbits were anesthetized with ketamine and nembutal and intracardially perfused with 0.1M phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1M PBS. Serial coronal and sagittal sections through the entire MGB were cut frozen at 40 um. Every third section was stained with 0.1% methylene blue while the remaining sections were processed immunocytochemically with monoclonal antibodies against PV or CB (SWANT). PV and CB immunolabeling display a remarkable complimentary staining pattern in the MGB : PV immunolabeling predominates in the ventral and medial division whereas CB+ cells characterize the dorsal and internal divisions. The *ventral* division is strongly PV immunoreactive due to dense neuropil labeling and moderately labeled PV+ somata. Sagittal sections reveal PV+ axons from the brachium of the inferior colliculus streaming into the posterior aspects of the ventral division. A CB+ *marginal zone* encapsulates the ventral MGB. Although CB immunoreactivity is distinctly lacking in the majority of the ventral division, the posteroventral portion exhibits compartments of CB somata where the PV neuropil labeling is reduced or absent. PV immunocytochemistry also reveals a small *medial* subdivision characterized by medium and large PV+ somata, thick PV+ axons and puncta. A large wedge-shaped *internal* subdivision, composed of densely-labeled CB+ cells, separates the dorsal and ventral divisions. The *dorsal* division contains large, multipolar CB+ neurons with radiate dendrites. A *deep dorsal* component is characterized by PV+ axons and terminals. These chemoarchitectonic methods will be useful for guiding future connectional studies of the MGB with the brainstem and auditory cortex. (Supported by NIH/NIDCD)

401.11

FINE STRUCTURE OF SYNAPTIC CONTACTS ON NEURONS LABELED BY RETROGRADE TRANSPORT FROM MEDIAL GENICULATE BODY IN THE RAT INFERIOR COLLICULUS. K. Ohtomo, G.E. Beckius, and D.L. Oliver*, Dept. Anatomy and Biology, College of Allied Medical Science, Akita University, Akita 010 Japan, and Dept. Anatomy, Univ. Connecticut Health Center, Farmington, CT 06030-3405.

Neurons in the inferior colliculus (IC) may project to one of several targets. The ipsilateral medial geniculate body (MGB) is the main target of the ascending pathway, but neurons also project to contralateral MGB, superior olive, and cochlear nucleus. Here, we examined the synaptic organization of IC neurons with different targets. Latex beads injected in MGB *in vivo* retrogradely labeled somata in both ipsi- and contralateral IC. Aldehyde-fixed slices, 150 μm-thick, were prepared from the IC (Hill & Oliver, '93, J. Neurosci. Meth. 46:59-68), and bead-labeled neurons were intracellularly injected with biotinylated Lucifer Yellow. Cells were processed to visualize biotin and flat embedded. The dendritic tree was sampled at regular intervals by cutting the warmed plastic (38°C) with a glass knife into 6 μm-thick sections. Reconstructions of neurons were made from camera lucida drawings and 6 μm thick sections. Thin sections, 80 nm-thick, were taken at the surface each 6 μm-thick section and examined with the electron microscope.

Seven injected neurons were studied, four with ipsilateral projections and three with contralateral projections. Most were in the central nucleus and had modest dendritic orientation ("less flat") or stellate dendritic morphology. The injected neurons could be differentiated according to their axosomatic synapses. Most neurons had relatively few axosomatic synapses, but others received many. Both types projected to ipsilateral and contralateral MGB. Neurons differed in the density of axodendritic synapses, but these differences were not easily correlated with axonal target or somatic synapses. All neurons received type 1, asymmetric, synaptic contacts from endings with round synaptic vesicles and type 2, symmetric, synaptic contacts from endings with pleomorphic vesicles. Usually type 2 synapses predominated. Neurons with projections to ipsilateral MGB tended to receive the greater proportion of type 2 synapses.

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401.12

MEDIAL GENICULATE BODY CHEMOARCHITECTONIC SUBDIVISIONS M. J. Campbell* and D.N. Pandya ENRMVAH, Bedford, MA, USA.

Monoclonal antibodies to the nonphosphorylated KSP segment of neurofilament protein (SMI-32) and to the calcium binding proteins, parvalbumin (PV), calbindin (CB), and calretinin (CR) were used in immunohistochemical studies of the medial geniculate body (MG) in the rhesus monkey. An examination of sections from brains cut perpendicular to the rostrocaudal (RC) axis of the brainstem revealed that the three main subdivisions are present along the entire RC extent of MG. The division of MG located ventrally (MGV) with respect to the brainstem can be divided into a peripheral sector (MGVp), and a more central region (MGVc). The density of SMI-32-immunoreactive (ir) profiles is less than that of PV-ir profiles in MGV, but both have greater numbers of ir neurons and a higher density of neuropil staining in MGVp relative to MGVc. There is a striking absence of CB-ir or CR-ir profiles in MGCv, with the exception of a small subpopulation of CB-ir neurons in MGVp. The division of MG located dorsally with respect to the brainstem (MGD) is characterized by a homogeneous neuropil staining with SMI-32. There are fewer PV-ir neurons in MGD relative to MGV, and MGD is distinct in its low density of PV-ir neuropil staining. In contrast to MGV, CB-ir neurons are distributed throughout MGD and CR-ir neurons are found in a large portion of MGD. In the medial of division of MG(MGmc), magnocellular neurons tend to be SMI-32-ir. PV-ir and CB-ir neurons are found in all parts of MGmc that contain smaller neurons. CR-ir cells are found only in small limited portion of MGCmc. These distinct distribution patterns provide clear criteria for subdividing MG, however when the functional properties of these chemically identified neuronal populations are defined, they may also provide insights into the functional organization of the MG. Supported by NINDS #5R29 NS-28571

401.13

CORTICOBULBAR SYNAPSES IN THE AUDITORY SYSTEM: A POSSIBLE SUBSTRATE FOR SELECTIVE ATTENTION. D. L. Weedman*, D. Vause, T. Pongstaporn, and D. K. Ryugo. Center for Hearing Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent studies have demonstrated the existence of direct projections from the auditory cortex to the auditory brainstem in rat (Feliciano et al, 1993, Soc. Neurosci. Abs.). Specifically, descending projections were shown to distribute in the superior olivary nuclei, nuclei of the trapezoid body, and the cochlear nucleus. We hypothesize that the cortex may be able to exert direct influence on both the olivocochlear (OC) efferent system and the initial processing of afferent information. In order to test this notion, we labeled corticobulbar fibers by injecting biotinylated dextran amine (BDA), an anterograde tracer, into auditory cortex in rat. After a one-week survival time, cholera toxin subunit-B (CT-B) was injected into the cochlea to retrogradely label OC cell bodies in brainstem. We examined double-labeled tissue with electron microscopy and found synaptic contacts between the descending cortical fibers and dendrites of neurons of the medial olivocochlear system. This disynaptic pathway from cortex to cochlea provides a mechanism for cortical control over the outer hair cells, and therefore over the responses of the basilar membrane itself. In a parallel study, we have found that cortical fibers synapse in the granule cell domains of the cochlear nucleus, a region which also receives afferent input from the outer hair cells (Brown et al, 1988, J. Comp. Neurol. 278: 581) and efferent collaterals from the superior olive (Brown et al, 1988, J. Comp. Neurol. 278: 591). This convergence of information may represent the first central site of cortical filtering of auditory input. These findings suggest an important role for cortex in descending control of ascending information, and reveal a possible substrate for mechanisms of auditory selective attention.

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401.14

3-D MAPPING OF HUMAN AUDITORY CORTEX IN STEREOTAXIC SPACE FROM MRI DATA VB Penhune*, RJ Zatorre, D MacDonald, T Fleischer and AC Evans; Montréal Neurological Inst & McGill University, Montréal PQ H3A 2B4.

Human primary auditory cortex is located principally on the surface of the transverse gyrus of Heschl (HG). Gross morphology of this area is highly variable, both between individuals, and between hemispheres. To measure this variation, high-resolution MRI scans (64 slices, 2 mm thick) of 19 young, healthy, right-handed subjects was transformed into the standardized stereotaxic space of Talairach & Tournoux (1988). HG was identified and labelled in the horizontal, sagittal and coronal planes using an interactive voxel-painting program to produce a stereotaxic voxel map. Averaging of individual maps results in a 3-D probability map for the location of HG. The area of 100% probability for both hemispheres differs from the stereotaxic atlas in the Z dimension, extending several mms dorsally and ventrally. Significant interhemispheric differences in the average volume of HG were observed ($L=20.3\pm7.5$ cm^2, $R=15.8\pm3.8$ cm^2; $p<.02$). HG was larger on the left by 10% or more in 14/19 brains. Absolute location in stereotaxic space did not differ between the two hemispheres. The probability map can be used to assess the extent of damage to primary auditory areas in patients with temporal-lobe lesions, and may be useful for identifying PET activation foci within auditory cortex.

401.15

LOCAL PROJECTION PATTERNS IN THE RAT CORTEX DEMONSTRATED WITH BIOCYTIN.

D.F. Siwek and P.B.Cipolloni E.N.R.M. VA Hospital, Bedford, MA 01730*

Small iontophoretic injections of biocytin into the auditory cortices of the rat labeled the somata and processes of neurons exposed to the injected marker. In many cases well labeled processes were followed for considerable distances to targets in the ipsi- and contralateral cortices. In some cases, the injection was placed quite superficially in the auditory cortex layer (I and II/III) and in others, it was placed deeper into layer V/VI producing different projection patterns.

The laminar pattern of local circuit projections is dependent on the depth of the injection in the cortex. For example, an injection restricted to the superficial laminae in rostral auditory cortex (area 41), labeled neurons and processes around the injection site. Labeled processes were distributed most heavily in layers I, II/III and V. A prominent fascicle of labeled axons descended into the subcortical white matter before turning abruptly dorsal, and then traveling to the corpus callosum. Bundles of labeled axons also passed horizontally through the cortex (not subcortical white matter), terminating in a columnar pattern in layers I, II/III and V. The plexus of labeled processes in layer I was heaviest, followed by that in layer II/III and then layer V. In contrast, when the injection involved the full thickness of cortex, the local projection plexes terminated in a column extending through all layers. Long distance projections to ipsilateral visual cortices frequently took the most direct path to their targets, passing through the cortical laminae at oblique angles. Labeled axons were also observed in the corpus callosum. These axons entered the subcortical white matter directly beneath the injection, ran anteriorly, and passed to the opposite hemisphere in the lower half of the callosum. The results suggest that horizontal interactions among the auditory cortices have a unique laminar distribution that is dependent on the cortical lamina from which it arises. Supported by Veterans Administration

401.16

THE MACAQUE MONKEY AUDITORY THALAMO-CORTICAL SYSTEM DEFINED BY CALCIUM BINDING PROTEIN IMMUNOREACTIVITY. T. Hashikawa*, M. Molinari, E. Rausell, M.E. Dell'Anna, M.G. Leggio, E.G. Jones.
Neural Systems Laboratory, Riken, Japan and Univ. of California, Irvine.

Immunoreactivity for calcium binding proteins is a useful marker to differentiate parallel thalamocortical projections in the monkey. The present study was aimed at analyzing the pattern of parvalbumin and calbindin immunoreactivity in the auditory cortex and thalamus of the Japanese monkey (*Macaca fuscata*) and to relate the parcellation of the cortex and thalamus thus obtained to reciprocal connectivity. The auditory areas of the supratemporal plane have been divided into 4 almost concentric zones of decreasing parvalbumin immunoreactivity (figure 1). Zone 1: intense staining corresponding to fields AI and RL ; Zone 2: moderate to dense staining corresponding to anteromedial (A-m), lateral (L) and posteromedial (P-m) paraauditory fields; Zone 3: weak staining located antero laterally to zone 2: Zone 4: weakest staining: anterior pole of the supratemporal plane. These zones appear to be related to the relative density of innervation by parvalbumin immunoreactive thalamocortical fibers. Retrograde tracing studies demonstrated that zones 1-3 receive inputs from different subnuclei of the medial geniculate (MG) complex. A direct correlation has been found between density of parvalbumin immunoreactivity in the cortex and that in MG nuclei providing thalamic afferents to an area. Zone 1 (AI + RL) receives projections from the ventral MG nucleus in which most cells are parvalbumin immunoreactive while zones 2 to 3 receive afferents from the anterodorsal and posterodorsal nuclei which show comparable differences in parvalbumin immunoreactive cell numbers. The present data demonstrate that parvalbumin immunoreactivity can differentiate parallel thalamocortical pathways of the monkey auditory system.

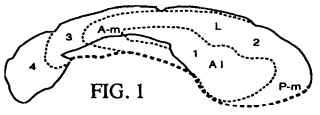

FIG. 1

401.17

DEVELOPMENT OF AUDITORY CALLOSAL CONNECTIONS IN NORMAL AND HYPOTHYROID RATS. R. A. Lucio*, J. R. Cerezo, P. Pacheco and P. Berbel. Depto. Histología e Instituto Neurociencias, Univ. Alicante (Spain), and CIF, Univ. Auton. Tlaxcala, INE, Univ. Veracruzana and IIB-UNAM (México).

Lack of thyroid hormones produces generalized brain damage, especially in the neocortex. Recent findings show that auditory callosal connections are severely damaged in adult hypothyroid (H) rats. In normal (C) rats, retrograde labelled neurons were found in cortical layers II-III to VI with a peak in layers II-III, but in H rats, they were mostly distributed in layers IV to VI. To test for an abnormal elimination/estabilization of transitory connections in H rats, the distribution of retrograde labelled callosal neurons in the auditory neocortex during development was compared with controls. For antithyroid treatment of H rats methimazole (Sigma) was orally adminestered from embryonic day 14 and a thyroidectomy performed on postnatal day (P) 6. At ages between P4 and P47, C and H rats were injected in the auditory cortex with HRP or WGA-HRP, and after 2 days, brain sections were processed with TMB. Data were analyzed with the "Neurograph" computer program. Until P35, the radial distribution of retrograde labelled neurons was similar in both groups. After P35, the number of labelled neurons falls dramatically in C rats, reaching an adult-like distribution by P47. Interestingly, their number and radial distribution in H rats appears not to change from P35 levels, even during adulthood. These findings indicate that the transitory callosal connections normally elliminated, were retained in H rats. DGICYT PB90-561 (PB); Prog. Coop. Iberoamer. (PB-PP); CONACYT-PADEP 30377 (RAL)

401.18

AUDITORY CORTICAL PROJECTIONS TO THE PONTINE NUCLEI IN THE CAT. J.J. Prieto*, R. Riquelme, M. Beneyto, and J.A. Winer. Department of Histology, University of Alicante, 03080-Alicante, Spain (JJP, RR, MB) and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720-3200 (JAW).

The pontine nuclei are a major source of brain stem mossy fibers to the cerebellar cortex. Little is known about how the auditory cortex influences the output of premotor nuclei in the brain stem. The sparse data available on corticopontine projections rely largely in studies which used autoradiography or silver degeneration methods. The goal of the present study was to demonstrate with more sensitive methods the pontine targets of corticofugal axons from four auditory cortical areas (AI, AII, PAF, and TE). Each was injected with WGA-HRP, and frozen sections were developed for TMB.

AI injections labeled axons in the dorsolateral, lateral, paramedian, and peduncular pontine nuclei. Injections in AII produced a lighter, but similar pattern of terminal labelling. With injections in PAF, most fibers terminated in the peduncular nucleus, while the projection to the dorsolateral and paramedian nuclei was scant. PAF was the only area that projected to the ventral pontine nucleus. TE was the origin of the heaviest projection to the pontine nuclei, and labeled fibers were located chiefly in the dorsolateral and paramedian nuclei. TE differed from the other areas since it alone projected contralaterally, to the paramedian nucleus, and also in that it sent axons to the median pontine nucleus.

These results demonstrate a convergence of auditory cortical afferents to the pontine nuclei, as well as other corticopontine projections that are specific to each auditory area. This suggests that multiple, descending corticofugal inputs to brain stem nuclei exist which may be analogous to parallel, ascending pathways to auditory cortex.

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GUSTATORY AND RELATED CHEMICAL SENSES

402.1

FOREBRAIN PROJECTIONS FROM THE GUSTATORY CORTEX OF THE SYRIAN GOLDEN HAMSTER. R.G.Webby*, J.deB. Zeiger, and J.A.London. Ctr. Neurol. Sci. and Dept. BioStructure & Function, UCHC, Farmington, CT 06030.

Biotinylated dextrans (10kDa, 5%, 0.2-1.0 μl, 6 animals) were pressure-injected into either the gustatory cortex, or into regions dorsally- or ventrally-adjacent to the gustatory cortex, of the Syrian golden hamster (*Mesocricetus auratus*). An injection into the gustatory cortex at the level of the genu of the corpus callosum demonstrated projections from this region to the ipsilateral infralimbic cortex, agranular insular cortex, piriform cortex, olfactory tubercle, amygdala, lateral hypothalamus, and contralateral homotypic gustatory cortex. An injection at a more rostral region of the gustatory cortex (approximately 500μ rostral to the genu of the corpus callosum) revealed a similar projection pattern, but with a relatively more robust projection to the ipsilateral infralimbic cortex. Control injections in somatosensory and piriform cortices, located 1 mm dorsal or 1 mm ventral to the gustatory cortex, respectively, resulted in projection patterns consistent with those reported for these regions, and quite different from that of the gustatory cortex. This work demonstrates that projections from the gustatory cortex of the hamster communicate primarily with limbic, olfactory, autonomic, and gustatory areas of the forebrain, and that this projection pattern is different from that of adjacent cortical areas.

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402.2

INVOLVEMENT OF THE INSULAR CORTEX IN NAUSEA: A *C-FOS* IMMUNOHISTOCHEMISTRY STUDY. P.A. Bryant*, B. Boot and I. S. McGregor Dept. Psychology, University of Sydney, NSW, 2006, Australia.

Lithium chloride (LiCl) is frequently used to examine the nature of emesis in animals. Although rats do not have an emetic reflex, the reduced food intake and conditioned taste aversions (CTA), induced by LiCl in rats suggests that this substance induces nausea in this species.

The insular cortex is a brain region containing both taste responsive and viscerosensory neurons and has been implicated in the acquisition and expression of taste-illness associations. Lesions of the insular cortex interfere with LiCl-induced CTA learning in rats while epileptic foci in the human insula are associated with nausea and vomiting. Lesions of the insular cortex in rats also cause aphagia and in body weight loss, and electrical or chemical stimulation of the insular cortex alters metabolic processes and feeding, suggesting overall involvement of this area in ingestive function (McGregor & Atrens, Behav. Neurosci., 105, 870-883) .

The present study determined whether LiCl injection would activate insular neurons as indexed by *c-fos* immunohistochemistry. Rats were injected i.p. with either LiCl (5mg/kg of 0.6M) or an equivalent volume of 0.9% saline. Two hours later, their brains were removed and processed immunohistochemically for the protein Fos (a marker of neural activation).

Rats injected with LiCl showed greater Fos expression in the insular cortex than rats treated with saline. This result suggests possible involvement of the insular cortex in nausea. Further research in our laboratory is aimed at understanding more fully this neural substrate and its role in nausea and CTA learning.

402.3

REPRESENTATION OF STIMULUS QUALITY AND INTENSITY IN A NETWORK MODEL OF BRAIN STEM GUSTATORY NEURAL CODING. P.M. Di Lorenzo* and F.W. Grasso (SUNY at Binghamton, Binghamton, NY 13902)

In the neural code for gustation, both the quality of a tastant, e.g. sweet, sour, salty or bitter, and the intensity of that tastant are conveyed by the same processing elements. Given that these elements are multisensitive across taste qualities and across concentrations within those taste qualities, it is difficult to conceptualize how a single neuron or type of neuron might convey an unambiguous signal about an unknown tastant. In this context we have devised a mathematical simulation of gustatory neural coding that demonstrates how homogeneous processing elements (analogous to neurons), differing only in the strengths of their interconnections, can collectively encode both quality and intensity of a tastant. Based on data derived from electrophysiological responses to taste recorded simultaneously in the nucleus of the solitary tract (NTS) and the parabrachial nucleus of the pons (PbN), a self-organizing neural network model, called the Gustatory Unit Stimulus Selective Topographical Organizer (GUSSTO), was constructed. The input from a "receptor" sheet was mapped with modifiable excitatory connections to processing units at the "NTS" level. Within the "NTS", processing was influenced by fixed lateral inhibitory connections. Units at the level of the "PbN" received fixed excitatory connections from the "NTS" and made plastic inhibitory connections within the layer. GUSSTO was trained with simulations of responses analogous to those in the chorda tympani nerve and permitted to self-organize. Results demonstrate that both the "NTS" and "PbN" layers acquire the ability to express different across unit patterns for different taste qualities and that these patterns are similar but distinguishable for different concentrations of the same taste quality. Individual processing elements retain the ability to respond broadly across stimuli.

402.4

MORPHOLOGY OF PROJECTION NEURONS IN THE RAT ROSTRAL NUCLEUS OF THE SOLITARY TRACT (rNST) AND EVIDENCE FOR A PROJECTION TO THE rNST FROM THE BASOMEDIAL AMYGDALA. M.S. King* and D.M. Murphy. Biology Dept., Stetson University, DeLand FL 32720.

The rat rostral nucleus of the solitary tract (rNST) contains second-order gustatory neurons, some of which project to higher brain centers via an obligatory synapse in the pontine parabrachial nucleus (PBN). The morphology of the projection neurons in the rNST has been described in rat (Lasiter and Kachele, '88, Lasiter, '91) and hamster (Whitehead, '90), with some conflicting results. The first part of the current study re-examined the morphology, number and location of rNST projection neurons visualized by stereotaxically injecting the fluorescent tracer DiI (Molecular Probes) unilaterally into the PBN in male Wistar rats (n=3) and examining the retrogradely labeled cells in the rNST. On average, each injection produced 200 retrogradely labeled cells that could be characterized morphologically. Sixty-three percent of the labeled cells were located ipsilateral to the injection. The labeled neurons were located throughout the rNST, with the highest percentage (33%) found in the rostral central subdivision. All neurons had either multipolar- or elongate-shaped somata with the majority (61%) being classified as elongate.

Evidence indicates that forebrain autonomic and limbic centers directly project to the rNST (van der Kooy et al., '84). As a preliminary step to double-labeling studies investigating the neurotransmitter within these projections, we unilaterally injected DiI into the rNST (n=3) and examined coronal sections through the forebrain under epifluorescence. Retrogradely labeled cell bodies were found bilaterally within the amygdala, but none were found in the hypothalamus or other limbic structures. Cells in the amygdala were most abundant in medial, and particularly, basomedial structures with few cells located in central or lateral regions. Therefore, the basomedial amygdala may have direct effects on the processing of gustatory information within the rNST.

402.5

RELATIONSHIPS BETWEEN THE STRUCTURE AND FUNCTION OF GUSTATORY NEURONS IN THE RAT BRAINSTEM. Z. Jin*, X. Zhang, J. Massey, L. Schweitzer and W.E. Renehan. Div. Gastroenterology, Henry Ford Health Sciences Center, Detroit, MI 48202 and Dept. Anat. Sci. and Neuro., Univ. of Louisville, Louisville, KY 40292.

Although the precise nature of the relevant neural code is still the subject of some debate, the results of recent investigations by other laboratories appear to support the hypothesis that independent taste information channels (analagous in many ways to those associated with other sensory systems) transmit data related to the gustatory environment through the central nervous system. As evidence for this theory accumulates, we have directed our attention to the potential morphologic substrates for these information channels. In the present investigation, glass microelectrodes filled with 2.0% Neurobiotin (Vector Laboratories) were used to physiologically-characterize and label individual gustatory neurons in the rat nucleus of the solitary tract (NST). If we restricted our analysis to the responses elicited by NaCl, HCl, quinine and sucrose, we found a marginally significant relationship ($p = 0.06$) between best tastant and the extent in the mediolateral plane, with NaCl-best neurons more widespread than those that were most sensitive to quinine ($\alpha = 0.05$). When we further restricted our analysis to those neurons that responded to only one of these four tastants, we found that both NaCl-only and HCl-only neurons were more widespread in the mediolateral plane than quinine-only cells. Furthermore, there was an indication that the quinine-only cells were more restricted in the dorsoventral axis. The difference between these groups was clearly revealed when the area of influence was considered. Quinine-only cells were smaller in the coronal plane than the cells that responded only to NaCl, HCl or sucrose. These data support the hypothesis that the response properties of gustatory NST neurons are related to the morphology of these cells. Our results suggest that the relationship between responsivity and dendritic architecture may be especially important. Supported in part by DC01074.

402.6

NEUROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF RAT NUCLEUS TRACTUS SOLITARII NEURONS THAT RESPOND TO STIMULATION OF UPPER AIRWAY CHEMORECEPTORS. R.D. Sweazey*, T.M. Todoran and J.A. Cook. Department of Anatomy, Indiana University School of Medicine, Fort Wayne, IN 46805.

Information from taste buds on the epiglottis and aryepiglottal folds initiates upper airway protective reflexes via a brainstem pathway that includes the nucleus tractus solitarii (NTS). Currently, little is known about the response properties and morphological characteristics of rat NTS neurons that receive upper airway chemosensory information. We recorded the responses of rat NTS neurons to stimulation of upper airway taste buds with 0.5 M KCl and NH_4Cl, 0.01 N HCl and distilled water. After determining neural response characteristics to these chemical stimuli, 1.5-2% biocytin was iontophoretically injected into the NTS to determine the morphological properties of cells near the recording site.

Chemoresponsive neurons were found 0.5 mm caudal to 1.0 mm rostral to obex, 0.5-1.2 mm lateral to the midline and 200-500 μm ventral to the brainstem surface. Most neurons were located in the medial, intermediate or interstitial NTS. The order of effectiveness for the four chemical stimuli was KCl > NH_4Cl > distilled water > HCl. Both KCl and NH_4Cl elicited sustained responses whereas the responses to water and HCl adapted quickly. The majority of biocytin-filled neurons had round to fusiform shaped soma and 2 primary dendrites. In general, these dendrites were oriented in the rostral-caudal or medial-lateral plane and were restricted to the NTS.

Our findings suggest that NTS neurons responsive to stimulation of upper airway taste buds exhibit different chemical sensitivities, but similar gross morphological properties when compared to more rostrally located NTS neurons that process oral cavity chemosensory inputs.

Supported in part by N.I.H. Grant DC00735

402.7

IN VITRO ANALYSIS OF GLUTAMATE AGONIST EFFECTS ON NEURONS IN THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT. X.Q. Shu* and R.M. Bradley. Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.

Glutamate receptor agonists have been shown to have excitatory effects on neurons in the caudal nucleus of the solitary tract in rats (Andresen and Yang, Am. J. Physiol. 259:H1307, 1990). Using whole cell recordings in brain slices of the rat medulla we have examined the effect of glutamate receptor agonists on neurons in the rostral gustatory area of the nucleus of the solitary tract (rNST). Recordings were made from 42 neurons with stable resting membrane potentials and a spike overshoot. Superfusion of the glutamate receptor agonists, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA), resulted in membrane depolarization usually accompanied by a decrease in membrane resistance. Neurons either increased their firing rate or became spontaneously active with the depolarizations. All neurons tested responded to NMDA and 83% responded to AMPA indicating that the majority of rNST neurons have both NMDA and AMPA receptors. Neurons responded to NMDA and AMPA in a dose dependent manner. NMDA was effective over a concentration range of 10 - 100 μM and AMPA was effective over a lower concentration range (0.25 - 10 μM). Reversal potentials for NMDA and AMPA were estimated from current-voltage relationships in control saline and in the presence of NMDA and AMPA at concentrations producing a maximal response. The NMDA reversal potential was 1.2 mV ± 10.2 (mean ± SE, n = 5) and the AMPA reversal potential was - 4.9 mV ± 7.1 (n = 7). These results, together with our previous studies on synaptic potentials in rNST neurons (Chem. Senses 18:647, 1993), indicate that glutamate is an excitatory transmitter at the first central synapse in the taste pathway. (Supported by NIDCD grant DC00288 to R.M.B.)

402.8

FUZZY SET ANALYSIS OF ORGANIZATION OF TASTE RESPONSES IN NTS OF RAT. R. P. Erickson*, P. M. Di Lorenzo, G. S. Doetsch and M. A. Woodbury (Department of Psychology, Duke University, Durham, N.C. 27708)

The diversity of response profiles of taste neurons in the rat NTS and PbN can be accounted for by the participation of the neurons and stimuli in grades of membership in as few as 3 general interactions; further, the temporal patterns of response are accounted for by grades of membership in as few as 4 temporal divisions of the stimulus portions of the general interactions (Erickson, R.P., Di Lorenzo, P.M and Woodbury, M.A., J. Neurophysiol. June 1994). That analysis was based on 4 stimuli at the PbN level (NaCl, HCl, sucrose and QHCl) and those plus KCl at the NTS level. The question remains whether these few parameters can account for data sets with larger numbers of stimuli. The analysis was performed on NTS neurons including the above 5 stimuli plus $NaNO_3$, Na_2SO_4, LiCl, Li_2SO_4, $CaCl_2$, NH_4Cl and HNO_3. It was found that 3 general interactions were still adequate. Further, it became clear that as few as 3 temporal courses of response were sufficient. The several known receptor processes may be considered to act in a coordinated fashion as 3 mechanisms; each stimulus may be considered to participate in each mechanism to various degrees, and each receptor cell may contain these mechanisms to various degrees. Graded membership in these mechanisms by both stimuli and receptors/neurons is a "fuzzy" classification method. Supported by Duke University and NIA/NIA.

402.9

CISPLATIN-INDUCED CONDITIONED TASTE AVERSION IN THE RABBIT IS NOT ATTENUATED BY AREA POSTREMA ABLATION. J.P. Messenger* and W.W. Blessing. Centre for Neuroscience, Flinders Medical Centre, Bedford Park, SA 5042, Australia.

Conditioned taste aversions (CTA) are food avoidance responses which develop in situations where malaise follows ingestion of a particular food, even though the food itself may not have been the actual cause of malaise. CTAs are a robust adaptive reaction of critical survival value. The neural mechanisms of CTAs in the rabbit are not known. New Zealand White rabbits were deprived of water overnight and the volume of water consumed in a 1h period was measured the next day. After 3 days the water was replaced with a 10% sucrose solution. After 1h the animals were given an i.v. injection of saline (n=2) or cisplatin (0.2 mg/kg). Three to 6 days later the sucrose solution was re-presented and the volume consumed was measured. In a further 4 animals the area postrema (AP) was ablated 10 days prior to CTA investigation. Under halothane anaesthesia (1% in O_2) the dorsal surface of the medulla was exposed by retraction of the atlanto-occipital membrane. The AP was removed by aspiration through a bevelled fine guage needle. The ablation was later verified histologically. Saline injection produced no CTA to the sucrose solution. Unoperated rabbits (n=7) consumed 99±9 ml sucrose water in 1h prior to cisplatin (0.2 mg/kg), but consumed only 28±6 ml when it was re-presented 3-6 days later. Animals which had the AP ablation consumed 63±9 ml sucrose water prior to cisplatin (0.2 mg/kg) but only 6±4 ml 3-5 days later. These results indicate that in the rabbit cisplatin-induced CTA is mediated by a neural pathway other than the chemosensitive AP.

402.10

INTERACTIONS BETWEEN SENSORY PETROSAL NEURONS AND CAROTID BODY CHEMORECEPTORS IN CO-CULTURE. H. Zhong and C.A. Nurse*, Dept. of Biology, McMaster University, Hamilton, Ontario, L8S 4K1.

We are interested in chemosensory transduction and synaptic mechanisms in the mammalian carotid body, an O_2-sensing organ that regulates breathing. Several studies indicate that the O_2-sensors are glomus or type 1 cells which transduce hypoxic stimuli by the closure of K^+ channels, followed by depolarization and neurotransmitter release at synapses formed with petrosal sensory terminals. To investigate chemosensory signaling we developed co-cultures of dissociated (rat) petrosal neurons and glomus cell clusters. Monitoring of membrane potential in isolated co-cultured petrosal neurons, before and after perfusion of hypoxic (Po_2=25-30 torr) solutions, revealed silent, as well as spontaneously-active neurons that showed no obvious response to hypoxia. A third, and most interesting category was juxtaposed to glomus cell clusters; 2 of 7 such neurons depolarized reversibly during hypoxia, from a typical resting potential of -55 to -70 mV, and this was accompanied by an increase in membrane conductance. These neurons are strong candidates for ones that develop *de novo* functional synapses with glomus cells, and will be useful for studying synaptic events during chemosensory signaling. Supported by MRC Canada.

402.11

CHARACTERISTICS OF ACTION POTENTIALS AND THEIR UNDERLYING OUTWARD CURRENTS IN MAMMALIAN TASTE RECEPTOR CELLS. M. Scott Herness*, Yushe Chen, and Sun Xiao-Dong, Indiana University School of Medicine, Muncie, IND. 47306.

Many rat taste receptor cells conduct action potentials (APs). APs had a mean threshold of -35 mV (n=61 cells) and a spike height of 52mV above threshold in current clamp (hold = -80mV). APs could be classified into two significantly different ($p<0.001$) groups - ***fast***, with short half-time durations and large outward currents (mean=1.3 ms and 2.7nA), and ***slow***, with long durations and small outward currents (mean=9.2 ms and 0.29nA). AP upstrokes were conducted by TTX-sensitive sodium currents whereas the downstroke by TEA-blockable outward currents. Voltage dependent analysis of outward currents separated transient and sustained components. The transient component was specifically blocked by 4AP (1mM). A calcium-dependent outward component was also revealed by modulating voltage and external calcium concentration. The fast recovery phase of the AP appears related to the sustained outward current whereas the after hyperpolarization (AHP) was blocked by 4AP suggesting a significant contribution of the transient component. Forskolin (FSK), which elevates cAMP, reversibly blocked the majority of the sustained current without influencing the transient. FSK greatly exaggerated the AHP without changing the spike height or duration. These data suggest that several components of the outward current contribute specifically to the gustatory AP and that the AP may be modulated by cyclic nucleotides. Supported by NIH DC00401.

402.12

INTRAGEMMAL AND PERIGEMMAL FIBERS IN TASTE BUDS: IMMUNOCYTOCHEMISTRY AND DIFFERENTIAL SENSITIVITY TO CAPSAICIN. T.E. Finger*, G.M. Nelson, B. Bryant, and P.A. Moore, Rocky Mtn. Taste & Smell Ctr., Dept. Cell. & Struct. Biol., U. Colo. Sch. Medicine, Denver CO 80262; Monell Chem. Senses Ctr., Philadelphia PA

Taste buds and the immediately adjacent epithelium are richly innervated both by specific gustatory fibers upon which taste receptor cells synapse and by free nerve endings. Classic anatomical studies have separated this rich nerve plexus into intragemmal and perigemmal classes according to whether the nerve fibers enter the taste buds. Immunocytochemical studies within the last two decades have demonstrated that many of the free nerve endings are immunoreactive (ir) for substance P (SP) and CGRP and that some of these enter the taste bud proper. Thus the designation of "intragemmal" and "perigemmal" probably does not exactly correspond to the physiological role of the particular fibers. Further, a rich synapsin- and synaptophysin-ir intragemmal nerve plexus is present and at least some of these fibers are post-synaptic to taste receptor cells. The present study on rats was undertaken to test whether the SP/CGRP-ir fibers and the synaptophysin-ir fibers represent distinct populations and whether either group is abolished following neonatal exposure of an animal to capsaicin. Co-localization experiments were carried out utilizing primary antisera raised in different species and simultaneous visualization with two different fluorescent probes. The synaptophysin-ir and SP/CGRP-ir fibers represent two largely independent and non-overlapping populations. In circumvallate papillae, a few scattered double-label fibers occur, while in the fungiform papillae, the two populations are mutually exclusive. Following perinatal treatment of rat pups with capsaicin (50 mg/kg i.p.), the SP/CGRP but not the synaptophysin-ir populations are abolished.

402.13

CHOLINERGIC RESPONSES OF TASTE CELLS IN *NECTURUS* TASTE BUDS. Douglas A. Ewald* & Stephen D. Roper Dept. of Anatomy & Neurobiology, Colorado State U., Fort Collins CO 80523 and the Rocky Mountain Taste & Smell Center, Denver CO 80262

Focal chemical stimulation of the apical tips of intact taste buds elicits receptor potentials in receptor cells and postsynaptic responses in Merkel-like basal cells (Ewald & Roper, J. Neurophysiol. **67:** 1316, 1992). There is evidence that serotonin is a neuromodulator in taste buds (Delay, Taylor and Roper, J. Comp. Neurol. **335:** 606, 1993; Ewald & Roper, J. Neurosci. May 1994), but the neurotransmitter in receptor cells that produces postsynaptic responses in basal cells and afferent nerve endings is unknown. In mammalian taste buds, acetylcholine (ACh) activates muscarinic receptors (Hwang *et al.*, P.N.A.S. **87:** 7395, 1990) and immunocytochemical evidence has shown the presence of choline acetyltransferase in taste buds (Kim & Roper, this volume). We have investigated the effects of focally applied ACh (10 µM to 10 mM) on cells in intact taste buds and on isolated taste cells from *Necturus*. Short pulses of ACh elicit hyperpolarizing responses in receptor cells (4.5 ± 1.3 mV; mean ± SE; N=8; ACh = 100 µM; V_m = -60 mV), accompanied by increases in input resistance (37 ± 13%). In intact taste buds these responses are enhanced by bath application of acetylcholinesterase inhibitor (10 µM neostigmine; 67 ± 6%; N=4). In isolated receptor cells, the responses are mimicked by oxotremorine (100 µM), a muscarinic agonist, blocked by atropine (30 µM), a muscarinic antagonist, and not mimicked by nicotine (100 µM). Responses are increased by hyperpolarization of the membrane potential. The zero intercept of this increase, in conjunction with an increased input resistance during the responses, suggests that ACh responses are primarily due to a transient decrease in resting Cl^- conductance. We speculate that cholinergic responses may be part of an efferent control circuit, or may play a role in lateral interactions between taste receptor cells. Support: NIH DC01238.

402.14

IN VITRO UPTAKE OF ^{3}H-GLUTAMATE AND ^{3}H-GABA IN *NECTURUS* TASTE BUDS. T. Nagai, R. J. Delay* and S. D. Roper. Dept. Physiol., Teikyo Univ. Sch. Med., Tokyo 173, Japan, Dept. Anat. Neurobiol., Colorado State Univ., Fort Collins, CO 80523.

Glutamate (Glu) and GABA are found in nerve fibers that innervate *Necturus* taste buds (Jain & Roper, '91; Lu & Roper, '93). To investigate whether Glu/GABA are involved in synaptic transmission in taste buds, we studied their uptake and release in taste cells. Lingual epithelium was incubated with ^{3}H-Glu (0.5, 5.0 µM) or ^{3}H-GABA (0.6, 6.0 µM) in amphibian physiological saline (APS) for 15 min., rinsed with fresh APS, fixed with 2% glutaraldehyde, postfixed with 2% OsO_4, embedded, and sectioned at 2.5 µm. Slides were coated with Kodak emulsion and exposed for 3-10 days. Background labeling for ^{3}H-Glu was seen throughout lingual epithelium. However, silver grains preferentially accumulated over some taste cells. Next, epithelium was incubated with ^{3}H-Glu and rinsed with APS containing 40 mM K^+. K-treatment reduced the number of heavily labeled taste cells, consistent with a depolarization-induced release of Glu. Rinsing tissues with 40 mM K^+ but in the presence of 20 mM Mg^{2+} and 0.4 mM Ca^{2+} did not reduce the number of labeled taste cells. When lingual tissues were incubated with ^{3}H-GABA, labeling was seen over cells lying outside of taste buds, including putative glial cells in the *lamina propria*. ^{3}H-GABA uptake was not affected by rinsing tissues with 40 mM K^+. We conclude that *Necturus* taste buds have uptake and Ca-dependent release mechanisms for Glu that may be related to synaptic actions. We did not find evidence for depolarization-dependent release mechanisms for GABA in taste buds.

402.15

IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN TASTE BUDS. Dae-Joong Kim and Stephen D. Roper* Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins CO 80523 and the Rocky Mountain Taste and Smell Center, Denver CO 80262

Cholinergic synaptic mechanisms in taste buds were proposed several years ago based on the finding that injecting cholinergic agonists and antagonists into the tongue modified taste responses. Additionally, acetylcholinesterase (AChE) has been localized in taste buds from a number of species. However, AChE can be associated with noncholinergic neurons. The presence of AChE is not a reliable indicator of cholinergic neurotransmission. In contrast, the localization of choline acetyltransferase (ChAT), the biosynthetic enzyme for acetylcholine, is considered direct evidence for cholinergic mechanisms in the nervous system. We have conducted immunocytochemical studies on lingual tissues from rats and mice to determine whether ChAT is localized to taste buds. Immunocytochemistry revealed ChAT in taste receptor cells of vallate papillae. Three to eight receptor cells per taste bud showed intense ChAT immunoreactivity in their cytoplasm. ChAT-immunopositive cells were elongate and their cell processes extended from the taste pore to the base of the taste bud. Immunostained cells were mainly located at the periphery of taste buds. On the basis of these data, as well as recent findings on the physiological actions of muscarinic agonists in taste buds (Hwang *et al.*, PNAS **87**: 7395, 1990; Ewald & Roper, this volume, 1994), we postulate that acetylcholine functions as a neurotransmitter in vertebrate taste buds.
Supported by NIH DC00374 and DC00244.

402.16

RELATIONSHIP OF TASTE BUD REGENERATION AND SALT DISCRIMINATION PERFORMANCE AFTER CHORDA TYMPANI TRANSECTION IN RATS. S.J. St. John, S. Markison, and A.C. Spector*. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Chorda tympani (CT) transection causes anterior tongue taste bud degeneration and severely disrupts the rat's ability to discriminate NaCl from KCl. We recently demonstrated that rats trained presurgically and tested starting 49 days postsurgically (CTX/49d) could discriminate the two salts, but those tested 8 days postsurgically (CTX/8d) could not. The CTX/49d rats had ~70% of the normal complement of anterior tongue taste pores (TPs), suggesting CT regeneration. As previously, rats in the present study were trained to maintain licking to one salt and suppress licking to the other. Performance was quantified as an overlap score (0-100%) of the response distributions, where 100% indicated no difference in licking to the two salts (i.e., no discrimination). The rats tested 7 days after unilateral CTX were only slightly impaired despite that these rats (n=4) had ~50% fewer TPs than controls. The rats tested starting 28 days after bilateral CTX (n=7) displayed variability in salt discrimination performance, ranging from normal to severely impaired (overlap score range: 6.7-77.9%). A third group (n=4) tested 49 days after CTX displayed competent discrimination. When the data from the present groups were combined with the data from the control, CTX/49d, and CTX/8d groups previously reported, the overlap scores correlated (r=-0.88) with the percentage of fungiform papillae containing a TP ($p<0.001$, n=27). Although some rats with a small number of anterior tongue TPs were partially competent, overall it appears that there is a strong relationship between the extent of taste bud regeneration and performance in the salt discrimination task. Supported by PHS grant DC-01628.

402.17

GLUTAMATE CHEMORECEPTION IN *PARAMECIUM*. J. L. Van Houten*, W.Q. Yang, F. Hecht, C. Braun**, and H. Plattner**. Dept. of Zoology, University of Vermont, Burlington, VT 05405 and **Universität Konstanz, Konstanz, Germany

Glutamate is an attractant stimulus to *Paramecium tetraurelia*. Cells in glutamate swim smoothly and relatively fast, which is characteristic of a relatively hyperpolarized cell. Direct measurements of membrane potential confirm this. The hyperpolarization appears to be associated with an increase in intracellular cyclic AMP (cAMP). H8, an inhibitor of Protein Kinase A among others, inhibits chemoresponse to glutamate but not NH_4Cl. To investigate whether the rise in cAMP were rapid enough to be part of a stimulus pathway as opposed to a desensitization pathway, we utilized a rapid mixing apparatus that allowed us to analyze whole cells that were stimulated for 30 msec to 5 sec with glutamate or low K hyperpolarizing conditions as a positive control. The 6 fold rise in cAMP was rapid, peaking at 300 msec and falling to a 3 fold elevated level of 118 pmole/mg protein. The level of cAMP approaches that of the baseline control by 5-10 min.

IMP is a repellent which depolarizes cells. The binding of and response to IMP can be inhibited by glutamate. Cyclic AMP levels decrease by 50% at 30 sec. Rapid kinetic measurements of cAMP in cells stimulated by IMP were inconclusive. However, cGMP appears to increase slightly in cells stimulated by IMP for 30 msec to 1 min, but the increases are much smaller than those caused by the high Ba depolarizing positive control.

MOTOR CORTEX: CORTICAL PHYSIOLOGY

403.1

SIMPLE AND COMPLEX RELATIONS OF MOTOR CORTICAL ACTIVITY TO GRIP FORCE IN THE ALERT MONKEY. H.-X. Qi, E.J. Huesler, I. Alig, M.-C. Hepp-Reymond*. Brain Research Institute, 8029 Zürich, Switzerland.

Neuronal correlates of force have been repeatedly shown in the primary motor cortex (M1). We wondered whether the neuronal activity in two ventral premotor regions (PMv) and M1 may code other features than just grip force.

We have analysed 242 finger-related cells, recorded from the 3 regions in 2 monkeys trained to produced isometric force in a step-tracking task. The trials varied in number of steps, in force range and direction, each alternative indicated by its own colour cue. The findings are: *1.* More than half of the neurons displayed positive or negative correlation between cell activity and force. In 70% of these significant cells, similar discharge patterns were found under all test conditions. The rest, and a large proportion of non-significant cells, had more complex firing patterns. Most of these neurons (**d1** cells) changed their force covariation at the highest force level, thus suggesting linearity only within a narrow force range. With high forces a few d1 cells also altered their phasic activity component during the force ramps. The minority of the neurons with complex force relation (**d2** cells) clearly changed their discharge patterns during the last hold period, regardless of the type of trial. *2.* The distribution of the 3 cell populations and their mean force sensitivity was comparable in the 3 explored regions. *3.* Most neurons with significant correlation coefficient responded to activation of deep (muscle and joint) receptors. The proportion of cutaneously activated cells was higher in the d2 population. *4.* Some PMv neurons with receptive fields on face and hand also showed significant covariation with force.

In conclusion, these findings suggest that most M1 and PMv neurons code force in simple manner, and that only a minority has activity related either to force within a narrow range or to other parameters or task features.

403.2

PRIMATE MOTOR CORTEX: NEURONAL ACTIVITY CODING FOR OPERATIONS IN EXTERNAL SPACE THAT ARE INDEPENDENT OF LIMB TRAJECTORY. L. Shen* and G.E. Alexander. Dept. Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322.

Two macaque monkeys were trained to perform a two-dimensional, delayed step-tracking task in which they used the right upper extremity to operate a joystick that moved a cursor on a video display screen. Single cell recordings were obtained from the shoulder and elbow regions of the primary motor cortex. The presentation of a fixation target on the center of the monitor signaled the beginning of a trial. Once the monkey moved the cursor into the fixation point, four peripheral targets were presented. One of the target subsequently dimmed briefly, signaling the target, and the monkey was to capture the target after a delay upon the dimming of the fixation point. Spatial mapping between the cursor and joystick movement was offset by either 0° or 90°. Hand trajectory was thus either parallel (0°) or perpendicular (90°) to the direction of displacement (from center) of a given target. The spacing of the targets was such that the set of movements towards all four targets was equivalent in the two conditions, allowing determination of whether neurons with directional preparatory or movement-related activity were preferentially tuned to the direction of target displacement or to the direction of hand movement. Among cells that were directionally tuned, two cell types were predominant. Tuning curves of the first type of cells were invariant with respect to hand trajectory. The second type showed directional activity that was invariant with respect to target direction. These results indicate that directional tuning of motor cortex neurons is not always representative of limb trajectory as neurons of the second type appeared to represent the intended direction of cursor movement rather than that of the limb.

403.3

CELL ACTIVITY IN MONKEY MOTOR CORTEX IS ALTERED BY CHANGES IN ARM POSTURE FOR MOVEMENTS WITH IDENTICAL HAND TRAJECTORIES. S. H. Scott* and J. F. Kalaska, Dépt. de Physiologie, Univ. de Montréal, Montréal, Québec, CANADA, H3C 3J7

The debate continues as to whether neuronal activity in the monkey motor cortex during reaching movements is better related to the extrinsic (i.e. hand trajectory) or intrinsic (i.e. muscle activity, joint moments) attributes of the motor task. We have trained a monkey to move a pendulum-like handle to visual targets using two different arm postures, but with identical hand paths. In the first posture (control), the monkey was allowed to perform the task in its preferred arm orientation (largely in the sagittal plane). In the second posture (abducted), the monkey had to abduct its arm approximately 90 degrees in order to grasp and move the handle. This perturbed arm posture changed the mechanical properties of the muscles (length and moment arm about the joints) and changed the EMG activity patterns of muscles that span the shoulder and elbow joints. We recorded the activity of a large sample of cells in the contralateral motor cortex during the motor task. In the control posture, the activity of individual cells were broadly tuned to the task. The vectorial sum of population activity was oriented approximately with the movement direction. In the abducted posture, the activity of the cells during the motor task was usually not identical to that recorded for the control posture; tonic cell activity often increased or decreased and the preferred direction often changed. The population vectors in the abducted posture varied systematically from the direction of hand movement; these vectors were skewed towards one of two possible directions in which maximal shoulder movement was required to generate the motor task. The sensitivity of cell activity in motor cortex to arm posture is inconsistent with the notion that motor cortex cells signal only hand trajectory in space. (Funded by MRC Group Grant in Neurological Sciences to JFK and MRC Post-Doctoral Fellowship to SHS).

403.4

MULTI-ELECTRODE RECORDING OF NEURONAL ACTIVITY IN THE MOTOR CORTEX: EVIDENCE FOR CHANGES IN THE FUNCTIONAL CONNECTIVITY BETWEEN NEURONS DURING A REACTION-TIME TASK J. Requin*, A. Riehle, J. Seal, B. Arnaud, M. Coulmance, R. Fayolle, and N. Vitton. Cognitive Neuroscience Laboratory, CNRS-LNC, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France

We have studied the changes in the functional connectivity between neurons, within a volume comparable in size to the modular organization of cortical structures, during the construction of a motor action. Two monkeys were trained in a reaction time (RT) task in which they performed a pointing movement with the arm, after a preparatory period (PP) of variable duration. A multi-electrode microdrive was used to transdurally insert into the cortex 7 independently driven microelectrodes, spaced 160 µm apart (Mountcastle et al., 1991). The activity of 780 neurons of the primary motor cortex was recorded during 255 sessions. In order to study the dynamic changes in interactions between sets of task-related neurons, joint peri-event time histograms (Aertsen et al., 1989) were then calculated on 265 pairs of simultaneously recorded neurons. The activity of 39 pairs of neurons (15%) was significantly synchronized during periods of time lasting 50 to 300 ms. Such periods of synchronization occurred just after the preparatory signal, at the end of the PP, during RT and/or during movement time. For 14 pairs, the neurons fired with a synchrony of less than 1 ms, whereas in the remaining 25 pairs, larger peaks of cross-correlograms were observed. Synchronized neurons were recorded with a mean horizontal distance of 377 µm (160 to 1980 µm) and a mean vertical distance of 464 µm (0 to 1810 µm). This study provides further evidence for dynamic changes in the functional connectivity within neuronal populations involved in the processing of sensorimotor information. Detailed analysis of cross-correlation data will be used in an attempt to decipher the functional organization of the basic processing unit of the cortical tissue and to investigate how cognitive functions are implemented in the microstructure of the cerebral cortex.

403.5

CHRONIC NEURAL RECORDING WITH MULTICONTACT SILICON MICROPROBES: EFFECTS OF ELECTRODE BIAS. E.M. Schmidt*, W.J. Heetderks and D.M. Camesi-Cole. Lab. of Neural Control and Neural Prosthesis Program (WJH), NINDS, NIH, Bethesda, MD 20892.

Silicon microprobes with integral flexible cables (Neurosci. Abstr. 1993) have been implanted in monkey supplementary motor area for over seven months and continue to record multi-unit activity with spike amplitudes that permit unit isolation using conventional multi-unit spike sorting methods. The recording sites on our probes are sputtered iridium that have been activated to produce an oxide film that lowers the impedance of the electrodes by a factor of ten. However, this electrode impedance is voltage dependent. In this report we demonstrate that the application of a bias potential to the electrodes has a significant effect on the electrode impedance and on the recorded signal amplitude of the spikes. Signal to noise ratios are repeatedly increased by a factor of three or more by the brief application of an anodic bias to the electrode. In a typical recording session, this results in a substantial increase in the amplitude of the larger spikes and the emergence of several smaller spikes from the background hash. After several months of implantation without bias, neural activity is greatly diminished and impedances are increased. A small positive bias no longer shifts the electrode to the low impedance state. In order to return the electrode to a low impedance value, a larger bias must be applied such that currents of approximately 10 na flow. When this occurs, the electrode impedance drops and spike amplitudes are increased. Two phenomena that appear to affect long term recordings are the impedance state of the electrode and an encapsulating sheath around the electrode that may be disrupted by the passage of currents in the nanoamp range.

403.6

PRIMATE PREFRONTAL CORTEX PLAYS A SIGNIFICANT ROLE IN PERFORMING SEQUENTIAL BEHAVIOR WITH AN IMPOSED DELAY. S. Funahashi*, M. Inoue, and K. Kubota. Dept. of Cognitive Sci., Grad. Sch. of Human and Environmental Studies, Kyoto Univ., Kyoto 606-01, and Primate Res. Inst., Kyoto Univ., Aichi 484, Japan.

To investigate the roles of the prefrontal cortex in generating sequential behavior, single neuron activity was examined in the periprincipal region while 2 monkeys performed a sequential hand-reaching task to two of three targets and a simple reaching task to one target as a control. In addition, to examine whether prefrontal neurons retain multiple target positions simultaneously, a 3-5 s delay period was imposed between the target presentation and the reaching behavior.

A total of 234 single neuron activities were analyzed. Among them, 138 exhibited task-related activity in at least one phase of either task; 85, 72, and 108 exhibited cue-, delay-, and response-related activity, respectively. A large proportion of these activities exhibited complex and context-dependent characteristics. For example, 36% of the delay-related activity was observed only when two specific targets were presented in a particular order during the cue period (pair- and sequence-dependent), and 18% of this activity was observed only when a specific target was presented in either the first or second position (position- and sequence-dependent). However, the remaining neurons exhibited task-related activity under simple conditions. For example, 8% of the delay-related activity was observed when the target was presented in a particular position, regardless of its order. A similar variety of response characteristics, from context-dependent to simple, was observed in response-related activity.

These results indicate that the prefrontal cortex plays an important role in executing sequential behavior. The prefrontal cortex may participate in complex sequential behavior by retaining target positions through a variety of activities, each of which can retain either simple information (e.g., a single position) or multiple and complex information (e.g., multiple positions and a particular order).

403.7

CROSS CORRELATIONS BETWEEN PAIRS OF IDENTIFIED CELLS IN MONKEY MOTOR CORTEX: IMPLICATIONS FOR THE ORIGIN OF POST SPIKE FACILITATION IN RECTIFIED EMG OF HAND MUSCLES. S.N. Baker, E. Olivier, R.N. Lemon*. Sobell Department of Neurophysiology, Institute of Neurology, Queen's Square, London, WC1N 3BG, UK.

The technique of averaging rectified EMG with respect to pyramidal tract neurones (PTN's) recorded from primary motor cortex (spike triggered averaging, STA) has been claimed to allow demonstration of monosynaptic cortico-motoneuronal (CM) connections. Such connections are assumed when the average shows a short latency and duration post spike facilitation (PSF). Critics have suggested that synchrony between the firing of the recorded cell and CM cells could produce facilitation in STA without direct connections from the trigger cell to the motoneurones innervating the recorded muscle (eg Kirkwood, Behav Brain Sci 1992, 15:766-767).

Using two parallel microelectrodes separated by 2 mm, we have recorded from 16 pairs of identified PTN's in the motor cortex (7 pairs on the same electrode) of 4 *Macaca nemestrina* monkeys trained to do the precision grip task, whilst simultaneously recording EMG from up to 9 hand and forearm muscles. For 6 pairs (5 on the same electrode), both cells showed clear PSF in STA of at least one common muscle. In all 6 such pairs, there was a significant central peak in the cross correlograms between the two cells, mean width 15.0 ms, range 9.5-24.5 ms, mean strength as measured by fraction of total spikes synchronised 0.053 (range 0.016-0.1135), suggesting a common input to the two cells.

These data have been combined with other information in the literature to produce a realistic computational model of the effect of synchrony on the production of PSF. A previous study (Smith and Fetz, Neurosci Lett 1989, 96:76-81) addressed the same problem using a spike selection method and a convolution method. Our model takes account of non-linearities inherent in using rectified EMG, and is able to investigate the consequences of synchrony amongst different numbers of cells, allowing more comprehensive quantitative analysis of the contribution of synchrony to PSF.

Supported by the Wellcome Trust, Action Research and the MRC.

403.8

MOTOR CORTEX AND INTERCEPTION OF MOVING TARGETS: SINGLE CELL ANALYSIS. N. Lindman Port*, W. Kruse, P. Dassonville and A. P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN.

Two monkeys were trained to use a 2D articulated manipulandum to intercept moving targets on a computer screen. In random order, 9 targets either accelerated, decelerated or traveled at a constant velocity. For each motion condition targets traveled for one of three target movement times: 0.5, 1 and 1.5 s. Targets appeared randomly in either the right or left lower corner of the screen, then traveled along a 45° path until they crossed the vertical meridian. The monkeys were required to intercept the moving target as it crossed the vertical meridian, making an upward movement (12 cm) from an initial hold position. An interception was considered successful when the movement entered, within 130 ms of the target, a 1 cm radius positional window centered on the point at which the target crossed the vertical meridian. The spike activity of 411 cells were recorded in the arm area of the motor cortex in one monkey during task performance. We found that the activity of various populations of neurons was modulated during the interception task. In most cases the change in cell activity was similar to that observed during upward movements toward a stationary target, in another task, but in other cases changes in cell activity differed between the two tasks. Moreover, cell activity during the last 200 ms of the reaction time was frequently modulated in relation to the temporal characteristics of the ensuing movement; for example, activity in 31% of cells differed significantly ($P<0.05$, ANOVA) among trials with different target velocities, irrespective of the kind of target motion (accelerating, decelerating or constant speed), whereas in 6% of cells it differed according to the kind of motion above, irrespective of the speed itself, and in 11% of cells both speed and kind of target motion had a significant effect. These results indicate that the motor cortex is involved with processing of the temporal characteristics of the interception movement. (Supported by NIH grant PSMH48185).

403.9

MOTOR CORTEX AND 3D ISOMETRIC FORCE: STATIC RELATIONS. J. Boline*, J. Ashe, M. Taira and A.P. Georgopoulos. Brain Sciences Center, Veterans Affairs Medical Center, and Dept. of Physiology, Univ. of Minn. Med. School, Minneapolis, MN

The relations between the steady-state activity of cells in monkey motor cortex and the direction and magnitude of isometric force were determined using a 3D isometric manipulandum (Massey et al., J. Neurosci. Methods 26:123-127, 1988) and a visually instructed task. Five repetitions of 24 constant force levels were employed in a randomized block design where each block consisted of 3 force magnitudes (100, 150 and 200 total gm-force) exerted in each of 8 XY directions (every 45 deg); force exerted in the Z dimension was monitored but not controlled. Data from electromyographic (EMG) activity of 7 muscles and impulse activity from 91 cells were analyzed using a repeated measures analysis of variance and a multiple linear regression of EMG and cell activity versus the X, Y, Z components of force. The magnitude of force had a significant effect ($P<0.05$) in 75% of muscles and 31% of cells studied, whereas the direction of force had a significant effect in all muscles and 70% of cells; significant magnitude x direction interactions were observed in 62.5% and 23% of muscles and cells, respectively. The multiple regression was significant in all muscles and 76% of cells. R^2 values (mean$\pm$SD) were 0.529 ± 0.203 and 0.239 ± 0.173 for the muscles and cells, respectively. Preferred force orientation vectors for cells and muscles were distributed throughout the XYZ directional continuum. (Supported by VA and NIH.)

403.10

MOTOR CORTEX AND INTERCEPTION OF MOVING TARGETS: NEURONAL POPULATION ANALYSIS. W. Kruse*, N. Lindman Port and A.P. Georgopoulos. Brain Sciences Center, Veterans Affairs Medical Center, Minneapolis, MN.

A monkey was trained to intercept a moving target on a planar working surface within 130 ms and 1 cm of its arrival at the 12 o'clock position on a computer monitor. In a trial, the target moved from either of two positions, at 3 or 9 o'clock, towards 12 o'clock in a linear trajectory. The target could be accelerating, decelerating or moving at a constant velocity; each one of these conditions was presented such that the total movement time of the target was 0.5, 1 or 1.5 s. The hand movement always started from the center of the clock and ended at 12 o'clock. Moreover, trials were included in which the monkey moved in 8 directions (every 45^0) towards targets on a circle. The spike activity of 212 directionally tuned cells recorded in the arm area of the motor cortex during task performance was analyzed. We calculated the population vector every 20 ms in all the conditions above; we also computed instantaneous velocity vectors of the ensuing trajectories. The population vectors pointed in the direction of the upcoming movement and did not seem to reflect the spatial motion of the stimulus. The change in the length of the population vector began -180 to -350 ms before the onset of movement, even though average reaction times (RT) varied from 345 ms (for the fastest decelerating target motion) to 1,280 ms (for the slowest accelerating motion). Assuming that information concerning stimulus motion is processed during the initial part of the RT, these results indicate that this stimulus processing may not be reflected in motor cortical activity. However, the time above by which the population vector preceded the onset of movement was systematically longer for slower speeds of target motion. This suggests that some aspects of visuomotor integration are processed in the motor cortex, most probably relating to the timing and temporal profile of the interception movement. (Supported by NIH grant PSMH48185.)

403.11

MOTOR CORTEX AND ISOMETRIC FORCE: DYNAMIC VS. STATIC PROCESSES. J. Ashe*, J. Boline, M. Taira and A.P. Georgopoulos. Brain Sciences Center, Veterans Affairs Medical Center, Minneapolis, MN 55417.

The hypothesis was examined that dynamic processes, effecting change in force, and static processes, involved in the maintenance of a constant force, are reflected separately in the patterns of neural activity of cells in monkey motor cortex. For that purpose cell activity was recorded while monkeys performed 3 visually instructed tasks using a 3D isometric manipulandum (Massey et al., J. Neurosci. Methods 26:123-127, 1988). The tasks involved the production of (a) purely dynamic isometric force pulses (task 1, n=430 cells); (b) pulse-and-step forces that were maintained for 0.5 s (task 2, three step forces; n=91); and (c) dynamic force pulses in the presence of a constant force bias (task 3, n=331). Results from tasks 1 and 3 showed that cell activity associated with dynamic force pulses was similar across different force biases. Results from tasks 1 and 2 showed that the time course of cell activity in task 2 resembled initially (i.e. during the reaction time) the pattern observed in the dynamic pulse task, and then changed to the pattern appropriate for the ensuing static force step. The replacement of the dynamic by the static pattern occurred approximately 150 ms before the attainment of the static force level. Moreover, the dynamic and static patterns were frequently directionally incongruent. (Supported by NIH and VA).

403.12

MOTOR CORTEX AND VISUOMOTOR MEMORY SCANNING. G. Pellizzer*, P. Sargent and A.P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417.

We trained a monkey in a memory scanning and a control task to exert a force pulse on a 2D semi-isometric handle in 8 different directions. In the memory scanning task, (1) 3 yellow stimuli were presented sequentially and stayed on the screen; (2) one of the stimuli, except the last one, changed from yellow to blue which identified it as the test-stimulus, and gave the go signal; (3) the correct response was to the stimulus that succeeded the test-stimulus during the initial presentation. In a control task, one stimulus was presented and the response was made to that stimulus after the go signal. We recorded the activity of 247 cells in the motor cortex during the two tasks. (a) When the test-stimulus was the 1st stimulus in the sequence, cell activity during the RT did not reflect the direction of that stimulus but instead the direction of the response; similarly, the neuronal population vector, calculated every 20 ms, pointed in the direction of the response. The lack of neural representation of the 1st stimulus in this case can be explained by the fact that, by the design of the task, the 1st stimulus in the sequence was never the target of the response. (b) When the test-stimulus was the 2nd in the sequence, cell activity during the RT reflected first the direction of this stimulus, and then switched to reflect the direction of the response; similarly, the neuronal population vector pointed first in the direction of the test stimulus and then abruptly switched direction and pointed in the direction of the response. These results suggest that memory scanning involves abrupt switching of direction, a process different from a rotation discovered previously in a mental rotation task (Georgopoulos et al. Science 243: 234, 1989). This is in accord with the results of psychophysical experiments which suggested different constraints in directional processing between mental rotation and memory scanning tasks (Pellizzer and Georgopoulos, Exp. Brain Res. 93: 165, 1993). (Supported by NIH NS17413).

403.13

CORTICOMOTONEURONAL (CM) POST-SPIKE EFFECTS ON SHOULDER, ELBOW, WRIST, DIGIT AND INTRINSIC HAND MUSCLES DURING A REACHING TASK IN THE MONKEY. B.J. McKiernan*, J.K. Marcario, J.H. Karrer and P.D. Cheney. Physiology Dept. and Smith MRRC, Univ. of Kansas Med. Ctr., Kansas City, KS 66160.

Spike triggered averaging of rectified EMG activity has proven to be an effective means of identifying motor cortex cells with a functional linkage to motoneurons (Cheney et al., Prog. Brain Res., 87: 213, 1991). Previous studies of forearm muscles have shown that many CM cells not only facilitate multiple agonist muscles of the forearm but also suppress the antagonist muscles (Kasser and Cheney, J. Neurophysiol. 53: 959, 1985). This reciprocal output pattern constitutes a functionally meaningful synergy raising the possibility that more complex functional synergies may also be represented in the synaptic outputs of single CM cells. The purpose of this study was to investigate 1) the possibility that during an arm reaching movement, individual CM cells may facilitate muscles at different joints; and 2) the extent to which the facilitated muscles constitute a functional synergy whose action would produce a specific phase of the movement. The spike discharges of single cells in motor cortex were used to compute spike-triggered averages of rectified EMG activity from 22 muscles of the shoulder, elbow, wrist and hand while the monkey performed an automated reaching task to retrieve a pellet from Kluver type food well. Stimulus-triggered averaging of EMG activity was used to map the location of output zones for the same 22 muscles. Of 108 motor cortex cells tested, 30 were identified as CM cells by their postspike effects in at least one of the muscles tested. Ten (33%) of these CM cells produced output effects in muscles at two or more joints. We conclude that some cells in motor cortex facilitate functional muscle synergies needed to produce specific phases of a coordinated arm reaching task. (Supported by NIH grant NS25646).

403.14

ACTIVITY OF MONKEY SENSORIMOTOR CORTICAL (SMC) AND NEOSTRIATAL (NS) NEURONS DURING HAND MOVEMENTS MADE UNDER UNPREDICTABLE CONDITIONS. R.J. Nelson*, J.M. Denton and M.A. Lebedev. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

The activity of neurons in monkey SMC and NS was studied in a paradigm where rewards for correct performance were given only 75% of the time, pseudorandomly. Monkeys made ballistic wrist flexions and extensions in response to palmar vibration (57 Hz) or visual go-cues. Animals actively held a steady position for 0.5-2.0s before the go-cue. The paradigm met NIH animal utilization guidelines. The activity during trials following rewarded trials (Regular trials) was compared to that during trials following ones in which reward for correct performance was withheld (After trials).

A total of 120/295 SMC neurons and 40/101 NS neurons were selected because their firing patterns changed before movement or with sensory cues. After trials mean reaction times (RTs) were significantly shorter regardless of cue type. Differences in RTs between visual and vibratory cued trials (Regular trials:~ 45ms; After trials : ~ 15 ms) were significantly reduced when reward delivery became unpredictable. This resulted largely from a decrease in visual trial RTs. Activity during active hold was significantly greater in After trials for both cueing conditions. Premovement activity (PMA) occurred closer to movement onset and was greater in magnitude during After trials as compared with Regular trials. These last differences were statistically significant for SMC but not NS neurons. PMA occurred significantly earlier during After trials when the monkey moved in the direction of the stimulated hand surface (flexion) as compared to away from it (extension). Premovement activity onsets for both SMC and NS neurons preceded movement onset by about 150ms while the earliest EMG activity recorded from nine forelimb muscles was about 90ms. SMC cue related neurons were significantly more responsive to vibration during After trials.

These observations suggest that monkeys are more attentive and that SMC and NS neurons are more responsive to peripheral and central inputs during unpredictable rewarding conditions . Thus, these neurons may be gated during predictable behavioral conditions. Supported by **USAF Gr AFOSR 91-0333** and **NIH Gr NS 26473**.

403.15

ACTIVITY OF NEURONS IN PRIMATE PRIMARY MOTOR CORTEX (MI) DURING TRAINED AND SEMI-AUTOMATIC OROFACIAL MOTOR BEHAVIORS. R.E. Martin*, Y. Masuda, P. Kemppainen, N. Narita, and B.J. Sessle. Toronto Hospital and Univ. of Toronto, Toronto, Canada.

This study aimed to determine whether tongue-MI neurons participating in trained tongue movements (Murray & Sessle, J. Neurophysiol. 92) also participate in semi-automatic movements such as those of swallowing and mastication. Extracellular single neuron recordings were obtained from tongue-MI (defined by intracortical microstimulation; $\leq 30\mu A$) while the awake monkey (M. fascicularis) (a) performed a trained tongue protrusion task, (b) swallowed a juice reward after successful tongue-protrusion trials, (c) swallowed juice from a syringe, and (d) masticated and swallowed apple. Electromyographic (EMG) activity was simultaneously recorded from various orofacial and laryngeal muscles for EMG confirmation of tongue protrusion, swallowing, and mastication. Of a total of 21 tongue-MI neurons tested to date, 12 (57%) showed alterations of firing during the trained tongue protrusion task. Of these, the activity of 92% was modulated in relation to swallowing of the juice reward and all showed modulated activity in relation to swallowing of juice from the syringe or swallowing apple. Of the neurons that showed altered activity during both trained tongue protrusion and swallowing, 89% also exhibited altered firing during mastication. Preliminary recordings more laterally in the "cortical masticatory area" of the same monkey indicate that some neurons in this area also may alter their activity in relation to both trained and semi-automatic orofacial movements. These findings suggest that neurons in tongue-MI and adjacent cortical regions may play a role in the control of semi-automatic as well as trained orofacial motor behaviors. Supported by Canadian MRC grant MT-4918.

403.16

SYNCHRONIZATION OF PRIMATE SENSORIMOTOR CORTEX NEURONS DURING 20-40 HZ FIELD POTENTIAL OSCILLATIONS. Y.N. Murthy* and E.E. Fetz. Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195.

In monkeys performing exploratory and manipulatory forelimb movements, local field potentials (LFPs) in sensorimotor cortex exhibit increased levels of oscillations in the 20-40 Hz range. Previous findings that LFP oscillations occurred synchronously at diverse sites have been extended to the associated changes in single/multi unit activity. Cycle-triggered histograms (CTHs) of unit activity aligned on cycles of LFP oscillations revealed modulation in two-thirds (176/268) of recorded units. Peak spike activity occurred 2.7 ms prior to peak LFP negativity, and the average relative modulation amplitude (amplitude of oscillatory component of CTH as percent of baseline) was 45 ± 25%. Cross-correlation histograms (CCHs) between pairs of ipsilaterally recorded units compiled with all spikes (during and outside oscillatory episodes) seldom revealed significant features. However, CCHs compiled selectively from spikes occurring during oscillatory episodes (OS-CCHs) revealed synchronization in 67 out of 134 pairs. The central peak in OS-CCHs had an average latency of 0.5 ± 13 ms; peak amplitude was 14 ± 11% of baseline. Simultaneous recordings in left and right motor cortex revealed significant features in OS-CCHs in 22 out of 42 pairs of bilateral units. Average peak latency (0.2 ± 8.0 ms), and average normalized peak (10 ± 8%) were similar to the values for ipsilateral pairs. These results indicate that oscillations transiently synchronize neural populations beyond the range of individual axonal projection. For pairs of ipsilateral precentral units, the probability of occurrence of significant OS-CCH features was the same whether both units of the pair were task-related (33/56), or only one unit was task-related (20/39) (χ^2 test, $p > 0.2$). Thus, oscillatory synchronization does not appear to preferentially associate task-related neurons. During oscillatory episodes the mean firing rates of single units tended to be restricted to values corresponding to the average rates just prior to or after the episodes: the coefficient of variation in firing rate during oscillations was significantly lower than that before ($p < 0.001$). This suggests that oscillatory modulation of neural activity does not involve a systematic change in the balance of excitatory and inhibitory inputs to the entrained cells. [Support: NS12542 & RR00166]

MOTOR CORTEX: ANATOMY

404.1

NEURONAL INPUTS TO RAT CORTICAL AREAS EVOKING CONTRACTIONS OF ANTAGONISTIC AND SYNERGISTIC MUSCLES. **X. Gu, W. M. Staines, and P. A. Fortier*. Dept. Anatomy & Neurobiology, Univ. of Ottawa, Ottawa, ON, Canada, K1H 8M5.**

The objectives were to determine whether primary motor cortical areas, evoking contractions in synergistic and antagonistic muscles, received inputs from common or separate sources. Intra-cortical microstimulation (ICMS) was used to map low-threshold areas capable of evoking limb movements. From this map, sites capable of evoking ankle flexion, ankle extension, hip flexion, and elbow flexion were selected for injection of fluorescent tracers. The procedures were to (1) inject fluorogold, fast blue, and propridium iodide into three separate motor output sites, (2) allow retrograde transport for 3-5 days, (3) section the brain, and (4) map distributions of differentially-labeled neurons. Computer controlled injections yielded 0.3-1 mm columns of densely filled tracer which was transported to common thalamic and cortical sites. There was limited overlap in the distribution of neurons labeled with different tracers when using small injections but this increased when using larger injections. Conversely, ventral thalamic areas did not show increased overlap with larger injections despite the presence of overlap of intralaminar thalamic areas with all injections. This study suggests that some cortical and thalamic areas provide a single command to the motor cortex for the control of coordinated muscle actions while other cortical and thalamic areas separately control individual muscle actions. [Funded by Canadian MRC]

404.2

LAMINAR DISTRIBUTION AND SYNAPTIC PATTERNS OF PARVALBUMIN- AND CALBINDIN-CONTAINING CELLS IN CAT MOTOR CORTEX. L.L. Porter* and A. Keller. Dept. Anatomy & Cell Biology and Neuroscience, USUHS, Bethesda, MD 20814.

Inhibitory interactions in the motor cortex are involved in shaping cortical output during movement. These inhibitory interactions are mediated by GABAergic neurons, that include several morphological and functional subclasses. We examined cells in cat motor cortex that contain parvalbumin or calbindin, calcium binding proteins that occur in most GABAergic cells. Immunohistochemistry revealed that parvalbumin neurons are more numerous than calbindin cells (2.6:1) and exhibit a more heterogeneous distribution throughout the cortical laminae: 53% in II-III, 29% in V, 18% in VI for parvalbumin, and 74% in II-III, 16% in V, 10% in VI for calbindin. The synaptic relationships of these neurons with axons intrinsic to the motor cortex were examined: Intrinsic axon collaterals were labeled following local injection of fluorescein-dextran, parvalbumin and calbindin cells were labeled by immonohistochemistry. Scanning confocal microscopy was used to determine the distribution and density of appositions between labeled axon terminals and labeled neurons. Similar proportions of the the parvalbumin and calbindin populations formed appositions with intrinsic axons (38% and 36%, respectively). The distribution of appositions on cells belonging to the two groups was also similar. The majority of cells formed axodendritic appositions, whereas a few cells in each group formed axosomatic appositions. The mean distance of axodendritic appositions from the somata of parvalbumin cells was 21 µm, while that for calbindin was 32 µm. Ultrastructural analysis is in progress to confirm that the appositions represent synaptic sites. Different classes of GABAergic neurons in motor cortex exhibit different densities and laminar distributions, but cells in both groups appear to receive similar patterns of input from intrinsic axons. The interactions between intrinsic axons and these interneurons may play a role in shaping the activity of functionally-defined populations of output neurons.
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404.3

INHIBITORY SYNAPSES WITH DIFFERENT CLASSES OF PROJECTIONS CELLS IN CAT MOTOR CORTEX. P.B. CIPOLLONI*, L. KIMERER, D. WEINTRAUB AND A. KELLER.
VA Medical Center, Bedford, MA 01730; Dept. Anat. & Neurobiol. and Neurol., Boston Univ. Med. School, Boston, MA 02118; Dept. Anat. & Cell Biol., USUHS, Bethesda, MD 20814.

The mechanisms by which neurons perform spatial and temporal integration are dependent on the numbers, types, and distribution of synapses they form with their afferents. To provide data pertinent to understanding these integrative mechanisms, the distribution of synapses with identified classes of cortical projection neurons was examined ultrastructurally. Pyramidal cells in the cat motor cortex projecting to the somatosensory cortex or to the spinal cord were labeled by the retrograde transport of HRP. The somata of corticocortical and corticospinal cells were examined using serial-section electron microscopy. The profiles of these somata and the synapses formed with each of these profiles were reconstructed from each thin section with the aid of a computer-aided morphometry system. All the synapses on the somata of these pyramidal neurons were of the symmetrical, presumably inhibitory type. These synapses were not homogenously distributed over the somatic membrane, but were clustered at several zones, particularly in the vicinity of the axon hillock. The nonhomogeneity in the distribution of axosomatic synapses indicates that analyses of synaptic innervations of neuronal somata require examination of the complete somatic surface area, and that studies in which small portions of these structures are randomly sampled may be misleading. The number, density and spatial distribution of symmetrical synapses were almost identical on the somata of different corticocortical cells. In contrast, different corticospinal cells formed varying densities of inhibitory synapses. These findings indicate that different classes of cortical neurons receive unique patterns of inhibitory inputs and support the hypothesis that the synaptic organization of the cerebral cortex is highly specific. Supported by PHS:NIH grant #NS31078 and Dept. Veterans Affairs.

404.4

INDEPENDENT ANATOMICAL CIRCUITS FOR REACHING AND GRASPING LINKING INFERIOR PARIETAL SULCUS AND INFERIOR AREA 6 IN MACAQUE MONKEY

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Recent physiological evidence showed that in the intraparietal sulcus (IPS) and inferior area 6 there are areas that have similar functional properties. Anterior intraparietal area (AIP), which lies in the lateral bank of IPS, and F5, located in the rostral part of inferior area 6, are both involved in plan and control of hand grasping and manipulation movements. Ventral intraparietal area (VIP), which lies in the fundus of IPS and F4, located in the caudal part of inferior area 6, are both involved in coding the peripersonal space possibly for reaching movements.

In order to see if these functionally similar parietal and frontal areas have direct and selective anatomical connections we made restricted injections of neural tracers (WGA-HRP, FB, DY and TB) in F4, F5, AIP and LIP, an area which lies just caudally to AIP, in three macaque monkeys. After injections in F4 the labelling was found only in the middle third of the fundus (VIP) and in the rostral tip of IPS. After injections in F5 VIP was spared and the labelling was almost completely confined to AIP. The selective anatomical linkage between AIP and F5 was fully confirmed by tracers injections in AIP. The retrograde and the anterograde labelling were mostly confined to the sector of F5 located in the arcuate sulcus. A caudal injection in the lateral bank of IPS (possibly LIP) produced labelling in prearcuate cortex. Inferior area 6 was devoid of any labelling.

The present data indicate that visuomotor integration for reaching and grasping are processed along independent, parallel parieto-frontal circuits.
(Supported by a Grant from Human Frontier Science Program)

404.5

TRAJECTORIES OF THE PREFRONTAL, PREMOTOR, AND PRECENTRAL CORTICOPONTINE FIBER SYSTEMS IN THE RHESUS MONKEY. J.D. Schmahmann* and D.N. Pandya, Massachusetts General Hospital and Harvard Medical School, Boston; ENRM Veterans Hospital, Bedford, MA.

We studied the course of the fiber systems from the frontal lobe to the basilar pons in 18 monkeys using the autoradiographic technique. **Prefrontal** fibers enter the most rostral anterior limb of the internal capsule (ICa), and progress caudally in its medial aspect with a nearly horizontal course. Fibers from lateral and dorsal prefrontal cortex (area 8B) lie within the upper third of the ICa; those from medial and ventral prefrontal cortices (areas 32 and 46v) are in the ventral third; and those from the superior frontal gyrus (area 9) occupy an intermediate location. At the genu all prefrontal fibers abruptly descend into the medial aspect of the cerebral peduncle. **Premotor** fibers enter the mid portion of the ICa and progress caudally. Ventral premotor and opercular fibers are situated laterally; dorsal premotor and supplementary motor fibers lie midway between the medial and lateral boundaries. All these fibers precipitously descend into the cerebral peduncle at the most rostral posterior limb of the internal capsule (ICp). Fibers from the lower part of the **precentral** gyrus (face area) obliquely enter the lateral sector of the caudal part of the ICa, and descend abruptly at the rostral part of the first quarter of the ICp into the middle of the cerebral peduncle. From the hand area fibers descend vertically through the white matter with an anteriorly convex course, enter the first quarter of the ICp, and descend in the first and rostral part of the second quarter, also situated laterally within the capsule. Fibers from the trunk area enter the ICp in its second quarter and descend in its second and third quarters with an intermediate mediolateral position. The dorsal precentral cortex (leg area) fibers descend with anterior bowing into the medial part of the second quarter of the ICp, and travel in the medial-intermediate compartment of the cerebral peduncle. The precise delineation of the organization of these fiber pathways provides an added dimension to the understanding of the frontal lobe and its subcortical connections. *Supported by NIH 16841 and the Veterans Administration.*

404.6

QUANTITATIVE ANALYSIS OF THE PROJECTIONS FROM THE PERICRUCIATE MOTOR CORTEX OF THE CAT TO THE PONTO-MEDULLARY REGIONS OF THE BRAINSTEM. K. Matsuyama* and T. Drew. National Institute for Physiological Sciences, Okazaki, Japan, and Dept. of Physiology, University of Montreal, Canada.

To quantitatively examine the zones of termination of the corticofugal pathway to the lower brainstem, Phaseolus vulgaris (PHA) was injected into either the forelimb (N=4) or hindlimb (N=2) regions of motor cortex (Area 4), or into the more dorsal (N=2) or more ventral (N=1) regions of the premotor cortex (Area 6). A light microscope, equipped with potentiometers and connected to a microcomputer system, was used to measure the X and Y coordinates of PHA-labelled terminals observed in every fourth transverse section of the ponto-medullary region (section thickness=50μm). In all cases, the greatest density of terminals was found in the ipsilateral pontine grey (PG). Injections into dorsal Area 6 resulted in labelling within the ventral part of the PG, while injections in the other three regions resulted in labelling in the more dorsal regions of the PG. In addition, both dorsal and ventral Area 6 projected heavily to the ponto-medullary reticular formation (RF). The projection was mostly bilateral, although with an ipsilateral predominance in the rostral pontine region. In the medullary RF, injections in ventral Area 6 resulted in a widely distributed pattern of labelling, while the labelling from the dorsal Area 6 injections was more restricted to the medio-ventral regions. Injections in Area 4 resulted in a similar distribution of labelled terminals, but with a weaker density of labelling than those produced by Area 6 injections. (Supported by the Canadian MRC and the FRSQ).

404.7

AN ELECTROPHYSIOLOGICAL STUDY OF THE CORTICORETICULAR PATHWAY IN THE INTACT, AWAKE CAT. K. Bouchra* and T. Drew Dept of Physiology, University of Montréal, Canada.

As part of a study to determine the functional organization of the corticoreticular pathway in the cat, we examined the projection patterns of 459 neurones within layer V of the pericruciate cortex (areas 4 and 6) of 4 cats, each of which was implanted, bilaterally, with 6 arrays (each of 6 wires) of microwire electrodes (50μm) in the medullary reticular formation (MRF) (P2-P11; L0.5-L1.5). In addition, microwire electrodes were inserted into the pyramidal tract (PT) at a caudal level (P14.5), close to the decussation. Within the premotor cortex (Area 6), 41/129 (32%) corticofugal neurones projected only as far as the MRF (corticoreticular neurones: CRNs); 17/129 (13%) neurones were activated both from the MRF and from the PT (CRN/PTNs); and 24/129 (19%) were identified only from the PT (PTNs). In area 4, in contrast, only 62/330 (19%) cells were CRNs; 88/330 (27%) were CRN/PTNs; and 154/330 (47%) were PTNs. Furthermore, 20/58 (35%) of the neurones in Area 6 and 45/150 (30%) of the neurones in Area 4 that projected to the MRF were activated from more than three stimulating electrodes. More than 70% of the CRNs in both areas 4 and 6 had a conduction velocity (CV) of less than 20m.s^{-1} (slow CRNs); in contrast, less than 25% of the CRN/PTNs were classified as slowly conducting. The results suggest that the major projection to the MRF from Area 6 is from slowly conducting CRNs, whereas the projection from Area 4 is both from slowly conducting CRNs and more rapidly conducting CRN/PTNs. (Funded by the FCAR and the MRC.)

404.8

A STUDY OF THE CORTICORETICULAR PATHWAY IN THE CAT USING RETROGRADE TRACERS. M-J. Rho*, T. Cabana, K. Matsuyama and T. Drew. Departments of Physiology and Biology, University of Montréal, Canada, and the National Institute for Physiological Sciences, Okazaki, Japan.

To examine the organization of the corticoreticular pathway, we injected aliquots of either 2% WGA-HRP (total 0.15-0.8μl: 5 cats) or 15% Texas Red (total 0.6-1.0μl: 6 cats) unilaterally into restricted regions of the medullary reticular formation (MRF) between P5 and P12.5 and L0.3-L1.7. Both tracers heavily labelled corticoreticular neurones (CRNs) in layer V of Area 6 and rostro-medial Area 4 (corresponding to the representation of the proximal forelimb). Approximately equal numbers of neurones were labelled ipsilaterally and contralaterally by most injections, except in the banks of the presylvian sulcus where more CRNs were labelled contralaterally. Labelling in more lateral parts of Area 4 (distal forelimb representation) and in the hindlimb region of the motor cortex was less dense. In addition, CRNs labelled by injections into the rostral MRF tended to be distributed more medially than those labelled by injections into more caudal regions. In 2 of the 6 cats, 15% fluorescein (total 0.8-1.0μl) was also injected caudal to the Texas Red injection. Texas Red and fluorescein single-labelled CRNs were heavily intermingled, and between 9% and 17% of the CRNs were double-labelled. Further, double-labelled CRNs were more numerous in the contralateral cortex. These results suggest some degree of specificity in the projection from the cortex to the MRF. (Supported by the FCAR.)

404.9

NEUROANATOMICAL EVIDENCE IN SI NEOCORTEX OF RATS THAT WIDESPREAD BACKWARD PROJECTIONS IN LAYER I FROM SII AND MI CONVERGE WITH LAYER VII PROJECTIONS UPON THE DISTAL DENDRITES OF CORTICO-BULBAR NEURONS: REDUCTION OF BACKGROUND FLUORESCENCE BY SODIUM BOROHYDRIDE ENHANCES DETECTION OF AXONS IN LAYER I. Barbara Clancy* and Larry J. Cauller, GR41, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

In our continuing study of afferents to layer I of SI neocortex, background auto-fluorescence has greatly limited the detectablility of fibers labelled with dextran tetramethylrhodamine (DTMR; 10k MW, Molecular Probes). Background fluorescence was reduced and detection of DTMR-filled axons in layer I was greatly enhanced by submerging sections in a solution of sodium borohydride (0.1% in phospate buffer). Using this method, abundant anterogradely labeled fibers extending > 1 mm in layer I of rat SI neocortex were observed after pressure injections (< 50 nl) of DTMR in MI and SII. A similar injection of DTMR in the deep layers of the superior colliculus retrogradely labeled profuse apical dendrites in layer I of SI extending from large pyramidal neurons restricted to layer Vb. In addition, surface applications of Fast Blue (FB) and Diamidino Yellow (DY), but not HRP or DTMR retrogradely labeled neurons in layer VII throughout SI. The absence of cells labeled in layer IV indicated these surface applications were restricted to layers I/II because injections into layer II did label layer IV cells. Therefore, layer I of SI receives widespread projections from layer VII of SI as well as from SII and MI. We propose that these backward cortico-cortical projections contribute to cortico-tectal influences by means of distributed processing in layer I of the primary sensory areas.

404.10

MORPHOMETRIC STUDY OF PROJECTION NEURONS IN THE CORTEX OF REELER MOUSE. R.Ichikawa* and Y.Inoue, Dept. of Anatomy, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan

The projection neurons within the reeler motor cortex, the corticocallosal(cc), corticospinal(cs) and cortico-thalamic(ct) neurons, were morphometrically analyzed using three parameters as follows: 1) the size of the soma, 2) the width of the main dendrite at a distance of 30 um from the center of the soma, and 3) the direction of the main dendrite. The entire image of each neuron, labeled with biocytin, was drawn by camera lucida and was input into a personal computer through a degitizer, followed by calculation corresponding to the three parameters. The soma size and dendritic shape of normal cc-, cs- and ct-neurons, which occurred within their own proper layers of the cortex, revealed significant diversity from each other. Conversely, within the reeler cortex, where the projection neurons aligned disorderly, the three parameters calculated varied significantly among the three types of neurons, and in addition, in each layer of the cortex (divided evenly into five layers from the pial site), cc-, cs- and ct-neurons revealed different characteristics in soma size and width and direction of the main dendrite, even though the synaptic environment was common to each other. The parameters measured in this study have been said to be strongly influenced with afferent elements surrounding the neurons. In the present reeler study, the afferent elements in contact with neurons might be regulated by where their axons project. Thus, the diversity in shape of the reeler neurons within the motor cortex possibly depends upon the different projection areas.

405.1

ATTENTION TO A VISUAL STIMULUS ENHANCES NEURONAL RESPONSES IN MONKEY PREFTONTAL CORTEX. **Y.Kodaka, A.Mikami* and K.Kubota, Dept. of Behavioral and Brain Sci., Primate Res. Inst., Kyoto Univ., Inuyama 484 Japan**

To investigate how selective attention modulates neuronal activities in the prefrontal cortex (PFC), we recorded neuronal responses to the identical visual stimuli in non-attentive and attentive conditions in the monkey PFC. In a visual fixation task (VFT), while the monkey gazed at a fixation point, a visual stimulus, to which no behavioral meaning was attached, was presented extrafoveally in its visual receptive field. In a visual peripheral cueing task (VCT), the monkey released the lever within 800ms after an identical stimulus was dimmed. Activities of 48 neurons in the prearcuate and periprincipalis areas were compared under these two conditions. In 38% of the neurons, the magnitudes of the responses in the VCT were significantly larger than those in the VFT. For each neuron, the visual response tended to be greater toward the center of the RF. In the remaining 62% of the neurons, the magnitudes of the visual responses in the VFT and VCT were similar. Neither eye movement nor lever release occurred when the visual stimulus appeared. Therefore, these enhancements were not causally related to either eye movement or lever release. These results suggest that enhanced responses due to focused attention to the visual stimulus in the PFC may facilitate the selection of a behaviorally significant stimulus within the RF.

405.2

THE HUMAN FRONTAL MESIAL CORTEX: A COMPARISON BETWEEN CYTOARCHITECTONIC, RECEPTOR AUTORADIOGRAPHIC, AND FUNCTIONAL ACTIVATION. K. Zilles*[1], G. Schlaug[2], S. Geyer[1], T. Schormann[1], R. Seitz[2], H. Steinmetz[2] and H.-J. Freund[2]. Brain Research Institute[1] and Department of Neurology[2], University of Düsseldorf, 40001 Düsseldorf, Germany.

Four supplementary motor areas (SMA proper, pre-SMA, caudal and rostral cingulate motor areas) are described in the literature. These areas were demonstrated by PET studies in the human frontal mesial cortex. The aim of our study is the identification of cytoarchitectonic areas representing these functionally defined regions and the analysis of the regional distribution patterns of 11 transmitter receptors by quantitative in vitro receptor autoradiography in these areas.

Several architectonically and receptor autoradiographically definable areas are found in the mesial frontal lobe and in the lower bank of the cingulate sulcus. By comparison with PET studies, the caudal-mesial part of Area 6 (mesial Area 6aα) is probably identical with SMA proper, the rostral-mesial part of Area 6 (mesial Area 6aβ) with pre-SMA, and the caudal or rostral parts of the cingulate areas showing large pyramidal cells with the caudal or rostral cingulate motor areas, respectively. Our study shows for the first time that within the human mesial frontal cortex multiple, probably supplementary motor areas are definable by architectonic characteristics and by receptor autoradiography. Additionally, gross anatomical landmarks are defined, which allow the approximate location of these areas in coregistered PET/MR images.

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405.3

2-DEOXY-GLUCOSE (2DG) UPTAKE IN THE MEDIAL WALL MOTOR AREAS OF BEHAVING MONKEYS. N. Picard*[1] and P.L. Strick[1,2]. [1]VA Medical Center and [2]Depts. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210.

The medial wall of the hemisphere contains multiple motor areas. They are the supplementary motor area (SMA), the Pre-SMA and three cingulate motor areas (CMAd, CMAv and CMAr) (Dum and Strick, 1991). We have begun to investigate the relative involvement of these motor areas in different aspects of motor function using the 2DG method. Two monkeys were trained to perform remembered sequences of reaching movements for a juice reward (REM task). Two other monkeys were trained on a Control task to sit in the primate chair and lick juice rewards delivered at time intervals comparable to that of the reaching monkeys.

Two areas of the medial wall showed significant activation in the REM animals and not in the Controls. For the REM task, 2DG labeling was particularly prominent caudally on the dorsal bank of the cingulate sulcus (CMAd). Focal activation was found in the region of the CMAd that projects to cervical segments of the spinal cord and to the arm area of the primary motor cortex. Additional activation was present on the superior frontal gyrus rostrally in the territory of the Pre-SMA. Labeling in other motor areas on the medial wall was either absent or less significant.

These observations suggest that, of all the motor areas on the medial wall, the CMAd and the Pre-SMA are preferentially involved in the internal guidance of sequential voluntary arm movements.

Supported by the VA Medical Research Service and USPHS 24238 (PLS), and Medical Research Council of Canada (NP).

405.4

NEURONAL ACTIVITY IN THE SUPPLEMENTARY AND PRE-SUPPLEMENTARY MOTOR AREA IN RELATION TO SEQUENTIAL PERFORMANCE OF MULTIPLE MOVEMENTS. K. Shima and J. Tanji*, Dept. of Physiology, Tohoku University School of Medicine, Sendai, 980, Japan

The purpose of the present study was to examine how neuronal activity in the supplementary motor area (SMA) and presupplementary motor area (pre-SMA) is involved in performance of multiple movements. We trained monkeys (Macaca fuscata) to perform three different movements (push, pull or turn a manipulandum) sequentially, separated by brief waiting periods. Each movement was triggered by a tone signal. After completion of individual movements, the manipulandum was mechanically returned to a neutral position within which the animal had to hold it and wait for next movement. In one condition, a visual signal (LED of three different colors) indicated what movement to be performed. In the other condition, correct sequences of three movements had to be remembered. EMG analysis showed that magnitudes of muscle activities were equal in the two conditions. Activity changes in forelimb muscles were not detectable during the waiting periods. A majority of SMA neurons were active during the waiting periods. The activity was generally greater when the sequence of the three different movements was remembered. By contrast, a majority of pre-SMA neurons exhibited greater activity when the movement was instructed by visual signals. In both areas, there was an abundance of neuronal activity that was related to the temporal order of the three different movements, rather than related to next movement to be performed. Such neuronal activity, useful for programming the sequence of multiple movements, was not observed in the primary motor cortex. (Supported by Japanese Ministry of Education, Culture & Science 06NP0101 &)

405.5

CINGULATE CORTEX SUBDIVISIONS IN THE HUMAN AND MONKEY REVEALED BY STAINING FOR PARVALBUMIN. A. Solodkin*[1], R.J. Morecraft[2] and G.W. Van Hoesen[1]. [1]Departments of Anatomy and Neurology. University of Iowa. Iowa City, IA 52242. [2]Department of Anatomy and Structural Biology. University of South Dakota, Vermillion, SD 57069.

The cingulate cortex has been implicated in a wide range of behaviors including motivation, attention, mechanisms relating to pain and motor control. Not unexpectedly, its structure is complex differing both in anterior-posterior and medial-lateral dimensions.

We have studied the topography of cingulate subdivisions in humans (N=15) and in the non-human primates (N=8) using immunohistochemical techniques for parvalbumin (SWant) and non-phosphorylated neurofilament protein (SMI-32, Stemberger). Krieg and Sarkissov's cytoarchitectural parcellation of 24a, b & c and 23a, b & c was used as a guide.

Our observations in the monkey revealed a clear correlation between differential cytoarchitecture and immunostaining patterns in terms of density and topography for both markers between the *indusium griseum* and areas 24a, 24b and 24c as well as for 23a, 23b and 23c. Also a clear difference was observed between areas 24 and 23, with the most prominent change between areas 24a and 23a. In the human, the subdivisions of area 24 showed a clear parallel to the cytoarchitectural and immunostaining characteristics seen in the monkey area 24.

Our observations indicate that parvalbumin immunostaining is an excellent method to delimit different subfields in the cingulate cortex in both humans and monkey. Since this calcium-binding protein coexist with a subpopulation of GABAergic neurons, its discrete distribution suggests probable functional differences between cingulate subfields.

Supported by NS 14944 and PO NS 19632.

405.6

Descending Projections to the Basal Ganglia, Red Nucleus and Pontine Nuclei from the Cingulate Motor Cortex (M3 or Area 24c) in the Rhesus Monkey. R.J. Morecraft[1]* and G.W. Van Hoesen[2,3]. Dept. of Anatomy and Structural Biology[1], Univ. of South Dakota, Vermillion, SD 57069 and Depts. of Anatomy[2] and Neurology[3], Univ. of Iowa, Iowa City, IA 52242

The ipsilateral corticostriate, corticorubral and corticopontine projections were studied in thirteen monkeys that received injections of tritiated amino acids into the cingulate (M3 or area 24c), supplementary (M2) and primary (M1) motor cortices. In M3 cases, light terminal labeling occurred over the head and anterior-third of the caudate nucleus. Heavy patches were observed over the anterior pole and ventrolateral part of the putamen, then posteriorly labeling shifted in the dorsolateral direction. In the red nucleus, labeling was found primarily over the ventromedial part of the parvicellular subdivision. At superior and mid-levels of the pons, labeling occurred over the ventromedial pontine gray matter. At inferior levels, patches of label formed an incomplete circle around the corticofugal axons traversing the pons.

These data were compared to the distribution of projections from M1 and M2. Some topographic differences were found, but regions of overlap occurred as well. For example, the projection from M1 favored the dorsolateral part of the putamen, M3 the ventrolateral part, and M2 an intermediate position. In the red nucleus, the densest distribution of labeled fibers terminated laterally from M1, medially from M3 and in between from M2. All three motor cortices projected heavily to the peripheral border of the medial and ventral portion of the basis pontis.

These observations show that M3 targets subcortical motor centers located at multiple levels of the neural axis. Judging from detailed comparisons, the highly interconnected cortical motor representations seem to be characterized as well, by overlap in their corticofugal projection zones. The terminal distribution of these pathways may provide opportunities of unity as well as diversity in terms of their influence on subcortical motor centers. (Support: NS 14944, USD Fac. Dev. Award)

406.1

CORTICAL STIMULATION INDUCES SELECTIVE PATTERNS OF JUN B EXPRESSION IN THE STRIATUM.

H. B. Parthasarathy*, S. Berretta, and A. M. Graybiel. M.I.T. Dept. of Brain and Cog. Sci., Cambridge, MA 02139.

It has been shown that electrical stimulation of the motor cortex in rats induces the expression of immunodetectable Fos-like protein (Fos-LI) in neostriatal neurons (L.Fu and R.M. Beckstead, Neuroscience 46(2):329-334). We have examined the effect of electrical stimulation of the motor cortex in awake and Nembutal-anesthetized rats (250 msec trains at 4 Hz of biphasic current pulses through parallel bipolar electrodes for 1 hr) on the induction of Jun B-like immunoreactivity (Jun B-LI). We show that Jun B-LI is induced in zones in the dorsolateral striatum that are in the same topographical location as Fos-LI-positive nuclei. However, with double-labeling techniques, we found that Fos-LI and Jun B-LI were only partially colocalized, i.e. some neurons expressed only Fos-LI, some only Jun B-LI, and some both. This result suggests that striatal neurons respond to cortical stimulation with heterogeneous gene expression profiles. Most Jun B-LI-positive nuclei detected were in projection neurons expressing enkephalin or DARPP-32. We found Jun B-LI-positive nuclei in a low to moderate percentage of parvalbumin- Chat- and NADPH-diaphorase-positive interneurons. The presence of Jun B-LI in parvalbumin-positive, presumably GABAergic, interneurons contrasts with the result obtained by chemical stimulation of the motor cortex (see Berretta et al SNA 94), after which Jun B-LI was never seen in parvalbumin-positive neurons. Such specific differences in the patterns of striatal gene induction by electrical and chemical activation of the cortex may be the result of indirect antidromic effects of electrical stimulation. They might also reflect the differences in cortical activity produced by direct synchronous activation of cortical neurons by electrical stimulation, in contrast to release of endogenous cortical activity by removal of inhibition. *Supported by NIH Javits Award NS25529.* We thank Drs. R. Bravo, H.Hemmings, and P. Greengard for antisera.

406.2

DIFFERENTIAL INDUCTION OF IMMEDIATE EARLY GENES (IEGs) BY CORTICAL ACTIVATION IN STRIATAL NEURONAL SUBPOPULATIONS.

S. Berretta*, H. B. Parthasarathy, and A. M. Graybiel. M.I.T. Dept. of Brain and Cog. Sci., Cambridge, MA 02139.

Local pharmacological blockade of GABAergic transmission in the motor cortex of awake rats was induced via chronic head-mounted wells (Berretta et al. '94). This treatment evoked discrete body movements and, at 2 and 4 hours, induced the expression of IEGs in localized brain regions. We immunocytochemically characterized striatal neurons expressing IEG protein products in this paradigm. Fos-, Fos B-, Jun B- and NGFI-A-like immunoreactivity (-LI) was induced in the ipsilateral dorsolateral striatum. There was also a weak contralateral IEG induction. Large numbers of Fos- and Jun B-positive nuclei were in striatal neurons expressing enkephalin or DARPP-32, proteins characteristic of striatal projection neurons. These results parallel similar findings obtained by electrical stimulation of the rat motor cortex (see Parthasarathy et al SNA 94). Of the striatal interneurons identified within the field of IEG induction, only a few somatostatinergic (NADPH-diaphorase-positive) neurons and even fewer Chat-positive neurons were positive for either Fos-LI or Jun B-LI. By contrast, large numbers (75%; n.cells= 420; n.rats=4) of parvalbumin-positive (GABAergic) interneurons were Fos-LI-positive. This distribution implies that the signal from neocortex to striatum, whether direct or indirect, can select for subset of striatal neurons within the activated field. Moreover, although cortical stimulation induced Fos-LI in parvalbumin-positive interneurons, Jun B-LI was never found in these cells. Thus the signal from activated cortex can select for particular IEGs in individual neurons of the striatum. Selectivity in the cortically-induced patterns of IEG expression in individual striatal neurons were further suggested by the fact that while many nuclei were found to express both Fos-LI and Jun B-LI, many others expressed only one of them. We thank Drs. R. Bravo, H.Hemmings, and P. Greengard for antisera. *Supported by NIH Javits Award NS25529.*

406.3

CORTICAL FOS EXPRESSION FOLLOWING DOPAMINERGIC STIMULATION: D1/D2 SYNERGISM AND ITS BREAKDOWN.

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While many recent studies have examined immediate-early gene expression in the basal ganglia following dopaminergic stimulation, few have focused on the cerebral cortex, the prime target (via the thalamus) of basal ganglia output. We have examined the areal and laminar distribution of Fos immunoreactivity in the cerebral cortex of rats following systemic administration of dopaminergic agonists with particular attention to interactions between D1 and D2 receptor systems. Combined, but not separate, D1 (SKF 38393 20 mg/kg) and D2 (quinpirole 3 mg/kg) agonist stimulation induced pronounced Fos expression in frontal and parietal, but not cingulate cortex in normal rats. Similarly, following amphetamine, cortical Fos could be blocked by *either* a D1 (SCH 23390, 0.5 mg/kg) *or* a D2 (eticlopride, 0.5 mg/kg) antagonist. By contrast, following a regimen of reserpine (1 mg/kg/day for 5 days) that is known to cause a breakdown in D1/D2 synergism by behavioral and basal gangliar Fos measures, independent stimulation of D1 or D2 receptors resulted in cortical Fos expression. Irrespective of treatment condition, Fos expression occurred in a distinct laminar pattern that appeared to vary somewhat with cortical field. Dopaminergic modulation of cerebrocortical Fos may occur at the level of the striatum as part of a cortico-striato-pallido/nigro-thalamo-cortical circuit; future studies will test this hypothesis. These findings show that the principles of D1/D2 synergism and its breakdown apply to the stimulation of cerebrocortical Fos by dopaminergic agents. Furthermore, this Fos expression is correlated with motor behavior.

406.4

TRUNCATED FosB IS RESPONSIBLE FOR THE LONG LASTING INCREASE IN STRIATAL Fos-LIKE IMMUNOREACTIVITY PRODUCED BY DOPAMINERGIC DENERVATION

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Using an antibody raised against the DNA binding region of Fos, we have reported that destruction of the nigrostriatal pathway by injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle produces a long-lasting (> 3 months) increase in striatal Fos-like immunoreactivity. In order to determine the nature of Fos immunoreactive protein(s) responsible for this increase, Western blots were performed on striatal extracts. Approximately six weeks after the 6-OHDA lesion, expression of a 38 kD Fos immunoreactive protein was dramatically enhanced. Since the molecular weight of the truncated form of FosB (ΔFosB) is 35 kD, we examined FosB and ΔFosB expression using two antibodies. One antibody recognizes both FosB and ΔFosB while the other recognizes just FosB. Western blotting revealed that dopaminergic denervation selectively increased expression of ΔFosB. This finding was confirmed by immunohistochemistry which demonstrated that the increase occured exclusively in medium-sized neurons of the denervated striatum. These results suggest that ΔFosB may participate in those intracellular events which maintain altered neuronal gene expression in the striatum after dopaminergic denervation. (Supported by grant MT-11539 from the MRC of Canada).

406.5

ELECTRONIC PUBLISHING OF A NEUROSCIENCE POSTER PRESENTATION ON THE INTERNET: THE START OF A DIGITAL NEUROSCIENCE LIBRARY AND DATABASE.

D.J. Woodward, A.B. Kirillov, C.D. Myre* and A. Worthen. Dept. of Physiol./Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27157, and Biographics, Inc., Winston-Salem, NC 27104.

A simple method now exists to electronically publish a Neuroscience Poster Presentation on the Internet. A user of a typical desktop computer, such as PC, Mac or UNIX workstation, desiring to access published information does so by installing the Mosaic viewer software available at *ftp.ncsa.uiuc.edu*. This abstract and the detailed presentation can then be accessed via Mosaic by using the universal resource locator (URL) address *http://biogfx.bgsm.wfu.edu/*, and the text with embedded images read through the viewer. Authors may make their documents available to the Internet community by converting computer-generated images to GIF format and scanning other images into GIF files, converting standard word processor files into HTML format and creating links to the corresponding GIF files. These files are then placed on a UNIX workstation set to operate as World Wide Web server. Instructions to perform all these operations are at the anonymous ftp site *biogfx.bgsm.wfu.edu*. Reprints and legacy documents after scanning and character recognition can be made available for public access using similar methods. There is a critical need for a public data base to index distributed neuroscience information. These procedures can provide, with a modest investment, immediate access to a wide range of neuroscience information. This procedure has been demonstrated as useful for dissemination of documents related to research on brain mapping and drugs of abuse. *Supported by DA2338. Poster is available on WWW server http://biogfx.bgsm.wfu.edu/*

406.6

EFFECTS OF METHYLAZOXY-METHANOL ACETATE ON THE FORMATION OF STRIATAL COMPARTMENTS.

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The striatum can be segregated into two compartments, the patch and matrix, based on afferent and efferent projections and certain neurochemicals (eg. calbindin, somatostatin). Lineage studies indicate that the neurons that comprise these two populations arise at two different time points from separate populations of developing neuroblasts. Neurons destined to be localized within the patch are born between E12 and 14 while matrix neurons are born between E16 and E18. In order to attempt to knockout the patch or matrix compartments, methylazoxy-methanol acetate (MAM) was injected intraparitoneally (25mg/kg) into E13 or E17 pregnant rats. MAM is a potent toxin which kills dividing neuroblasts. It is effective for 24hrs. post-injection with effects peaking after 8hrs. Pups obtained from the pregnant mothers were allowed to grow to adulthood. The striatum, globus pallidus, entopenduncular nucleus and substantia nigra were then examined using immunocytochemistry for calbindin, somatostatin and parvalbumin. Preliminary data indicates that animals injected with MAM at E13 have fewer and smaller patches than control animals. There also appears to be a reduction in the size of the patch associated regions of the striatal output centres. The overall size of the striatum in animals injected at E18 is unaltered. The density of neurons in the matrix compartment, however, is reduced. These results indicate that MAM injections at the appropriate developmental time can alter the patch/matrix compartment ratio. Research supported by NATO grant 900303, MRC and Huntington's Society of Canada.

406.7

EXCITOTOXIC LESIONS OF RAT STRIATUM: TIME COURSE OF DNA DAMAGE ASSESSED BY IN SITU NICK TRANSLATION. Y. Bordelon, L. MacKenzie, B-A. McLaughlin, and M-F Chesselet* Department of Pharmacology, U. of Penn, Philadelphia, PA 19104.

We have used in situ nick translation to identify increases in DNA strand breaks in individual neurons of the striatum after local injections of the NMDA receptor agonist quinolinic acid (QA). Male Sprague Dawley rats (250-300 gms) received a unilateral injection of QA (60 nmol in 0.5 µl) over 5 min. in the striatum. This procedure induces a massive loss of striatal efferent neurons with relative preservation of striatal interneurons 2 weeks later (Qin et al. Exp. Neurol. 115, 1992,200-211). Controls received saline injections. Rats were sacrificed 6,8,10, 12, 24 , and 48 hrs after surgery by transcardial perfusion with 4% paraformaldehyde under deep anesthesia. Striatal sections (10 µm) were cut on a cryostat, incubated with ^{35}S-dTTP in the presence of DNA polymerase I for 30 min at 37° C, and processed for emulsion autoradiography. In rats sacrificed 6 and 8 hrs post-surgery, densely labelled pycnotic cells were only detected along the needle tract and were present in both QA- and saline-injected rats. In QA-injected rats, labelled cells were observed in the striatum away from the needle tract 10 hrs after surgery. These included densely labelled pycnotic cells, and neurons with minimal morphological alteration at the light microscopic level. Labelled cells were found near unlabelled neurons with or without morphological alterations. The number of labelled cells, and the intensity of labelling, increased dramatically between 10 and 12 hrs post-lesion. Numerous labelled cells were still observed in the striatum 48 hrs after surgery. The data indicate 1) that there is a delay in the appearance of DNA strand breaks after excitotoxic lesion of the striatum but that they can precede major morphological alterations in the neurons destined to die; 2) a differential vulnerability to DNA damage among striatal neurons after QA injections; 3) in view of the presence of numerous labelled cells at both 12 and 48 hrs, that cells do not die rapidly once DNA strand breaks occur after excitotoxic injury. Supp. by PHS grant NS 23390.

406.8

GLUTAMIC ACID DECARBOXYLASE (GAD 67) mRNA LEVELS IN THE GLOBUS PALLIDUS: 6-OHDA-INDUCED INCREASES ARE ABOLISHED BY LESIONS OF THE SUBTHALAMIC NUCLEUS. J.M. Delfs*, V.M. Ciaramitaro, T.J. Parry, M.F. Chesselet, Inst. of Neurological Sci. and Dept. of Pharmacology, Univ. of Pennsylvania, Phila., PA 19104.

The globus pallidus (GP), one of the main output nuclei of the striatum, receives an inhibitory GABAergic projection from the striatum and an excitatory glutamatergic input from the subthalamic nucleus. Lesions of the nigrostriatal pathway result in increased expression of the mRNA encoding glutamic acid decarboxylase (GAD 67) in the GP (Soghomonian and Chesselet 1992; Kincaid et al. 1992). To determine whether this increase results from excitatory glutamatergic inputs from the subthalamic nucleus, rats received either a unilateral 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra (SN), a unilateral kainic acid lesion of the subthalamic nucleus or a combination of the two lesions. Unilateral 6-OHDA lesions of the SN resulted in contralateral rotation when animals were challenged with apomorphine (0.5 mg/kg, s.c.) 7-8 days after the lesion whereas kainic acid lesions of the subthalamic nucleus resulted in ipsilateral rotation. When the two lesions were combined no rotational behavior was observed in response to systemic apomorphine. Confirming previous reports, 6-OHDA lesions increased GAD 67 mRNA levels in the GP ipsilateral to the lesion. Unilateral kainic acid lesions of the subthalamic nucleus resulted in a slight decrease in GAD 67 mRNA levels in the GP which may be related to a loss of tonic excitatory input from the subthalamic nucleus. Lesions of the subthalamic nucleus completely abolished the 6-OHDA lesion-induced increases in GAD 67 mRNA in the GP. These results suggest that increases in GAD 67 mRNA levels in the GP are triggered by excitatory inputs from the subthalamic nucleus. Supported by PHS grants MH 44896 and MH 17168.

406.9

PSA-NCAM IN THE DEVELOPING STRIATUM: ULTRASTRUCTURAL LOCALIZATION, EFFECTS OF EARLY POST-NATAL 6-HYDROXYDOPAMINE LESIONS, AND OF THE WEAVER MUTATION. K. Uryu*, S. Roffler-Tarlov, G. Rougon, and M-F Chesselet, Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104. Tufts Univ.Sch. Med., Boston, MA 02111, and U. of Aix-Marseille, Luminy, France.

We have shown that loss of immunoreactivity for the immature, highly polysialylated form of the Neural Cell Adhesion Molecule (PSA-NCAM-IR) occurs at the end of the third postnatal week in rat striatum (Szele et al., Neurosci. 1994). Ultrastructural studies show that, at postnatal day 7 (P7), PSA-NCAM-IR is associated both with axonal growth cones, and with the cytoplasmic membrane of intrinsic striatal neurons. At post-natal day 18, PSA-NCAM-IR was still associated with intrinsic striatal neurons, but labelling of the cytoplasmic membrane was much more discontinuous than at P7. In addition, PSA-NCAM-IR immunoreactivty was associated with both pre and post-synaptic elements of asymmetrical synapses on dendritic spines. The results confirm that during late post-natal development, PSA-NCAM-IR is still associated with striatal neurons, as well as with striatal inputs, likely originating from the cerebral cortex and/or the thalamus. In a second set of experiments, we examined the role of dopaminergic inputs to the striatum on PSA-NCAM maturation. Lesions of the dopaminergic pathway by bilateral injections of 6-hydroxydopamine in the striatum at P2 resulted in a profound loss of tyrosine hydroxylase immunoreactivity in the striatum, but did not affect the time course of loss of PSA-NCAM-IR. In contrast, in weaver mice, which exhibit a profound loss of dopaminergic inputs to the dorsolateral striatum postnatally, the loss of PSA-NCAM-IR in striatum was delayed by approximately a week compared to wild type mice. These results suggest that dopaminergic inputs do not play a critical role in the shift from immature to mature forms of NCAM in the striatum, and that other factors than the loss of nigro-striatal neurons play a role in the delayed maturation of PSA-NCAM in weaver mice. Supp. by NS29230.

406.10

PSA-NCAM IN THE DEVELOPING STRIATUM: EFECTS OF EARLY POST-NATAL LESIONS OF THE CEREBRAL CORTEX. A.K. Butler*, G. Rougon, and M-F Chesselet, Inst. of Neurol. Sci., and Dept of Pharmacol., U. of Penn. Phila, PA19104 and U. Aix-Marseille, Luminy, France.

Previous studies (Szele et al. Neurosci. 1994) have shown that expression of highly polysialylated Neuronal Cell Adhesion Molecule (PSA-NCAM) remains high in the dorsolateral striatum of rat pups until the end of the third post-natal week. The loss of immunoreactivity for PSA-NCAM is one of the latest developmental events recorded so far in the striatum and follows the development of nigro-striatal dopaminergic inputs, the formation of cortico-striatAL synapses, and the emergence of spontaneous activity in the corticostriatal pathway (see Tepper and Trent, Prog. in Brain Res. 99 ,1993, 35-50). This study examined the hypothesis that excitatory inputs from the cerebral cortex play a role in the loss of PSA-NCAM in the developing rat striatum. Unilateral cortical lesions were performed in 14 day old pups by thermocoagulation of the pial blood vessels over the frontoparietal cortex (see Salin and Chesselet, PNAS 89,1992, 9954-9958). Animals were sacrificed at postnatal day 25, a time when PSA-NCAM is undectable in the dorsolateral striatum of control rats, and striatal tissue processed either for immunohistochemistry or immunoblotting with an antibody recognizing specifically the alpha 2-8 linked sialic acid units associated with NCAM (Rougon et al. J.Cell Biol. 103, 1986, 2429-2437). With both techniques, the data showed a higher level of expression of PSA-NCAM in the dorsolateral striatum ipsilateral to the cortical lesion than in the contralateral striatum or in control pups. These results suggest that, in contrast to the dopaminergic input (see Uryu et al, this meeting), the corticostriatal input may play a role in NCAM maturation in the developing rat striatum. Supp. by an NSF training grant (AKB) and PHS grant NS23390 (MFC).

406.11

GLUTAMATE DECARBOXYLASES GENE EXPRESSION IN THE STRIATUM AND PALLIDUM OF PARKINSONIAN MONKEYS. J.-J. Soghomonian, S. Pedneault, A. Parent and R. Boucher *, Centre de recherche en Neurobiologie, Fac. of Med., Univ. Laval, Québec (Qc.), Canada, G1K 7P4

The mRNA levels encoding for the two isoforms of the enzyme glutamate decarboxylase (GAD65 and GAD67) were measured in different sectors of the caudate and putamen and in the internal (GPi) or external (GPe) segment of the pallidum in control (n=5) and parkinsonian (n=7) monkeys (*Saimiri sciureus*) after administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Cryostat-cut sections of the brains were processed for *in situ* hybridization histochemistry with ^{35}S radiolabeled cRNA probes and the radioautographic labeling was quantified on X-ray films or emulsion radioautographs. The extent of dopaminergic fiber loss in MPTP-treated monkeys was measured after 3H mazindol binding and was shown to be widespread throughout the putamen and the caudate. In MPTP-treated primates, an increase in both GAD67 and GAD65 mRNA levels was measured in the striatum. At a coronal rostral level, this increase was significant only in the dorsolateral sector of the putamen. At more caudal, post-commisural level, GAD67 mRNA-levels were increased in both the dorsal and ventral putamen and the caudate. Caudally, the increase in GAD65 mRNA levels reached significance only in the ventral putamen. In MPTP-treated monkeys, individual GPe neurons exhibited an increase in GAD67 but not GAD65 mRNA levels. In individual neurons of the GPi of MPTP-treated monkeys, both GAD67 and GAD65 mRNA levels were increased. The present results demonstrate that gene expression of both GAD isoforms is significantly altered throughout the basal ganglia of parkinsonian monkeys. In the striatum, increased GAD67 and GAD65 expression appears prominent in sensorimotor territories such as defined by cortical afferents. The results also support the hypothesis of an increased activity of striatal and pallidal output GABAergic neurons in parkinsonism [Supported by FRSQ, the Parkinson Foundation of Canada and NSERC-0155607].

406.12

REGULATION OF mRNA LEVELS ENCODING FOR TWO ISOFORMS OF GLUTAMATE DECARBOXYLASE AND FOR PREPROENKEPHALIN IN THE RAT STRIATUM BY DOPAMINERGIC RECEPTORS. N. Laprade and J.-J. Soghomonian*, Centre de Recherche en Neurobiologie, Fac. Med., Univ. Laval, Quebec (QC.) G1K 7P4.

The role of D1 and D2 dopamine receptor subtypes on the regulation of messengers RNAs (mRNAs) encoding for the two isoforms of glutamate decarboxylase (GAD65 and GAD67) and preproenkephalin (PPE) in rat striatum was investigated. The mRNA levels encoding for GAD65, GAD67 and PPE were dectected by *in situ* hybridization histochemistry with radioactives cRNA probes and quantified by computerized densitometry. Chronic treatment (10 days) with the D1/D2 dopamine receptor agonist apomorphine or with the D1 agonist SKF 38393 significantly increased GAD65 mRNA levels but did not change GAD67 or PPE mRNAs levels. Chronic treatment (10 days) with the D2 dopamine receptor agonist quinpirole decreased GAD67 and PPE mRNAs levels without changing GAD65 mRNA levels. On the other hand, chronic blockade (14 days) of D1 and D2 dopamine receptors with haloperidol or D2 receptors with sulpiride increased GAD67 and PPE mRNA levels but did not change GAD65 mRNA labeling. Chronic blockade (14 days) of D1 receptors with SCH 23390 induced a slight decrease in PPE mRNA levels but did not significantly alter GAD67 or GAD65 mRNA levels. These results show that gene expression of each isoform of GAD is differentially regulated in the rat striatum. Gene expression of GAD67 or PPE appears to be under the inhibitory control of D2 dopamine receptors while GAD65 gene expression would be under the excitatory control of D1 dopamine receptors. The results also suggest that each GAD plays a distinct role in the regulation of GABA synthesis in striatal neurons (Supported by FRSQ, Parkinson Foundation of Canada and NSERC-0155607).

406.13

INTRASTRIATAL INJECTIONS OF QUINOLINIC ACID SHOW MARKED CHOLINE ACETYLTRANSFERASE AND NADPH-DIAPHORASE CELL LOSS. S.M. Walker, W.C. Perryman, and G.K. Rieke*, Department of Anatomy and Cell Biology, School of Medicine, University of North Dakota, Grand Forks, North Dakota.

The debate as to whether the striatal pathology observed in the Quinolinic Acid (QA) rat model of Huntington's Disease (HD), as it demonstrates a selective sparing of aspiny interneurons, is unresolved. QA is an endogenous, excitatory amino acid that is known to spare fibers of passage. It is the intent of this study to define cell types and the associated loss within the HD model. Alzet mini-osmotic pumps were utilized for a 14 day, 340.8 mg/ml, Ph 7-8, QA 0.5 ul per hour injection period. Choline Acetyltransferase (ChAT) immunohistochemistry and NADPH-diaphorase (NADPH) histochemistry were used to determine cell sparing and cell loss. ChAT and NADPH cell counts were determined in adjacent sections. The results indicate marked cell loss. There is an overall 27% decrease in diaphorase-positive cells and a 36% decrease in ChAT-positive cells. Thus, preliminary results suggest a significant loss of intrastriatal ChAT and NADPH aspiny interneurons. Supported by BSRG.

406.14

LOCALIZATION OF "FLIP" AND "FLOP" SPLICE VARIANTS OF AMPA-SELECTIVE GLUR2 AND GLUR3 EXCITATORY AMINO ACID RECEPTOR SUBUNITS ON STRIATAL PROJECTION NEURONS. S.J. Tallaksen-Greene* and R.L. Albin. Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

We have previously shown that the majority of striatal projection neurons are immunostained using an antibody recognizing an epitope conserved on GluR2, 3 and 4c subunits and common to both "flip" and "flop" splice variants. In order to determine the relative expression of GluR2 and 3 splice variants on striatonigral and striatopallidal neurons we used *in situ* hybridization in combination with the retrograde transport of the fluorescent tract-tracer Fluoro-Gold (FG).

Unilateral injections of FG were made into either the substantia nigra or globus pallidus of adult male rats. After 12 days, the animals were killed by decapitation. Twelve µm sections were processed for *in situ* hybridization using ^{35}S-labeled oligonucleotide probes against "flip" and "flop" splice variants of GluR2 and GluR3 mRNAs.

GluR2 flop was the splice variant expressed by the greatest percentage of both striatonigral and striatopallidal neurons: approximately 50% of labeled striatonigral neurons and 30% of striatopallidal neurons expressed GluR2 flop. The remaining splice variants were each expressed by approximately 10% of striatopallidal neurons. About 12% of striatonigral neurons expressed GluR2flip and GluR3flip and 20-25% expressed GluR3flop.

These findings indicate that splice variants of AMPA receptor subtypes are differentially expressed by striatal projection neurons.

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406.15

OPPOSITE EFFECTS OF NIGROSTRIATAL OR SUBTHALAMIC LESIONS ON MITOCHONDRIAL ENZYME ACTIVITY IN THE BASAL GANGLIA

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In Parkinson's disease (PD), the striatal dopaminergic denervation induces complex changes in the functional circuitry of the basal ganglia which result, ultimately, in an overactivity of the output nuclei, substantia nigra pars reticulata (SNr) and medial globus pallidus (MGP). Beside the loss of GABA-ergic inhibition from the striatum, the SNr and MGP hyperactivity seems to be related to an increased firing of the subthalamic nucleus (STN), a structure playing a pivotal role in the regulation of the basal ganglia output.

The aim of this study was to investigate mitochondrial enzyme activity, a reliable marker for synaptic activity, in different nuclei of the basal ganglia of rats following a selective stereotaxic lesion of the nigrostriatal pathway or the STN. For this purpose, a group of rats received a unilateral 6-hydroxydopamine lesion of the substantia nigra pars compacta and medial forebrain bundle, whereas another group had the STN selectively ablated by means of an injection of 25 nmoles of N-methyl-D-aspartate. Succinate dehydrogenase and cytochrome oxidase activities were assayed histochemically on brain sections previously mounted on polylysine-coated slides. A densitometric comparison between the enzymatic staining in the lesioned and the unlesioned side was then carried out.

The rats with the nigrostriatal depletion showed, ipsilaterally to the lesion, an increased enzymatic activity in the projection nuclei of the STN: SNr, entopeduncular nucleus, and globus pallidus (the rodent homologues of MGP and lateral globus pallidus, respectively). Conversely, the enzymatic activity in these areas was reduced, ipsilaterally, by the complete ablation of the STN. These data lend further support to the more recent view of the basal ganglia circuitry organization focusing, in particular, on the modulatory activity that the STN neurons, excitatory (glutamatergic) in nature, play in the regulation of the basal ganglia output.

406.16

INTERACTION OF DOPAMINERGIC AND GLUTAMATERGIC SYSTEMS IN THE REGULATION OF STRIATAL CHOLINERGIC ACTIVITY Christopher J. Schmidt* and Vicki L. Taylor, Marion Merrell Dow Research Institute 2110 E. Galbraith Road, Cincinnati, OH 45215

The balance between dopaminergic and glutamatergic activity within the corticostriatothalamic pathways (CST) is an important determinant of sensory input through the thalamus. Disturbances of this balance in favor of glutamate or dopamine are believed to produce the symptoms of Parkinson's disease or schizophrenia, respectively. The cholinergic interneurons of the striatum are regulated by long feedback loops involving dopaminergic and glutamatergic afferents from the midbrain and the thalamus, respectively. The strategic position of these neurons allows changes in tissue concentrations of acetylcholine (ACh) to be used to assess effects of dopaminergic and glutamatergic agents on striatal function and potentially the CST pathways. Consistent with a reduction in cholinergic/striatal activity, high doses of the uncompetitive NMDA antagonist MK-801 produced a modest accumulation of striatal ACh while the D_2 agonist quinpirole produced a robust increase in tissue concentrations of the transmitter. Pretreatment with quinpirole augmented the elevation of ACh produced by MK-801 in a synergistic fashion. The D_1 antagonist, SCH 23390 alone produced only a small increase in ACh yet completely prevented any further elevation of ACh by MK-801. The D_2 antagonist haloperidol produced the expected decrease in striatal ACh and also completely blocked the effect of MK-801. Thus inhibition of cholinergic/striatal activity with the D_2 agonist potentiated the effect of MK-801 whereas treatments which increase striatal/cholinergic activity prevent any further effect of MK-801. These observations suggest that glutamatergic feedback from the thalamus may be important in determining the magnitude of the MK-801 effect. To test this hypothesis, amphetamine was used to enhance thalamic activity and thereby increase thalamic feedback to the striatum. Although amphetamine alone was without effect, the combination of amphetamine plus MK-801 produced a large increase in striatal ACh concentrations. These results suggest that the effects of dopaminergic agents on the striatal cholinergic interneurons are a composite of the direct striatal effect of such drugs and of their ultimate effect on the glutamatergic thalamostriatal feedback loop.

406.17

DECREASED NUMBER OF SOMATOSTATIN mRNA CONTAINING NEURONS IN STRIATUM OF HALOPERIDOL TREATED RATS.

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For the first time and by stereological cell counting it has been possible to demonstrate a reduction in the *number* of somatostatin (SS) mRNA containing neurons in the *dorsal* striatum of rats with dyskinetic symptoms caused by longterm treatment with a dopamine receptor blocking drug.

Adult rat were treated with haloperidol for 6 months and the behaviour video-taped. Five rats, which developed serious dyskinetic symptoms and five rats, which did not, together with five control rats were anesthetized and perfused. One serie of vibratome sections of striatum were in situ hybridized for SS mRNA using an alkaline phosphatase labeled oligonucleotide probe. The Total *number* of neurons expressing SS mRNA in the *dorsal* striatum was estimated with the optical fractionator technique. The mean *number* of SS mRNA containing cells in the treated rats with dyskinetic symptoms was significantly less than for the rats with no symptoms and the control rats.

406.18

EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR MGLUR5 BY SPECIFIC RAT STRIATAL CELL POPULATIONS. C.M. Testa*, D.G. Standaert, G.B. Landwehrmeyer, J.B. Penney and A.B. Young, Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Glutamate has a central role in basal ganglia regulation of motor behavior, and is the main afferent neurotransmitter to the striatum. Glutamate acts via many different striatal receptors, including metabotropic glutamate receptors (mGluRs). Striatal mGluRs are involved in motor behavior regulation, long term depression, and N-methyl-D-aspartate (NMDA) excitotoxicity. Several lines of evidence suggest that mGluR5 is particularly important in the striatum.

We used a double label in situ hybridization technique to determine if subpopulations of striatal neurons differentially express mGluR5. Digoxygenin labeled probes targeting enkephalin, somatostatin and acetylcholinesterase mRNAs were used in conjunction with radiolabeled probes specific for mGluR5 mRNA. We observed that mGluR5 mRNA is strongly expressed by enkephalinergic striatal neurons, but is relatively absent from at least two types of striatal interneurons: somatostatin-containing medium aspiny neurons and cholinergic neurons. Differential mGluR5 expression in striatal neuron subpopulations may underlie mGluR-mediated effects on striatal output pathways, plasticity and susceptibility of neurons to injury. Supported by USPHS grants NS19613 and NS31579. DGS is an HHMI Fellow and recipient of an AAN Research Fellowship Award in Neuropharmacology.

407.1

DOPAMINERGIC REGULATION OF THE EXPRESSION OF m1 AND m4 MUSCARINIC RECEPTOR mRNAS IN THE RAT DORSAL STRIATUM. N. Kayadjanian, R. Heavens, M.J. Besson, D. Tritsch* and D.J.S. Sirinathsinghji. Merck Sharp and Dohme Research laboratories, Neuroscience Research Center, Harlow, U.K. Institut des Neurosciences, CNRS URA 1488, UPMC, 75005 Paris, France.

In the rat neostriatum, cholinergic interneurons comprise about 1-2% of the total population of neurons but play an important role in the regulation and maintenance of striatal functions e.g. in the modulation of dopamine (DA) transmission. In vitro release data indicate that DA inhibits acetylcholine (ACh) release and receptor autoradiography shows a decrease in 3H-pirenzepine (a pharmacologically M1muscarinic receptor antagonist) binding sites after unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal DA tract. In this study, we examined the influence of striatal DA on the expression of m1 and m4 muscarinic receptor mRNAs in rats with unilateral 6-OHDA lesions of the nigrostriatal DA tract. In situ hybridization experiments of m1 and m4 mRNAs were performed using ^{35}S-labelled oligonucleotidic probes, and densitometric analysis of the autoradiogramms were then assessed. Three weeks following 6-OHDA lesion there was a 15% reduction in the levels of both m1 and m4 muscarinic receptor mRNAs in the dorsal striatum. These results indicate that dopamine exerts a tonic excitatory influence on the expression of the two main muscarinic receptor mRNAs (m1 and m4) expressed in the striatum. We are now investigating the role of the glutamatergic cortico-striatal pathway in the regulation of the muscarinic receptor mRNAs expression in the striatum.

407.2

LOW vs. HIGH DOSE AND ACUTE vs. SUBCHRONIC HALOPERIDOL TREATMENT INDUCES DISTINCT PATTERNS OF IMMEDIATE-EARLY GENE EXPRESSION IN THE STRIATUM. B. Elibol, R. Moratalla, N. Hiroi, and A.M. Graybiel*. Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

The extrapyramidal side effects of the typical neuroleptic haloperidol (Hal) are thought to be related, in part, to its regulation of striatal activity by antagonism of D2-like dopamine receptors. Consistent with such striatal effects, neural activity mapping with immediate-early gene protein (IEGP) immunohistochemistry has shown in rat that typical neuroleptics, more than atypical neuroleptics, induce IEGPs in the sensorimotor striatum. We have examined this acute effect and also have studied the effects of repeated Hal exposure on regulation of members of the Fos-Jun family (Fos, FosB, Fras, JunB, detected by immunostaining).

Rats received either saline or one of four doses of Hal (0.25, 0.5, 2 and 5 mg/kg, i.p., at least 2 rats per treatment group) acutely or daily for 4 or 5 days and were perfused 3 hr after the last injection. Acute administration of Hal induced striatal IEGP expression with clearly different topographies at different doses. Low-doses of Hal (0.25 and 0.5 mg/kg) induced nearly homogeneous expression of the IEGPs throughout the mediolateral axis of the striatum. Higher doses of Hal not only induced increased expression, but also produced a clear predominance of expression in the lateral (sensorimotor) caudoputamen. Medial expression was weak and often patchy. These dose-related patterns changed markedly with repeated Hal exposure. After subchronic treatment, high-dose Hal induced the IEGPs in a pattern similar to that induced by acute low-dose Hal: the IEGPs had a nearly even mediolateral expression pattern. These results imply differential up-regulation of the IEGPs medially and down-regulation of the IEGPs laterally in the striatum. Subchronic low-dose Hal diminished striatal IEGP expression throughout the caudoputamen. The induction of IEGPs in the striatum by neuroleptics may be mediated not only through modulation at the postsynaptic level, but also by presynaptic actions on dopaminergic and glutamatergic afferents. We did not distinguish among these possibilities, but our evidence does suggest that repeated exposure to neuroleptics can bring about major shifts of expression patterns in the striatum. (Supported by NIH Javits Award NS25529 and Dystonia Medical Research Foundation). We thank to Drs R. Bravo and M.J. Iadarola for their gifts of the antiserum.

407.3

ACUTE AND CHRONIC PSYCHOMOTOR STIMULANTS INDUCE DIFFERENT SETS OF IMMEDIATE-EARLY GENES IN THE STRIATUM. R. Moratalla*, B. Elibol, A.M. Graybiel, MIT, Brain and Cognitive Sciences, Cambridge, MA 02139.

The molecular mechanisms by which psychomotor stimulants produce behavioral changes in animals and humans remain unknown. However, treatments with cocaine and amphetamine have been found to induce long-term changes of specific proteins in target neurons (a form of neural plasticity). Transcription factors, including members of the Fos and Jun families, are expressed in neurons in response to treatment with these drugs, suggesting that plasticity at the transcriptional level may underlie some of the changes in protein expression observed.

In the present study, we investigated the effects of acute and chronic treatments with cocaine and amphetamine on the induction in the rat striatum of immunohistochemically detected protein products of the immediate-early genes (IEGs) *fos*, *jun* B and *fra*. For chronic treatment, cocaine (25 mg/kg) or amphetamine (5 mg/kg) was administered twice a day for 8 days. For acute treatment, saline was administered for 7 days and cocaine or amphetamine was given on the 8th day. Brains were taken for processing 2 hours after the last treatment. The acute cocaine and amphetamine treatments resulted in the induction of Fos-, Jun B- and Fra-like immunoreactivity (LI) in the striatum in anatomical patterns specific for each drug, as reported previously. In animals treated with chronic cocaine or amphetamine, Jun B- and Fra-LI were still detectable in these specific patterns: cocaine induced broad expression, amphetamine a striosome-selective pattern. By contrast, Fos-LI was almost completely absent after chronic cocaine treatment, and was markedly down-regulated after chronic amphetamine treatment. These experiments suggest that Jun B and Fra, by having maintained responsiveness, may be involved in mediating some of the long-term effects of chronic exposure to psychostimulants. Supported by NIDA 5RO1 DA08037 and Dystonia Med. Res. Fdtn. We thank Drs. R. Bravo and M. J. Iadarola for their gifts of antiserum.

407.4

DOPAMINE RECEPTORS AND CALCIUM CHANNEL ACTIVATION REGULATE PHOSPHORYLATION OF CREB IN ORGANOTYPIC CULTURES OF STRIATUM. F.-C. Liu* and A.M. Graybiel. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Phosphorylation of CREB (cAMP response element-binding protein) by activation of cAMP or voltage sensitive calcium channels (VSCC) can regulate expression of the immediate-early gene *c-fos* in PC 12 cells. In the present study, we tested whether phosphorylation of CREB was associated with Fos induction in an organotypic culture of neonatal striatum (Liu et al., 1993). Stimulation of cultured slices for 30 min with SKF-81297 (D1 agonist, 100 nM), forskolin (adenylate cyclase agonist, 10 μM) or BAY K 8644 (L-type VSCC agonist, 1 μM) resulted in phosphorylation of CREB in cultured striatum as detected by immunostaining with an antiserum recognizing Ser^{133}-phosphorylated CREB-like protein (PCREB) (Ginty et al., 1993). The expression of PCREB was also suggested by Western blotting, which showed induction of a 43 kD protein by forskolin. The association of PCREB and Fos-like protein (FLP) expression in cultured striatum was shown by 1) their correlated patterns of spatial expression, both being more pronounced in DARPP-32-positive developing striosomes after SKF-81297 stimulation and being striosome-matrix inclusive after forskolin and BAY K 8644 stimulation; 2) the parallel reduction of SKF-81297-induced PCREB and FLP expression by D1 antagonists (SCH-23390, 1 μM) and 3) the lack of PCREB and FLP expression in response to D2 agonists (quinpirole, 10 μM) and protein kinase C activators (phorbol ester, 80-800 μM). However, pretreating SKF-81297- or forskolin-treated slices with the kinase inhibitor H7 (50 μM) abolished FLP expression without affecting PCREB expression in the cultured striatum and with only 13% reduction in protein synthesis. Uncorrelated expression of PCREB and FLP was also observed in BAY K 8644-treated slices pretreated with the VSCC antagonist, nifedipine (1 μM), in which the decrease of PCREB expression was more pronounced than that of FLP expression. These results suggest that expression of PCREB and FLP may be subject to differential regulation in the developing striatum. We thank Drs. D.D. Ginty and M.E. Greenberg for providing PCREB antiserum. Supported by NIH 1 R01 HD28341 and grant #3244A from The Council for Tobacco Research-U.S.A., Inc.

407.5

INCREASE OF DOPAMINE RELEASE BY STIMULATION OF THE CHOLINERGIC PEDUNCULOPONTINE NUCLEUS STUDIED BY INTRASTRIATAL MICRODIALYSIS.

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The pedunculopontine nucleus (PPN) is large cholinergic neurons located bilaterally in the lower midbrain and pons extending from the caudal pole of the red nucleus to the parabrachial nucleus. Anatomically, the projection from the PPN to the substantia nigra (SN) is well known. Since constant ipsiversive circling behavior was observed after the chemical stimulation of the unilateral PPN of the rat, activation of the mesencephalic dopaminergic neurons by the cholinergic PPN was hypothesized.

Adult male Wistar rats were used under the anesthesia with urethane. A probe for microdialysis was inserted into the striatum and samples of each 10 min were assayed with HPLC. For the stimulation of the PPN, ibotenic acid (IBO) (7.5μg/0.75μl) was injected with a Hamilton syringe. In order to verify the activation of mesencephalic dopaminergic neurons to be mediated by the cholinergic transmission, scopolamine (200μg/2μl) was injected into the SN with a Hamilton syringe. One week after the experiment, the rats were perfused and the location of the probe and the injection syringes were histologically examined.

DOPAC in the striatum increased gradually and reached plateau about 2 or 3 hours after the operation. IBO was injected at this stage. After the injection of IBO into the PPN, DOPAC increased about 60%, while 5HIAA remaind in the baseline. Then, scopolamine was injected to the SN. After the injection of scopolamine, DOPAC in the striatum decreased transiently.

It is concluded that the ascending cholinergic pedunculopontine projection to the SN modulates the striatal dopaminergic release.

407.6

BLOCKADE OF NMDA AND MUSCARINE RECEPTORS IN THE NUCLEUS ACCUMBENS CAUSE MICE TO ROTATE IN OPPOSITE DIRECTIONS. A. Svensson*, M.L. Carlsson and A. Carlsson, Department of Pharmacology, University of Göteborg, S-413 90 Göteborg, Sweden.

The effects on rotational behaviour of glutamate blockade and acetylcholine blockade in the nucleus accumbens were studied in male NMRI mice with different tones in the dopaminergic system.

We have previously shown that a unilateral injection into the nucleus accumbens of the competitive N-methyl-D-aspartate (NMDA) receptor antagonist AP-5 causes the animals to rotate. The rotation is predominantly ipsilateral in animals with intact dopaminergic systems. In contrast, dopamine-depleted mice, pretreated with reserpine and α-methyl-tyrosine, rotate exclusively contralaterally. We have proposed that the ipsilateral rotation is due to interference with the direct striatothalamic pathway of the basal ganglia motor circuit, whereas the contralateral rotation is due to interference with the indirect striatothalamic pathway.

In contrast to AP-5, the muscarine receptor antagonist methscopolamine induced predominantly contralateral rotation both in dopamine-depleted and intact animals, when injected unilaterally into the nucleus accumbens. Consequently, it seems as if acetylcholine in the nucleus accumbens primarily interferes with the indirect striatothalamic pathway of the basal ganglia motor circuit.

407.7

REGULATION OF Na^+,K^+-ATPase ACTIVITY BY PROTEIN KINASE C PHOSPHORYLATION IN RAT NEOSTRIATUM. G. L. Snyder*[1], G. Fisone[1], A. Nishi[1,2], M. J. Caplan[3], A. Aperia[4] and P. Greengard[1]. [1]Lab. of Mol. and Cell. Neurosci., The Rockefeller Univ., NY, NY 10021; [2]Dept. of Pediatrics, Kurume Univ. School of Medicine, Kurume, Japan; [3]Dept. of Cell. and Mol. Physiol., Yale Univ. School of Medicine, New Haven, CT 06510 and [4]Dept. of Pediatrics, Karolinska Institute, Stockholm, Sweden.

The ion pump Na^+,K^+-ATPase is a ubiquitous integral membrane protein formed by a catalytic α subunit and a β subunit, which transports Na^+ from the inside to the outside of the cell, in exchange for K^+. This enzyme is of fundamental importance in creating and maintaining the electrochemical gradient across the plasma membrane underlying resting and action potentials in neurons. Previously, we showed that, *in vitro*, phosphorylation of purified Na^+,K^+-ATPase α subunit by either cAMP-dependent protein kinase or protein kinase C (PKC) significantly inhibits its activity. Three different isoforms of Na^+,K^+-ATPase α subunit have been identified in neurons, their distribution differing in the various brain regions. We detected both α1 and α3 isoforms in primary cell cultures from rat striatum. We found that addition of the activator of protein kinase C, phorbol 12,13-dibutyrate (PDBu) (5 μM), to ^{32}P-prelabeled slices from rat striatum stimulates by several-fold the phosphorylation of the α1 subunit and also increases the phosphorylation of the α3 subunit. In agreement with the data obtained *in vitro*, treatment of striatal neurons with PDBu inhibited Na^+,K^+-ATPase activity by 25%. These data provide evidence for regulation of Na^+,K^+-ATPase activity, and hence neuronal excitability, by PKC phosphorylation in the striatum. [Supported by USPHS grant MH 40899].

407.8

INTRASTRIATAL NMDAR1 GLUTAMATE RECEPTOR SUBUNIT ANTISENSE OLIGONUCLEOTIDES INDUCE IPSILATERAL ROTATION

D.G. Standaert*, C.M. Testa, Z. Hollingsworth, G. Rudolf, J.B. Penney, Jr., and A.B. Young. Dept. of Neurology, Mass. Gen. Hosp., Boston, MA 02114.

NMDA glutamate receptors, composed of subunits from a family of at least twelve structurally related proteins, have an established role in the regulation of motor behavior by the basal ganglia. Several of the NMDA receptor subunits exhibit anatomically restricted patterns of expression, so that the components of the basal ganglia have distinct NMDA receptor subunit mRNA phenotypes (J. Comp. Neurol. 343:1-16 (1994)).

We have used *in vivo* intrastriatal injection of synthetic antisense oligonucleotides to examine the roles of particular NMDA receptor subunits in the regulation of motor behavior. Injection of 15 nmoles of a 20mer oligo targeted to the NMDAR1 subunit (a region common to all of the observed isoforms) induced ipsilateral rotation. This effect was maximal between 18 and 24 hours after a single injection. Smaller doses did not lead to spontaneous rotation, but prominent ipsilateral rotation was observed after administration of D-amphetamine. *In situ* hybridization demonstrated dose-dependent reduction in NMDAR1 mRNA in the injected striatum, while NMDAR2B and actin mRNA were not reduced. Conversely, injection of an antisense oligo targeted to the NMDAR2B subunit reduced the striatal content of mRNA for that subunit, without affecting NMDAR1 mRNA. NMDA-displacable [3H]-glutamate ligand binding sites were not altered by injection of NMDA subunit antisense oligos in this paradigm.

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407.9

ELECTROCHEMICAL EXAMINATION OF THE CONNECTIONS BETWEEN THE MESOPONTINE TEGMENTUM, VTA AND NUCLEUS ACCUMBENS IN THE RAT. C.D. Blaha, L.F. Allen, W.L. Inglis, S. Das, M.P. Latimer, S.R. Vincent and P. Winn* Depts. Psychol. (CDB) & Psychiat. (SD, SRV), Univ. British Columbia, Vancouver B.C., V6T 1Z4, Canada and School of Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU (LFA, WLI, MPL, PW)

The effects of cholinergic stimulation of ventral tegmental area (VTA) on DA efflux in the nucleus accumbens (NAcc) (measured by *in vivo* chronoamperometry: *Blaha and Jung, 1991, J. Electroanal. Chem. 310: 317-334*) were investigated in intact, pedunculopontine tegmental nucleus (PPTg) lesioned and laterodorsal tegmental nucleus (LDTg) lesioned rats under urethane anesthesia. Using stearate-modified electrodes, a dose-dependent increase in oxidation current in NAcc corresponding to DA efflux was found following microinjection into VTA of nicotine (0.2 mM, 132±4% [maximal increase as a percentage of baseline]; 2.0mM, 276±14%). Increasing extracellular concentrations of ACh in VTA by infusion of the anticholinesterase neostigmine (NEO) also increased NAcc DA efflux (0.5mM, 580±36% 1.0mM, 1044±49%). Rats with ibotenate (IBO) lesions of PPTg showed no difference to controls in their NAcc DA response to stimulation of VTA with NEO (lesion: 523±22%; control 580±36%) but rats with IBO lesions of the LDTg showed a significant ($p<0.001$) attenuation of NAcc DA efflux in response to stimulation of VTA with NEO (lesion: 235±10%; control 580±36%). These studies show the existence of a functional cholinergic innervation of the VTA from the LDTg but not PPTg. Since lesions of the PPTg have been shown to attenuate DA efflux in the caudate-putamen after injections of NEO into the SNC (with an increase in the response to nicotine - *Blaha and Winn, 1993, J. Neurosci. 13: 1035-1044*) we can conclude that cholinergic neurons in the mesopontine tegmentum are indirectly involved in regulating DA activity in the striatum by virtue of connections in the midbrain: PPTg (Ch5) with SNC, VTA (Ch6) with VTA.

407.10

NADPH DIAPHORASE EXPRESSION IN FRESHLY DISSOCIATED RAT CAUDATE-PUTAMEN NEURONS. Jonathan E. Freedman*, Gabriela J. Greif and Yong-Jian Lin. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

It is known that only about 2% of the neurons in the rat caudate-putamen express NADPH diaphorase *in situ*. In the course of characterizing freshly dissociated rat striatal neurons which express a dopamine-modulated K^+ channel in patch-clamp recordings (accompanying abstract), we have stained the dissociated cells for NADPH diaphorase using the nitro blue tetrazolium method. About 60% of "large" dissociated cells were stained for diaphorase, and about 40% were immunoreactive for GABA. In preliminary double-labeling studies, there were no obvious cases of "large" cells labeled for both markers. The diaphorase-containing cells tended to be somewhat rounded and pyramidal-like in shape, whereas the GABA-containing cells tended to be more angular, similar to the unfixed cells expressing the dopamine-modulated K^+ channel. Among "small" cells, about 60% expressed diaphorase and about 60% expressed GABA, and we observed some doubly-labeled cells. Our results indicate either that the dissociation procedure differentially selects for diaphorase-containing cells, or induces this enzyme, or both. Interestingly, this high level of NADPH diaphorase expression in the dissociated cells resembles that seen in the brains of patients with Huntington's disease. (Supported by MH-48545.)

407.11

IMMUNOCYTOCHEMICAL PROPERTIES OF RAT CAUDATE-PUTAMEN NEURONS EXPRESSING DOPAMINE-MODULATED K^+ CHANNELS. Gabriela J. Greif*, Yong-Jian Lin, June-Chih Liu, Barbara L. Waszczak and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

We have previously used patch-clamp recording to describe an 85 pS K^+ channel which is modulated by D_2-like dopamine receptors on freshly dissociated rat corpus striatum neurons. We are now using some cytochemical methods to identify the cells expressing this channel. Under phase-contrast optics, dissociated cells could be placed in two broad morphologic categories: "large" multipolar cells with diameters ≥10 μm, and "small" unipolar cells <10 μm. The 85 pS channel was observed in about 26% of cell-attached patches from "large" cells, mainly on cells with a characteristic angular shape, and never on "small" cells. Virtually all "large" cells were immunoreactive for neuron-specific enolase (NSE) and negative for glial fibrillary acidic protein (GFAP); 70% of the "small" cells expressed NSE and 30% expressed GFAP. Many of the angularly-shaped "large" cells were immunoreactive for GABA. In preliminary studies, some "large" cells of this morphology were retrogradely labeled with fluorescent microspheres injected into the substantia nigra. We conclude that the dopamine-modulated K^+ channel is selectively expressed on a subpopulation of striatal neurons, some of which may be GABAergic medium spiny projection neurons.

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407.12

GAT-1 GABA TRANSPORTER mRNA IN RAT BRAIN: CELLULAR CO-EXPRESSION WITH GAD67 mRNA AND PARVALBUMIN mRNA.

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The re-uptake of GABA into pre-synaptic nerve terminals terminates GABA signalling. Several GABA transporter cDNAs have been cloned including GAT-1, which display micromolar affinity for GABA when transfected *in vitro* (1,3). It has been suggested that cells which tonically release GABA may utilise more GAD67 than GAD65; two forms of the GABA synthetic enzyme (2). Expression of the calcium-binding protein parvalbumin (PV) is associated with fast-firing cells in the rat brain. This study aimed to determine if GAT-1 mRNA was expressed by GAD67 cells, and if PV cells represented a subgroup of GAD67-GAT-1 cells.

Cellular sites of (i) GAD67 and GAT-1 mRNAs, and ii) GAD67 and PV mRNAs were visualised using a combination of alkaline phosphatase (AP)- and 35S-labelled oligonucleotides. Strong hybridization signals for these three probes were detected in discrete cells within most brain regions including the cerebral cortex, basal ganglia, hippocampus and cerebellum. Examination of silver grain deposits overlying AP-positive cells showed that most high-GAD67 cells were enriched in GAT-1 mRNA, although the expression of GAT-1 mRNA was more widespread; a divergence of the two signals was seen in the reticular thalamus and inferior colliculus. PV mRNA was detected in some high-GAD67 cells. Detailed analysis of the striatum will be presented. These data suggest strongly that GAT-1 is a pre-synaptic marker of most high-GAD67 GABA cells *in vivo* and that tonically active striatal GABA/PV cells may utilise GAT-1 for re-uptake.

(1) Borden *et al.*, 1992 J Biol Chem 267 21098-21104; (2) Erlander *et al.*, 1991 Neuron 7 91-100; (3) Guastella *et al.*, 1990 Science 249 1303-6.

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407.13

PARTIAL LESIONS OF THE NIGROSTRIATAL DOPAMINE PATHWAY ALTER SUBSTANCE P BUT NOT ENKEPHALIN mRNAs IN THE RAT STRIATUM. L.K. Nisenbaum*, S.T. Kitai, W.R. Crowley, and C.R. Gerfen. Depts. of Anatomy/Neurobiology and Pharmacology, Univ. of Tennessee, College of Medicine, Memphis, TN 38163, and Sec. of Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

The expression of striatal peptide mRNAs is regulated by the nigrostriatal dopamine (DA) pathway. For example, near total depletion of striatal DA levels results in an increase in enkephalin and a decrease in substance P mRNAs. However, it is unknown whether partial depletions of striatal DA content produce similar changes in these peptide mRNAs. To test whether compensations in DA synthesis and release following partial DA depletion prevent the lesion-induced alterations in enkephalin and substance P mRNAs, varying concentrations of 6-OHDA were injected unilaterally into the substantia nigra. Seven days after injection of 6-OHDA (2-16 µg) or vehicle, *in situ* hybridization was employed to examine tyrosine hydroxylase mRNA in the substantia nigra and enkephalin and substance P mRNAs in the striatum. The extent of the DA depletion was determined by measuring striatal DA tissue content. Subjects were divided into three groups based on the extent of striatal DA depletion: <50%; 50-90%; and >90%. The decrease in tyrosine hydroxylase mRNA closely paralled the change in striatal tissue DA content in all groups. Although no significant change in substance P mRNA was detected in rats with <50% DA depletion, a 24% and 68% decrease was observed in the 50-90% and >90% depleted groups, respectively. In contrast, a significant increase in enkephalin mRNA was not detected until a >90% depletion of striatal DA was produced. In summary, alterations in tyrosine hydroxylase mRNA in the substantia nigra are well correlated with decreases in striatal tissue DA levels. In addition, whereas partial lesions of the nigrostriatal DA pathway produce a decrease in substance P mRNA, a near total depletion of striatal DA levels is necessary to increase enkephalin mRNA. *Supported by USPHS grant NS26473, the United Parkinson Foundation and the Human Frontiers Program.*

407.14

IDENTIFICATION OF STRIATAL NEURONS SHOWING POTENTIATED *ZIF268* EXPRESSION TO COMBINED D1-D2 DOPAMINE RECEPTOR STIMULATION. C.R. Gerfen* and K.A. Keefe Section of Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

Activation of D1-dopamine receptors causes induction of immediate early genes in striatum. Numerous studies have shown that this induction occurs predominately in D1-containing striatonigral neurons. We previously have shown that stimulation of D2-dopamine receptors causes a decrease in basal expression of *zif268* in striatum (Keefe & Gerfen, *Soc. Neurosci. Abstr*, 1993). Combined stimulation of D1 and D2 receptors, however, produces enhanced expression of immediate early genes in the dopamine-depleted striatum relative to that seen in response to D1-receptor stimulation alone. This manifestation of D1-D2 receptor synergy is a consequence of D1- and D2-receptor activation in striatum. Because D1 and D2 receptors are differentially expressed by striatonigral and striatopallidal neurons, respectively, we are interested in the identity of striatal neurons showing decreased *zif268* expression in response to D2-receptor stimulation and increased expression to combined administration of D1- and D2-receptor agonists. A double-labeling *in situ* hybridization approach is being used with ribonucleotide probes complementary to mRNAs for the D1-dopamine receptor, the D2-dopamine receptor, preproenkephalin, and *zif268* labeled with either ^{35}S or digoxigenin. Co-localization of the signal for *zif268* with selective markers for striatonigral and striatopallidal pathways should allow us to determine whether striatopallidal neurons show decreased expression of *zif268* in response to D2-receptor stimulation, and whether the enhanced expression seen in response to combined D1-D2 receptor stimulation occurs in those neurons that respond to stimulation of the D1 receptor alone or reflects induction in a novel population.

407.15

DIFFERENTIAL INVOLVEMENT OF NMDA RECEPTORS IN STRIATUM IN D1-DOPAMINE RECEPTOR-MEDIATED BEHAVIOR AND IMMEDIATE EARLY GENE EXPRESSION. K.A. Keefe* and C.R. Gerfen. Section of Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

Dopamine's effects in striatum often are thought to result from its ability to modulate the response of striatal neurons to other afferents. For example, activation of D1-dopamine receptors increases conductance through the NMDA subtype of glutamate receptor. D1-receptor activation also increases expression of the immediate early genes *zif268* and *c-fos* in striatum. To further understand interactions between dopamine and glutamate in the regulation of striatal function, we examined the contribution of NMDA receptors to immediate early gene expression in the dopamine-depleted striatum by infusing NMDA receptor agents into the striatum of freely moving rats. Expression of *zif268* and *c-fos* was determined by *in situ* hybridization histochemistry. Infusion of NMDA (1 mM) for 20 min via a microdialysis probe induced contralateral rotation and increased expression of *zif268* uniformly throughout the striatum, as well as the cortex, on the side of the infusion. The induction in striatum and cortex, as well as the behavioral effects of NMDA, were blocked by co-infusion of the NMDA receptor antagonist CPP (1 mM). Such infusion of CPP into striatum also blocked the contralateral rotation and decreased the expression of *c-fos* induced by the D1 agonist SKF 38393 (2 mg/kg, i.p.). CPP did not, however, affect SKF 38393-mediated induction of *zif268* under the same conditions. The data indicate that D1-mediated changes in striatal output are dependent, at least in part, on ongoing excitatory input to the striatum, as evidenced by CPP blockade of D1-induced contralateral rotation and its partial reduction of D1-mediated *c-fos* induction. However, D1-mediated gene regulation in these neurons is not completely dependent on NMDA receptor activity, as evidenced by the incomplete blockade of *c-fos* induction and lack of effect on *zif268* induction.

407.16

DYNORPHIN OPIOID INHIBITION OF D1 DOPAMINE RECEPTOR-MEDIATED INDUCTION OF IMMEDIATE-EARLY GENES IN THE STRIATUM. H. Steiner* and C. R. Gerfen. Section of Neuroanatomy, NIMH, Bethesda, MD 20892.

Dynorphin is an opioid peptide contained in striatonigral projection neurons. In such neurons, dopamine agonists produce rapid induction of immediate-early genes (IEGs), such as c-*fos* and *zif 268*. Recent studies showed that IEG induction by the indirect dopamine receptor agonist cocaine in striatum (1) is inversely related to striatal dynorphin expression, and (2) can be suppressed with systemic and intrastriatal administration of the dynorphin (kappa opioid receptor) agonist spiradoline. These results suggest that dynorphin is involved in the regulation of dopamine input to striatonigral neurons, directly and/or indirectly, through kappa opioid receptors located in the striatum. In the present study, we examined whether dynorphin exerts direct influence on striatonigral neurons by analyzing the effects of the dynorphin agonist spiradoline (1-10 mg/kg) on IEG induction by stimulation of D1 dopamine receptors which are expressed in these neurons. Gene expression was assessed with *in situ* hybridization histochemistry. Spiradoline partially suppressed IEG induction by the selective D1 receptor agonist SKF-38393 in the dopamine-depleted striatum, while the lesser IEG response in the intact striatum was completely blocked. Striatal regions with higher levels of dynorphin/kappa receptor expression (ventral regions) showed greater inhibition of IEG induction than regions with lower levels of these mRNAs (dorsal striatum). These results suggest that kappa opioid receptors on striatonigral neurons participate in dynorphin-mediated inhibition of dopamine input to these neurons.

CONTROL OF POSTURE AND MOVEMENT VI

408.1

CHARACTERISTICS OF THE REPRESENTATION OF HAND IN SPACE FOR 3-D TACTILE LOCALIZATION. P. Dassonville* & A. P. Georgopoulos, Minneapolis VAMC Brain Sciences Center and Physiology Dept., University of MN, Minneapolis, MN 55455.

To encode the location of a tactile stimulus in 3-D space, information of the stimulus' somatotopic location must be combined with a representation of limb position. Previously, we investigated the time course of the representation of hand in space by instructing subjects to point, in complete darkness, to the 3-D location of a tactile probe presented to the fingertip during a previous arm movement (Dassonville et al. 1993). On average, subjects perceived the probe to be at the location occupied by the hand approximately 100 ms after probe onset, indicating that the dynamic representation of hand in space does not compensate for cutaneous, kinesthetic, and/or motor delays. Moreover, this representation is seen in some subjects to change with a velocity different from that of the hand itself.

In the present study, two additional problems were investigated as follows: (1) Two subjects performed the task under normal illumination. The pattern of errors observed in each subject was similar to that observed when tested in complete darkness. Thus, the internal representation of hand in space does not have an appreciable visual component. (2) Four subjects were instructed to simply state whether the probe was presented "before", "during" or "after" the movement. Probes presented <38 ms before movement onset were perceived as occurring *during* the movement, whereas those presented <145 ms before movement termination were perceived as occurring *after* the movement. Across subjects, these temporal shifts were highly correlated with the temporal shifts measured in the original localization task. Thus, the subjects' perception of movement onset and termination appears to rely on the same internal representation of hand in space that is used for 3-D tactile localization.

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408.2

REACHING ERRORS RESULTING FROM THE DEGRADATION OF AN INTERNAL MODEL. C. A. Buneo*, J. Boline, J. F. Soechting, and R. E. Poppele. Dept.of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

We investigated, by means of simulations, possible mechanisms responsible for the systematic directional errors exhibited by deafferented patients during reaching movements (Ghez et al., 1990). Two aspects of altered feedforward control were evaluated: the inability to sense initial conditions and the degradation of an internal model. The first simulation demonstrated that the pattern of directional errors exhibited by deafferented patients could not be explained by assuming inadequate information regarding initial arm configuration. In contrast, a second simulation, which introduced random errors in torque production, produced results that corresponded more closely to the general pattern of errors exhibited by deafferented patients. We conclude that these directional errors do not result simply from a failure to compensate for inertial anisotropies, but are consistent with a degraded internal model which manifests as increased variability in the mapping between joint torques and motion.

408.3

SHORT-LASTING CONTROL SIGNALS UNDERLIE THE FASTEST SINGLE-JOINT MOVEMENTS. A.G. Feldman, S.V. Adamovich, R. Forget*, M.F. Levin. Research Ctr., Rehabilitation Inst. of Montreal, Quebec. Canada H3S 2J4

We tested two versions of the hypothesis that movements are produced by shifts in the system's equilibrium state for the fastest elbow flexions in humans: 1) that shifts end near the peak velocity of movement and 2) that shifts proceed until the end of movement. The first predicts that movement time may be significantly reduced by opposing loads without changes in the control pattern. Subjects flexed their elbows about 65° (control trials) and, in random test trials, movements were opposed by loads generated by a torque motor. Subjects had no visual feedback and were instructed not to correct arm deflections when perturbations occurred. At the end of the movement, the load was removed leading to a secondary movement to the same final position as that in control trials (equifinality). The static arm positions before unloading were related to load torques. This finding and equifinality implies that subjects could reproduce the same control patterns regardless of perturbations. Test movements opposed by a high load ended near the peak velocity of control movements. Phasic and tonic EMG patterns were load-dependent. Results indicate that the control pattern is of short duration which suggests that rather than being pre-programmed, EMG signals represent long-lasting dynamic responses of the system to the short-duration control pattern, external forces and proprioceptive feedback.

408.4

ADAPTIVE CHANGES IN TORQUE CONTROL DURING REACHING OF YOUNG INFANTS. J. Konczak*, M. Borutta, J. Dichgans. Dep. of Neurology, Univ. of Tübingen, 72076, Tübingen, Germany. (Spon: EBBS)

When newborns attempt to produce goal-directed arm movements they are faced with the problem to actively control their muscular forces with respect to external and reactive forces. We report cross-sectional data of 19 young infants between the ages of 4-15 months. They performed reaching movements to a stationary target presented at shoulder height of the infant. Time-position data of the hand, shoulder, and elbow were collected at a sampling rate of 100 Hz using an optoelectric measurement system (ELITE). We analyzed the intersegmental dynamics of 348 successful reaches to investigate how changes in torque control of the shoulder and elbow joint determined the kinematics of hand trajectory formation. Our results show: A) As expected, kinematic profiles improved over age. With increasing age the number of movement units and the curvature of the hand trajectory decreased. B) The ranges of mean peak muscular and motion-dependent torques (relative to body-weight) did not change significantly with age, while systematic changes in the relative timing of torque peaks were observed. We conclude that coordination emerges not primarily by regulating force **amplitude** but by modulating the correct **timing** of dynamic force production.

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408.5

CONTROL OF INTERACTION TORQUES DURING REACHING IN NORMAL AND CEREBELLAR PATIENTS. AJ Bastian*, MJ Mueller, TA Martin, JG Keating & WT Thach. Dept of Anatomy, Program in Physical Therapy, The IWJ Inst. for Rehab. Research, Wash. U. Sch. of Med., St. Louis, Mo. 63110.

Recent studies (Goodkin et al 1993, Thach et al 1992) support Gordon Holmes' observations that while cerebellar lesions affect simple movements at one joint and compound movements across several joints, the latter are affected disproportionately. From a mechanical standpoint, a multijointed movement is more complex than a summed combination of single-jointed movements due to interaction torques (e.g. inertial, centripetal, and Coriolis) generated by one linkage moving on the other. We have studied normal and cerebellar subjects performing two-jointed reaching movements under two conditions. For the "accurate" condition, seated subjects were asked to make a self-paced reach to touch a 1 cm target on a 4 cm ball hanging in front of them. For the "fast" condition, seated subjects were instructed to move as fast as possible and touch any part of the 4 cm ball. Subjects were videotaped with markers at the index finger, shoulder, elbow, and wrist joints. Marker positions were digitized (60 Hz) and joint angles and trajectories calculated. Inverse dynamic equations (Soechting and Lacquaniti 1981) were used to estimate 1) net torques and 2) interaction torques at the elbow and shoulder joints. Preliminary data indicate that, under the "accurate" condition, cerebellar subjects moved in a manner that reduced the complexity of torques by decomposing the reach and/or slowing it down. Slowing the reach also permitted use of peripheral feedback to help shape the ongoing movement. Under the "fast" condition, cerebellar subjects produced abnormal torque profiles and often overshot the target. Fast reaching movements increase the magnitude of interaction torques (Soechting and Lacquaniti 1981) and normally require subjects to account for them in a predictive manner. We are currently addressing whether abnormalities in the "fast" movements made by cerebellar patients reflect the inability to account for only interaction torques or increasing magnitudes of all torques. We speculate that the cerebellum plays a role in generating appropriate commands to account for the complex nature of the torque components during fast, multijointed movements (NIH grant NS12777).

408.6

INADEQUATE COMPENSATION FOR MECHANICAL COUPLING BETWEEN LIMB SEGMENTS UNDERLIES MISDIRECTIONS OF UPPER LIMB MOVEMENTS IN A SUBSET OF SUBJECTS WITH UNILATERAL BRAIN LESIONS. R.F. Beer*, J.P.A. Dewald and W.Z. Rymer. Depts. of Biomedical Engr. and Physiology, Northwestern University, Chicago, IL 60611

Previously we reported [1] that a subset of subjects with unilateral brain lesions exhibited a disturbance of reaching movements performed with the contralateral limb while the initiation phase of retractive movements was relatively undisturbed. In the aforementioned study, ballistic movements were performed in a horizontal plane from a central starting point to each of 16 targets located equidistantly around the circumference of a circle. Subsequent simulation studies indicated that the disturbances in hand paths occurred for target directions for which the initial direction of movement was most sensitive to a failure to adequately compensate for the mechanical coupling between limb segments in the motor command.

We have completed a second set of experiments in which the subjects were required to perform movements from the peripheral target locations to the central target. This "centripetal" paradigm resulted in more extended starting positions for the retractive movements and hence, substantially increased the magnitude of the inertial coupling torque at the elbow in comparison to movements made during the "radial" paradigm. Conversely, in relation to the radial paradigm, reaching movements were initiated from a more flexed elbow position which decreased the inertial coupling torque at the elbow.

Consistent with the change in coupling torques, retractive movements exhibited a significant misdirection in the centripetal paradigm while the misdirection of reaching movements was reduced. These results support the hypothesis that the observed disturbances of voluntary movement have their basis in an inadequate compensation for the mechanical coupling between limb segments rather than the use of specific muscle combinations. The origins of such inadequate compensation will be discussed.

This work was supported by NS 19331 to WZR.

[1] Beer et al. Soc. Neurosci. Abstr., 1993.

408.7

COORDINATION OF PLANAR TWO-JOINT ARM MOVEMENTS IN DIFFERENT DIRECTIONS. N. Virji-Babul', J.D. Cooke,. Faculty of Applied Health Science, University of Western Ontario, London, Canada N6G 1H1.

In order for coordinated movement to occur, it is generally assumed that the CNS must in some manner play an active role in counteracting the effects of interactional torques. Very few studies have directly examined the influence of such torques and identified the movement characteristics that are preserved under such compensatory conditions during two-joint movements. We examined EMG-movement relations during planar elbow and wrist movements in which the two joints moved in different directions. i.e elbow flexion - wrist extension and elbow extension - wrist flexion. Elbow and wrist movements of different amplitude combinations were performed during a visual, step-tracking task in which subjects were specifically required to attend to the initial and final angles at each joint.

Elbow kinematics were generally unaffected by concurrent wrist movement. In contrast, wrist movement trajectories were variable and wrist movement duration increased as elbow amplitude increased. Qualitative changes were also observed in the pattern of muscle activation at the wrist joint. Specifically, wrist antagonist activity preceded wrist agonist activity in conditions where elbow reaction torques could potentially produce a larger than required wrist movement.

The variability observed in wrist trajectories suggests that the CNS may not plan movement of the distal joint in terms of kinematic variables. Rather, the observed kinematic and EMG changes at the wrist joint appear to be the direct result of a strategy used by the CNS to compensate for reaction torques resulting from elbow movements, ensuring the production of movements of designated amplitudes.

408.8

DELAYED VISUAL INFORMATION SLOWS DOWN THE TIME COURSE OF PRISM ADAPTATION IN HUMAN. S. Kitazawa*, T. Kohno and T. Uka. Neuroscience Section, Electrotechnical Laboratory, Tsukubashi, 305, JAPAN.

Accurate pointing is initially disturbed when the visual field is displaced by prisms, but gradually recovers. To test if the improvement in the movement requires visual error signals that correlate in the time domain with the motor output, the rate of prism adaptation was studied with delayed visual information.

Nine subjects were trained to point rapidly at a target that appeared randomly in a square area (40x40 mm) on a tangent screen (400 mm away). Vision of the hand and the arm was always blocked during the movement by liquid-crystal shutters, and allowed 0, 50, 100, 500 or 5000 ms after the index finger touched the screen. One experiment consisted of 3 sets of 30 trials. In the first set, the subject wore no prisms and visual information was allowed after 0 ms delay. In the second, the visual field was displaced to the right or to the left by prisms (15 diopter), and visual information was available only after one of the 5 delay periods while the subjects maintained their final pointing position. One of the 2x5 conditions was used through the second set. Ten experiments, covering all conditions, were carried out on each subject. Initially the subject misreached the target by about 60 mm in the direction of visual displacement. Errors decreased with trials by an amount proportional to the error in the preceding trial (r=0.76±0.17). The rate of decrease of error was generally largest (10-40%) when the delay was 0 ms, became significantly smaller when 50 ms (median of the ratio to rates of 0 ms = 0.65) and showed little change thereafter (0.53-0.67). In the third set, the subject performed pointing without prisms and without visual delay. Initial misreaching in the direction opposite to the displacement of the second sets, reflecting the amount of adaptation in the second set, was largest with no delay: mean error was 51.2 mm with 0 ms delay and 19-43 mm with longer delays.

These results suggest that the rate of prism adaptation depends critically on visual information that closely correlates with the motor output in the time domain.

408.9

SELECTIVE PROCESSES IN THE DEVELOPMENT OF REACHING. I. SINGLE ARM TRAJECTORIES. E. Thelen*, D. Corbetta, and J. P. Spencer, Dept. of Psychology, Indiana University, Bloomington IN 47405

Developmental studies provide a window on the neural mechanisms of hand trajectory control in reaching and grasping. It is well known that during the first year, human infants' hand trajectories become straighter, smoother, and more accurate. But reaches do not develop in isolation; rather, infants learn reaching movements within a context of continuous and variable non-reaching movements of the hands and arms. Here we describe reaching development as infants' discovery of stable trajectory parameters from a wider range of movement possibilities.

We observed 4 infants (3 boys and 1 girl) weekly from 3 to 30 weeks and biweekly thereafter until 52 weeks as they reached for a toy at midline. Reaches were embedded in 14 s trials, and hand trajectories were monitored by WATSMART motion analysis at 150 Hz before, during, and after the reach. Infants first reached at 12, 15, 21 and 22 weeks and showed a dramatic improvement in trajectory straightness, smoothness and velocity modulation at 30, 32, 30, and 36 weeks, respectively. Prior to the transition, each infant had a month-long epoch of high velocity reaches, which in turn, disrupted the reach path stability. The speed increases were not unique to reaching, however, but were also characteristic of other non-reaching movements. After this active period, trajectory parameters stabilized.

Reaching improvement is not steady, linear and encapsulated. Rather, reaches are "carved out" of ongoing movements and are influenced by the ongoing movement context. Development consists of discovering consistent solutions after exploring a wide range of movement parameters.

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408.10

SELECTIVE PROCESSES IN THE DEVELOPMENT OF REACHING. II. PATTERNS OF MUSCLE ACTIVATION. J. P. Spencer* and E. Thelen, Dept. of Psychology, Indiana University, Bloomington, IN 47405

For decades, researchers have been searching for neuromotor constraints that limit the possible muscle activation patterns underlying multi-joint reaches. Developmental studies offer a unique window into the processes of exploration and selection through which suitable neuromotor constraints are assembled. Here we ask 1) is there selection of muscle activation patterns across the transition from pre-reaching to reaching; 2) is there a subset of activation patterns that is specific to reaching; and 3) what is the temporal organization of these "reaching" patterns.

We observed 4 infants weekly from 3-30 weeks and biweekly from 30-52 weeks as they reached for toys at midline. We collected EMG data (750Hz) from the following muscles: biceps, triceps, anterior deltoid, and upper trapezius. We computed the percentage of time spent in each muscle "state", where the state space consisted of all possible muscle combinations.

Several muscle states occurred prior to reaching onset but not after reach onset: TRI/BI, BI/TRAP, TRI/BI/TRAP. Conversely, DELT/TRAP did not occur prior to reach onset but increased dramatically after onset. This specific muscle state might serve to lift the arm (DELT) while stabilizing the head and shoulders (TRAP). In addition, the percentage of TRI/DELT, TRI/DELT/BI, TRI/DELT/TRAP increased as reaching skill improved. These states were specific to the reaching segment. DELT activity was distributed across the reaching segments, while TRI activity was specific to the final third of the reach. TRI activity likely functions to extend the arm toward the toy while counteracting DELT activity just prior to toy contact. These results suggest that 1) constraints on the patterns of muscle activation underlying reaching develop during the first year, and 2) these constraints are specific to the reaching task.

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408.11

SELECTIVE PROCESSES IN THE DEVELOPMENT OF REACHING. III. INTERLIMB COORDINATION. D. Corbetta* and E. Thelen. Department of Psychology, Indiana University, Bloomington, IN 47405.

Early patterns of interlimb coordination associated with the development of reaching follow highly unstable and rapidly fluctuating forms over time. Reaching patterns alternate between periods of one- and two-handedness, and, during one-handed periods, no clear hand preference emerge. One interpretation is that developmental fluctuations reflect steps of the maturing neuromotor system. Alternatively, we propose that developmental fluctuations emerge as "self-organized" properties of the system in interaction with the task.

We report the development of four infants that we followed weekly from 3 to 30 weeks and biweekly until 52 weeks as they were reaching for small toys at midline. Each week, we recorded the endpoint kinematics of both arms during multiple 14s trials using a WATSMART motion analysis system. Thus, we collected data including both reaching and non-reaching activity. Specific movement analyses allowed us to capture the interlimb patterns of both the reach itself and the ongoing activity from which the reach emerged.

We show that fluctuating patterns in reaching emerge from similarly fluctuating patterns in the general non-reaching activity. We show that the general non-reaching responses modulate as general movement speed changes: when speed increases interlimb patterns become more synchronous and reaches more bimanual; when speed decreases, synchronous and bimanual patterns dissolve. We finally show that decreases in movement speed coincide with the emergence of interlimb asymmetries, revealing a shift toward an increased right arm activity. We argue that the emergence of lateral, one-handed reaching is progressively carved out of the general movement activity, through dynamic and selective processes.

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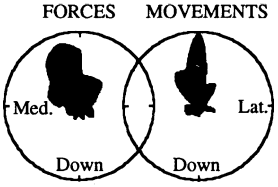
408.12

DIRECTIONAL PROPERTIES OF ARM MUSCLE ACTIVATION FOR DYNAMIC ISOMETRIC FORCES. J.J. Pellegrini* and M. Flanders, Dept. of Physiology, Univ. Minnesota, Minneapolis, MN 55455.

Recently we described how the timing and intensity of arm muscle electromyographic (EMG) activity vary with the direction of reach. After subtraction of postural activity, phasic EMG exhibited classic triphasic burst patterns. The intensity of phasic activity was a multimodal function of movement direction, rather than a unimodal cosine function.

In the current study, we examined the patterns of activity in the same muscles during dynamic isometric forces. The surface EMG of 9 elbow and shoulder muscles was recorded while human subjects rapidly exerted 10N of force at the wrist in one of 20 directions in either a sagittal or frontal plane. The onset of activity in a muscle varied with force direction: agonist bursts occurred in one range of force directions, while antagonist bursts generally occurred in the opposite range. As with reaching movements, the directional tuning of EMG intensity was multimodal. The frontal plane polar plots above illustrate that brachioradialis showed lobes of intense EMG activity (greater distance from center) for ranges of directions including and in addition to straight up. Data are from one subject; left plot from forces, right plot from 30 cm movements. Dynamic isometric forces will be useful for the study of the generation of complex motor patterns at the level of single motor units.



408.13

TEMPORAL PARCELLATION OF MOVEMENT KINEMATICS IN THE MUSCLE ACTIVITY OF MONKEYS. J. D. Coltz*, Q. -G. Fu and T. J. Ebner, Graduate Program in Neuroscience and Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Previous electrophysiological studies of the motor cortices in our laboratory (Fu et al., 1993; Fu et al., 1994, in press) revealed timing differences in the encoding of movement parameters including direction, distance, and x-y position. Specifically, these parameters are encoded separately and sequentially, with direction-related discharge occurring first, x-y position-related discharge second, and distance-related discharge last. Our paradigm required monkeys to make reaching movements in the horizontal plane, from a centrally located start position to 48 targets in 8 different directions and at 6 distances. This study extends these observations to muscle activity during the same reaching task. Intramuscular EMG's were recorded in 15 muscles and fitted to a multivariate time regression model. An ANOVA and subsequent Tukey's Test were done on a subset of 14 muscles and revealed a clear temporal ordering of parameter latency ($F=35.68$, $p<.0001$; all three means significantly different). Direction-related discharge occurred first (67± 68 ms following movement onset), followed by x-y position-related discharge (264±151 ms), and distance-related discharge (497±165 ms). Latencies for each kinematic parameter were longer, and peak R^2 values were smaller, than those for cortical cells. In addition, muscles were assigned according to their actions into upper arm, lower arm, and wrist/hand groups. Tukey's Test revealed no significant differences in discharge latency among the muscle groups for each parameter. These preliminary findings suggest that the temporal parcellation of movement parameters observed in the motor cortices is preserved at the level of muscle activation. Supported by NIH grants R01-NS18338 and R01-NS31530.

408.14

THE DYNAMICS OF GENERALIZATION OF LEARNING ACROSS DIFFERENT COORDINATION SYSTEMS. P.G. Zanone*, J.A.S. Kelso, and C. Brown III. Program in Complex Systems & Brain Sciences, Center for Complex Systems, Florida Atlantic University, Boca Raton, Fl. 33431.

Previous work [1] on perceptuomotor coordination in humans has shown that learning a new behavioral pattern can be captured as specific alterations of the underlying coordination dynamics: The to-be-learned pattern (i.e., a frequency-locked pattern between oscillating limbs with a specific relative phase) becomes an attractive state of the system's dynamics.

Here we report *spontaneous generalization* of learning between two different coordination systems. Subjects learned a visually-specified phase relation either between the arms or between the legs. Systematic probes of the arm and leg coordination conducted before and after practice showed that a) both initial dynamics are qualitatively comparable; and b) a phase relationship learned with one system (e.g., arms) is transferred spontaneously to the (untrained) other (e.g., legs), and vice-versa.

These results *across* coordination systems are in line with previous findings [2] showing automatic transfer of learning *within* the same coordination system. They suggest that learning of relative timing patterns is independent of the actual components performing the task and occurs at the abstract level of coordination dynamics. Such dynamics reflect the correlated spatiotemporal activity of the CNS and may be at the origin of flexibility in learning and performing behavioral patterns.

Research supported by NSF grant DBS-9213995 and NIMH grant MH42900.

[1] P.G. Zanone, J.A.S. Kelso, *J. Exp. Psychol.: Hum. Perc. & Perf.* (1992).
[2] P.G. Zanone, J.A.S. Kelso, *Soc. Neuroscience Abstracts* (1991).

408.15

THE SUPPRESSION AND ACTIVATION OF DEGREES OF FREEDOM IN TRAJECTORY FORMATION. J.J. Buchanan*, G.C. DeGuzman, J.A.S. Kelso and P.J. Treffner. Program in Complex Systems and Brain Sciences, Center for Complex Systems, Florida Atlantic Univ., Boca Raton FL 33431.

How does the CNS spontaneously recruit/suppress biomechanical degrees of freedom (*df*) during the performance of goal directed acts? We examined the ability of subjects to trace a sequence of six arcs, each with a different curvature, displayed on a computer screen. The arcs were presented in a decreasing (most to least curved) and increasing (least to most curved) series. The task was to rhythmically trace the arcs with the hand in the sagittal plane at a fixed frequency. Kinematically monitored motions were restricted to flexion and extension of the wrist, elbow and shoulder. Only a few coordinative patterns among the three joints were observed, indicating the existence of constraints. For the most curved arc, wrist flx-ext was coordinated with elbow flx-ext with small amplitude (if any) motion of the shoulder (pattern 1). For the least curved arc, two different patterns were observed: wrist flx-ext was coordinated with elbow ext-flx with small amplitude (if any) motion of the shoulder (2a); or elbow flx-ext was coordinated with shoulder flx-ext, with a reduction or loss of wrist motion and an increase of shoulder motion (2b). On some occasions, the frequency ratio between the shoulder and elbow was 2:1, a pattern never observed between the wrist and elbow. In the decreasing series, transitions from pattern 1 to pattern 2(a or b) were observed. In the increasing series, transitions from pattern 2(a or b) to pattern 1 were observed. All coordinative patterns and their dynamics, including the suppression and activation of *df*, are accommodated by a nonlinear oscillator model that treats trajectory curvature as a control parameter and the relative phase and amplitude relations among the joints as collective variables.

This work was supported by ONR Grant N00014-J-1904 and NIMH Grant MH42900.

408.16

UPPER LIMB FLEXOR REFLEX TORQUE RESPONSES IN HEMIPARETIC STROKE SUBJECTS. J P.A. Dewald*, J.R. McGuire, R.F. Beer, J.D. Given and W.Z. Rymer. SMPP, R.I.C., and Dept. of PM&R, Northwestern Uni., Chicago, IL 60611.

In earlier studies of voluntary muscle activation in hemiparetic stroke subjects, we observed a significant increase in stereotypic coactivations of muscles acting at the elbow and shoulder. To determine the relation between sensory input and disturbed muscle synergic relations in hemiparetic stroke, flexion reflexes were compared in the impaired and unimpaired upper extremities of 3 hemiparetic stroke subjects. The effects of mildly noxious electrical stimuli (500 Hz; pulse duration 1 ms; train duration 20 ms) delivered to the index finger, thumb and little finger were studied using EMGs from 12 arm muscles along with elbow and shoulder torques measured at the wrist with a six degree of freedom load cell. A quantitative analysis of torque and EMGs responses was performed.

Earlier torque and EMG onsets were found in the unimpaired limb relative to the impaired arm in all subjects. Muscles were also recruited proximal to distal. In the impaired limb, EMG onsets were delayed in all muscles. Furthermore, segmental muscle recruitment was lost resulting in simultaneous activation of proximal and distal muscles. Static mechanical measurements in all anatomical degrees of freedom permitted a complete characterization of the flexion withdrawal response.

The flexion withdrawal response in both normal subjects and in the unimpaired limb of stroke subjects consisted of elbow flexion combined with shoulder adduction, extension, and internal rotation torque responses. The reflex response in the impaired side was significantly altered at the shoulder. There were torque reversals in two of the three shoulder torques: a change from adduction to abduction along with shoulder flexion torque generation instead of shoulder extension. The responses were consistent regardless of stimulation location. The obligatory coupling between certain elbow and shoulder muscles in the impaired limb as observed under voluntary conditions would result in the torque reversals observed during flexor reflex activation of the limb. Since the cutaneous responses were similar to those recorded during voluntary activation, it follows that cutaneous and other sources of tonic sensory input may strongly influence muscle coactivation patterns in the impaired upper extremity of hemiparetic stroke subjects. Supported by NS 19331-11 and NIDRR R&TC (Stroke) HI33B30024.

408.17

EYE-ARM COORDINATION IN PATIENTS WITH PARIETAL LOBE DYSFUNCTION. S.H.Brown*[1], K.R.Kessler[2], H.-J.Freund[2]. Ctr for Human Motor Research, Kinesiology, Univ. Michigan, Ann Arbor, Michigan[1] & Neurologische Klinik, Heinrich-Heine-Univ., Duesseldorf, Germany[2].

Recent studies have shown that the performance of eye-arm tracking tasks is impaired in cerebellar patients (Brown et al., 1993). To further investigate the neural basis of oculomanual coordination, similar experiments were performed on patients with parietal lobe dysfunction. Elbow movements were studied under 4 conditions: tracking with the eyes only, eye-arm tracking with normal visual feedback of handle (arm) and target position (NVF), and tracking during either random blanking of handle (HB) or target (TB) position. The contralateral arm was tested in all cases. No signs of major visual field defects, optic ataxia or spatial neglect were clinically apparent at the time of testing. Eye movements were recorded using infrared techniques and arm movements were recorded by potentiometers mounted below the elbow.

Mean saccadic onset times were not significantly different from control values and, in contrast to cerebellar patients, were unaffected when coupled with arm movements. Onset times were, however, significantly prolonged under TB compared to NVF conditions. Arm movement onset was delayed compared to control values but was unaffected by different visual feedback conditions. However, removal of handle position information (HB) resulted in significant end point errors (undershooting), decreased peak velocities and prolonged acceleratory phases.

Preliminary findings revealed no evidence of specific disturbances in the temporal linkage of coordinated eye and arm movements. However, visual feedback-dependent changes in movement kinematics support current views of parietal cortex function in the transformation of visual and limb proprioceptive signals into spatially coded limb motor commands. (Supported by DFG,SFB A4)

408.18

LEFT HEMISPHERE MOVEMENT CONTROL. H. Poizner*, A. Merians, M. Clark, B. Macauley, L. Rothi, K.M. Heilman. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102; VA Medical Center and University of Florida, Gainesville.

Many investigators have explored the differential roles of the left and right hemispheres in planning and executing skilled, learned movements, although few have analyzed the kinematic properties of these movements. It has been suggested that the left hemisphere is critical for the control of skilled gestures but nonetheless, errors in movement performance have also been reported in subjects with right hemisphere damage. To further clarify the differing roles of the two hemispheres in skilled movement control, 3D motion analyses were performed on the trajectories of repetitive "slicing" gestures made to verbal command by subjects with either right or left hemisphere lesions. Four subjects with left hemisphere lesions and limb apraxia, six subjects with right hemisphere lesions and seven neurologically intact subjects participated. Left-lesioned apraxic subjects, but not right-lesioned subjects, showed marked movement deficits. The apraxic subjects exhibited decoupling of the spatial and temporal aspects of the wrist trajectories, deficits in intersegmental joint synchrony, deficits in apportioning the relative arm angles and deficits producing the appropriate phase relations among arm angles. In contrast, the right-lesioned subjects in general produced normal patterns of joint kinematics and showed the normal coupling of spatial and temporal attributes of their wrist trajectories. However, both groups of brain lesioned subjects differed from control subjects in the 3D plane of the wrist motion. Whereas control subjects produced tightly overlapping sagittally oriented planes of motion, the apraxic subjects oriented their movement in the frontal plane, while the right-lesioned subjects had a major component of the movement in the horizontal plane. The specific deficits of the right-lesioned subjects in controlling the plane of motion may reflect a deficit in the representation of external space, since the plane of the hand motion depends on the position and orientation of an object in extrinsic space. The deficits of the left-lesioned apraxic subjects in both joint and wrist kinematics instead seem to reflect a deficit in these left-lesioned subjects for the plan of the movement itself.

408.19

PRODUCTION OF VOLUNTARY FINGER MOVEMENTS BY THE SUPPLEMENTARY MOTOR AREA WITHOUT PARTICIPATION OF THE PRIMARY MOTOR CORTEX. C.D. MacKinnon*, S. Kapur, M.C. Verrier, S. Houle, W.G. Tatton. Departments of Physiology and Physical Therapy, University of Toronto, and the PET Centre, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1W5

Prominent direct projections from the supplementary motor area (SMA) to the spinal cord in monkeys have been hypothesized to drive movements independently of the primary motor cortex (M1) (Dum & Strick, 1991). We tested this hypothesis by determining the location and time course of intracerebral activity time-locked to the generation of voluntary finger movements by collecting EEG for dipole source analysis (DSA) and positron emission tomography (PET) simultaneously. Experiments were conducted to directly compare unilateral serial, repetitive finger opposition movements (SMs) performed at 2 Hz to the same movements performed intermittently (IMs) at 0.2 to 0.08 Hz. EEG epochs were obtained during the IMs by back-averaging 2.5 sec. prior to the onset of EMG activity and 0.5 after EMG onset. $H_2{}^{15}O$ PET scans were performed during the collection of the EEG epochs and foci coregistered on MRI with the DSA solutions. PET data demonstrated that the SMs involved significant activation of the contralateral M1 together with other structures in the cerebello-thalamocortical pathways and minimal activation of the SMA. DSA and PET both showed that the IMs were accompanied by activity in the SMA with minimal activation of M1. The SMA dipole orientation suggested that the IMs were generated by neuronal populations in the dorsal tier of the cingulate sulcus. We hypothesize that IMs are generated via basal ganglia-thalamocortical pathways which do not involve M1 and are separate from the pathways generating the SMs. Both the SMs and IMs are presently being investigated in patients with Parkinson's disease and neuroleptic induced Parkinsonism. (C.D.M. supported by Ontario Ministry of Health Fellowship #04515.)

408.20

SCHEMA LEARNING IN MULTIJOINT PULLING. W.A. Lee*, A.M. Russo, Y-C Pai and D. Schena. Prog. in Physical Therapy & Inst. for Neuroscience, Northwestern Univ. Med. School, Chicago, IL, 60611 USA.

We studied whether perceptual-motor schema or exemplars are learned when subjects practice a multijoint pulling task that involves sagittal-plane movement of the whole body. Ten freely-standing subjects practiced impulse-like pulls against a load cell to three force targets (20, 40 and 80% of maximum) on four days, receiving feedback with decreasing frequency. On a fifth day, subjects pulled to both the training and novel (10, 50, 60 and 95%) targets, receiving no feedback. On half of the trials each day, subjects estimated the force produced before getting feedback. On Days 1 and 5, sagittal plane body motion (11 markers) was recorded, from which force components associated with the location (F_{grav}) and acceleration ($F_{inertial}$) of the body's center of mass were derived. Schema learning predicts similar actual and estimated force errors and similar F_{grav} and $F_{inertial}$ proportions for training and novel pulls, while exemplar learning predicts larger errors for novel pulls.

Actual and estimated force errors decreased significantly with practice. Higher correlations between actual and estimated forces on Day 5 suggest parallel improvements in force production and perception. Errors for the training and novel pulls were comparable on Day 5, except for 10% pulls which had greater errors than all other pulls. The regression equations between force components and pulling force were similar across conditions. These results support schema learning, but also suggest that much of the improvement may have been due to subjects' learning to parameterize an already familiar kinetic schema for force production.

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409.1

Development of gonadotropin hormone releasing (GnRH) systems in the terminal nerve and brain of the clearnosed skate, Raja eglanteria. L.S.Demski*, J.A.Beaver and J.J.Sudberry, Div. of Natural Sciences, New College of the Univ. of South Florida, Sarasota Fl, 34243.

GnRH-ir (immunoreactivity) was similar in older animals (16, 32 weeks and adult). The TN has well developed ganglia of bipolar and multipolar cells that in the smaller fish span the length of olfactory bulb. The TN may enter the brain via dorsal and ventral roots. At 10 weeks TN fascicles can be traced into the ventromedial telencephalon, from here bundles of ir-fibers extend into the septo- preoptic area and continue into the basal hypothalamus. Many fibers approach the infundibulum and portal vessels. A large ir- nucleus of bipolar and multipolar cells extends most of the length of the midbrain in all stages. In older animals, ir-beaded fibers are present in the neurohypophysis and most areas of the CNS; some may extend to the ventricular surfaces. A few scattered ir-fibers are present in the olfactory epithelium. Few ir-fibers are seen anywhere in the younger fish (7-8 weeks).

409.2

INCREASED LEVELS OF NEUROPEPTIDE Y (NPY) AND DOPAMINE (DA) IN THE MEDIAN EMINENCE OF CHICKS SHOWING EARLY GONADAL DEVELOPMENT. W.J. Kuenzel*, K. Macko Walsh, M.M. Abdel-Maksoud and J.P. Advis[1]. Dept. Poultry Sci., Univ. of Maryland, College Park, MD 20742 and [1]Dept. Animal Sci., Rutgers Univ., New Brunswick, N.J. 08903.

Early gonadal development in chicks can be induced via: 1) parasagittal hypothalamic knife-cuts, 2) chronic intracerebroventricular injection of NPY, and, 3) feeding sulfamethazine (SMZ). An experiment was conducted to determine the neural effects of feeding 0.2% SMZ to chicks beginning at one week of age. At 3 weeks of age 4 SMZ-treated chicks and 4 controls were anesthetized, perfused with saline followed by 4% paraformaldehyde and prepared for immunocytochemistry using antibodies to gonadotropin releasing hormone (GnRH) and NPY. A second group, 6 experimentals and 6 controls, was cervically dislocated, brains removed, frozen and 5 brain areas micro-dissected and prepared for radioimmunoassay (RIA) of NPY: bed nucleus (n.) of the pallial commissure (nCPa), paraventricular n. (PVN), septal area, intergeniculate n. and median eminence/infundibular n.(ME). A third group (n=5/trt) was sacrificed at 4 weeks of age. Similar to the second group, brains were frozen and 5 brain areas micro-dissected for HPLC-EC analysis of biogenic amines. Immunocytochemical results showed no significant changes in GnRH immunoreactivity (ir) in the nCPa or anterior lateral thalamic n. but significantly more NPY ir in perikarya within the ME of SMZ-treated chicks. Chicks fed SMZ also showed significantly higher NPY concentrations within the ME (1.5 ± 0.25 vs. 0.9 ± 0.09) as determined by RIA. Significantly higher concentrations dopamine and serotonin within the ME and PVN, respectively, in chicks fed SMZ. Early sexual maturation appears to be associated with elevated levels of NPY and DA within the infundibular n. and median eminence of developing chicks. Supported by USDA Competitive Grant #90-37240-5506 and MAES Comp. Grant POUL-94-061.

409.3

SEXUAL DIMORPHISM IN THE HYPOTHALAMUS AND AMYGDALA OF THE TOKAY GECKO. L.L. Bruce* and H.A. Manley. Dept. Biomedical Sciences, Creighton Univ., Omaha, NE 68178.

Sexually specific behaviors are frequently associated with sexually dimorphic brain areas. In particular, the ventromedial hypothalamic nucleus (VMH) has been associated with the female display of receptivity in lizards. We wished to determine if the VMH and two amygdalar areas that project to the VMH, the lateral amygdala (LA) and medial amygdala, have sexually dimorphic volumes or cell sizes in a lizard, the Tokay gecko (Gekko gecko). Data was collected from 3 adult males and 3 adult females. Borders of the nuclei and somata were drawn on photographs of brain sections, then traced on a digitizing tablet linked with a computer to calculate areas and volumes. Only the values for the volume of the medial subdivision of the VMH were statistically significant, being larger in females than males. The values for the volumes of the lateral VMH and total VMH approached statistical significance with larger volumes in females. Measurements of somal areas revealed a marked sexual dimorphism, with females containing larger cells in the medial and lateral subdivisions of VMH and in the lateral amygdala. Somal areas of the other nuclei were not sexually dimorphic. Our data is consistent with previous findings in lizards and mammals that associated the VMH with sexual dimorphism and female receptivity. However, a sexually dimorphic telencephalic nucleus with larger somata in females has not previously been reported. The larger somal areas in the LA and VMH of female geckos and the strong projection from the LA to the VMH suggest that both the LA and VMH are involved in female-specific behaviors.

409.4

UNIQUE DISTRIBUTION OF CRH-IR RELATIVE TO AVT-IR IN THE BRAIN OF THE SADDLEBACK WRASSE: A NOVEL CHARACTERISTIC OF SEX REVERSING VS. GONOCHORISTIC FISH. A.A. Ghazanfar* and M.S. Grober. Department of Biological Sciences, University of Idaho, Moscow, ID 83844-3051.

The saddleback wrasse *Thalassoma duperrey* is a sequential hermaphrodite that begins life as a subordinate female or male and may then transform into a terminal male via sex or role change. Terminal male fish aggressively dominate subordinate fish and this social stress may function to inhibit sex/role change. In fish, corticotropin-releasing hormone (CRH) and arginine vasotocin (AVT) are the primary hypothalamic secretagogs in the stress response. CRH and AVT cells in teleosts show a robust and overlapping distribution in the magno- and parvocellular preoptic area (POA). Immunocytochemical studies on the saddleback wrasse showed that the distribution of AVT-ir cells reflects this general teleost pattern. Conversely, CRH did not conform to this pattern: in all but one individual, CRH-ir somata were entirely absent from the POA. However, there were a unique group of CRH-ir cells located in Hypothalamus ventralis (Hv). A comparative study using goldfish (*Carassius auratus*), a gonochoristic teleost, was conducted to assess the specificity of our results. Intense CRH and AVT immunolabeling in the magno- and parvocellular POA indicated that 1) the goldfish is representative of the general teleost pattern, and 2) our results with *T. duperrey* represent a novel feature of a sex reversing fish. While the paucity of CRH-ir somata in the POA may be indicative of negative feedback from stress-induced elevation in cortisol, the unique group of cells found in Hv may play a role in stress-mediated control of sex reversal. The present results underscore the importance of these fish as a model system for investigations of neuroendocrine control of vertebrate sexual plasticity. Supported by NSF (IBN-9309555 to MSG and REU to MSG/AAG) and a University of Idaho Seed Grant.

409.5

GLUTAMATE RECEPTOR (GluR) SUBUNIT LOCALIZATION IN PIGEON TELENCEPHALON. C.L. Veenman*, Q. Chen, and A. Reiner. Dept. Anatomy & Neurobiology, Univ. Tennessee, Memphis, TN 38163.

We used specific antibodies (AB) recognizing the GluR1, GluR2/3, and GluR4 subunits of the AMPA receptor family (Chemicon) and the GluR5,6&7 subunits of the kainate receptor family (Pharmingen) to localize these ionotropic glutamate receptor subunits in pigeon telencephalon. The GluR1 AB labeled medium and large neurons throughout the densely labeled neuropil of dorsal and ventral striatum, while the neuropil and large projection neurons of dorsal and ventral pallidum remained unlabeled. Scattered neuronal perikarya were labeled for GluR1 throughout the pallium, while the pallium externum, archistriatum dorsale, nucleus taenia, hippocampus, prehippocampal area, and hyperstriatum dorsale and ventrale stood out by their densely labeled neuropil. The GluR2/3 AB labeled many medium and large neurons throughout the well labeled neuropil of the striatum and lightly labeled large cells in the pallidum. All pallial regions possessed very abundant populations of GluR2/3 neurons, particularly the ectostriatum and field L, while the hippocampus also possessed a densely labeled neuropil. The GluR4 AB labeled large cells throughout the striatum and the pallidum, and many neurons scattered throughout all pallial regions. The GluR5,6,7 AB labeled large and medium neurons in striatum and pallidum, including small populations of intensely labeled cells in medial striatum. GluR5,6,7 neurons were labeled throughout all pallial regions, including intensely labeled cells in the hippocampus and prehippocampal area. The distributions of these GluR subunits in the avian telencephalon share many similarities with those found in mammals, indicating similarly important roles for glutamatergic transmission in the avian pallium, and also for pallial and diencephalic inputs to the avian basal ganglia. Supported by NS-19620, NS-28721 (A.R.), & Neuroscience Center for Excellence (Q.C.)

409.6

CHOLINERGIC PROJECTION FROM THE BASAL FOREBRAIN TO THE CORTICAL AREAS IN PIGEONS. L. Medina*, C.L. Veenman, C.A. McCandlish, & A. Reiner. Dept. Anat. & Neurobiol., College of Medicine, University of Tennessee, Memphis, TN 38163.

A cholinergic projection from the basal forebrain to the cortex is present in several mammalian species, and is related to basic cognitive operations such as learning and memory (1). To investigate the presence of a similar projection in birds, White Carneaux pigeons were injected with Fluorogold (FG) in either the Wulst or the external part of the dorsal ventricular ridge (DVRe, called pallium externum in our previous works), which are avian cortical regions known to be densely innervated by cholinergic fibers (2). After FG injections in either the Wulst or the DVRe, numerous retrogradely labeled cells were observed ipsilaterally in basal forebrain structures known to contain cholinergic neurons (2), such as the paleostriatum primitivum, the intrapeduncular nucleus, the fasciculus prosencephalicus lateralis, and less frequently in the ventral paleostriatum. Immunohistochemistry for choline acetyltransferase (ChAT) revealed that about 60-75% of the retrogradely labeled cells were also ChAT immunoreactive. Our results provide evidence for the presence of a cholinergic as well as a non-cholinergic projection from the basal forebrain to the Wulst and DVRe in pigeons, and add proof for the similarity of the telencephalic cholinergic systems of birds and mammals. As suggested in mammals (1), the cholinergic input from the basal forebrain to the Wulst and DVRe in birds may play a role in the involvement of these cortical structures in plasticity and learning.

(1) Woolf (1991) Prog. Neurobiol., 37:475-524.
(2) Medina and Reiner (1994) J. Comp. Neurol., 342:497-537.

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409.7

IMMUNOHISTOCHEMICAL ORGANIZATION OF THE FOREBRAIN IN THE WHITE STURGEON. C. Piñuela and R. G. Northcutt*. Neurobiology Unit, Scripps Institution of Oceanography, and Dept. of Neurosciences, School of Medicine,Univ of Calif., San Diego, La Jolla, CA 92093-0201.

To determine the pallial and subpallial divisions in ray-finned fish, the distribution of leucine-enkephalin (LENK), substance P (SP), tyrosine hydroxylase (TH), serotonin (5HT) and dopamine (DA) was determined in the forebrain of an actinopterygian, *Acipenser transmontanus* (Chondrosti). Immunoreactive TH, SP, LENK, 5HT and DA perikarya were observed in different nuclei of the area ventralis telencephali, preoptic area, periventricular nucleus of the posterior tuberculum (PPT), and inferior lobe of the hypothalamus. In the ventral thalamus, few SP+ and TH+ somata are seen, whereas in the median nucleus of the posterior tuberculum only cells positive for TH were observed. A high number of SP+, LENK+ and TH+ fibers were located in the nuclei of the area ventralis telencephali, whereas DA+ and 5HT+ fibers were confined to the dorsal nucleus (Vd) of this area. All five substances were present in fibers innervating various diencepahlic areas. Earlier studies suggested that the pallial-subpallial boundary was located between the medial (Dm) and the dorsal (Dd) zones of the dorsal telencephalon, and that Dm may be homologous to the striatum of land vertebrates. The present results do not support this view, since Dm showed poor content of SP+ and LENK+ fibers compared to the nuclei of the area ventralis telencephali. In this respect, Vd appears highly similar to the striatum, with a very high concentration of SP+, LENK+, 5HT, and DA+ fibers. This is supported by the presence of DA+ cells in PPT, a presumed homolog of the substantia nigra of amniotes, which could be the source of DAergic input to Vd. Supported by NIH grant NS24869 to R.G.N. and Fulbright FU93 07857019 to C.P.

409.8

RAPHE NUCLEI IN THE BRAINS OF THREE CARTILAGINOUS FISHES. S. Stuesse*, D. Stuesse, Kevin Kaut, and W.L.R. Cruce. Neurobiology Department, N.E. Ohio Univ. Col. of Med., Rootstown, OH 44272.

Many nuclei that are present in other groups of vertebrates are present in cartilaginous fishes. We identified and describe raphe nuclei in three cartilaginous fishes, *Squalus*, *Heterodontus*, and *Hydrolagus*. Antibodies made in rabbit against serotonin (5HT) and enkephalin (ENK) were localized in fish brains by means of the PAP technique. Raphe cells were identified by topographic position, reaction with the antibodies, and cytoarchitectonics. The cytoarchitectonics of 5HT+ raphe cells in cartilaginous fishes are remarkably similar to that described in mammals. Raphe nuclei were clustered in inferior and superior cell groups, but within these groups, individual nuclei were identified. From caudal to rostral these nuclei are raphe (ra.) pallidus, ra. obscurus and ra. magnus in the inferior group and ra. dorsalis, ra. centralis superior, and ra. linearis in the superior group. A ra. dorsalis may not be present in *Hydrolagus*. The majority of 5HT+ cells are found in the superior group, but 5HT+ cells are present from spinal cord to caudal mesencephalon. The only sections devoid of 5HT+ cells are just caudal to cranial nerve VII. The numbers and distribution of 5HT+ and ENK+ cells are similar to each other. (Supported in part by grant NS2585 from NIH).

409.9

A MARINE CHAMELEON: THE SANDLANCE, LIMNICHTHYES FASCIATUS. J. D. Pettigrew and S. P. Collin*. Vision, Touch and Hearing Research Centre, University of Queensland, St Lucia 4072 and Department of Psychology, University of Western Australia, Nedlands, 6009, Australia.

The sandlance, *Limnichthyes fasciatus* (Creediidae, Teleostei), behaves like a marine chameleon, with independent movements of its turret-like eyes and rapid strikes from camouflage for swimming prey. The optical system has a fixed circular pupil, a deep pit fovea and a flattened lens unlike any other teleost lens so far described. The convex, laminated structure of the cornea is also unparalleled in a teleost and suggests that the cornea plays a refractive role. This suggestion has been supported by four independent sets of observations: i. Purkinje images formed underwater by the cornea; ii. Measurements of the magnification of intracorneal iridophores viewed through the corneal lenticle; iii. Measurements of the dissected corneal lenticle and lens when viewed over a grating; iv. Ray tracing experiments comparing the degree of refraction produced by both the lens and corneal lenticle. All four sets of observations confirm that the cornea of the sandlance has a substantial refractive power of approximately 200 D compared with a lens power of 550 D. This is the first report of a teleost cornea with a significant refractive role. The optical system of lens plus cornea, in combination with the deep pit fovea, may be more suitable for the detection and accurate depth localisation of small, moving prey than the conventional, wide-field, spherical lens system of teleosts. The evolutionary convergence of this marine optical system and lifestyle with those of the chameleon is remarkable given the constraints imposed by underwater optics.

409.10

IDENTIFICATION OF GLOBULAR AND ASYMMETRIC FORMS OF AN INTERMEDIATE CHOLINESTERASE IN AMPHIOXUS: IMPLICATIONS FOR THE EVOLUTION OF THE CHOLINESTERASES IN THE VERTEBRATES. L.Pezzementi*, M. Sanders, M. Miller, K. Glaese and L.A.Shannon. Division of Science and Mathematics, Birmingham-Southern College, Birmingham, AL 35254.

In vertebrates, the cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), are found in a variety of molecular forms. The globular forms, G_1, G_2, and G_4 are composed of one, two, or four globular subunits; the asymmetric forms A_4, A_8, and A_{12} consist of one, two, or three globular tetramers covalently linked to a collagenous tail. Only globular forms have been described in invertebrates. As part of an investigation of the evolution of the cholinesterases, we characterized the cholinesterase activity of the invertebrate chordate, amphioxus. The kinetic and pharmacologic characteristics of the enzyme were found to be intermediate to AChE and BuChE. These biochemical findings are supported by DNA sequence data. The cholinesterase was found in two molecular forms, sedimenting at 5.5 and 17 S on sucrose gradients. The smaller form was partially purified by affinity chromatography, labeled with [^{3}H]DFP, analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and identified as the disulfide-linked dimeric form G_2. The migration of the dimer on nondenaturing gels was modified by digestion with phosphatidylinositol-specific phospholipase C, indicating that it possessed a glycolipid anchor. The larger form was found to be collagen-tailed A_{12} asymmetric cholinesterase: collagenase digestion of the form in crude extracts or from preparative sucrose gradients increased the sedimentation rate of the enzyme. A high-salt-soluble form of G_2 was also observed. We discuss the implications of these findings for the evolution of the cholinesterases. Supported by NIH AREA 1 R15 NS249343-01 and NSF RUI BNS-9010710 to L.P.

409.11

ARE THE AMPHIBIAN "STRIATUM" AND THE REPTILIAN ANTERIOR DORSAL VENTRICULAR RIDGE HOMOLOGOUS? M.R. Braford, Jr.* Biology Department, Oberlin College, Oberlin, OH 44074

In amphibians the cell masses of the ventrolateral telencephalon are traditionally homologized to the striatum of amniotes. Comparative cytoarchitectonic studies of developing and/or adult forebrains of representatives of all three amphibian orders, together with topological, histochemical and connectional data, suggest the following new hypothesis. The "striatum" of amphibians is pallial rather than subpallial and is homologous to the anterior dorsal ventricular ridge (ADVR) of reptiles. This hypothesis is a more parsimonious interpretation of the data than the prevailing one, and it suggests that the amphibian homolog of the amniotic striatum may lie in the ventromedial telencephalic wall. It further follows that the telencephalon of all anamniotic vertebrates may be subject to a similar reinterpretation. Taken together with the idea that the reptilian ADVR is homologous to the mammalian basolateral amygdala (Neary and Bruce, 1993), the possibility is raised that a thalamo-recipient pallial amygdala is a significant and common feature of anamniotic forebrains. (Support: NSF BNS-8820858 and Oberlin College)

409.12

CAVUM SEPTUM PELLUCIDUM (CSP) IN TREATMENT RESISTANT SCHIZOPHRENIA. R. Conley*, S. Daviss, C. Gounaris, C. Tamminga. Maryland Psychiatric Research Center, Univ. of MD, Baltimore, MD 21228

A number of investigations have focussed on differences in clinical characteristics between people with schizophrenia who respond well to treatment and those who do not. Schizophrenics who fail to respond to typical neuroleptics may represent a separate population, with a distinctly different etiology and/or neuropathophysiology. Compared to treatment-responsive patients, one CT study found smaller ventricles in treatment-resistant patients (Ota et al., 1987), while another found greater prefrontal sulcal prominence in these patients (Friedman et al., 1991). Examination of the neuroanatomy in these two patient populations may provide further evidence to support a biological distinction between them. We examined MRI scans from treatment-resistant and treatment-responsive schizophrenics to determine if the treatment-resistant population had a higher prevalence of CSP. A total of 28 patients with schizophrenia who had completed MRI scans were identified. As part of a larger study, they were categorized as either treatment-responsive or treatment-resistant, as defined by the Multicenter Clozapine Study criteria (Kane et al., 1988). Of the 16 treatment-responsive patients, 2 (12.5%) had a CSP rating of "probable", and 3 (18.8%) had a rating of "definite". Of the 12 treatment-resistant patients, 4 (33.3%) had a "probable" CSP, and 5 (41.7%) had a "definite" CSP. The findings in this preliminary report suggest that schizophrenics who respond poorly to neuroleptics may have evidence of neuropathology which distinguishes them from patients who respond well to these medications. In our sample, this is reflected by the presence of a CSP in 75% of treatment-resistant patients, compared to 31% of treatment-responsive.

409.13

HIGH RESOLUTION NMRI ATLAS OF AN INFANT MOUSE LEMUR (MICROCEBUS) BRAIN OBTAINED AT 12 T.

Mark O'Dell*, Pratik Ghosh, Russell Jacobs, and John Allman. Department of Computation & Neural Systems* and Division of Biology, Beckman Institute, Caltech 139-74, Pasadena, CA 91125.

In preparation for doing functional brain imaging, we have constructed a 3D registered digital atlas of the head of a deceased infant Mouse Lemur (Primate M. *Mimurinus*) using a Bruker AMX500 12 Tesla Nuclear Magnetic Resonance Imaging system (^1H = 500 MHz). We obtained very high spatial resolution data (60 μm isotropic voxels) with strong intrinsic contrast (T_2-weighted Spin Echo 3D), making it possible to see fine anatomical detail. We show 3D volume renderings of this atlas on videotape, anatomical identifications on 2D prints, and details of data acquisition and processing.

Data was acquired using TE/TR = 100ms/1.3s with the sample at 2° C.. While some MRI contrast is likely to be different from what would be obtained from a living sample, all familiar anatomical structures are clearly visible. Fine fiber tracts, the laminations of corticies, and details of the inner ears are especially striking. Mouse Lemurs were selected because they have a highly developed visual system and are small enough to fit inside our imaging system.

409.14

MORPHOMETRIC VARIABILITY IN 3D HIGH RESOLUTION DIGITAL POSTMORTEM ANATOMY AND IN VIVO MRI

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We examined morphometric variability of selected neuroanatomic structures in high resolution digital anatomy from cryosectioned whole human head and brain and in vivo MRI. Postmortem whole human head and brain were cryosectioned and high resolution color serial images were digitized from the blockface. Serial digital images were reconstructed to 3D and aligned along the bicommissural plane as described by Talairach and Tournoux (1988). Caudate, putamen, thalamus, globus pallidus, anterior commissure, cerebellum, corpus callosum, hippocampus, and ventricular system were analyzed for surface area (SA), volume (VOL), center of mass (COM), principle axes and distance from a known landmark. We were able to use the Talairach stereotactic coordinate system as a reference for alignment and analysis of 3D reconstructed data within and between subjects and modalities. Data were in general agreement with less morphometric variability demonstrated between R-L hemispheric structures than intersubject or separate modality anatomy. Sulcal anatomy demonstrated greater variability than subcortical structures. Measures for principle axes and COM were less reliable for geometrically complicated structures such as the limbic system and hippocampus. The higher resolution of cryosectioned anatomy enabled us to localize and delineate a far greater number of neuroanatomic structures than those in MR data sets. These results indicate the potential of high resolution postmortem cryosectioned anatomy of human head and brain as a standard for digital human brain atlasing.

BRAIN METABOLISM AND BLOOD FLOW: NITRIC OXIDE

410.1

CEREBRAL BLOOD FLOW (CBF), AND NEUROLOGIC OUTCOME IN RATS SUBJECTED TO TRANSIENT FOREBRAIN ISCHEMIA (TFI) FOLLOWING CHRONIC NITRIC OXIDE SYNTHASE (NOS) INHIBITION.

V.L. Baughman, D.A. Pelligrino*, and Q. Wang. *Dept. of Anesthesiology, Univ. of Illinois-Chicago, Chicago, IL 60612.*

It is unclear if the increased presence of NO during and following cerebral ischemia is protective (e.g., via promoting vasodilation) or neurotoxic (e.g., through production of highly reactive O_2 species). These opposing actions of NO could account for some of the variability in findings present in the literature. In this study, we subjected rats to TFI, using right common carotid occlusion + hemorrhagic hypotension, and examined the effects of NOS inhibition, with nitro-L-arginine (L-NA), on: ***a)*** the CBF changes accompanying TFI, and ***b)*** neurologic outcome postischemically. In order to differentiate the potentially protective vasodilating actions of NO from other (perhaps toxic) NO actions, 2 groups of L-NA-treated rats were studied-- one (*L-NA1*, n=10) where the level of hypotension was matched to control (*CTL*, n=7) and the other (*L-NA2*, n=10) where blood withdrawal was altered to produce a CBF drop similar to that observed in *CTL*. L-NA-treated rats received daily ip injections over 3 days (300 mg/kg total) so as to achieve >95% inhibition of brain NOS (confirmed in pilot studies). On the 3rd day, under fentanyl/N_2O anesthesia, the rats were subjected to TFI. CBF (laser-Doppler) was recorded continuously. After 30 min of ischemia, the rat's blood was reinfused, the carotid occlusion was released and CBF monitoring was continued for 15-20 min. The anesthetic was then discontinued. Rats were analyzed for neurologic function over 3 days. During ischemia, the CBF reductions measured were 65%, 95%, and 70% in the *CTL*, *L-NA1*, and *L-NA2* groups, respectively. The corresponding MABP values were 31, 31, and 52 mmHg. L-NA treatment was accompanied by a greater post-ischemic hyperemic response than seen in *CTL* (160%, 253%, and 193% of baseline in *CTL*, *L-NA1*, and *L-NA2*, respectively). Neurologic outcome in the *L-NA1* group was markedly worse than *CTL*. However, the mean score in the *L-NA2* group suggested improved outcome vs *CTL*, although the difference was not significant. These results suggest that NO is beneficial during TFI, presumably via an intra-ischemic vasodilating action. That benefit may be moderately opposed, but not negated, by a neurotoxic effect of NO. The greater post-ischemic CBF in the L-NA group indicates that, in this model, post-ischemic hyperemia is not mediated by NO.

410.2

L-ARGININE REVERSES HYPOTHALAMIC VASOCONSTRICTION INDUCED BY CHRONIC NITRIC OXIDE SYNTHASE INHIBITION IN RATS.

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Chronic oral administration of the nitric oxide synthase inhibitor N^G-nitro-L-arginine-methyl-ester (L-NAME) results in increased hypothalamic vascular resistance (HVR) (Benyó et al.; Society for Neuroscience Abstracts, Vol. 19, p. 1660, 1993). The aim of the present study was to investigate whether this phenomenon can be reversed by L-arginine.

Adult male Wistar rats were treated with L-NAME dissolved in the drinking water. After a week of treatment the animals were anesthetized with ip. Urethan, and hypothalamic blood flow (HBF, H_2 gas clearance method) and mean arterial blood pressure (MAP) were determined before, and 10, 20, 30 and 40 minutes after the start of iv. L-arginine administration (30mg/kg bolus followed by 10 mg/kg/min infusion).

Results	Control	10 min.	20 min.	30 min.	40 min.
HBF (ml/g/min)	0.68±0.04	0.82±0.04	0.83±0.06	0.85±0.06	0.82±0.06
MAP (mmHg)	150±10	130±11	121±11	115±10	107±10
HVR (MAP/HBF)	225±20	162±19	151±20*	141±19*	134±16**
$apCO_2$ (mmHg)	35.2±0.7	36.3±0.9	36.5±0.9	35.5±1.1	34.1±0.9
apO_2 (mmHg)	83.6±2.4	84.0±1.4	84.5±1.8	86.8±1.7	88.6±2.2
apH	7.35±0.02	7.33±0.02	7.32±0.02	7.31±0.02	7.31±0.02

*=p<.05, **=p<.01 vs. Control; ANOVA with Dunnett's test

The results indicate that the hypothalamic vasoconstrictor effect of nitric oxide synthase inhibition induced by chronic oral L-NAME treatment can be reversed by an excess amount of exogenous L-arginine.

(Supported by OTKA I/3-1371 and OTKA F 013241 Grants)

410.3

NITRIC OXIDE SYNTHESIS INHIBITION AND THE PRODUCTION OF EARLY HYPOPERFUSION DURING SPREADING DEPRESSION IN ANESTHETIZED RATS.

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Inhibition of nitric oxide synthase (NOS) by Nω-nitro-L-arginine methyl ester (L-NAME) is known to be associated with the appearance of a transient hypoperfusion prior to the advancing wave of hyperperfusion which accompanies spreading cortical depression (SCD) in awake rats. It is assumed that this hypoperfusion is the result of potassium-induced vasoconstriction that would normally be balanced by NO-EDRF mediated vasodilation. Because cerebral blood flow (CBF) responses to SCD are known to be dependent on resting vascular tone, this process was reexamined in anesthetized rats. Rats were anesthetized with pentobarbital, 50 mg/kg i.p. Body temperature was maintained and arterial pressure, pH, gas tensions, and hematocrit were monitored. L-NAME, 30 mg/kg i.v., was given 20 minutes before SCD was induced by needle penetration of the frontal cortex. Local CBF was monitored by continuous laser-Doppler flowmetry using a Vasamedic BPM^2 0.8 mm probe placed on a parietal bone window away from dural vessels. In separate rats, regional CBF was measured 90 and 120 s after induction of SCD using the diffusible tracer [14C]isopropyliodoamphetamine and quantitative autoradiography. Continuous laser-Dopper flowmetry was unable to detect the preceding hypoperfusion during SCD in L-NAME treated rats. However, 3-dimensional reconstruction of serial autoradiographic sections reliably demonstrated this hypoperfusion when L-NAME was prepared immediately prior to injection. If the L-NAME solution (30 mg/ml in normal saline) was used more than 2 days after preparation the preceding hypoperfusion was absent despite preservation of the global reduction in CBF known to accompany NOS inhibition. This disparity in the ability of L-NAME to produce resting and stimulated changes in regional CBF suggests that differential sensitivity to nitric oxide, different degrees of NOS activity, or different forms of NOS are involved in the static and dynamic regulation of blood flow in rat brain. (Supported by PHS NS24109)

410.4

NITRIC OXIDE PLAYS DIFFERENT ROLES IN CEREBRO-VASODILATION EVOKED BY HYPERCAPNIA OR BASAL FOREBRAIN STIMULATION

F. Zhang* and C. Iadecola, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Inhibition of nitric oxide (NO) synthase (NOS) attenuates the increases in cerebral blood flow (CBF) elicited by electrical stimulation of the basal forebrain (BF) cholinergic system or hypercapnia. We sought to determine whether the effect of NOS inhibition can be reversed by re-establishing basal levels of NO. In halothane-anesthetized and ventilated rats the NOS inhibitor nitro-L-arginine (L-NA; 1 mM) was superfused on the neocortex. CBF was monitored at the site of superfusion by a laser-Doppler probe. Inhibition of NOS activity was verified using the assay of Bredt and Snyder. With Ringer superfusion, BF stimulation (100μA; 50Hz) and hypercapnia (pCO_2=55±1 mmHg) increased CBF by 214±6% (n=6) and 139±11% (n=6), respectively (p<0.001). Superfusion with L-NA reduced resting CBF by 33±4% (p<0.05), attenuated the increase in CBF elicited by BF stimulation (-37±2%; p<0.001) or hypercapnia (-65±4%; p<0.001) and reduced NOS catalytic activity by 95±3%. After L-NA, superfusion with the NO donor SIN 1 (0.1-0.5mM) re-established resting CBF (p>0.05 from before L-NA) and restored the CBF response to hypercapnia (p>0.05 from before L-NA). However, SIN 1 failed to counteract the L-NA-induced attenuation of the increase in CBF elicited by BF stimulation (p<0.05 from before L-NA). We conclude that both the CBF responses to hypercapnia and BF stimulation have NO-dependent components. However, the role of NO in these responses differs: In hypercapnia, unlike BF stimulation, the NO-dependent component can be fully re-established by a NO donor despite near total inhibition of NOS catalytic activity. The data suggest that the CBF response evoked by BF stimulation requires NOS activation, probably via cortical release of acetylcholine, whereas the hypercapnic cerebrovasodilation requires a basal level of NO for its full expression. (Supported by NS 31318 and the AHA)

410.5

NITRIC OXIDE SYNTHESIS IS REQUIRED FOR FUNCTIONAL HYPEREMIA IN CEREBELLAR CORTEX J. Li*, X. Xu and C. Iadecola, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Functional brain activation is associated with increases in cerebral blood flow (CBF) restricted to the active areas. Although the mediators of the vascular response remain to be elucidated in full, there is evidence that nitric oxide (NO), a potent vasodilator released by active neurons, may participate. We used the parallel fiber (PF) system of the cerebellar cortex as a model to investigate the role of NO in the increases in CBF elicited by neural activation. Rats were anesthetized with halothane and ventilated. The cerebellar vermis was superfused with aerated Ringer. PF were stimulated electrically (100 μA; 30 Hz) and the associated changes in cerebellar cortex blood flow (BF_{crb}) monitored by laser-Doppler flowmetry. Field potentials evoked by PF stimulation were recorded using microelectrodes. During Ringer superfusion (n=7), PF stimulation increased BF_{crb} (+52±4%). Topical application of the nitric oxide synthase (NOS) inhibitor nitro-L-arginine (L-NA; 1 mM) attenuated the increases in BF_{crb} by 50±4% (n=9; p<0.001; analysis of variance) and inhibited NOS catalytic activity, assessed by L-arginine-to-citrulline conversion, by 95±9% (p<0.001). L-NA did not influence the increase in BF_{crb} evoked by topical application of the NO donor SIN-1 (p>0.05) or the field potentials evoked by PF stimulation. D-NA (1 mM; n=6), the inactive stereoisomer of L-NA, did not attenuate the BF_{crb} response (p>0.05). The guanylyl cyclase inhibitor and NO scavenger methylene blue (1 mM; n=7) reduced the response by 41±9% (p<0.01). Thus, the increases in BF_{crb} evoked from the PF are, in part, mediated by NO. NO could be produced either in PF terminals or in post-synaptic neurons, probably, interneurons. The data suggest that NO participates in the "coupling" of functional activity to blood flow in cerebellum and that this agent is a critical mediator of functional hyperemia in the central nervous system.

(Supported by NS 31318 and by the American Heart Association)

410.6

INHIBITION OF NITRIC OXIDE SYNTHASE INDUCES A MARKED DECREASE OF THE CEREBROVASCULAR RESPONSIVENESS TO HYPERCAPNIA IN THE AWAKE RAT. G. Bonvento*, J. Seylaz, P. Lacombe *Laboratoire de Recherches Cérébrovasculaires, CNRS UA 641, Université Paris VII, 75010 PARIS, France*

The widely accepted notion that inhibition of nitric oxide synthase (NOS) attenuates the cerebral blood flow (CBF) increase elicited by hypercapnia relies mainly on studies performed in anesthetized animals (JCBFM 14:175-192, 1994). In this study, we sought to determine whether NO mediates the CO_2 responsiveness in awake rats. Regional CBF was measured in 4 groups of 5 awake, gently restrained rats, using the quantitative [^{14}C]iodoantipyrine technique and brain tissue sampling. CBF was determined 1h following NOS inhibition via i.v. injection of 30 mg/kg N^{ω}-nitro-L-arginine methyl ester (L-NAME; n=10) or saline (n=10), during normocapnic ($PaCO_2$: 34-46 mmHg) or hypercapnic ($PaCO_2$: 51-69 mmHg) conditions. L-NAME significantly decreased CBF in all 11 brain regions studied (-17 to -49%) and produced a moderate hypertension along with bradycardia. In control conditions, CO_2 responsiveness, expressed as the slope of the regression lines of the $PaCO_2$-CBF relationship, ranged from 1.3 ± 0.4 in the hypophysis to 6.4 ± 0.6 ml/100g/min/mmHg in the parietal cortex. Under L-NAME, the reactivity to hypercapnia was significantly decreased in all structures, the magnitude of the reduction ranging from 57% in the medulla (lowest density of NOS-containing neurons) to 74% in the cerebellum (highest density of NOS-containing neurons). This result suggests that neuronal NO, in addition to endothelial NO, is a mediator of the hypercapnic vasodilation in the awake rat.

410.7

THE EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON CARDIAC OUTPUT AND REGIONAL ORGAN BLOOD FLOW IN PIGLETS.
Lohe AS, Kuluz JW*, Gelman B, Prado RJ, Schleien CS. Department of Pediatrics, University of Miami School of Medicine, Miami, FL 33101.

We examined the effects of low dose N-nitro-L-arginine methyl ester HCl (LN) 0.1 and 1.0 mg/kg and subsequent reversal with L-Arginine (L-Arg) 11.0 mg/kg on cardiac output and regional blood flow in piglets. Under pentobarbital/fentanyl anesthesia, piglets were ventilated via tracheostomy and catheterized for measurement of cardiac output by thermodilution and regional blood flow using microspheres. Measurements were made after a 30 min stabilization, 15 min after LN 0.1 and 1.0 mg/kg, and 20 min after L-Arg. LN significantly decreased cardiac index (CI), myocardial (MBF), and renal (RBF) blood flow, without affecting jejunal (JBF) and cerebral blood flow (CBF). These changes were only minimally affected by L-Arg.

	Baseline	LN 0.1	LN 1.0	L-Arg 11.0
HR (bpm)	212	215	209	218
MAP (mm Hg)	115	121	131	129
CI (%)	100	85.9	61.1*	68.9*
PVRI (%)	100	134.2	201.3*	146.4
SVRI (%)	100	122.4*	190.9*	166.4*
CBF (%)	100	107.2	85.4	111.8
MBF (%)	100	101.6	71.3*	91.7
RBF (%)	100	62.5*	32.8*	39.9*
JBF (%)	100	107.5	75.6	82.4

Low dose LN does not affect CBF but causes deleterious effects on cardiac output and regional organ blood flow in piglets. We sepculate that the reduction in cardiac output was due to a direct effect on the myocardium and not a secondary effect of increased afterload, becuase cardiac output decreased so rapidly after LN administration, without a significant change in MAP.

410.8

NITRIC OXIDE SYNTHASE (NOS)- CONTAINING NEURONS INNERVATE HUMAN CEREBRAL MICROVESSELS. W.H.Zhang(1), I.Divac , M.O.Lopez-Figueroa(2) and L.Edvinsson(1)*. Dept. of Exp. Res. , Malmö Gen. Hosp. , The Univ. of Lund, S-214 01, Malmö, Sweden [1] and Dept. of Med. Physiol. , Panum Inst., The Univ.of Copenhagen, Denmark[2].

Nitric Oxide(NO) is a prominent vascular and neuronal messenger molecular generated from L-argine by NOS. Although data indicate that NOS is important in the modulation of human cerebral blood vessel relaxation, there is little information about the localization of NOS in the human cerebral circulation. In the present study, we have used NADPH-diaphorase (NADPH-d) histochemistry and NOS immunocytochemistry to human tissue in order to examine the existence and distribution of cerebrovascular neurons containing NOS. Four blocks of human temporoparietal cortex were cut on a vibratome at 50 μM. Two of five series were reserved for NADPH-diaphorase staining, two of five series were processed for immunocytochemistry for NOS reaction by a monoclonal antibody against the neuronal isoform of the enzyme and the remains was used for anatomical demonstration of neurons with Nissl staining.We found that NADPH-d and NOS positive staining is located in cells and nerver fibers surrounding cerebral vessels. Differential NADPH-d and NOS cellular and nerve fiber staining was noted at various sites of the cerebrovascular bed. In some cases, NADPH-d and NOS positive neurons in the cortex were seen close to and sending processes to the neighboring vessels; in other cases, the cell bodies were located far from the microvessels, sending processes to the blood vessels. NADPH-d/NOS positive cellular body were also situated on the wall of blood vessels and had processes that parallel with longitudinal direction of the vessels. Part of the vessel wall with stained fibers contained varicorsities. Cell bodies were oval, fusiform and triangular with one to three primary dendrites of variable length. Some of primary dendrites followed by second dendrites. The identity of neurons and fibers containing NOS in human microvessels provides morphological evidence for a role of NO in modulating human cerebral blood flow.

410.9

THE EFFECT OF 7-NITROINDAZOLE, AN INHIBITOR OF BRAIN NITRIC OXIDE SYNTHASE (NOS), ON BASAL CORTICAL BLOOD FLOW AND ON VASODILATION INDUCED BY WHISKER STIMULATION.Y.Zagvazdin[1]*, G.Sancesario[2], M.E.C.Fitzgerald[1] and A.Reiner[1]. Department of Anatomy & Neurobiology, University of Tennessee, Memphis, TN 38163[1], Universita di Roma "Tor Vergata", Rome, Italy[2].

The brain vasculature is regulated by nitric oxide (NO) produced by endothelial and neuronal NOS isoforms. While the vasodilatory action of endothelially produced NO is well documented, the role of neurally derived NO in circulatory control is still uncertain. Recently, an apparently selective inhibitor of neuronal NOS,7-Nitroindazole (7NI), has been described (Moore et al., 1993, Br.J.Pharmacol.,110). The goal of this study was to investigate the effects of neuronal NOS inhibition by 7NI on basal cerebral blood flow (CBF) and on the vasodilatory responses in somatosensory cortex associated with neuronal activation elicited by vibrissal stimulation. Male Sprague-Dawley rats were anesthetized with urethane and a-chloralose. Body temperature, blood gases and blood pressure were monitored. CBF was measured by a Laser Doppler flowmeter probe. Barrel cortex was activated by manual deflection of the contralateral vibrissae for 15-30 seconds. There were no significant changes in basal CBF or in dilatory responses induced by vibrissal stimulation following vehicle (i.p. oil) injection. Basal CBF decreased significantly (p<0.005) in 30 minutes after 7NI injection (i.p. in 3ml of peanut oil) with maximal reduction 71% (n=8) and 59% (n=3) of pre 7NI baseline at doses of 50 mg/kg and 75 mg/kg respectively. A slight (10-13 mm Hg) but significant increase (p<0.05) in blood pressure was observed after 7NI injection. The magnitude of the dilatory responses were decreased after 7NI injection at 50 mg/kg (88%) and 75 mg/kg (42%), but the reductions were not significant (the latter case probably due to the small number of animals yet tested). These studies revealed that 7NI reduces basal cerebral blood flow presumably via inhibition of neuronal NOS and has a slight vasopressor effect. Less NOS inhibition is required to affect basal cerebrovascular tone than to affect dilatory responses associated with neuronal activation.

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410.10

VASODILATORY ACTIONS OF CALCITONIN GENE-RELATED PEPTIDE AND NITRIC OXIDE IN PARENCHYMAL MICROVESSELS OF THE RAT HIPPOCAMPUS. A. Fergus, Y. Jin, Q.A. Thai, N.F. Kassell, K.S. Lee*. Dept. of Neurological Surgery and The Neuroscience Graduate Program, Univ. of Virginia, Charlottesville, VA 22908.

Calcitonin gene-related peptide (CGRP) and nitric oxide (NO) are known to exert vasodilatory actions in a variety of vascular beds. Recent evidence suggests that CGRP may mediate some aspects of the vasodilation elicited by NO. The present studies examined the responses of parenchymal microvessels in the rat hippocampus to CGRP and an inhibitor of nitric oxide synthase. Hippocampal slices were prepared from adult male Sprague-Dawley rats. Microvessels in the neuropil of submerged slices were examined using computer-assisted videomicroscopy. Drugs were administered by addition to the medium superfusing the slices.

The resting diameter of vessels analyzed in this study ranged from 12 to 25 micrometers. Treatment with the nitric oxide synthase inhibitor, N-nitro-L-arginine (NNLA; 100 μM), constricted vessels to 51.1±7.7% of resting diameter (n=9). Application of CGRP (10 nM) in the presence of NNLA resulted in the dilation of the preconstricted vessels to 97.0±9.6% of their resting diameter (n=4). These findings suggest that NO participates in the regulation of microvascular function by providing a tonic dilatatory influence. The ability of CGRP to dilate vessels in the presence of NNLA suggests that CGRP-induced dilation is not mediated by increased production of NO. Ongoing experiments are investigating the possible role of CGRP in mediating NO-induced vasodilation.

410.11

NITRIC OXIDE SYNTHASE (NOS) INHIBITION DOES NOT AFFECT LEUKOCYTE-ENDOTHELIUM INTERACTION AND BLOOD BRAIN BARRIER (BBB) INTEGRITY IN THE PIAL MICROCIRCULATION OF THE RAT. U.Lindauer, J.Dreier, W.Oertel*, A. Villringer, U.Dirnagl. Dept. of Neurology, Humboldt University Berlin, FRG.

NOS inhibition causes leukocyte activation and disturbs endothelial barrier integrity in the intestinal microcirculation (Circ Res 73:164-171;1993). We investigated whether NOS inhibition activates leukocytes in the brain and disrupts the BBB. In pentobarbital anesthetized male Wistar rats under full physiological control a closed cranial window (dura removed) was implanted over the parietal cerebral cortex. For labelling of circulating leukocytes, Rhodamin 6G was injected i.v. (200µg/ml, 1ml as a bolus, followed by the same dosage per hour continuous infusion). We used a confocal laser scanning microscope (Biorad MRC 600) and studied leukocyte rolling and sticking in pial veins and arteries before and after NOS blockade. The NOS inhibitor L-NA was applied systemically (sys) (10mg/kg i.v., n=5) or topically (top) (1 mM, n=5). At the end of the experiment, Na-fluorescein was injected i.v. (2mg/100g b.w.) to test BBB integrity. The results are summarized in the table:

	baseline (60 sec interval)			60 min (60 sec interval)		
X ± SD *p<0.05 to baseline	roller /100µm vein	stickers /100µm vein	SAP mmHg	roller /100µm vein	stickers /100µm vein	SAP mmHg
control (n=3)	0,80 ± 0,66	0,91 ± 0,35	110 ± 17	1,86 ± 0,34	1,03 ± 0,14	102 ± 10
L-NA sys (n=5)	0,64 ± 0,94	0,76 ± 0,24	104 ± 9	1,21 ± 0,99	1,19 ± 0,23	141 ± 15*
L-NA top (n=5)	0,44 ± 0,55	0,84 ± 0,42	108 ± 3	0,67 ± 0,66	1,21 ± 0,54	100 ± 10

Systemical or brain topical L-NA application did not alter leukocyte-endothelium interaction significantly and caused only discrete Na-fluorescein leakage in one animal of each group. No rolling and sticking of leukocytes was observed in arteries. These results suggest that NO, in contrast to its action in peripheral microvascular beds, does not affect leukocyte endothelium interaction and BBB integrity in the pial microcirculation . Supported by the DFG and the Wilhelm Sander Stiftung.

410.12

NITRIC OXIDE SYNTHASE (NOS) BLOCKADE DOES NOT AFFECT CORTICAL SPREADING DEPRESSION (CSD) IN RATS: A LASER DOPPLER (LD) AND NEAR INFRARED SPECTROSCOPY (NIRS) STUDY. T. Wolf, U. Lindauer, C. A. White*, J. Dreier, H. Obrig, A. Villringer, U. Dirnagl. Dept.of Neurology, Humboldt University Berlin, Germany; and Harvard Medical School, Boston, USA

In barbiturate anesthetized, ventilated rats under full physiological control, we measured continuously: regional cerebral blood flow (rCBF) by LD; oxyhemoglobin (HbO), hemoglobin (Hb) and cytochrome a/a3 oxygenation ($CytO_2$) by NIRS; and cortical DC potential. CSD was induced before and 60 minutes after i.v. application of the NOS-inhibitor L-NA (10 mg/kg) 10 mm from the measurement site. The results are summarized in the table :

n=7 X±SD *p<.05	SAP (mmHg)	basal rCBF (%)	CSD velocity (mm/min)	CSD hyperperfusion (%)	CSD $\Delta CytO_2$ (arb.units)	CSD ΔHbO (arb.units)	CSD ΔHb (arb.units)
control	104 ± 15	100	3.4 ± 0.1	+360 ± 196	-4.6 ± 0.3	+26.1 ± 6.3	-15.4 ± 4.0
L-NA	131 ± 15*	82 ± 19*	3.4 ± 0.3	+433 ± 228	-4.8 ± 0.3	+27.9 ± 3.9	-14.7 ± 6.0

NOS inhibition did not alter significantly the quantitative changes or curve patterns in rCBF, DC potential, hemoglobin and cytochrome a/a3 oxygenation due to CSD. Particularly, there was no brief phase of hypoperfusion preceding the CSD hyperperfusion after NOS inhibition as reported by Duckrow (Brain Res, 618:190-195; 1993). We conclude that NOS inhibition in the anesthetized rat does not affect the coupling of cerebral hemodynamics and oxygen metabolism in CSD. NIRS is useful for noninvasive monitoring of hemodynamic and metabolic changes associated with CSD in rats and possibly in humans. Supported by the DFG.

COGNITION II

411.1

MISMATCH-NEGATIVITY IN DIFFERENT BRAIN STRUCTURES - A TOPOGRAPHICAL AND DIMENSIONAL STUDY. M. Molnár*, V. Csépe., I. Winkler, J.E. Skinner+ and G. Karmos
Institute for Psychology, Hungarian Academy of Sciences, Budapest, Hungary; +Totts Gap Medical Research Laboratories, Bangor, USA.

The purpose of the present investigation was twofold: 1) In animal experiments an attempt was made to record the mismatch-negativity (MMN) in other brain structures than the auditory cortex, the latter being the hypothesized generator for the MMN. In chronically implanted freely moving cats auditory evoked potentials were recorded from the auditory and association cortices, dorsal hippocampus, amygdala, dorsal raphe nucleus and vertex. Auditory stimuli were short tones of 4 kHz frequency ("standards") randomly interrupted by 2 kHz "deviant" ones (probability: 10%). 2) In human studies the mathematical tools of non-linear dynamics was used to analyze data collected in MMN-experiments from different scalp locations. The MMN, elicited by randomly appearing 1100 Hz deviant stimuli within series of 1000 Hz standards was recorded from Fz, Cz and Pz. The point-correlation dimension (PD2) of EEG epochs were calculated.

In the animal studies it was found that in the primary auditory and association cortices, hippocampus and vertex the MMN manifested itself with almost identical latencies of about 50 ms, but it started to develop with the shortest latency in the hippocampus. The MMN had a longer latency in the secondary auditory cortex. No MMN was recorded in the other structures studied. It is suggested that the hippocampus probably plays an important and early role in the stimulus comparison process the result of which is the MMN. In the human studies the occurrence of the MMN was accompanied by PD2 decreases most conspicuously in the frontal region indicating the activity of a possible frontal MMN-generator.

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411.2

COMPARATIVE ANALYSIS OF CORTICAL GENERATORS OF MISMATCH-NEGATIVITY IN THE CAT AND MONKEY. G. Karmos*, I. Ulbert, D.C. Javitt+, M. Molnár, V. Csépe, Zs. Pincze and C.E. Schroeder+.
Inst. for Psychology of the Hungarian Academy of Sciences, Budapest, Hungary, +Dept. of Neuroscience, Albet Einstein College of Medicine, Bronx, NY 10461.

Human source localization techniques indicated an auditory cortical origin of the mismatch-negativity (MMN), which is a negative component of the scalp recorded auditory evoked potential (AEP). It is elicited by infrequent, "deviant" stimuli, presented randomly within sequences of repetitive "standard" auditory stimuli.

MMN appeared also in cats and monkeys as a negative component in the epidural AEPs in the latency range of 40-120 ms. To study its intracortical generators the field potentials and multiple unit activity were recorded from the auditory areas of awake cats and macaque monkeys by intracortical multielectrodes. One dimensional current source density (CSD) analysis was used to localize intracortical sinks and sources. Surface maps in cats indicated the spread of the frequency MMN to both AI and AII auditory areas. In the middle layers of the auditory cortex of cats large amplitude local positive field was elicited by the standards in the latency range of the MMN while this positivity appeared with much smaller amplitude to the deviants. CSD analysis and the increased unit response elicited by the deviant stimuli suggested that the MMN represent a local disinhibition in the upper layers. In the AI area monkeys surface negative MMN to deviant stimuli was accompanied by an increased lamina II/III sink.

The data indicate that the MMN generators are localized in the supragranular layers of the auditory cortex.

Supported by OTKA Grant I/3/2595 and by the J.S. McDonnell Foundation.

411.3

THE GENERATOR QUESTION: A COMPARISON OF EVENT RELATED POTENTIAL (ERP) PROFILES WITH RECONSTRUCTIONS FROM CURRENT SOURCE DENSITY (CSD) ANALYSES. **C.E.Tenke* and C.E.Schroeder, Dept. Biopsychology, NYS. Psychiatric Institute, NY,NY and Depts. Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY**

One-dimensional CSD profiles of intracranial ERPs detail the temporal and spatial patterns of transmembrane current flow which underlie the local field potential, and allow inferences about the likelihood that the sampled activity is recordable at a distance. In contrast, inverse models (e.g. dipole models) simplify a scalp topography by indicating locations and waveforms for putative generators based on statistical and geometric inferences. A synthesis of these complementary approaches would provide a distinct advantage for the study of ERP generators. We recorded the Flash-ERP in the monkey within and above the lateral geniculate nucleus (LGN), and reconstructed the ERP profile from computed CSD profiles. CSD profiles were computed at low or high spatial resolution to bias the profiles toward open or closed field activity, respectively (Tenke et al.,1993). Weighting functions for these forward solutions assumed uniformly distributed cylindrical generators (Nicholson and Llinas,1971). Reconstructed and empirical profiles were compared and residual waveforms computed from their difference. Low resolution methods produced a better overall fit to the empirical profile. High resolution methods produced an oscillatory residual, reflecting closed field activity. Factors common to empirical and reconstructed profiles were extracted using a principal components analysis, to summarize them and to provide a direct linkage between local generators and the ERP recorded within and superficial to LGN. This approach shows promise for computing waveforms of volume conducting, regional dipoles directly from measurable properties of a region.

411.4

COMBINED MEG AND ERP MEASURES OF VISUAL SPATIAL SELECTIVE ATTENTION IN HUMANS. G.R. Mangun[1], M. Sams*[2,3], R. J. Ilmoniemi[3], G.V. Simpson[4] (1) University of California, Davis, USA; (2) University of Tampere, Finland; (3) Helsinki University of Technology, Finland; (4) Albert Einstein College of Medicine, USA

Mangun et al. (1993) investigated event-related potentials (ERPs) to attended and ignored stimuli using topographic current density analysis, and proposed that spatial selective attention first modulated visual processing in extrastriate cortex in humans. In the present study we used combined electrical (7-channel ERP) and magnetic (122-channel MEG) recording to index the processing of stimuli flashed to the four quadrants of the visual field when attended and ignored. In normal subjects (N=3) the ERPs showed significant attention-related amplitude increases of the P1 (110 ms) and N1 (190 ms) components at occipital scalp sites. No attention effects were observed for the earlier NP90 (90 ms) component thought to be generated in striate cortex. MEG recordings for the three time periods corresponding to the NP90, P1 and N1 components were analyzed and modeled as equivalent current dipoles in order to localize their brain generators. In the time period of the electrical NP90, a source was identified in the calcarine sulcus of the contralateral striate cortex that showed inversions in dipole orientation for upper versus lower field stimuli; this magnetic component was not affected by attention. Later attention-sensitive MEG components, in the time range of the electrical P1 were localized to regions of extrastriate visual cortex and were attention sensitive. Longer latency activity in the N1 range (160-190 ms) was localized to parietal and lateral temporal cortex. These findings provide further support for the idea that spatial attention first begins to affect visual processing at the level of extrastriate cortex in humans.

411.5

VISUALLY EVOKED ELECTRIC AND MAGNETIC RESPONSES: SIMULTANEOUS WHOLE-SCALP RECORDINGS. G. V. Simpson*[1], R. J Ilmoniemi[2], S.P. Ahlfors[2], M.S. Hämäläinen[3], J.J. Foxe[1]. [1]Depts. Neurology and Neuroscience, Albert Einstein Coll. Med., [2]Low Temperature Laboratory and [3]Neuromag Ltd, Helsinki University of Technology, 02150 Espoo, Finland.

The electric currents in brain areas which are active during sensorimotor and cognitive processes produce electric and magnetic fields outside the skull which can be measured in real-time as event-related electric potentials (ERPs) and magnetic fields (ERFs) respectively. The accuracy of localizing active brain sources may be improved by combining ERP and ERF data since they are complementary. Prior to recent technological advances, it has not been possible to simultaneously record both electric and magnetic field data over the whole scalp in a single experimental session. We report here, simultaneously recorded electric and magnetic responses to visual stimuli with large sensor arrays (122 magnetometers and 32 electrodes) over the whole scalp. A series of retinotopic, pattern discrimination and attention experiments were conducted. The retinotopic results are presented here. ERPs and ERFs were recorded to small checkerboard wedge stimuli delivered to each quadrant. Source configurations for the initial responses were estimated with spatiotemporal dipole analyses and minimum norm estimation. The source locations for ERP and ERF converged, and followed the expected retinotopic pattern for striate cortex, i.e., upper right, lower right, lower left and upper left quadrant stimulation activated lower left, upper left, upper right and lower right striate cortices respectively.

411.6

VALIDATION STUDIES OF A PROBABILISTIC SOLUTION TO THE EEG INVERSE PROBLEM. **D.M. Rice[1]* D. Khosla[2], M. Singh[2]. Andrus Gerontology Center[1] and Dept.of Biomedical Engineering[2], Univ. of Southern California, Los Angeles, CA 90089-0191.**

The EEG inverse problem is to reconstruct the distribution of currents within the brain which generate the scalp measured potential distribution. This problem has an infinite number of exact solutions, so a probabilistic solution would seem to be the most reasonable solution to this problem. The approach employed here maximizes the entropy of the source distribution subject to a system of linear equations which require that this distribution fits the scalp measured potential field and which ensure that the joint probability of sources in opposite directions at the same location is everywhere equal to zero. With this formulation, this maximum entropy solution is the probability distribution for the source(s) of the measured potential field, and it is also the most likely source distribution for the measured potential field. In addition, the current probability distribution can be easily derived from this source probability distribution. The present studies were to provide inital validation for this approach. With simulated potential fields produced by a small number of equivalent current dipoles in a homogeneous sphere model, the current probability distributions approximated the actual equivalent current dipoles which generated the potential field. With a potential field measured at the human scalp during visual checkerboard stimulation which strobed at 3 Hz., the current probability distribution computed through a four sphere model was largely concentrated in the posterior regions of the brain including a region roughly corresponding to the primary visual cortex.

411.7

TOMOGRAPHIC MAPPING OF TEMPORAL LOBE P300 ACTIVITY IN THREE DIMENSIONS: ASSESSMENT OF RELIABILITY AND ACCURACY. C. C. Kessler*and R. L. Lloyd. Psychiatry Department, 116A, V.A. Medical Center, Sepulveda, CA 91343.

A noninvasive interpolative computer graphics algorithm for imaging the distribution of the P300 event-related potential within the temporal lobe is described. This technique takes advantage of the fact that the primate temporal lobe may be noninvasively surrounded by 4 electrodes forming a tetrahedral array consisting of three surface leads (C4, T6, and T4) and one nasopharyngeal lead (Pg2). It is then possible to calculate the potential distribution within the enclosed volume of the temporal lobe given that the field strength Ep for any point p within the tetrahedron may be represented by the sum of the field contributions to or from the surrounding vertices, weighted by the distance between point p and each of the vertices. As part of an effort to assess the reliability and accuracy of this technique, monopolar recordings were obtained from the tetrahedral array in several squirrel monkeys (*Saimiri sciureus*) using a passive oddball paradigm. Simultaneous monopolar recordings were made at indwelling electrodes from an initial depth of 10mm to a final depth of 22mm within the temporal lobe, and these measurements were used to adjust the imaging algorithm to account for variations in impedance and conductance via a linear least-squares technique. When applied to other subjects in a cross-validation procedure, the adjusted algorithm was found to yield accurate and reliable measurements at various depths which were highly and significantly correlated with simultaneous measurements obtained from indwelling electrodes at corresponding sites.

411.8

THE TIME COURSE OF REGIONAL CEREBRAL ACTIVATION DURING COGNITIVE TASKS: RESULTS OF DIRECT CORTICAL RECORDING IN HUMANS. N.E. Crone, J. Hart, R.P. Lesser* and B. Gordon. Depts. of Neurology, Neurosurgery, and Cognitive Science, and The Zanvyl Krieger Mind/Brain Institute, The Johns Hopkins University, Baltimore, MD 21287

Language and related cognitive functions are considered to be the product of distinct underlying processing stages, which are often serially-connected. However, little is known about the dynamics of processing within stages, or the time relations between stages of processing. We therefore examined the onset and duration of activation in different cerebral regions during cognitive tasks, using spectral changes in human cortical EEG as indices of functional activation.

Subjects were evaluated with indwelling subdural electrode arrays placed for clinical purposes (Lesser et al., 1994). Arrays consisted of 48 or more electrodes (2.3 mm exposed surface), embedded (10 mm center-to-center) in silastic sheets. Recordings were made during the following cognitive tasks: visual object/nonobject discrimination; visual-motor decision; reading words aloud; visual object naming; and auditory word repetition. Changes in EEG spectral power (18 frequency bands; 8-100 Hz) from pre- to post-cognitive stimulus presentation, in overlapping 200 msec time segments, were used as markers for regional cerebral activation at each electrode.

In general, within a given task, cerebral activation appeared to occur in close sequence, and often overlapped considerably in time, among the different regions involved in cognitive processing. These data provide further support for cascade models (McClelland, 1979) of information flow in human cognitive processes.

411.9

BRAIN ACTIVATION WITH A MAZE TEST: AN EEG COHERENCE ANALYSIS STUDY IN HEALTHY SUBJECTS. M. Tremblay, D. Lacroix*, V. Fraïle, Y. Chaput, R. Lamer and J.-M. Albert. Département de Psychiatrie, Hôpital Notre-Dame, Université de Montréal, 1560 Sherbrooke E., Montréal, CANADA H2L 4M1.

The maze test is a complex cognitive task involving visuo-perceptual abilities and executive functions, such as planning and foresight. EEG coherence analysis is performed on the electrical signal of two electrodes and it's value is analogous to a correlation coefficient between the two signals. EEGs were recorded from 20 healthy subjects (mean age ± S.D. : 27.4 ± 5.23) during a maze test (3 mazes from the WISC and 2 from the Porteus) and during two baseline conditions: 1- rest with eyes opened; 2- mazes with dotted lines showing the way out. 19 electrodes were used according to the 10/20 system. Signals were referenced to averaged signals from both ear lobes (TC 0.3s, Filter 35 Hz). Interregional coherence (IRC) refers to coherence between each electrode paired to each of the remaining electrodes and may reflect dynamic physiological processes between brain regions during cognitive activation. Statistical probability mapping were used to show the regional distribution of changes. The results show that: 1- compared to the eyes opened condition, the maze test provoked increases of IRC mainly located between paired posterior electrodes (mostly parietal P3 and P4 sites) and decreases between all fronto-frontal sites; 2- compared to IRC of the 2nd baseline condition, the maze test provoked increases of IRC mainly between paired posterior electrodes in θ and β1 bands and between the left prefrontal F3 site paired with frontal or posterior sites in β band and provoked decreases between centro-parietal sites paired with frontal sites in α. EEG coherence analysis detects electrical changes in regions known to be involved in visuo-analytic, visuo-practic and executive functions (posterior, central and frontal sites respectively).

411.10

EVENT-RELATED POTENTIALS DURING COMPREHENSION OF SYNTAX IN SPANISH SPEAKING SUBJECTS F.Ostrosky*, C.Rigalt, M.Pérez, F.Cruz, J.Marcos and A.Ardila Dep.de Psicofisiología, Facultad de Psicología. Universidad Nacional Autónoma de México. México D.F. 04510.

Event-related brain potentials (ERPs) were recorded during a syntactic decision task. Twenty-five right-handed young adults were presented with a total of 198 eight-word sentences, that were divided in three different groups: 66 Pseudo-Cleft Agent, (LO QUE UN ELEFANTE EMPUJO FUE UN OSO; "WHAT AN ELEPHANT PUSHED WAS A BEAR"); 66 Pseudocleft Pasive (UN ELEFANTE FUE LO QUE EMPUJO UN OSO; "AN ELEPHANT WAS WHAT A BEAR PUSHED") and 66 sentence type without a syntactic order that were used as control (ELEFANTE UN FUE EMPUJO QUE LO UN OSO; ELEPHANT AN WAS PUSHED WHAT A BEAR) Sentences were presented randomly intermixed. Subjects had to select the agent in each sentence. Scalp electrical activity was recorded from 32 derivations of the 10/20 international system. ERPs were registered to the seventh and eight word. Peak amplitudes were visually identified by windows of 200 msec each one (from 50 to 900 msec). ERP measures associated with each sentence type were subject to ANOVA and repeated measures. Wave differences between ERPs were obtained. A slow negative response between 300-500 msec largest over anterior regions of the left hemisphere. These data reflected an negative component around 500 msec elicited by syntactic decision.

411.11

APPEARANCE OF FRONTO-PARIETAL MIDLINE (FPm) THETA ACTIVITY IN GOOD AND POOR LEARNERS

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Fronto-parietal theta activity (FPm,4-7Hz) was investigated during learning process of problem solving task. In a simulated traffic situation the subject had to learn to find the right way for driving a car through a set of roads by trial-and error. Two interdependent decisions had to be made at two crossroads guided by two traffic signs. After learning the level of performance was tested in a practising situation. Feedback about quality of performance was given. EEG was recorded from Fz and Pz.

In the learning situation the amount of appearance of theta activity did not differ either on the frontal or on the parietal area between the good learners and the poor learners. In the practising situation poor learners showed a significantly higher amount of theta than good learners in the frontal and parietal area.

The results demonstrate a relation between the appearance of fronto-parietal midline theta activity and the quality of performance.

411.12

ERP CORRELATES OF ATTENTION DURING THE CUE-STIMULUS INTERVAL. A.L. Keyes & R.G. Eason*. U. of NC at Greensboro, Greenbsoro, NC 27412

This study examined the effects of a cue-directing stimulus and the length of the cue-target interstimulus interval (ISI) on: (1) ERPs recorded prior to and following the occurrence of relevant (validly cued) and irrelevant (invalidly cued) target stimuli appearing in a given visual field; and (2) on rapidity (RTs) and accuracy (d-primes) of responses to cued stimuli. On a given trial a foveally-presented arrow cue directed to subject to attend (without moving the eyes) to either the right or left visual field. After a brief delay a target appeared randomly 8 degrees peripherally in either field. The subject responded as quickly as possible when the target appeared in the cued field. ERPs were recorded under one of three ISI conditions during an averaging run: 1000; 1400; or (3) a randomly presented mixture of 1000 and 1400 ms. Late ERP components occurring during the ISI interval, especially the CNV, were sensitive to the attention directing cue stimulus, length of the ISI, and degree of uncertainty as to when the target stimulus might appear (after 1000 or 1400 ms). These pretarget effects were consistent with the ERP and behavioral data obtained to the target stimulus.

411.13

CORTICAL EVOKED POTENTIALS DURING A VISUAL CONTINUOUS PERFORMANCE TASK. A. Tekok-Kilic, E. Sargent, D.W. Shucard*.
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The Continuous Performance Task (CPT) was originally developed for the behavioral assessment of sustained attention in brain damaged patients (Rosvold et al., 1956). Different types of CPTs have been used to investigate attention in psychiatric and neurologic patient groups. It has become important to clarify the behavioral, perceptual and cognitive factors measured by the CPT in normal subjects. One of the variations of the CPT is the visual "A-X" paradigm (Halperin et al., 1988) in which subjects are asked to respond as fast as possible to the target letter "X" only if it is preceded by "A". Nontarget letters preceded by "A" and "X"s not preceded by "A" (nontarget "X"s) are also presented. In order to study the physiological aspects of sustained attention, we examined the relationship between a cortical evoked potential component (P300) and the CPT "A-X" paradigm. P300 is an endogenous component that is elicited by stimuli that are relevant to the subject. We expected that the P300 component would have the highest amplitude for both target "X"s and for other letters immediately preceded by "A". Eleven letters were visually presented as stimuli in a random sequence of 400 letters, with 40 "A-X" pairs (p=.10) and 68 "A-nontarget" pairs (p=.17), stimulus duration was 200 msec and ISI was 1500 msec. Electroencephalographic activity was recorded at 12 scalp electrode sites of the 10-20 system, referenced to linked earlobes.

As reported in previous studies, the P300 component was most prevalent in the midline scalp sites. As predicted, the highest amplitude responses were observed for both target "X"s and other letters that were preceded by "A"s. These results are consistent with findings showing that task relevance is an important antecedent condition of P300 amplitude. The electrophysiological data provide information not attainable through behavioral observation about possible cognitive events underlying CPT performance.

411.14

THE EFFECTS OF PRECEDING INTERSTIMULUS INTERVAL ON BEHAVIORAL DISCRIMINATION AND MISMATCH NEGATIVITY. M.G.Woldorff* and M.Matzke, Univ. of Texas Health Science Center, Research Imaging Center, San Antonio, TX 78284- 6240.

The mismatch negativity (MMN) is elicited by infrequent, physically deviant sounds in a sequence of repetitive auditory stimuli. It has been asserted that this wave reflects a strongly automatic feature-analysis and mismatch-detection process, in which each stimulus is compared to the sensory template formed by the repeated standard-tone stimulus. This framework asserts that the MMN is larger at shorter interstimulus intervals (ISIs), due to the comparison template formed by the previous standard(s) being stronger. For similar reasons, this framework also predicts discriminability of the deviant stimuli should be better at shorter ISIs.

To explore this relationship more directly, subjects were monaurally presented with tone pips at ISIs ranging randomly from 120-920 msec, with 10% of the tones either slightly fainter in intensity or slightly lower in pitch (different blocks). On half of the runs, subject tried to discriminate the deviant stimuli; on the other half, subjects read a book. Preceding ISIs were divided into 4 subranges, and target discrimination and ERPs were subdivided as a function of the ISI subrange of the preceding standard tone. The discriminability of the deviant target tones was strongly affected by preceding ISI, with subjects doing progressively worse at the shorter ISIs. Preliminary ERP results suggest that the reading-condition MMNs and the active condition MMN/DRN (deviance-related-negativity) also decreases with shorter ISIs. These data do not fit into a framework of increasing standard-tone template strengthening with decreasing ISI. Rather, as suggested by Woldorff and Hillyard (EEGJ, 1991), a type of neural refractoriness appears to play a major role in these phenomena at shorter ISIs.

411.15

VEP ESTIMATION OF INTERHEMISPHERIC RELAY TIME. C. Potvin, C.M.J. Braun and A. Achim*. Laboratoire de Neuroscience de la Cognition, UQAM, Montréal, Canada,H3C 3P8

Lines, Rugg & Milner (1984) dissociated a non sensory from a sensory component of interhemispheric relay (IR) by showing that the asymmetry in N160 latency of visual evoked potentials (VEP) to contralateral and ipsilateral stimuli is affected by stimulus intensity only at occipital sites (about 11 and 18 msec for bright and dim stimuli), not at central sites (about 3 msec). It appears possible, however, that the latter 3 msec delay simply represents different shifts in the apparent peak latency of a synchronous symmetrical source, due to superimposed asymmetrical potentials of occipital origin, the central N160 peaking during the rising phase of the occipital N160. To allow more detailed topographic analysis, we obtained VEP from 21 scalp sites in 10 subjects in a simple reaction time task to yellow squares on a blue background, about 10° left or right of fixation. Preliminary N160 latency analysis shows a strong antero-posterior gradient, from 154 msec at F3/F4 to 190 msec at O1/O2. The IR estimates show a parallel gradient from 7.0 msec at F3/F4 to 15.9 msec at O1/O2, with a value of 10.3 msec at C3/C4. The latter, with a standard error of the mean of about 3.2, is a significantly larger asymmetry than those reported earlier at C3/C4. The notion of simply two levels of IR in VEP appears to be an oversimplification.

411.16

ACTIVATION OF DURATION-SENSITIVE AUDITORY NEURONS IN HUMANS. C. Alain*, D. L. Woods, and D. Covarrubias. Department of Neurology and Center for Neuroscience, UC Davis, Northern California System of Clinics, Martinez, CA 94553.

Auditory neurons vary in their duration tuning: some neurons fire to tones of short duration, whereas others require longer duration tones. In the current study, auditory evoked potentials (AEPs) were recorded to signals varying in duration (8, 24, or 72 ms) and frequency (250, 1000, or 4000 Hz) in an effort to characterize temporal integration functions (amplitude/latency changes with changes in tone duration) for different AEP components. High-rate sequences were used in which tones were delivered randomly to the left and right ears. Subjects attended to a designated location and frequency to detect occasional long- or short-duration targets. Subjects were more accurate and faster in detection of long- than short-duration targets. Paralleling this, AEP components increased in amplitude and decreased in latencies with increased signal duration. Since all signals had identical rise-fall times and peak-to-peak amplitudes, duration-specific difference waves could be isolated by subtracting AEPs to short-duration tones from AEPs to longer duration tones at the same location and frequency. Duration difference waves varied in amplitude and scalp distribution for tones of different frequencies. These results are compatible with different integration time for different frequencies and generators, with tonotopically organized AEP generators generally having longer temporal integration times.

411.17

EVENT-RELATED DIFFERENCES IN EEG SPECTRAL POWER (ERDISP)
D. L. Woods*, C. Alain, D. Covarrubias, and M. P. Remler, Dept. of Neurology and Neurosciences Center, UC Davis, Northern California System of Clinics, Martinez, CA 94553.

Recent studies using narrow band filtering techniques have shown changes in alpha (8-13 Hz) and gamma (30-50 Hz) frequency bands of the EEG in asociation with the sensory and cognitive analysis of signals. A simple technique is described for analyzing Event-Related Differences in EEG Spectral Power (ERDISP) over the entire EEG frequency spectrum. Power spectra from brief EEG epochs preceding stimulus delivery are subtracted from power spectra following the stimulus. The selection of the epoch duration is based on a trade-off between temporal and frequency resolution: short EEG epochs provide good temporal resolution but poor frequency resolution, whereas long EEG epochs provide good frequency resolution but poor temporal resolution. An analysis strategy using multiple epoch durations is proposed. ERDISPs reflect both sensory and cognitive processes. ERDISPs relating specifically to higher cognitive processes can be isolated in ERDISP difference waves (Δ-ERDISPs) using multiple subtractions. For example, the effects of selective attention on ongoing EEG rhythms can be analyzed by subtracting ERDISPs to nonattended stimuli from ERDISPs to the same stimuli when attended. Δ-ERDISPs in selective attention tasks show alterations in several EEG frequency bands. The relationship between ERDISPs and event-related brain potentials (ERPs) and between ERDISPs and other techniques for analyzing event-related changes in EEG power will be discussed. Supported by NS32893 and DC01049.

411.18

OPERANTLY CONDITIONING THE P200 PEAK OF THE HUMAN SEP: EFFECTS ON PAIN SENSITIVITY AND THE R3 REFLEX. R. Dowman*, Dept. Psychology, Clarkson Univ. Potsdam N.Y., 13699-5825.

The goal of this experiment was to replicate and extend previous work suggesting that operantly conditioned changes in the amplitude of the P200 peak of the human somatosensory evoked potential (SEP) alters pain sensitivity. The SEP, R3 reflex, compound nerve action potential (CAP) and magnitude ratings were elicited by stimulation of the sural nerve. Twenty-one subjects (conditioning group) were rewarded for increasing (up-training) and decreasing (down-training) the P200 peak evoked by innocuous stimulation. Magnitude ratings and R3 reflexes elicited by innocuous and noxious stimulation were obtained immediately following each training session. An additional 12 subjects served as no-training controls.

P200 amplitude was larger during up-training than down-training in the conditioning group but not the control group. There were little or no changes in the other peaks of the SEP. There were no changes in stimulus current or CAP amplitude across the training sessions, which implies that the conditioned change in P200 amplitude was due to some change within the CNS. Magnitude ratings and the R3 reflex were the same in up-training as down-training in both the conditioning and control groups. Thus, conditioned changes in the SEP that are specific to the P200 peak do not alter pain sensitivity.

411.19

SPATIO-TEMPORAL CORRELATIONS IN HUMAN GAMMA BAND ELECTROCORTICOGRAMS.
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Animal electrocorticogram (ECoG) studies have shown that coherent spatial patterns in the gamma band ($> 20Hz$) reflect perceptual categorization. Spatio-temporal correlations were investigated in the $20-50Hz$ range in search for similar phenomena in human ECoG. The ECoG was recorded in a somatosensory discrimination task from a 64-electrode subdural grid array with spacing of $1cm$ between electrodes, overlaying somatosensory, motor, and superior temporal cortices in two patients with intractable epilepsy.

Stimulus and response related modulations of the μ, β, *and* γ bands of the $1/f$ spectrum were observed over intervals of $500ms$ in the somatosensory and motor cortices but not in the temporal cortex. No evidence was found for globally coherent activity related to behavior either in the narrow (e. g. 40 Hz) or broad bands. Instead, spatially and temporally intermittent synchronization was observed between pairs of electrodes with high variability within and across trials. The distribution of correlation coefficients differed substantially from noise levels for separations of $1cm$ and $1.4cm$ but not $2cm$ or more. The average correlation duration for $1cm$ separation was about $150ms$ and the average recurrence rate of significant correlation peaks was $1/s$.

The findings suggest that domains of spatial coherence in human gamma band ECoG are limited to $< 2cm$, that the intermittent synchronization observed across separations of $1cm$ and $1.4cm$ is not solely due to volume conduction, and that the search for perceptual categorization in human gamma band ECoG needs to be at a smaller spatial scale.

411.20

SPIKE SPACE SYNCHRONIZATION OF NEURONAL ACTIVITY AS MECHANISM OF QUICK SHORT MENTAL PROCESSES P.D.Perepelkin* Pavlov Inst. of Physiol., St.Petersburg, Russia./ Barrow Neurological Inst. 350 W. Thomas Rd., Phoenix, AZ 85013/

Spike space synchronization (SSS) of neuronal ensemble activity of the human brain was investigated. Gold electrodes were implanted in the deep structures for therapeutic purpose. The impulse activity of 14 distant ensembles were recorded simultaneously. It has been established that there were concrete SSS for definite phase's of psychological tests. This investigation was completed by chronic experiments. Monkeys (M.mulatto) were trained to differentiate colored geometric figures. The impulse activity of 6-10 neuronal ensembles was recorded simultaneously in associative, temporal and frontal cortex of the animals during their operant conditioning. It was show the existence, the reliability and the importance of SSS. There was a nonlinear character dependency between discharge frequency charges of impulsation and spike's synchronization. The experiment showed that SSS correlated with definite phases of the monkeys behavior. The interaction of neuronal processes which have been investigated allow that such space interaction as SSS could be a special mechanism for quick short phases of integral mental processes in the primate brain and that spatial - temporal information stimulations by means of many implanted electrodes lead to modify behavior.

COGNITION III

412.1

RECOGNITION MEMORY AND CUED RECALL IN PATIENTS WITH FRONTAL CORTEX LESIONS: ERP AND BEHAVIORAL FINDINGS. D. Swick* & R.T. Knight, Dept. of Neurology & Center for Neuroscience, UC Davis, VAMC, Martinez, CA 94553.

Frontal patients reportedly show deficits in recall but not in recognition. The first experiment examined the role of frontal cortex in verbal recognition memory and the generation of late positive potentials in this task. Words and pronounceable nonwords repeated after one of 3 delays: immediate (3 sec), 1-3 intervening items (6-12 sec), or 9-19 items (30-60 sec). Stimuli were categorized as new or old. Subjects were 11 stroke patients (10 Left, 1 Right) with lesions centered in dorsolateral prefrontal cortex and 11 age-matched controls. In controls, first presentations of words and nonwords elicited a large centro-parietal positivity (LPC) peaking at 700 msec. Immediate repetitions elicited the largest (10-14 μV) and earliest (500 msec) LPC. LPC was still enhanced at lag 1-3 but not at lag 9-19, unlike the longer-lasting effect seen in young subjects. In frontal patients, reductions in LPC were visible at most sites and for most conditions, while the repetition effects themselves were relatively preserved. Effects were dependent on lesion size and location; focal negativities were observed in some patients. These ERP changes were primarily accounted for by patients with the most posterior lesions (n=5), although behavioral impairments were observed in all patients: frontals were less accurate across all conditions ($p<.05$). The ERP results in posterior frontal patients imply the loss of a neural generator and/or an input to the generators of LPC, while the performance decrement suggests that frontal cortex is important for aspects of recognition memory. An overlapping set of patients with lesions in areas 8, 9, 10, and/or 46 (5 Left, 5 Right) were run on a cued recall task. Words were presented in a semantic study condition and again at test as 3-letter stems. As a group, these patients were not significantly impaired in cued recall, although two patients (1 L, 1 R) scored more than 2 SD below controls. These results will be discussed in the context of recent PET activation studies in young subjects.

412.2

AGE-RELATED PREFRONTAL CHANGES DURING AUDITORY MEMORY
L.L. Chao* and R.T. Knight; Department of Neurology & Center for Neuroscience; U.C. Davis, VAMC, 150 Muir Road, Martinez, CA 94553-4685.

The current study examined the effects of aging and prefrontal damage on auditory memory for environmental sounds. Event-related potentials were recorded from young (mean age = 21 ± 1) and elderly (mean age = 71 ± 11) control subjects and elderly patients (mean age = 70 ± 6) with lesions due to infarcts in the dorsolateral prefrontal cortex (centered in Brodmann areas 9 and 46). Digitized environmental sounds were binaurally presented in four blocks (111-114 sounds per block, 700 msec duration, 1200 msec SOA). Twenty percent of the sounds were presented once and 80% were presented twice. Sounds that were presented twice repeated at delays of either 1.9 seconds (short delay) or 4 to 12 seconds (long delay). Subjects indicated whether they had heard the sounds before by pressing a "yes" or "no" button.

Young control subjects generated a prominent sustained frontal negativity to all stimuli. The negativity was larger for longer recognition delays. Aging was associated with decreases of the sustained frontal negativity for stimuli at all recognition lags. Prefrontal lesions resulted in further decrements of the frontal negativity. At all stimulus intervals, young, old, and frontal lesioned subjects generated comparable posterior P300 components. Changes in prefrontal activity appear to underlie age-related alterations in auditory memory processing.

412.3

THE WISCONSIN CARD SORTING TASK SHOWS LITTLE SENSITIVITY OR SPECIFICITY FOR THE FRONTAL LOBE.

Z. Caramanos* and M. Petrides. Cognitive Neuroscience Unit, Montreal Neurological Institute and McGill University, Montreal, Canada H3A 2B4.

The Wisconsin Card Sorting Task (**WCST**) measures an individual's ability to form, maintain, and switch categories based on external feedback. The WCST is widely used in clinical and experimental neuropsychology, and deficits on it are widely accepted as a sign of frontal-lobe disturbance. It has recently been suggested, however, that impairments on the WCST are not necessarily indicative of frontal-lobe damage. The present study examines: (a) whether or not group differences are found in the WCST performance of focal epilepsy patients with unilateral frontal- or temporal-lobe excisions, and (b) whether or not performance on this task can be used to classify such patients according to the side, the lobe, and/or the side-lobe combination of their lesion.

As part of standard clinical neuropsychological testing, patients with well-localised epileptic foci were given the WCST before, and both two weeks and at least one year after undergoing unilateral excision from the frontal- (n=23 to 34) or the temporal-lobe (n=89 to 139). The left-frontal group was statistically impaired on several WCST measures in the pre- and early post-operative period. Nevertheless, considerable between-group overlap was found on all measures at all stages of testing. Furthermore, the locus of neural disturbance in these patients could not be predicted pre-operatively based on WCST performance, either by using established cut-off scores or cross-validated linear discriminant analyses. Similarly, their site of cortical excision could not be predicted either on the basis of early post-operative or late follow-up testing. In conclusion, these findings suggest that the WCST is not effective at localising neural disturbance in frontal- and temporal -lobe epilepsy patients. Furthermore, they warn against its use as an unequivocal indicator of frontal-lobe integrity in other populations.

412.4

NON-CONSCIOUS AUTONOMIC SIGNALLING ADVISES THE AVOIDANCE OF RESPONSES WITH NEGATIVE FUTURE OUTCOMES. A.Bechara*, H. Damasio, A. R. Damasio, and D.Tranel. Dept. of Neurology, Univ. of Iowa, Iowa City, IA, 52242.

In a new task, subjects must choose one card at a time from one of four decks. In two decks, choosing a card is followed by a high gain of play money, but at unpredictable points, the selection of a card is followed by a high penalty. In the other two decks, the immediate gain is smaller but the future loss is also smaller. After sampling and encountering losses in each deck, normal subjects begin to avoid the decks with high immediate gain (bad decks), and generate anticipatory skin conductance responses (SCRs) prior to selecting a card from these decks. Prefrontal patients do neither. Since these patients choose disadvantageously, even when they know which decks are good or bad, the somatic signalling indexed by anticipatory SCRs must be critical for the implementation of behavior. But does this mean that somatic signalling is triggered by "knowing" which decks are good or bad, or do the SCRs arise prior to such knowledge ? SCRs were recorded from 6 normal subjects while performing the card task. At various points, subjects were briefly interrupted and asked what they understood of the game. In the pre-punishment period (before any penalty cards), anticipatory SCRs averaged 0.1 μS in all decks, and subjects made an average of 13 selections from the good versus the bad decks. In the pre-conceptual period (after penalty cards, and before knowing good from bad decks), SCRs rose to 0.45 μS in the bad decks, and remained 0.1 μS in the good decks. These SCRs represented an average of 10.5 cards selected from the bad decks, and16.7 cards from the good decks. In the post-conceptual period (after realizing which decks are good or bad), SCRs and number of cards were 0.5 μS and 11.5 cards for bad decks, and 0.15 μS and 20.8 cards for good decks. These results go counter to the idea that only pure reason is the basis for wise decisions, and supports the notion that at least part of our advantageous behavior can be guided by covert processes.

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412.5

FRONTAL LESIONS AFFECT PURSUIT AND MIRROR DRAWING PERFORMANCES. M.J. Chouinard*, J. Rouleau. Lab. de Neuroscience de la Cognition, Université du Québec, Montréal, Qc, H3C 3P8.

As part of a larger study of the role of the frontal lobes in sensori-motor performances and learning, we examined performance of patients with frontal lesions in two tasks requiring continuous regulation of movement on the basis of sensory information. We tested 5 patients with a unilateral frontal lobe lesion (FL) and 8 patients with a unilateral temporal lobe lesion (TL) on two sensori-motor tasks: 1) Rotary Pursuit and 2) Mirror Drawing. In the Pursuit task, subjects had to keep a light detector on a target ($4cm^2$) moving at a constant speed (15rpm, 30rpm, or 45 rpm) around a circle in three 20 sec. trials. In the Mirror Drawing task, the subjects had to trace a starlike path seen through a mirror. On the Rotary Pursuit task, FL spent less time on the target than TL at 30 and 45 rpm, suggesting a deficit in movement correction. On the Mirror Drawing task, FL showed longer total tracing time although their performance without the mirror was equivalent to that seen in TL. This slowing was mainly due to more frequent episodes of non-progressive tracing (jitter) for FL than for TL. However, line-crossing errors were not more frequent in FL. The performance of FL on both tasks suggests a difficulty in the control of movement under sensory guidance, especially under challenging conditions.

412.6

MECHANISMS UNDERLYING ORAL AND WRITTEN PRODUCTION DIFFERENCES IN APHASIC PATIENTS. H.L. Lutsep* and N.F. Dronkers. Center for Neuroscience, UC Davis, Davis, CA 95616; and VA Medical Center, Martinez, CA 94553.

Oral and written language abilities often differ in aphasic patients. This study examined this difference in six patients with aphasia following left hemisphere infarction. All had impaired fluency, as reflected in Western Aphasia Battery (WAB) fluency scores ranging from one to six on a ten-point scale. Using 24 nouns and 24 verbs matched for length, frequency and imageability, oral and written picture naming were compared with oral and written spelling of dictated words. Oral and written naming of five different letter sounds were compared to dictation, or repetition, of the five letters themselves. Spontaneous oral and written descriptions of the WAB "picnic scene" were also obtained. The picture naming, letter dictation and spontaneous scene description tasks elicited better oral than written performances. The dictated words and letter naming, however, were more proficiently written than orally spelled. In these aphasic patients, the tasks which required involvement of letter codes appeared to be better written than spoken, whereas those tasks which bypassed letter codes were more readily orally produced.

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412.7

LOSS OF GLOBAL PROCESSING AFTER RIGHT HEMISPHERE ATROPHY

Alessandra Schiavetto*[1], Johannes Stauder[2], Laurent Mottron[2] Phillippe Robaey[2]and Maryse Lassonde[1]

[1] Department of Psychology, Université de Montréal, and [2]·Ste-Justine Hospital for Chidren, Montréal, Québec. CANADA

The purpose of this study was to investigate the global and local processing abilities in a patient with a right hemispheric atrophy restricted to the temporal lobe. This atrophy resulted from a viral infection, herpes encephalitis, when the patient was 9 years old. As a result of this illness, the patient became prosopagnosic, color agnosic and presented evidence of associative visual agnosia. Recent MRI (Jan. 1993) revealed discreet left temporal lesions and a right temporal atrophy, including the hippocampal region. In the present study, the patient was tested on two choice reaction time tasks in which hierarchical processing was manipulated: a global-local letter identification task and a figure parsing task. Ten age-matched controls were also tested on the same tasks. Analysis of A.R.'s response accuracy shows that she could never distinguish global patterns. Moreover, reaction times in the global-local task show that, unlike normals, she did not have interference effect from the global to the local level. Indeed, her reaction times were slowed when congruent stimuli were presented (local vs. global level). Similarly, in the parsing task, she did not show the usual advantage for Gestalt fit items. In contrast to normals, her reaction times were significantly slower for the Gestalt items These data suggest the involvement of the right temporal lobe in global processing. Furthermore, they may offer insight into the deficits resulting from visual agnosia.

412.8

FRONTAL LESIONS INCREASE ATTENTIONAL SUPPRESSION IN RAPID SERIAL IDENTIFICATION. F. Richer*, M. Lepage. Lab. de Neuroscience, Université du Québec, Montréal, QC, H3C-3P8.

While attentional problems have long been associated with frontal lesions in humans, the mechanisms underlying these deficits are still poorly understood. We have previously shown that frontals can have problems finding target stimuli in sequences (Richer et al., 1993) but it is not clear whether the processes of target detection or identification are different in frontal patients. To test this, we examined the effect of a first identification on a subsequent one in six patients with frontal lesions and six normals. Subjects were asked to name the two white letters embedded in rapid sequences of 15-24 black letters presented centrally on a video monitor at rates of 6 or 8/sec. After 20 practice trials, 60 sequences were presented in which the first target letter (T1) appeared randomly at positions 9 through 15 and in which the second target letter (T2) appeared randomly at positions +1, +3, +5, or +7 following T1. Both frontals and normals showed near perfect identification of T1. In the case of T2, normals showed near perfect performance at all positions while frontals showed a poorer identification of T2 at T1+1 and T1+3 (50% correct), but not at T1+5 and T1+7 at a rate of 8 letters/sec. Frontals did not show this deficit at the slower presentation rate. These results indicate that target identification produces a more pronounced or longer lasting interference on subsequent processing in frontals than normals.

412.9

SOUND LOCALIZATION PERFORMANCES BEFORE AND AFTER A THERAPEUTIC HEMISPHERECTOMY. P. Poirier*, M. Lassonde, J.G.Villemure, F. Lepore, Groupe de Rech. en Neuropsy. Exp., Univ. de Montréal, Qc, Canada, H3C-3J7.

We previously reported that localization of sound sources in hemispherectomized patients was less accurate than that of the controls in the hemifield contralateral to their removed hemisphere. Moreover, their performances obtained with fixed sources were generally more precise than those obtained with moving sources (Neuropsychologia, 1994 in press). In order to precisely evaluate the effects of the hemispherectomy on free-field sound localization performances, the present study examined response accuracy to auditory targets in one patient, shortly before and 6 months after a functional right hemidecortication was performed. We also evaluated the performances of three matched controls. Listeners reported sound positions by pointing with their dominant hand to the apparent sound location in an anechoic chamber. Two conditions were tested: (i) localization of a fixed-sound source and (ii) localization of the beginning and the end of a simulated moving stimulus. Prior to the operation, the patient was performing almost as well as the controls when fixed sources were used; however, she was completely unable to perform the task with moving sources. After the operation, the patient's responses to fixed sources were less accurate than those of the controls in all positions tested in the hemifield contralateral to the removed hemisphere. Unexpectedly, the patient was now able to perform the moving task. These results are discussed in terms of the effects attributable to the surgery per se and they suggested the existence of a differential involvement of cortical and subcortical structures in the processing of stationary and moving sounds (*supported by FCAR and CRSNG*).

412.10

SPATIAL SELECTIVE ATTENTION IN PATIENTS WITH PARIETAL AND TEMPORAL-PARIETAL LESIONS. A.P. Iha[1,2], A.F. Kingstone*[2], G.R. Mangun[1,2]. (1) University of California at Davis Department of Psychology, Davis, CA 95616. (2) Center for Neuroscience, Davis, CA 95616.

There has been growing evidence on the role of the parietal lobes in spatial selective attention. In detection paradigms, patients with parietal damage are slower to respond to invalidly cued contralesional targets than invalidly cued ipsilesional targets. Though the relative reaction time (RT) benefits of cue validity were maintained in these patients, they were impaired in their ability to "disengage" attention from the current focus and move it to the contralesional location (Posner et al., 1984). Here we used a discrimination task, wherein d' measures were utilized to understand the role of perceptual sensitivity in the disengage deficit. Normal and age-matched controls, and patients with parietal and temporal-parietal lesions performed a task in which central arrow cues gave predictive information about where in space (left or right of fixation) targets were likely to occur. A go/no-go response was required, with both targets and nontargets occurring with equal probability. The d' results in normal and age-matched controls indicated that perceptual sensitivity was higher for validly than invaldily cued targets. Preliminary data from temporal-parietal patients showed this pattern as well. Parietal patients, however, did not show differences in d' as a function of spatial cueing, though their overall d' scores were comparable to those of the temporal-parietal patients.

412.11

VISUOSPATIAL ATTENTION SHIFT IN CEREBELLAR DISORDER. S. Yamaguchi*, H. Tsuchiya and S. Kobayashi. Third Div. of Internal Medicine, Shimane Med. Univ., Izumo, 693 Japan.

Cerebellum has been suggested to be involved in attentional shift mechanism. The present study investigated contributions of cerebellum on visuospatial attentional ability in a trial-by-trial cueing task involving covert orientation of visual attention. Event-related potentials (ERPs) and reaction times (RTs) were measured in patients with cerebellar degenerative disorders and age-matched controls. The cerebellar group had slower RTs to both valid and invalid targets. The effects of cue validity on RTs were larger for the cerebellar group in both the central and peripheral cue experiments, suggesting efficiency of the attention shift process in the cerebellar group. ERP data over the cue-target interval (i.e. 800msec) in the central cue experiment demonstrated comparable effects of voluntary attention shift in both groups, the effects which were reflected in negative potential shifts at contralateral parietal and temporal sites in the early stage and at central and frontal sites in the late stage. Peripheral cues also generated comparable effects of the early automatic and late voluntary attentional shift on ERPs in the cerebellar and control groups. The only difference in ERPs was CNV amplitudes, which were significantly diminished in the cerebellar group. These results suggest a preserved ability of visuospatial attention shift in cerebellar disorder.

412.12

COGNITIVE DEFICITS FOLLOWING CEREBELLAR LESIONS IN HUMANS: STUDIES OF ATTENTION AND VERBAL FLUENCY. L.L. Helmuth* and R.B. Ivry. Dept. of Psych, Univ. of California at Berkeley, Berkeley, CA 94720.

The human cerebellum may participate in a number of cognitive skills. Akshoomoff & Courchesne (1992) have reported that children with cerebellar damage show deficits on attention shifting tasks. Fiez, et al. (1992) have suggested that the cerebellum plays a role in error detection. These studies have involved either case reports or children with cerebellar disorders. We have tested these hypotheses on a group of adults with cerebellar pathology. One group has bilateral cerebellar atrophy, with some showing involvement of brainstem structures. Patients in the other group have unilateral focal lesions. Subjects were tested on the Posner cueing task, a semantically cued attention shifting task, a verbal fluency task, and a verbal discrimination task. Few differences were found between age-matched controls and cerebellar subjects from either group. Performance on these cognitive tasks was relatively spared despite a wide range of differences in the patients' deficits on motor tasks. Finally, we will compare the performance of cerebellar patients on attention shifting and error detection tasks with their performance on neuropsychological measures of frontal lobe function.

412.13

BRAIN VOLUME AND NERVE CONDUCTION VELOCITY: CORRELATIONS WITH IQ. J. C. Wickett*[1], P. A. Vernon[1], D. H. Lee[2], J. D. Brown[3], D. W. Snow[3] and B. K. Rutt[2]. [1]Dept. of Psychology, Univ. of Western Ontario, London, ON N6A 5C2; [2]Diagnostic Radiology and Nuclear Medicine, Univ. Hospital, London, ON N6A 5A5; [3]Dept. of Clinical Neurological Sciences, Univ. of Western Ontario, Univ. Hospital, London, ON N6A 5A5.

Recent research in the field of intelligence has witnessed a resurgence in the search for biological explanations. The current study examines two such explanations: brain volume and nerve conduction velocity. This study is composed of two phases. In Phase I, 40 healthy adult female subjects (ages 20 to 30 years) underwent magnetic resonance imaging (MRI) and nerve conduction velocity (NCV) procedures. It was found that brain volume was significantly correlated with IQ (r=.395, p<.05), but that NCV measures (taken along the median nerve of the arm) were not so correlated (rs=-.12 and .02, both *ns*). A reanalysis of this and past NCV findings indicated a possible sex difference with NCV being related to IQ in males but not in females. Finally, it was found that brain volume and NCV were not correlated (rs from .10 to .19, all *ns*). Phase II of this study, which is currently under way (36 subjects collected to date, with new subjects being obtained at the rate of about 3 per week), expands on these findings with a sample of healthy adult male siblings. Beyond a simple replication, Phase II uses MRI procedures that allow for advanced multi-exponential calculations of white matter relaxation times in addition to assessment of brain volume, and also incorporates central, in addition to peripheral, NCV. The use of siblings allows for assessment of within as well as between family effects, and so can illuminate the types of genetic and/or environmental factors at work in obtained correlations.

412.14

CENTRAL AUDITORY PROCESSING IN A FAMILY WITH 'DYSARTHRIA'. D M Daly*, Box 210855, Dallas, TX 75211-0855

'Dysarthrias' include various disorders involving control of movements in speaking. Using sets of computer synthesized sounds[1] we examined auditory processing in a 6 yr. old boy referred with a diagnosis of 'functional dysarthria' and seven other members from three generations of his family. All had appropriate audiometric thresholds for age. One sibling, mother, and maternal grandmother reported similar problems: their speech is difficult for unaffected individuals to understand, yet they understand each other with less difficulty; they have difficulty drawing circles, turning a screwdriver/rolling hair curlers, or pedaling a bicycle; they are athletically challenged; they also have difficulty 'carrying a tune'. Two generations have received school based speech therapy; all three generations reported that speech improved spontaneously at 10-11 yr. All performed at or above grade level on core subjects. Unaffected individuals are free of these problems.

On sets of time varying synthesized sounds, neither proband, mother nor maternal grandmother distinguished BW. Mother and maternal grandmother reported GY as vocalic *e* and *ye* with clear transition 20-30 msec less than controls. The affected sibling also differed significantly from controls. On sets of frequency varying sounds (BDG) mother and sons were less aberrant. Paternal grandmother, father, paternal aunt, and proband's unaffected brother had clear, well-defined transitions. Concurrent testing of affected and unaffected members confirmed marked perceptual differences; LRCS measures of divergence from individual and control composites substantiated pairwise intelligibility ratings. Distribution in the pedigree is compatible with autosomal dominant expression.

1 J Neurophysiol (44:1, 200, 1980). Testing contributed by inventor who retains all proprietary rights and interests.

412.15

A VERBAL ABSTRACTION DEFICIT IN MULTIPLE SCLEROSIS. W.W. Beatty*, K.A. Hames, C.R. Blanco, R.H. Paul and S.L. Wilbanks. Dept. Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190.

Impairments on nonverbal tests of abstraction by patients with multiple sclerosis (MS) have been reported frequently, but findings on the verbal Similarities test from the WAIS are equivocal. To reexamine the status of verbal reasoning in comparison to nonverbal reasoning in MS we administered the Shipley Institute of Living Scale (SILS), the Wisconsin Card Sorting Test (WCST), and a shortened version of the Free Sorting part of the California Card Sorting Test (CCST) to 100 MS patients and 32 age- and education-equated control subjects. Patients achieved fewer categories on the WCST, fewer correct sorts on the CCST, and lower scores on the Abstraction scale from the SILS than did control subjects. Patients also scored lower on the Vocabulary Scale from the SILS, but because they also attained lower Conceptual Quotients, their poorer performance on the verbal abstraction cannot be attributed solely to lower verbal ability. Perseverative responding by patients was clearly elevated on the WCST, marginally increased on the CCST, and did not occur on the SILS, suggesting that difficulties in generating and identifying concepts are the major reasons MS patients often have difficulty on tests of problem solving or abstract reasoning.

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412.16

DISORDERS OF PROSODY IN PARKINSON'S DISEASE
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Disturbances of intonational patterns of speech are some of the most common signs in Parkinson's disease (PD). If deficits in movement planning are responsible for this deterioration, we should then observe greater impairment in PD as the speech production tasks become more difficult. In this study we attempted to determine whether PD patients are more impaired, relative to controls, in the production of simple vowel syllables in increasingly more complex intonation contexts. Twenty non-demented PD patients (mean age = 68.6) and 15 controls matched for age and education (all subjects ≤ 75 yr and no other neurologic or psychiatric disorder) were asked to produce the vowels /a, e, o/ in four intonation conditions: two stable contexts (low and high intonation) and two unidirectional change contexts (ascendent and descendent intonation) for a period of five seconds. Subjects were recorded individually in a soundproofed room and speech was then digitized to extract indices of fundamental frequency (F_0). ANOVAS were conducted on F_0 measures, for each intonation condition, to determine the extent of group differences. Except for the low stable intonation condition, analyses revealed main effects of Group, suggesting a significantly more restricted F_0 range in PD patients when producing vowels in stable high, as well as in ascending and descending intonation conditions (all p's < .001). The results suggest that the subcortical structures affected in idiopathic movement disorders are also involved in disorders of prosody. These structures are implicated in deficits in planning and sequential execution of movement and cognitive acts.

412.17

VISUOSPATIAL DYSFUNCTION AND PROBLEM SOLVING IN PARKINSON'S DISEASE. A. Cronin-Golomb*, A.E. Braun. Department of Psychology, Boston University, Boston MA 02215.

Impaired performance by subjects with Parkinson's disease (PD) on Raven's Coloured Progressive Matrices (RCPM) contrasts with their normal performance on other problem-solving tasks and suggests an underlying visuospatial deficit. We analyzed RCPM results in 59 subjects with PD (9 demented), 45 subjects with Alzheimer's disease (AD) (19 with extrapyramidal signs), and 39 healthy age-matched control subjects. The PD and AD groups all performed deficiently, especially the demented PD group. For the nondemented PD group, scores on the RCPM subtest that mainly assessed visual closure ability (RCPM-A) correlated consistently with scores on other visuospatial tests but not non-visuospatial tests. The results support the view that there is a visuospatial deficit in PD, exacerbated by dementia, and that this deficit may adversely affect problem solving. The brain basis of the visuospatial dysfunction may be the posterior parietal lobes, a major component of the basal ganglia-thalamocortical circuit that also includes the dorsolateral prefrontal cortex.

412.18

AGE-RELATED CHANGES IN THE ELECTROPHYSIOLOGICAL RESPONSE TO VISUAL STIMULUS NOVELTY: A TOPOGRAPHICAL APPROACH. K.M.Thomas*, C.A.Nelson, and S.M.Malone. Institute of Child Development, University of Minnesota, Minneapolis, MN 55455.

In this study we examined age-related changes in the scalp distribution and topography of the novelty response in a visual event-related potential (ERP) paradigm in order to examine the possible neural substrates underlying such responses. ERP data were collected from neurologically normal adults (mean age=24) and children (mean age=8). Participants completed a variation of the oddball paradigm with frequent non-target (70% probability), infrequent target (15% probability) and infrequent novel (15% probability) stimuli. Two female faces posing neutral expressions served alternately as the target or non-target stimulus. Novel stimuli consisted of non-repeating (trial-unique), easily recognizable scenes, objects and animals, as well as abstract patterns which were difficult to label verbally. Participants were required to press a button in response to the target stimuli while disregarding all other stimuli. An incidental memory task at the conclusion of the session assessed memory for the irrelevant novel stimuli. Preliminary results indicate age-related differences in the amplitude of the N200 and the latency of the P300 components to the target and novel stimuli. Topographical maps of activity to the novel stimuli show a pattern of frontal activation in children not seen in adults. Results are compared to previous work with infant subjects and are discussed in terms of differences in attentional capacity, as well as the role of hippocampal and frontal lobe development in mediating the novelty response.

412.19

DOES IMPAIRMENT OF WORKING MEMORY PRECEDE DEVELOPMENT OF DEMENTIA IN EARLY ALZHEIMER'S DISEASE? S. Murtha, H. Chertkow*, H. Bergman, N. Phan. Bloomfield Center for Studies in Aging, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada.

Working memory (the store capable of holding information in memory while another cognitive task is performed) is impaired in subjects with dementia of the Alzheimer type (DAT) compared with elderly control subjects. We wished to examine whether working memory impairment might be demonstrable in elderly subjects not yet meeting criteria for dementia but having Age Associated Cognitve Decline (AACD).

Working memory was assessed with the Brown/Peterson task on 15 DAT, 17 AACD, and 12 elderly control subjects. On this task, consonant trigrams (three letters presented visually) are recalled over periods of short duration (0, 5, 10 seconds (s)). During the time interval the subject is either allowed to rehearse the material with no distraction or is distracted with subsidiary tasks (articulation or digit reversal).

The results indicated that the average percentage of trigrams recalled by the three groups differed the most for the digit reversal distraction condition. The DAT subjects showed a moderate decline after 5s (55%) and 10s (42%) delay intervals, and the AACD subjects showed a mild decline at both the 5s (83%) and 10s (77%) interval. Elderly controls showed considerably less decline in both conditions.

The percentage of trigrams recalled with digit reversal distraction after 5s indicated that the control subjects never showed greater then a 20% decline from baseline performance. 4/17 of the AACD subjects, and 10/15 of the DAT subjects demonstrated a decline below this performance level. These results demonstrate that working memory is not only impaired in DAT, but also in AACD subjects.

It is known that up to 2/3 of AACD patients eventually progress to dementia. Since working memory impairment can be demonstrated in a percentage of the AACD subjects it is possible that this might be a cognitive marker for future development of dementia.

412.20

IMPAIRED AUTONOMIC RESPONSES TO EMOTIONALLY SIGNIFICANT STIMULI IN ALZHEIMER'S DISEASE. C-C. Chu,* D. Tranel, A. R. Damasio. Dept. of Neurology, Univ. Iowa, Coll. Med., Iowa City, IA 52242

Pathological changes in Alzheimer's disease (AD) selectively involve layer V neurons in Brodmann's area 25, posterior orbitofrontal cortex, and anterior insula, which project directly to subcortical autonomic centers. Disruption of these cortico-autonomic projections would be expected to compromise autonomic responses. We hypothesized that autonomic responses elicited by emotionally significant pictures would be impaired in AD patients. Color slides with high (target) or low (nontarget) emotional significance were shown for 6-s each while skin conductance and heart rate were measured. Target pictures significantly increased heart rate and skin conductance in non-demented age-matched controls (N=18). However, in mild-moderate AD patients (N=18), this effect was completely absent for heart rate responses and severely diminished for skin conductance responses. The autonomic response defect in the AD patients was specific to emotionally-charged stimuli. The initial cardiac deceleration during perception was intact, as was the ability to generate skin conductance responses to deep breathing and loud noises. Heart rate change during withheld deep breathing was also normal. The results demonstrate defective autonomic responses to emotionally charged stimuli, which may be attributed at least in part to the disruption of cortico-autonomic projections in AD patients. The findings are consistent with our proposal that activation of somatic states which play an important role in decision-making and social behavior depends on ventromedial cortices.

413.1

SIMULTANEOUSLY RECORDED NEURONS IN THE LATERAL AMYGDALA OF FREELY MOVING RATS DURING FEAR CONDITIONING. G.J. Quirk*, P. Tao, J.C. Repa, and J.E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

To understand the neural mechanisms underlying the processing of an acoustic conditioned stimulus (CS) paired with a footshock unconditioned stimulus (US) during fear conditioning, we recorded simultaneously isolated cells in the lateral nucleus of the amygdala (LA) with a movable bundle of microwires in freely moving rats. Responses to the CS (2 sec pure tone) were compared during trials involving sensitization (unpaired CS and US), conditioning (CS-US pairing), and retention (CS alone) trials. For 12/16 neurons recorded from the dorsal LA and the immediately adjacent amygdalo-striatal transition area, CS elicited responses at the end of conditioning increased an average of 5 times above sensitization. One hour post-conditioning this increase was maintained or increased for 7 cells. About one-third of the cells exhibited increases within the first 20 ms of the CS, while the increase occurred at latencies of 20-40 ms in the others. Early responses are most likely pre-cortical, while later responses may involve cortical areas. Cells in ventral part of LA or in the caudate-putamen were tone-responsive but failed to show conditioned responses. Cross-correlations between the spontaneous spike trains of cell pairs revealed examples of synchronous firing, recurrent inhibition, and monosynaptic excitation. In some cases, conditioning-induced changes in these measures were observed, suggesting plasticity in local connections. NIMH 38774, 46516, 00956.

413.2

EFFECTS OF CORTICAL LESIONS ON GENERALIZATION OF LEARNED FEAR RESPONSES. J.L. Armony*, D. Servan-Schreiber and J.E. LeDoux. Center for Neural Science, New York Univ. and Dept. of Psychiatry, Univ. of Pittsburgh School of Medicine.

Auditory information reaches the lateral nucleus of the amygdala by way of two parallel pathways. The first one involves a direct projection from the auditory thalamus, whereas the second pathway involves transmission over thalamo-cortical routes, ultimately to the amygdala. Either pathway is sufficient for eliciting fear responses to a single auditory conditioned stimulus (CS) paired with a footshock (Romanski & LeDoux, 1992). Nevertheless, the two pathways have different physiological properties, which suggest that the cortical input to the amygdala may be necessary for an accurate representation of complex sensory stimuli. In order to further investigate the processing limitations of the direct pathway, we began a series of studies involving lesions of the auditory cortex in rats. Two weeks after aspiration of most of primary auditory cortex, animals were conditioned to a single tone paired with a 1mA footshock in a conditioned suppression of operant responding paradigm. Following conditioning, auditory stimuli of different frequencies were presented and a stimulus generalization gradient (SGG) was obtained. These lesions did not have a significant effect on the SGG. If confirmed by larger lesions, including damage to auditory association cortices, these results would suggest that the auditory cortex may not be essential for some aspects of frequency analysis. These results are consistent with predictions of a computational model of the neural circuit involved in fear conditioning (Armony et al., 1993, 1994). Supported by MH38774, MH 46516 and MH00956.

413.3

FACILITATION OF AMPA RECEPTORS ACCELERATES CLASSICAL FEAR CONDITIONING IN RATS. M. Rogan*, U. Staubli, J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003

Ampakines are a class of drugs that facilitate AMPA receptor-mediated synaptic responses. This has the effect of increasing NMDA receptor-mediated currents in cells containing both NMDA and AMPA receptors. Ampakines have a documented memory-enhancing effect on several learning tasks, and also accelerate LTP induction *in vitro* and *in vivo*. Since fear conditioning has been found to be disrupted by interference with NMDA function, the possible enhancing effect of ampakines on fear conditioning was assessed in the present study. Rats were trained on a task that consisted of 2 pairings a day of an acoustic conditioned stimulus (CS: 10KHz, 80 dB, 20s) with a footshock unconditioned stimulus (US: 0.3mA, .5s) for 2 consecutive days. Thirty minutes prior to training on each of the 2 training days, the ampakine group (n=6) received an i.p. injection of CX-516 (50mg/kg), and the control group (n=3) received the equivalent volume of vehicle (cyclodextrin 33%). On the third day, both groups received injections of vehicle 30min prior to 2 trials of the CS alone. Learning was assessed by measuring the time accounted for by freezing during the 20s CS. The control group displayed normal acquisition of CS conditioning, freezing 6s after 1 day of training, and 11s after the second day of training. The ampakine group froze twice as much as the controls after one day of training (13s), a level that controls required 2 days of training to reach. That this effect is not reflective of a general increase of fearfulness, nor simply a hyper-reactivity to the CS, is indicated by the fact that neither group displayed any unusual behavior, nor froze to the CS or context prior to the first CS/US pairing. These findings are consistent with ampakine-induced acceleration of the formation of the aversive association between the CS and the US. That ampakine significantly accelerated, but did not significantly increase the ceiling, of learning is consistent with data on ampakine effects on LTP. Supported by MH38774, MH46516, MH00956.

413.4

ETHANOL (ETOH) PRE-TREATMENT SELECTIVELY IMPAIRS CLASSICAL CONDITIONING OF CONTEXTUAL CUES: POSSIBLE INVOLVEMENT OF THE HIPPOCAMPUS. K. Melia?K. Corodimas, A. Ryabinin, M. Wilson and J. LeDoux, Ct. For Neural Science, N.Y.U., NY, NY, 10003, and Dept. Neuropharm., Scripps Res. Ins., La Jolla, CA, 92037.

ETOH pre-treatment significantly attenuates the induction of c-fos mRNA by restraint stress in hippocampus but not cortex, suggesting that ETOH intoxication impairs information processing at the level of the hippocampus (Ryabinin et al., Neurosci. Abs., 1993). This finding led us to hypothesize that ETOH exposure prior to classical conditioning would impair acquisition of hippocampal dependent associations, such as conditioning to context, but not hippocampal independent associations, such as conditioning to a tone. To test this hypothesis animals were injected with NaCl or 1.0, 1.5, or 2.0 g/kg 16% ETOH (n=6/group) 10 min prior to the presentation of 10 tone (80 dB)/foot shock (0.5 mA) pairings. Conditioned freezing to context and to the tone were measured (20s/trial) 48 hours later. Time spent freezing to context was significantly and dose dependently decreased in animals pre-treated with ETOH (NaCl = 16s; ETOH1.0 = 12s; 1.5 = 7s; 2.0 = 2s), while freezing to the tone was not affected except at the highest ETOH dose (NaCl = 18s; ETOH 1.0 = 17s; 1.5 = 17s; 2.0 = 13s).

These findings indicate that ETOH intoxication selectively impairs the acquisition of contextual conditioning. In light of our previous finding, it is possible that this impairment is due to inhibition of information processing at the level of the hippocampus. Experiments to determine whether ETOH pre-treatment alters the induction of c-fos mRNA in the hippocampus by this classical conditioning paradigm are currently underway. Supported by: MH38774; MH46316; MH00956; NSF IBN 9209646, and NARSAD.

413.5

POSTTRAINING LESIONS OF PERIRHINAL CORTEX DISRUPT CLASSICAL FEAR CONDITIONING: CONTRIBUTION OF CONTEXTUAL CUES. K.P. Corodimas* and J.E. LeDoux Center for Neural Science, New York University, NY, NY 10003.

Posttraining lesions of the rostral perirhinal cortex (rPRh) disrupt conditioned fear responses to auditory and visual stimuli when presented in a novel context. We examined whether rPRh lesions have similar effects in the training context. Two days after male rats received classical fear conditioning, involving the pairing of an auditory conditioned stimulus (CS) with footshock, bilateral electrolytic lesions were produced in the rPRh. Five days later, conditioned fear (freezing) was measured to a 60 sec CS in a novel context and then in the training context. There were three major findings. First, lesioned animals froze significantly less than controls to the CS in the novel and in the training context. Second, lesioned animals also froze less to the training context itself. Third, rPRh-lesioned animals froze more to the CS in the training context than in the novel context. These findings suggest that rPRH lesions affect conditioning to contextual and discrete stimuli, and despite deficits in contextual processing, rPRh-lesioned animals were able to use contextual information to facilitate retrieval of memories about the aversive CS. We conclude that rPRh lesions affect processes related to information storage or retrieval. Supported by MH46516, MH38774, MH00956 and NSF9209646.

413.6

MEDIAL ORBITAL CORTEX LESIONS INCREASE RESISTANCE TO EXTINCTION BUT DO NOT AFFECT ACQUISITION OF FEAR CONDITIONING. M.A. Morgan,* J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003

As part of an ongoing series of experiments to examine the role of medial prefrontal cortex in fear, we examined the effects of lesions of medial orbital cortex (MO) in a fear conditioning task. Rats received lesions of MO (n=9) or sham lesions (n=12). Two weeks after surgery, they received 2 pairings a day of a tone conditioned stimulus (CS: 10KHz, 80 dB) with a footshock unconditioned stimulus (US: 0.5mA, 0.5sec.) for 2 consecutive days (acquisition). The following days were the same but no US was presented (extinction). Testing continued until extinction criterion (5 sec or fewer freezing during the CS and pre-CS on two consecutive days) was met. Conditioning to both the CS and to the context in which conditioning took place were examined. MO lesioned rats did not differ significantly from controls during acquisition to either the context or the CS. They did, however, take significantly longer to extinguish to both stimulus types. It is unlikely that this effect is due to a general increase in learned fear, given that there is no apparent increase in freezing during acquisition. Instead, the effects may reflect an emotional equivalent of the cognitive perseveration found after frontal cortex damage in humans and other primates. This conclusion is consistent with findings from single cell recordings in monkey orbitofrontal cortex indicating that these cells play a crucial role in updating the brain as to the present reward value of a stimulus. Supported by NSF 9209646, MH46516, MH38774, MH00956.

413.7

SEPTAL LESIONS POTENTIATE FREEZING BEHAVIOR TO CONTEXTUAL BUT NOT TO PHASIC CONDITIONED STIMULI.
P.D. Sparks* and J.E. LeDoux. Department of Psychology and Center for Neural Science, New York University, NY,NY 10003.

Damage to the hippocampal formation interferes with the conditioning of fear responses to contextual but not phasic conditioned stimuli. Given the anatomical connections and functional relationship between the hippocampus and the septum, we examined the effects of septal lesions on the conditioning of fear reactions to phasic and contextual stimuli. In the first experiment, rats with electrolytic lesions (n=9) or sham operations (n=9) of the septum were studied. Two weeks after surgery, blocks of two conditioning trials consisting of a tone (10kHz, 75 dB, 20 sec) paired with a foot shock (500 msec, 0.5 mA) were presented on two consecutive days. Tone alone trials were presented each day thereafter until extinction criterion was met. The amount of freezing elicited by the context but not by the tone was greater in the septal lesioned animals than in controls. The lesions involved the lateral and to a lesser degree the medial septum. In experiment two, quisqualitic acid (27.7 mg/ml, 550 nl; n=4) or vehicle (n=6) was infused into the septum. The rats were treated as above. These lesions, which mainly involved the lateral septum, also potentiated the freezing elicited by the context but not the tone. Neurons in the septum thus appear to be involved in the acquisition and/or expression of defensive reactions to a dangerous context. Supported by NSF9209646, MH38774, and MH00956.

413.8

C-FOS mRNA EXPRESSION FOLLOWING FOOTSHOCK STRESS AND CONTEXUAL FEAR CONDITIONING.
J. B. Rosen[1]*, M. S. Fanselow[2], S. L. Young[2] and S. Maren[2]. [1]Biological Psychiatry Br., NIMH, Bethesda, MD 20892, [2]Psychol. Dept., Univ. of Cal, Los Angeles, CA 90024-1563.

Rats display conditional freezing behavior to contextual cues associated with footshock. Although the amygdala plays a prominent role in the expression of fear, and the hippocampus plays a role specific to contextual fear, little is known about other forebrain regions involved in fear. Therefore, this study examined regional expression of *c-fos* mRNA as a marker for cellular activity elicited by footshock and by context conditioning. Rats were given either a footshock immediately or 3 minutes after placement in a chamber. Other rats were placed in the chamber without receiving footshocks or handled without exposure to the chamber. Half of the rats in each group were sacrificed 15 minutes after exposure. The other half were re-placed in the chamber without shock 24 hours later for a retention test and sacrificed 15 minutes later. Only rats receiving shock 3 min after placement in the context showed significant levels of freezing. *In situ* hybridization with a cRNA probe complimentary to *c-fos* mRNA was conducted. Image analysis revealed increased *c-fos* mRNA in the medial nucleus of the amygdala following both types of shock, but no increases after the retention test. No differences were found in the dorsal hippocampus. *C-fos* mRNA was increased in the pyriform cortex in both shock groups after both test situations. Cingulate and parietal cortices of all groups exposed to the context had increased *c-fos* mRNA levels relative to handled controls. The results demonstrate a complex pattern of activity of forebrain regions in response to shock and contextual fear conditioning.

413.9

HIPPOCAMPAL LESIONS BLOCK LATENT INHIBITION BUT NOT NEGATIVE TRANSFER BY A NON-NMDA RECEPTOR MEDIATED PROCESS.
S. L. Young*, S. Maren and M. S. Fanselow. Dept. of Psychol., Univ. of Cal., Los Angeles, CA 90024-1563.

Latent inhibition describes a Pavlovian learning deficit caused by preexposure to the conditional stimulus (CS). Negative transfer is also a learning deficit but is caused by prior pairing of the CS with a weak unconditional stimulus (US). The two phenomena have traditionally been considered the result of a similar CS processing deficit. Evidence that both negative transfer and latent inhibition are context specific support this speculation. Latent inhibition is reversed by hippocampal lesions but the hippocampus dependence of negative transfer has never been tested. Experiment 1 examines the possibility that negative transfer is hippocampus dependent like latent inhibition. Hippocampal lesions blocked latent inhibition but had no impact on negative transfer. These results provide evidence that latent inhibition and negative transfer are different. The second set of experiments examined the role of NMDA receptors in latent inhibition. Application of a competitive NMDA receptor antagonist (APV, 5 μg/rat) had no impact on hippocampus and context dependent latent inhibition. These results are surprising because NMDA receptor mediated processes in the hippocampus are important for learning to recognize contextual cues. Supported by NIMH 1F31MH10383-01 (SLY) and NSF BNS 9008820 (MSF).

413.10

SYNAPTIC PLASTICITY IN PROJECTIONS FROM THE ENTORHINAL CORTEX TO THE BASOLATERAL AMYGDALA IN ANESTHETIZED RATS: AN EXTRACELLULAR FIELD POTENTIAL ANALYSIS.
S. Maren* and M. S. Fanselow. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Pavlovian fear conditioning to contextual conditional stimuli (CSs) in rats requires the hippocampus for CS representation, but not CS-unconditional stimulus (US, footshock) association. CS-US association is presumed to occur in the amygdala and may be mediated by an activity-dependent synaptic plasticity in anatomical pathways that convey context information to the amygdala. One putative context CS pathway consists of monosynaptic projections from the lateral entorhinal cortex (LEA) to the basolateral nucleus of the amygdala (BL). In the present study, we sought to determine if this pathway is capable of physiological plasticity and, if so, the nature of the plasticity. Urethane-anesthetized adult male Long-Evans rats were implanted with a bipolar stimulating electrode in the LEA and a recording electrode in the posterior BL. Single-pulse stimulation (0.1 - 2.0 mA) of LEA evoked a reliable biphasic extracellular field potential (FP) in BL, which consisted of a negative-positive-negative waveform (peak-to-peak amplitude ≈ 1 mV). LEA-evoked unit firing in BL occurred with a short latency (5 - 8 ms) and was correlated with the initial negativity of the FP. High stimulation intensities (>500 μA) revealed a sharp negative-going potential, possibly a population spike (PS), that was superimposed on the initial negativity of the FP. Paired-pulses (10-100 ms ISI) or short (10 ms) trains of high-frequency stimulation (HFS, 50 or 100 Hz) revealed short-term plasticity of LEA-BL field potentials, which was manifest as an increase in the amplitude of the FP peak negativity. Longer trains of HFS (10 200 ms bursts of 100 Hz delivered at 1 Hz; series repeated four times at 5 min intervals) revealed a long-term potentiation (LTP) of LEA-BL responses. In contrast, the same number of pulses delivered at low frequency (1 Hz) produced a long-term depression (LTD) of the responses. Synaptic plasticity such as that reported here may underlie CS-US association during fear conditioning. Supported by NSF grant BNS-9008820 to MSF.

413.11

SEX DIFFERENCES IN HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) AND PAVLOVIAN FEAR CONDITIONING IN RATS.
M. S. Fanselow*, S. Maren and B. De Oca. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

An emerging body of evidence indicates that the hippocampus is required for some forms of classical conditioning, particularly Pavlovian fear conditioning to contextual conditional stimuli (CSs). Lesions placed in either the fornix, dorsal hippocampus, or entorhinal cortex produce marked impairments in contextual fear conditioning. Additionally, recent data indicate that manipulations which affect hippocampal LTP, such as NMDA receptor antagonists or water deprivation, also affect context conditioning. Because there are sex differences in the performance of many hippocampus-dependent learning tasks in rats, we sought to determine if there are corresponding sex differences in hippocampal LTP and contextual fear conditioning. Pentobarbital-anesthetized rats of three ages (20, 35, and 60 days) were implanted with a bipolar recording electrode in the hilus of the dentate gyrus and a stimulating electrode in the perforant path. There was a robust sex difference in the magnitude of LTP induced in both 35 and 60 day old rats (20 day old rats did not show LTP) with males showing significantly greater LTP than females. This sex difference in LTP was correlated with a similar sex difference in levels of NMDA receptor activation during LTP induction. In addition to sex differences in LTP, we also observed a significant sex difference in fear conditioning to contextual cues. When tested in a chamber that was paired 24 hours earlier with footshock, male rats showed substantially greater levels of freezing, a species-specific fear response, than their female counterparts. This sex difference in freezing was specific to contextual fear conditioning because fear conditioning to a discrete tone CS was not different in male and female rats. The positive correlation between sex differences in hippocampal LTP and contextual fear conditioning provides further evidence for a linkage between the two. Supported by NSF grant BNS-9008820 to MSF.

413.12

DIFFERENTIAL EFFECTS OF VENTRAL AND DORSAL PERIAQUEDUCTAL GRAY (PAG) LESIONS ON DEFENSIVE RESPONSES OF RATS TO CATS, SHOCK AND TASTE AVERSION.
B. De Oca, J. P. DeCola, J. C. Liebeskind* and M. S. Fanselow. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Anatomical structures within the midbrain have been implicated in fear-related and defensive behavior. The present experiments showed that lesions of the midbrain that included damage to the ventral portions of the periaqueductal gray (PAG) reduced the defensive freezing response of rats to the presence of a cat. Lesions that spared the ventral PAG did not affect freezing to a cat even if they produced extensive damage to the dorsal PAG, lateral PAG and superior colliculus. We also examined freezing conditioned by exposure to electric footshocks. Massed shocks produced less freezing than spaced shocks. This difference was eliminated by midbrain lesions because ventral PAG lesions reduced freezing under both conditions and dorsal-lateral PAG lesions selectively enhanced freezing in the massed condition. Midbrain lesions did not influence taste aversions to a saccharin solution that was associated with the gastrointestinal malaise produced by LiCl. Supported by NIMH grant MH-39786 to MSF.

413.13

LESIONS OF THE AMYGDALA BLOCK CONDITIONED EXCITATION BUT NOT CONDITIONED INHIBITION OF FEAR MEASURED WITH FEAR-POTENTIATED STARTLE. W.A. Falls* & M. Davis, Dept. of Psychiatry, Yale Univ. Med. Sch., 34 Park St., New Haven, CT 06508.

Although much is known about the neural systems responsible for the acquisition and expression of conditioned fear, little is known about the neural systems responsible for the inhibition of fear. We have recently detailed a behavioral procedure for producing conditioned inhibition of the fear-potentiated startle effect. Following training in which a light is repeatedly paired with shock (i.e., light+) and a light-noise compound is presented without shock (i.e., noise→light-), the noise acquires the ability to inhibit fear-potentiated startle to the light in a noise→light summation test (Falls & Davis, 1993; *Neurosci. Abs.* vol. 19, p.372, #155.6).

Amygala lesions performed after either moderate or extensive training block fear-potentiated startle. However, if lesions are made after extensive training, fear-potentiated startle can be re-acquired. Because of this, it is possible to use a summation test to assess whether lesions of the amygdala would disrupt previously acquired conditioned inhibition. Rats were given 15 light+shock pairings on each of 2 days followed by conditioned inhibition training consisting of 5 light+shock trials intermixed with 15 noise→light no shock trials on each of 5 days. Testing occurred 1 day later. All rats showed conditioned fear to the light as defined by greater startle amplitude in the presence of the light than in its absence. All rats showed conditioned inhibition of fear, defined as a reduction in fear-potentiated startle to the light when accompanied by the noise. One day later, half of the rats were given amygdala lesions and half were sham operated. All rats were tested 1 week later. As expected, lesions of the amygdala blocked fear-potentiated startle to the light. On each of the next 5 consecutive days, the rats were retrained with light+shock pairings with no further conditioned inhibition training. Testing occurred 1 day later. Amygdala lesioned rats re-acquired fear-potentiated startle to the light. Importantly, the noise conditioned inhibitor retained its ability to inhibit fear-potentiated startle to the retrained light.

These results indicate that areas of the amygdala critical for initial performance of fear-potentiated startle are not critical for the expression of conditioned inhibition.

413.14

CHOLINERGIC SYSTEMS AND THE CONSOLIDATION OF CONTEXTUAL AND AUDITORY FEAR CONDITIONING IN INFANT RATS. Jerry W. Rudy* and Rachael Hermance, Department of Psychology, Univ. of Colorado, Boulder, Co 80309

Rats 23 days old that experience a pairing of an auditory cue and shock (1 mA) 24 hrs later show conditioned freezing to both the conditioning context and the auditory cue. Scopolamine hydrobromide (1.0 mg/kg) given prior to conditioning, however, blocked both forms of fear conditioning. Yet, the peripheral antagonist, methylscopolamine had no effect on either contextual or auditory cue fear conditioning. Scopolamine also retroactively influenced both forms of conditioning: Rats administered the drug either 10, 30, or 120 min after the shock also showed reduced conditioning. Yet, when the drug was administered 24 hrs after training, it had no effect. A 2-min exposure to the context 24 hrs prior to conditioning, however, protected the animals against the effects of the cholinergic blockade on contextual fear conditioning. These results suggest that central cholinergic systems play an important role in the consolidation of the memory for both contextual and auditory fear conditioning in infant rats. They also suggest that cholinergic systems contribute to contextual fear conditioning by mediating the consolidation of a memory representation of context.

413.15

DIFFERENTIAL EFFECTS OF AMYGDALA LESIONS ON ANTINOCICEPTIVE AND CARDIOVASCULAR RESPONSES TO CONDITIONAL AND UNCONDITIONAL AUDITORY STRESSORS. F.J. Helmstetter*, G. Gale & S.A. Tershner, Department of Psychology, University of Wisconsin, Milwaukee, WI 53201

Exposing rats to a tone that has been paired with shock or to a novel loud noise will provoke a set of fear-related defensive behaviors which includes a simultaneous increase in arterial blood pressure (ABP) and decrease in sensitivity to pain. In the present study we directly compared the expression of conditional and unconditional fear responses using these two dependent measures in animals with small lesions of the basolateral amygdala (BLA). Groups of rats were first exposed to a series of paired (or unpaired) presentations of a tone CS (60sec, 72dB) and shock and then prepared with arterial catheters and lesions. During subsequent testing all rats were exposed to both the CS and white noise (60 sec, 95dB) while recording ABP and radiant heat tail flick (TF) latencies. Both auditory stimuli produced significant inhibition of TF in sham-operated animals that had received paired training. As reported previously, amygdala lesions blocked both forms of hypoalgesia. Both stimuli also produced large time-dependent elevations in ABP with the noise-evoked response being considerably larger. However, animals with BLA damage that blocked hypoalgesia showed normal or potentiated ABP responses to both stimuli. These results suggest that neural systems responsible for the expression of learned versus unlearned fear responses may not differ although the control of ABP and TF may be dissociable within the amygdala.

413.16

MK801 ATTENUATES BOTH STARTLE PREPULSE INHIBITION AND CONDITIONED FEAR SENSITIZATION. J. Cranney*, T. Haralambous, G. Paxinos and M. Kiernan. School of Psychology, The University of New South Wales, Sydney, 2052, Australia.

The non-competitive NMDA receptor antagonist dizocilpine (MK-801) is known to decrease prepulse inhibition in the auditory startle paradigm (Mansbach and Geyer, Neuropsychopharmacology, 1989, 2: 299-308). The present study replicates and extends these observations by measuring freezing (an index of fear sensitization) in the prepulse inhibition paradigm as well as in a startle alone paradigm. Rats were administered with either MK-801 (0.1 mg/kg s.c.) or saline and presented 20 min later with 97 startle-eliciting (122-dB) white noise stimuli. For rats in the prepulse inhibition paradigm, 48 of the 97 startle stimuli were preceded (100 msec earlier) by a low intensity (80-dB) white noise stimulus (the prepulse). For all groups the intertrial interval was varied between 10 and 20 sec (mean = 15 sec).

One hundred and five mins after the completion of the 97 trial session, all rats were subjected to another 10-trial session of startle alone (no prepulse) stimuli with the intertrial interval varied between 30 and 90 sec (mean = 60 sec). As expected, the group injected with MK-801 showed less prepulse inhibition than did the saline group. Additionally, the MK-801 group showed less freezing than did the saline group, consistent with the suggested role of NMDA receptors in the acquisition of conditioned fear. Responding to startle alone trials was similar in both the startle alone and the prepulse (excluding prepulse trials) paradigms, whereas MK-801 appeared to increase responding to startle alone trials irrespective of paradigms.

It is concluded that MK-801 produces deficits in prepulse inhibition but also in conditioned fear sensitization.

Supported by ARC and NH&MRC.

413.17

A PRIMARY ACOUSTIC STARTLE REFLEX CIRCUIT: ROLE OF AUDITORY ROOT NEURONS AND THE NUCLEUS RETICULARIS PONTIS CAUDALIS. Y. Lee*, D. López, E. Meloni, & M. Davis. Dept.of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Our laboratory proposed a primary acoustic startle circuit that consisted of the auditory nerve, posteroventral cochlear nucleus (VCP), an area ventral and medial to the ventral nucleus of the lateral lemniscus (VLL), the nucleus reticularis pontis caudalis (PnC), and motoneurons in the spinal cord (Davis et al.1982; Cassella & Davis 1986). While lesion and stimulation data generally supported this circuitry, the relatively large lesion size and the nonselective nature of electrical stimulation made it difficult to identify the acoustic startle circuit more precisely. For example, lesions of VLL in the original study often included the paralemniscal zone (PL), ventro lateral tegmental area (VLTg), and the caudal extension of these areas. Therefore, the present studies re-evaluated the role of structures previously implicated in the primary acoustic startle circuit using discrete bilateral electrolytic and chemical lesion techniques.

Small electrolytic lesions of VLL, PL and VLTg failed to eliminate the startle reflex, whereras large electrolytic lesions including the area vental to VLL and the caudal extension of this area (ventrolateral PnC) substantially blocked the startle reflex (88%). Furthermore, NMDA lesions restricted to the rostral part of ventrolateral PnC failed to eliminate the startle reflex, whereas NMDA lesions of the full extension of ventrolateral PnC or the lateral PnC plus the ventrolateral PnC blocked the startle reflex (88%,90% respectively), suggesting only the ventrolateral PnC is critically involved.

In rodents, neurons have been found embedded in the cochlear nerve, named cochlear root neurons (RN), that receive input from the cochlea, and have very thick axons that give off collaterals terminating contralaterally in the ventrolateral PnC (López et. al., 1993). Because it has been suggested that RN may be involved in acoustic startle (Lingenhöl & Friauf,1994), we tested their role in the acoustic startle reflex. Kainate lesions of RN reduced the acoustic startle reflex by 90%, despite the fact that these animals still oriented to sudden noise, and had intact evoked potentials recorded from the VCN using 65-87 dB white noise pulses or 10 kHZ tones.

Although the role of the VCP is yet to be fully determined, we now think that the acoustic startle pathway in the rat may be simpler than we had originally thought, consisting of only three synapses onto 1) RN; 2) neurons in the ventrolateral PnC; 3) motoneurons in the brain stem and spinal cord

413.18

HABITUATION OF PREPULSE INHIBITION OF THE STARTLE RESPONSE WITH WEAK AUDITORY PREPULSES. J.C. Gewirtz, D.L. Walker* and M. Davis. Dept. of Psychology and Psychiatry, Yale University Sch. of Med., 34, Park St., New Haven, CT 06508.

In a variety of mammalian species, the startle response elicited by a strong stimulus (the pulse) is reduced when preceded by a weak stimulus (the prepulse) which itself does not elicit startle. The phenomenon of "prepulse inhibition" (PPI) is often viewed as the mechanism of "sensory gating", so that an impairment of PPI would result in an attentional deficit. However, PPI itself can be affected by attention. Therefore deficits in PPI may simply represent a marker for attentional deficits, rather than the mechanism which causes them.

If PPI is an early attentional filter, it should not habituate with repeated presentation of the prepulse. Consistent with this, some existing evidence indicates that the magnitude of PPI is not decremented by repeated presentation of the prepulse.

The present study re-evaluated habituation of PPI, using long intervals between startle-eliciting pulses to reduce habituation of baseline startle, because changes in baseline startle are problematic for interpreting changes in PPI. To maximize habituation of PPI and minimize dishabituation that could result from presentation of the intense (115 dB) startle-eliciting pulse, a large number (150) of auditory prepulse-alone trials were intermixed with a small number (9 pulse alone and 9 prepulse-pulse) test trials. A control group received an identical number of PPI test trials using the auditory prepulse, but received intervening presentations of a prepulse in a different modality.

When the auditory prepulse was very weak (2.5 dB above background noise), PPI showed reliable habituation. However, when the auditory prepulse was more intense (13 dB above background), PPI did not habituate, consistent with the literature.

Our findings indicate that some habituation occurs with a prepulse, only when it is close to auditory threshold. Interestingly, the disruptive effects of dopamine agonists on PPI primarily occur with weak prepulses, raising the possibility that these two sets of observations may be related.

413.19

EFFECTS OF ENTORHINAL CORTEX ASPIRATIONS ON ODOR- AND CONTEXT-GUIDED FEAR CONDITIONING. T. Otto* and K.M. Schiller. Program in Biopsychology and Behavioral Neuroscience, Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

Previous work has demonstrated that hippocampal lesions abolish contextual fear conditioning but spare fear conditioned to a discrete tone CS (Phillips & LeDoux, 1992; Kim & Fanselow, 1992). The present study was conducted to determine the effects of damage to entorhinal cortex on contextual vs. cued fear conditioning using an odor as a CS. One group of male Sprague-Dawley rats received bilateral aspiration of the entorhinal cortex (ENT, n=12); a second group served as operated controls (SHAM, n=11). Ten days after surgery all subjects received 6 pairings of an odor (cis-3-Hexenol) and footshock (0.6mA, 2sec) in a distinctive operant chamber. On each of the 6 trials, the odor was presented for 20sec (delivered by means of a flow-dilution olfactometer and blown through the roof of the chamber) and the footshock overlapped with the final 2sec of odor delivery; the ITI was 4min. Twenty-four hours later conditioning to the context was assessed by placing subjects into the operant chamber in which training took place and measuring freezing behavior for 5min using a time-sampling method (5sec inter-sample-interval). Conditioning to the odor was assessed by placing subjects into a novel operant chamber and measuring freezing for 6min; the odor CS was presented during the second minute and remained in the chamber throughout the final 5min of sampling.

Histological verification of lesion placement has not yet been completed. However, preliminary analyses suggest that, using this paradigm, entorhinal cortex aspiration attenuates conditioning to both the context and the odor CS as indicated by significantly less freezing behavior for ENT subjects. This pattern of findings is consistent with the effects of hippocampal damage on contextual fear conditioning, but suggests that there may be a degree of sensory specificity in the role of the entorhinal cortex/hippocampal system in cued fear conditioning.

413.20

NEURAL SUBSTRATES OF APPETITIVELY CONDITIONED BRADYCARDIA. C.M. Gibbs~ and K.L. Watson. WJB Dorn Veterans' Hospital & University of South Carolina, Columbia, SC 29201.

Aversive Pavlovian training procedures induce phasic, bradycardiac responses (CRs) in many mammalian species, and we have established that appetitive Pavlovian training elicits similar heart rate (HR) CRs. The present studies sought to compare the neural substrates for appetitive and aversive cardiac conditioning in rabbits. We first showed that peripheral muscarinic receptor antagonism profoundly disrupts the development of discriminative, bradycardiac CRs during differential appetitive training (CS^+ paired with water, CS^- presented alone). Next, we evaluated the development of HR CRs in sham-operated animals vs. those with bilateral lesions of the medial prefrontal cortex (PFCm), the amygdaloid central nucleus (ACe) or both the PFCm and ACe during differential appetitive-aversive training (CS_{ap} paired with 0.5M sucrose, CS_{av} paired with eye-shock, CS^- presented alone). PFCm lesions reduced the magnitude of the bradycardiac CRs to the CS_{ap} and CS_{av} but did not affect their discriminative character; whereas, ACe lesions abolished bradycardiac responses to the CS_{ap} and CS_{av} and significantly attenuated HR discrimination. Finally, PFCm/ACe lesions abolished the development of discriminative HR CRs. These findings indicate that (a) appetitive and aversive HR CRs involve similar peripheral and central mechanisms and (b) the PFCm and ACe are differentially involved in their development and/or expression. (Supported by VA Institutional Research Funds)

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS VI

414.1

PREVENTION OF THE RABBIT TRACE-CONDITIONING NICTITATING MEMBRANE LEARNING BY INACTIVATION OF THE INTERPOSITUS NUCLEUS. Pezhman Eliaszadeh, Georges Tocco*, Michel Baudry and Richard F. Thompson. University of Southern California, Neurosciences Program, Los Angeles, CA 90089-2520.

While the cerebellum is necessary and sufficient for the learning of the classical conditioning of the nictitating membrane in a delay paradigm, the hippocampus is required for the learning in a trace paradigm when at least 300 msec separate the end of the CS from the US onset. However the importance of the cerebellum in a trace paradigm is not well understood. While the interpositus nucleus is absolutely necessary for the performance of a previously learned trace conditioning task, its importance during the acquisition phase is not well understood. In an attempt to answer this question, we produced reversible inactivation of the cerebellum by muscimol infusion in the interpositus nucleus during the acquisition phase for a trace paradigm.

Male new Zealand white rabbits were implanted with a cannula in the interpositus ipsilateral from the stimulated eye. Rabbits were then trained on trace paradigm (500 ms trace interval) and infused with muscimol before each training session. The rabbits were trained with muscimol infusion for 12 sessions after which they were infused with saline for the following sessions. The animals did not show any CRs during the first 12 training sessions and gradually developed CRs thereafter as would naive animals.

This work was supported by NSF IBN 9110377 to MB and NSF BNS-8718300, NIH (NIA) AG05142 and a Sankyo grant to RFT.

414.2

ROLE OF CEREBELLAR CORTICAL NEURONS IN CLASSICAL CONDITIONING. P.G. Shinkman*, A.J. Annala, R.A. Swain, R.E. Clark, and R.F. Thompson. Neurosciences Program, Univ. Southern California, Los Angeles, CA 90089-2520.

Classical conditioning of discrete motor responses of the facial and neck musculature has been shown in rabbits implanted with stimulating electrodes in cerebellar cortex. Following threshold measurements for the elicitation of discrete motor responses, subthreshold stimulation was used as the CS and suprathreshold stimulation through the same electrode served as the US. In this preparation, we observed conditioning following paired (but not randomly unpaired) CS-US trials, extinction following CS-alone trials, and reacquisition with savings, as well as a significant posttraining increase in local cortical excitability. These and earlier findings point to critical roles for both cerebellar cortex and deep cerebellar nuclei in classical conditioning, and suggest that such conditioning is mediated by an interaction between cortical and subcortical cerebellar circuits. The present experiment was designed to further elucidate the nature of cortical participation in the conditioning process. New Zealand white rabbits were implanted chronically with bipolar electrodes placed within the cortical layers of lobule HVI, and a cannula located 0.75 mm dorsally. Following training acute local injections of muscimol, but not saline, reversibly abolished conditioned responding. Since the muscimol selectively inactivated cell bodies at the injection site, these results strongly implicate cerebellar cortical neurons, and hence their efferent connections to the deep nuclei, in the neuronal plasticity manifested in classical conditioning. (Supported by NSF BNS 8718300, ONR N00014-88K-0112, and the McKnight Foundation, to R.F.T.)

414.3

INVOLVEMENT OF THE RED NUCLEUS IN ACCURATE TIMING OF THE RABBIT'S CLASSICALLY CONDITIONED EYEBLINK RESPONSE. D. J. Krupa*, J. Weng, & R. F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089.

Numerous studies have demonstrated that during classical conditioning of the eyeblink reflex, subjects learn to time the conditioned response (CR) so that the peak occurs at about the time the US is normally delivered. The present study was designed to investigate whether the red nucleus might play a role in accurate timing of the CR. Two groups of rabbits were implanted with cannulae aimed at the contralateral red nucleus (RN). Following recovery, all rabbits received 6 training sessions in which a tone CS (650 ms) was paired with a coterminating airpuff US (100 ms) so that the interstimulus interval was 550 ms. All rabbits were then given 7 more training sessions in which the ISI was shifted to 200 ms. Prior to the first 3 of these 200 ms ISI sessions, one group was infused with muscimol into the RN while the other group received vehicle alone infusions into the RN.

Consistent with previous studies, all rabbits learned to time the CR during the first 6 sessions so that the peak occurred about 500 ms after CS onset (as measured on CS alone test trials). When switched to the 200 ms ISI, the rabbits which were infused with saline (n=6) rapidly learned to retime the CR so that after the first session, there was a significant reduction in peak CR latency and by the end of the third session, the mean peak latency was 250 ms. In marked contrast, the rabbits infused with muscimol into the RN (n=6) performed no CRs during the 3 infusion sessions with the 200 ms ISI. On the fourth session (first session without muscimol) the mean peak CR latency did not differ from the peak latency obtained during the last session with a 550 ms ISI (session 6). During subsequent sessions, these rabbits learned to retime the CR at the same rate as the saline controls. In sum, inactivation of the RN (which previous studies have shown to have no effect on the ability to acquire the CR with a 250 ms ISI) disrupts the animal's ability to properly retime a previously learned CR. [Support: NSF(IBN-9215069); NIH(AG05142); Sankyo.]

414.4

INACTIVATION OF THE SUPERIOR CEREBELLAR PEDUNCLE PREVENTS EXPRESSION BUT NOT ACQUISITION OF THE RABBIT'S CLASSICALLY CONDITIONED EYEBLINK RESPONSE. R. F. Thompson* & D. J. Krupa. Neurosciences Program, University of Southern California, Los Angeles, CA 90089.

Previous studies have shown that inactivation of a critical region of the cerebellum encompassing the dorsal anterior interpositus nucleus prevents acquisition of the classically conditioned eyeblink response (CR) while inactivation of the contralateral magnocellular red nucleus prevents expression but not acquisition of the CR. The present study was designed to extend these findings. Three groups of rabbits were implanted with cannulae in the ipsilateral superior cerebellar peduncle (SCP); one group was also implanted with stimulating electrodes in the ipsilateral lateral reticular nucleus (LRN). One group received 9 training sessions with an auditory CS (350ms) paired with a coterminating airpuff US (100ms). The second group also received 9 training sessions but with electrical microstimulation of the LRN as the CS (350ms, 200Hz, 50μA). Prior to each of the first 6 sessions, 0.9ng tetrodotoxin (TTX) was infused into the SCP. Controls were infused with TTX and placed in the behavioral recording apparatus for one hour but received no stimuli during the first 6 sessions. These rabbits then received 3 sessions of training (auditory CS) without infusion.

Rabbits trained with either the auditory CS or the LRN stimulation as CS on the first 6 sessions performed no significant number of CRs during these infusion sessions. On session 7 (first session without TTX) these rabbits performed the CR at asymptotic levels; they had fully learned the CR. The control rabbits performed the CR at significantly lower levels on session 7 and subsequently learned the CR during the following 2 sessions. Infusions of TTX on session 10 completely abolished the CR in all groups. These results indicate that cerebellar output projections (via the SCP) are not essential for acquisition of the CR. Further, this result is true for auditory CSs as well as other stimulus modalities which have previously been shown to serve as effective CSs.

Support: NSF(IBN-9215069); NIH(AG05142); Sankyo.

414.5

FUNCTIONAL ANATOMY OF HUMAN EYEBLINK CONDITIONING DETERMINED WITH RELATIVE GLUCOSE METABOLISM AND POSITRON EMISSION TOMOGRAPHY C. G. Logan* and S. T. Grafton. Dept. of Neurology, University of Southern California, Los Angeles, CA 90033.

Relative cerebral glucose metabolism (rCMRglu) was examined with positron emission tomography (PET) as a measure of neuronal activation during performance of the classically conditioned eyeblink response in 12 young adult subjects. Each subject received three sessions: 1) an unpaired control session with PET scan; 2) a paired training session; and 3) a paired test session with PET scan. Stimuli were tones and corneal airpuffs (ISI=500 ms, mean ITI=28 s on paired days). During the unpaired control session, subjects received the same number of tones and airpuffs in the same period of time, but with no predictive relation between the two. ^{18}FDG was injected immediately prior to the last 6 blocks of training on sessions 1 and 3. PET scans following the training blocks measured rCMRglu.

Brain regions showing potential learning-related changes in activity were identified as those areas which showed significant differences in rCMRglu between the unpaired control condition (session 1) and well-trained state (session 3) in the 9 subjects who met learning criterion. Areas of significant activation included bilateral sites in the cerebellum; anterior cerebellar vermis*; ipsilateral hippocampal formation; ipsilateral anterior middle temporal gyrus*; and caudate nucleus bilaterally*. Among all 12 subjects, metabolic changes in the ipsilateral cerebellar deep nuclei and * sites correlated significantly with degree of learning. These results are consistent with studies in humans and other species which have demonstrated that the cerebellum plays a critical role in eyeblink conditioning, and they suggest that additional striatal and cortical sites may also be involved.

414.6

MULTIPLE UNIT RECORDINGS FROM THE TRIGEMINAL COMPLEX. EYEBLINK CONDITIONED UNIT RESPONSES DURING INTERPOSITUS OR RED NUCLEUS INACTIVATION VIA COOLING. Robert E. Clark,* Elizabeth B. Gohl, and David G. Lavond. Departments of Psychology and Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

The trigeminal complex is of considerable interest to researchers of the conditioned eyeblink response for primarily two reasons. (i) The unconditioned response pathway involves the trigeminal system. (ii) Other research has suggested that the trigeminal complex may also participate in the generation of the behavioral conditioned response or even in the formation of the essential plasticity for the learned response.

Recordings were obtained from the trigeminal complex with the aid of a moveable electrode in rabbits classically conditioned with a tone conditioned stimulus (CS) and an airpuff unconditioned stimulus (US). Units were examined over a block of 9 trials during normal training. These unit recordings were then examined over a block of 9 trials during reversible cooling lesions of either the interpositus or red nucleus.

Unit responses to the CS and US were frequently observed as were responses that modeled the learned behavioral response. The model responses were found in various regions of the trigeminal complex, but most prominently in the principal sensory nucleus and pars oralis of the spinal trigeminal. Cooling either the interpositus or the red nucleus completely abolished the unit models but did not abolish the responses evoked by the CS or US. We conclude that the learning related activity in the trigeminal complex is driven by the interpositus via the red nucleus. (Supported by NSF BNS 8718300, ONR N00014-88K-0112, and the McKnight Foundation, to R.F.Thompson)

414.7

FUNCTIONAL LOCALIZATION AND NEUROANATOMICAL CONNECTIVITY OF NEURAL SUBSTRATES WITHIN THE ANTERIOR INTERPOSED NUCLEUS INVOLVED IN EXPRESSION OF THE NICTITATING MEMBRANE REFLEX. S.A. Bartholomew*, M.L. Webster, J.R. Bloedel, V. Bracha. Barrow Neurological Institute, Phoenix, AZ 85013.

The purpose of the present study was to analyze the neuroanatomical connectivity of subregions within the anterior interposed nucleus (AIN) which are critical for expression of the classically conditioned nictitating membrane response (NMR) in the rabbit. Animals were chronically implanted with a matrix of three guiding cannulae in the region of the AIN and then conditioned in the standard delay paradigm. A functional map of the AIN was reconstructed from the behavioral effects of systematic muscimol nanoinjections (20 ng in 50 nL) along the three injection tracks in trained animals. In each animal, the AIN subregion in which muscimol nanoinjections most effectively blocked the conditioned response performance was electrophoretically injected with anterograde (PHA-L) and retrograde (CTB) tracers.

The neuroanatomical data indicate that besides the traditionally considered AIN connections with the red nucleus, the pontine nuclei, and the inferior olives, several other projections could also be relevant to AIN involvement in control of NMR expression: 1) a direct afferent projection from the spinal trigeminal nucleus; 2) direct efferent connections to the mesencephalic periaqueductal and reticular accessory oculomotor regions; and 3) an efferent projection to the pontine nuclei.

NIH Grants NS 30013 and NS 21958.

414.8

CONDITIONING-SPECIFIC MODIFICATION OF THE RABBIT'S UNCONDITIONED NICTITATING MEMBRANE RESPONSE Bernard G. Schreurs*, M. Matthew Oh, Chie Hirashima, and Daniel L. Alkon Laboratory of Adaptive Systems, NINDS, NIH, Bethesda, MD 20892, USA.

Acquisition of a classically conditioned response was shown to modify subsequent responding to the unconditioned stimulus, a phenomenon we have called conditioning-specific reflex modification. Procedurally, unconditioned responses to different values of periorbital electrical stimulation varying in intensity (.1, .25, .5, 1.0, 2.0 mA) and duration (10, 25, 50, 100 ms) were assessed within subjects before and after a between-subjects manipulation of one, three, or six days of paired, explicitly unpaired, or no presentations of tone and periorbital electrical stimulation. After three days of paired presentations (Experiment 1), conditioned responding had reached an asymptote of 94% and, on posttest, there was a significant increase in the latency to the peak of the unconditioned response ($p < .01$). The conditioning-specific increase in peak latency was replicated ($p < .05$) in a second experiment in which animals received six days of paired presentations (Experiment 2). In addition, the six days of pairings produced a significant increase in the amplitude of the unconditioned response ($p < .01$). Finally, there were no significant changes in unconditioned responses as a function of the minimal levels of conditioning produced by one day of pairings (Experiment 3). Taken together, the data show that after acquiring asymptotic levels of conditioned responding, rabbits elicited unconditioned responses that were as much as 50% larger and timed to reach a peak 20% later than before training. These results indicate that processing of the unconditioned stimulus is modified as a function of the acquisition of a conditioned response. At a neural level, the data suggest that classical conditioning induces learning-specific changes in pathways mediating the unconditioned stimulus/unconditioned response as well as those mediating the conditioned stimulus/conditioned response.

414.9

DESTRUCTION OF NEURONS IN THE VENTRAL POSTERIOR MEDIAL THALAMUS WITH IBOTENIC ACID PREVENTS CLASSICALLY CONDITIONED HEART RATE IN RABBITS. P.M. McCabe*, M.D. McEchron, E.J. Green, and N. Schneiderman. Behavioral Neuroscience Program, Department of Psychology, University of Miami, Coral Gables, FL 33124.

In the heart rate (HR) conditioning paradigm, an unconditioned corneal air puff stimulus (US) is paired with an acoustic conditioned stimulus (CS). Previous evidence suggests that the ventral posterior medial thalamus (VPM) receives inputs from the trigeminal nucleus, and that VPM is involved in the sensation of tactile- and nociceptive-facial information, such as a corneal air puff. Although, VPM is involved in the processing of trigeminal information, it is not clear if VPM is part of a circuit that relays US information to other critical sites of sensory convergence in the HR conditioning pathway. Therefore, the present study examined the role of VPM in the acquisition of HR-conditioned responses (CRs) and the expression of HR-unconditioned responses (UR). Rabbits were given bilateral ibotenic acid lesions in VPM and subjected to one Pavlovian HR conditioning session which consisted of 28 trials of the CS (560 Hz; 90 dB; 2 s duration) paired with the US (15 N/cm^2; 0.5 s duration). Four CS-test trials were used to measure the initial (rate of first 16 beats) bradycardiac CR, and 5 US-test trials were used to measure the initial (rate of first 26 beats) tachycardiac UR. The results of the present study demonstrate that the destruction of cell bodies in VPM reduces HR-CRs to the level of a pseudoconditioning control without affecting HR baseline, or orienting responses to the CS. Lesions of VPM also significantly augment the tachycardiac UR above the level of control lesioned animals. These data suggest that VPM lesions prevent conditioning by altering the somatosensory processing of the US. These results are consistent with the notion that VPM may participate in a circuit which relays US information to other important areas of conditioning such as the medial geniculate and the amygdala. Supported by NSFIBN9222194 and NIHHL07426.

414.10

CROSS-MODALITY CORTICAL PROCESSING: SPATIO-TEMPORAL PATTERNS OF OLFACTORY, VISUAL, AUDITORY, AND SOMATIC EEGs IN PERCEPTION BY TRAINED RABBITS

J.M. Barrie, W.J. Freeman*, and M. Lenhart. Dep't of Molecular and Cell Biol., University of California at Berkeley, CA 94720

Stimulus-induced, perceptually related events are accessed in paleo- and neocortex by recording the endogenously generated spatial patterns of the EEG. They take the form of amplitude modulation in spatial coordinates of a broad spectrum, aperiodic carrier wave. This finding has been extended to characterize the evolution of such patterns across time and across sensory modalities. Arrays of 64 electrodes (8x8 at 0.79mm spacing) were fixed onto the epidural surface of either the prepyriform, visual, auditory, or somatic cortex of 18 rabbits classically conditioned to discriminate between pairs of stimuli (CS+ and CS-) not related to the cortical implant site. The 64 EEG traces were decomposed by FFT, PCA, RMS, and spectral entropy analysis for 120millisecond windows stepped at overlapping 20mSec intervals. Pattern differences were expressed as Euclidean distances in 64-space. Decomposition and successful classification of the EEG patterns shows that sensory information is shared between all primary cortical sensory processing areas during lagged time periods after stimulus arrival. Long term EEG recordings have also shown that basal state spatio-temporal patterns are inconstant and continually evolve at a constant rate corresponding to perceptual drift. A temporal frequency analysis of the EEG has demonstrated no relationship between periods where percepts are formed and an increase or decrease in the power spectral density within narrow bands of the temporal spectra. We conclude that cortical function in perception is an endogenous process in which percepts are *constructed*, not *filtered*, by chaotic nonlinear dynamics. Funded by the National Institute of Mental Health - MH06686.

414.11

THE ABSENCE OF INTERHEMISPHERIC COMMUNICATION IN THE NORMAL RABBIT. L. Steele Russell*, Anatomy, Texas A&M University, College Station, TX. 77843-1114.

The present report examines the role of the cerebral cortex to inhibit interhemispheric communication in normal rabbits.

Intact normal rabbits (N=40) were tested for visual interocular transfer (I.O.T.) using a variety of visual tasks in a two choice situation. They were first trained monocularly to criterion. Following this they were trained with the other eye. No signs of IOT were seen in any animal. All animals had to be retrained from chance and showed no savings. This suggests that the normal intact rabbit functions as a junctional "split brain" animal, i.e. the left hemisphere does not communicate with the right hemisphere.

To test this, a group of rabbits were monocularly trained on a pattern discrimination task. After reaching criterion the animals were hemidecorticated such that half of them had the hemisphere removed contralateral to the trained eye, and the others had the ipsilateral hemisphere lesioned. The results showed that following monocular learning a unilateral memory trace is established in the normal rabbit. Perfect retention of learning was found following ipsilateral hemidecortication; whereas no retention was evident when the lesion was contralateral.

These findings suggest that the contralateral optic projections play an important role in collateral inhibition of the ipsilateral projections and thus prevent the formation of a bilateral memory record. The lack of such a record would also explain the absence of I.O.T. in the rabbit. Hemidecortication prior to learning was used to disrupt this inhibitory mechanism. Such hemidecorticate rabbits when trained monocularly showed a bilateral memory record and perfect IOT. These findings argue strongly that the rabbit has a cortical inhibitory control over the extent interhemispheric communication required during monocular viewings.

414.12

DISRUPTION OF CEREBELLAR MATURATION BY AN ANTIMITOTIC AGENT IMPAIRS THE ONTOGENY OF EYEBLINK CONDITIONING IN INFANT RATS. J.H. Freeman Jr.[1]*, S. Barone, Jr.[2] and M.E. Stanton[1,3] [1]Dept. Psychology, UNC, Chapel Hill, NC, 27599-3270; [2]ManTech Environmental Technology, RTP, NC 27709; [3]Neurotoxicology Div., US EPA, RTP, NC 27711.

In rats, eyeblink conditioning develops dramatically between postnatal day 17 (PND17) and PND24 (Stanton, Freeman, & Skelton, 1992, *Behav. Neurosci., 106,* 657-665.). There is dramatic cerebellar growth during this period (Altman, 1969, *J. Comp. Neurol., 136,* 269-294) and the cerebellum is critical for the acquisition of eyeblink conditioning in adult animals (Thompson, 1988, *TINS, 11,* 152-155). These observations suggest that the ontogeny of eyeblink conditioning depends upon the maturation of the cerebellum. In the present study, we experimentally manipulated cerebellar maturation by exposing neonatal rat pups to the antimitotic agent methylazoxymethanol (MAM) during cerebellar cortical neurogenesis. Pups were then trained with eyelid conditioning procedures at different ages. In Experiment 1, pups were given either saline or MAM (20 mg/kg) on PND4 and 7 and then given six 100-trial sessions of either delay conditioning or unpaired training on PND24 and 25 (3 sessions/day). A subset of the animals were then trained on T-maze delayed alternation on PND27. In Experiment 2, pups were given the same dosing treatment and trained on delay conditioning on days 17-18, 20-21, or 31-32. Staining for Nissl and glial fibrillary acidic protein immunoreactivity revealed greatly reduced volume, disrupted cellular organization, and induction of reactive gliosis within the cerebellum, respectively. There was no evidence of damage in other postnatally developing brain systems. This treatment impaired eyelid conditioning at all ages tested without affecting unconditioned responding or delayed alternation in a T-maze. This study suggests that cerebellar maturation is critical for the ontogeny of eyeblink conditioning.

414.13

ONTOGENY OF THE CONDITIONED EYEBLINK RESPONSE IN RATS: ACQUISITION OR EXPRESSION. G.D. Fox,[2] C.S. Carter,[2] & M.E. Stanton,[1,2]* [1]Neurotoxicology Div., US EPA, RTP, NC 27711; [2]Psychology Dept., UNC, Chapel Hill, NC, 27599.

Previous work in our laboratory (Stanton, Freeman, & Skelton, 1992, *Behav. Neurosci.*, 106, 657-665) has determined that the classically conditioned eyeblink response in the rat pup develops between Postnatal Day 17 (PND17) and PND24. The current study sought to explore the possibility that acquisition does occur at PND 17, but can not be expressed until a later age. Animals were trained in a Pavlovian eyeblink preparation utilizing a tonal CS and a periorbital shock US. Tones of two different frequencies, 2.8 and 9 kHz, were used because some of our recent findings have indicated that CS frequency can be an important variable in the ontogeny of conditioning in this preparation. A "savings" design was used in which acquisition on PND20 was examined as a function of prior training on PND17. Group P-P received 300 trials of paired training on PND17 and again on PND20. Performance of this group was compared with that of control groups that received no treatment (N-P) or unpaired training (U-P) on PND17, followed by paired training on PND20. A final control group (N-U) received no treatment on PND17 and unpaired stimulus presentations on PND20. Response acquisition on PND20 was found to differ reliably as a function of the animals' experience on PND17. Group P-P showed accelerated acquisition of the conditioned eyeblink response on PND20 relative to groups U-P and N-P, indicating that some unexpressed associative learning does occur during paired training on PND17. A surprise finding was that unpaired exposure with the 2.8 kHz tone had a facilatory effect on PND20 acquisition, while unpaired exposure with the 9 kHz CS resulted in a significant retardation of day 20 acquisition. These findings suggest that maturation of fibers efferent to the cerebellum may play a role in the developmental appearance of eyeblink conditioning in the rat.

414.14

AMYGDALA STIMULATION FACILITATES THE EYEBLINK RESPONSE IN RAT. T. Canli* and T.H. Brown. Depts. of Psych. and Cell. & Mol. Physiol., Yale University, New Haven, CT 06520.

The rat eyeblink reflex is an attractive model system for studying associative learning. The neural circuitry underlying its fast component (R1) involves a three-synapse arc between the (5th) sensory nerve and the orbicularis occuli (o.o.) muscle, which is responsible for the eyelid closure. In the rabbit, the nictitating membrane response can be associatively modulated by an amygdala-dependent mechanism (Weisz et al., 1992). We were interested in knowing more about the relationship between the amygdala and the eyeblink circuitry in the rat, which is more convenient for *in vitro* cellular neurophysiology.

Here we show that stimulation of the rat amygdala facilitates the R1 component of the EMG recorded in the o.o. muscle. Twelve rats were implanted with a bipolar nerve cuff placed around the 5th nerve to elicit eyeblink and a monopolar stimulating electrode targeted at the amygdala. Both nerve cuff stimulation (NCS; 0.5-2.0 mA, 0.2 ms) of the 5th nerve and electrical brain stimulation (EBS; 100-400 μA, 0.2-0.8 ms) of the amygdala consisted of a single biphasic square wave pulse. All electrode placements were histologically verified. We found that amygdala stimulation facilitates R1 maximally when EBS and NCS were presented simultaneously, suggesting that the projection from the amygdala to the site of reflex modulation must be fairly direct.

We are presently using confocal microscopy to trace projections from the amygdala to the facial nucleus, using *in vivo* injections of anterograde fluorescent tracers into the amygdala and tetramethyl-rhodamine into the orbicularis occuli muscle to retrogradely identify motoneurons in the facial nucleus (Lofthus and Brown, 1993).

414.15

CRITICAL IMPORTANCE OF THE HIPPOCAMPUS FOR LOCAL NAVIGATIONAL LEARNING IN YOUNG HOMING PIGEONS. V. P. Bingman*, P. Ioalé, G. Casini, P. Bagnoli and R. Strasser. Dept. Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

Among the deficits in spatial cognition produced in homing pigeons following hippocampal (HF) ablation, none are as robust as those observed in young birds learning to navigate near the loft. Local navigation near the loft is based on young birds learning to use familiar landmarks to identify goal locations. Even loft identification, an ostensibly non-spatial task, is based on young birds learning about a loft's location rather than its features (e.g. color). Compared to young controls, young birds with HF lesions: (1) display less loft fidelity (e.g., they are more likely to move between lofts within a loft complex), (2) are more likely to get lost flying near the loft during training, and (3) are less likely to return home after being experimentally released the first time from short distances (20 km). Taken together, these findings emphasize the critical importance of HF for local (familiar landmark) navigational learning in homing pigeons.

414.16

THE EFFECTS OF BILATERAL HIPPOCAMPAL-AREA PARAHIPPOCAMPALIS LESIONS ON VISUAL DELAYED MATCHING-TO-SAMPLE BEHAVIOR IN PIGEONS. M. Colombo*, N. Swain, D. Harper, and B. Alsop. Department of Psychology, University of Otago, Dunedin, New Zealand.

Damage to the hippocampus and area parahippocampalis (Hp-APH) in pigeons is known to impair spatial memory. Whether similar lesions impair visual memory is unclear. Although Sahgal [Behavioural Brain Research (1984), 11, 47-58] showed that Hp-APH lesions may impair visual memory in pigeons, the delayed matching-to-sample (DMS) procedure used to evaluate visual memory required the pigeons to execute responses to different spatial locations, and thus the impairments may have arisen not from impairments in visual memory but rather from impairments in processing spatial information. The purpose of the present study was to examine whether bilateral Hp-APH lesions in pigeons impairs visual memory using a visual DMS procedure which eliminated this spatial confound.

In Experiment 1, we generated retention gradients with delays of 0, 1.5, 3, 6 and 12 sec for three pigeons experimentally sophisticated with respect to visual DMS behavior. Bilateral Hp-APH lesions had no effect whatsoever on visual retention. In Experiment 2 we compared the ability of five Hp-APH lesioned pigeons and five unoperated pigeons to learn the visual DMS procedure with a variety of different delays. Again, there was no difference in the speed at which the visual DMS task was acquired by the two groups. Furthermore, there was no difference between the Hp-APH pigeons and unoperated pigeons when tested for visual retention with delays of 0, 3, 6, 12, and 24 sec. These results support the view that the hippocampus in pigeons functions mainly in the processing and retention of spatial, rather than visual, information.

414.17

THE AVIAN HIPPOCAMPUS AND MEMORY FOR CUED LOCATION. T. Fremouw*. Dept. of Psychology, University of Utah, Salt Lake City, UT 84112.

The avian hippocampus is similar to the rat hippocampus in that it processes various types of spatial information. Avian hippocampal lesions have produced deficits in place learning, cached food recovery, sun compass learning, and homing performance. All these tasks have emphasized spatial relations and the integration of spatial information (e.g., as in a cognitive map). The present study explored the role of the avian hippocampus in a task that does not involve spatial relations or a cognitive map. Pigeons (Columba livia) were trained to criterion on a spatial, delayed matching-to-sample task in which the pigeons saw a sample on the right or left key, experienced a delay during which they pecked the center key, and then were rewarded for pecking the key on which the sample had appeared. After the pigeons reached criterion, they were given either hippocampal lesions or sham surgery. Multiple sample durations and delays were examined. Hippocampal lesions did not appear to impair performance compared to shams. Thus, in a fairly simple and non-integrative spatial location task, pigeons with hippocampal lesions do not appear to have a memory deficit. Therefore, the avian hippocampus seems to be critical for integrating and remembering spatial information rather than processing simple features of space in isolation. Alternatively, these results can be interpreted within an allocentric versus egocentric spatial framework: previous results suggest that the avian hippocampus is critical for allocentic spatial processing and now the present results suggest that it is not critical for egocentric spatial processing.

414.18

SUN COMPASS ORIENTATION IN THE FOOD-FINDING BEHAVIOUR OF THE BLACK-CAPPED CHICKADEE. S.J.Duff and D.F. Sherry*. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

Previous research indicates that food-storing birds use distal landmarks to remember the location of previously stored food (Herz et al. *Anim Behav* [in press]; Vander Wall *Anim Behav* 1982 30, 84-94). Recent evidence, however, suggests that food-storing birds that have cached food in the presence of the sun and distal landmarks, preferentially use sun compass information to relocate their stored food (Balda & Wiltschko *Condor* 1991 93, 1020-1023; Wiltschko & Balda *J Comp Physiol A* 1989 164, 717-721). The present experiment examined the role of sun compass and distal landmark information in the food-finding behaviour of the black-capped chickadee (*Parus atricapillus*). Chickadees were trained to food sites on one side of an octagonal aviary under several cue conditions: outdoors, indoors with no view of the sun, indoors with a view of the sun through floor-to-ceiling windows, and in an outdoor aviary with a view of the sun through mesh walls of the enclosure. After reaching criterion, birds were given a 6-hour counter-clockwise, or back, clockshift. Following the clockshift, birds trained and tested outdoors rotated their search patterns 90 degrees clockwise from the original trained location, as predicted if the birds were using sun compass information to locate food sites. This occurred despite conflicting landmark information. In the remaining three conditions, in which sun compass information had been eliminated or in which landmarks may have been more salient, birds did not exhibit rotated search patterns and instead maintained their pre-clockshift search patterns. The results indicate that black-capped chickadees can use both sun compass information and distal landmarks to find familiar food sites and use sun compass information preferentially under some conditions.

414.19

SEX DIFFERENCES IN SPATIAL MEMORY AND HIPPOCAMPAL SIZE DO NOT OCCUR IN FOOD-STORING BLACK-CAPPED CHICKADEES. K. Petersen, D.F. Sherry and E. Hampson*. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

Several recent studies have described sex differences in the relative size of the hippocampus that are correlated with ecological differences in the use of space. Male polygynous voles and female brood-parasitic cowbirds both possess relatively large hippocampal formations compared to female polygynous voles and male cowbirds, respectively (Jacobs et al. *PNAS* 1990 87, 6349-6352; Sherry et al. *PNAS* 1993 90, 7839-7843). We wished to determine whether sex differences in the size of the hippocampus also occur in the absence of ecological differences in the use of space and whether the previously described correlations could be adventitious.

Black-capped chickadees (*Parus atricapillus*) store food and retrieve their scattered caches using spatial memory for cache sites. The present study examined sex differences in caching, cache retrieval, spatial memory, and relative hippocampal size in wild-caught chickadees. Birds were habituated to an indoor aviary for two days, followed by five days observation of caching and cache retrieval. Because black-capped chickadees are monomorphic, observations were blind with respect to sex. Sex was determined by dissection at the time of perfusion. Brains were stained with cresyl violet and volume of the hippocampus and telencephalon were determined by digital video image analysis. Behavioural results indicated no sex difference in caching, cache retrieval, or the accuracy of memory for cache sites. Neuroanatomical results indicated no sex difference in relative hippocampal size. Thus, in the absence of ecological differences between the sexes in the use of space for food caching, and in the absence of a difference in memory for spatial locations, no sex difference in the relative size of the hippocampal formation was observed.

414.20

THE DORSOCAUDAL NEOSTRIATUM OF THE CHICK: A STRUCTURE WITH HIGHER ASSOCIATIVE FUNCTIONS ? J. Bock, S. Braun* and K. Braun, Federal Institute for Neurobiology, 39118 Magdeburg and Institute for Zoology, 64287 Darmstadt, FRG.

In the forebrain of domestic chicks several brain regions are involved in early filial imprinting. While the intermediate hyperstriatum ventrale (IMHV) seems to play a dominant role in visual imprinting, a system of interconnected forebrain areas such as the medio-rostral neostriatum/ hyperstriatum ventrale (MNH), the lobus parolfactorius (LPO) and parts of the caudal neostriatum (Nc) is involved in auditory imprinting. Since a natural imprinting object combines visual and acoustic features, we postulate that forebrain regions must exist, in which the two sensory modalities are processed on a higher associative level. By using the 2-deoxyglucose (2-DG) autoradiography we compared the activation patterns of acoustically imprinted chicks with those of visually imprinted chicks. We conducted two different imprinting procedures: i) individual chicks were imprinted on rhythmic 400 Hz tone pulses without presenting visual stimuli, ii) individual chicks were imprinted on a rotating red flashing light without presenting auditory stimuli. During the 2-DG experiment imprinted chicks of both groups were exposed to either the visual or acoustic imprinting stimulus, respectively. A subregion in the dorsal part of the Nc, which we termed dorsocaudal neostriatum (Ndc), was strongly labelled in the acoustically as well as in the visually imprinted chicks. In order to rule out that the Ndc-activation is caused by emotional stress during the 2-DG experiment, we investigated the activation patterns of naive, unimprinted chicks, which were distressed. Besides other brain areas a subregion in the Nc was activated, which was localised medial from and partly overlapping with the above described Ndc.

Our results together with recent evidence for dopaminergic innervation in the Ndc (Schnabel & Braun, this meeting, Metzger et al., this meeting) strongly suggest that this area acts as a higher associative structure comparable to the mammalian prefrontal cortex.

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414.21

DOPAMINE IN LEARNING RELEVANT FOREBRAIN AREAS OF THE DOMESTIC CHICK: A COMBINED IMMUNOHISTOCHEMICAL/TRACING STUDY M. Metzger, H. J. Bischof* and K. Braun, Federal Institute for Neurobiology, Brenneckestr.6, 39118 Magdeburg & Dept. of Biology, University of Bielefeld, FRG.

As demonstrated by 2-deoxyglucose autoradiography, acoustically imprinted domestic chicks develop an increased metabolic activity of several telencephalic areas, including the medio-rostral neostriatum/hyperstriatum ventrale (MNH), the Lobus parolfactorius (LPO) and the dorsal neostriatum caudale (Nc). Since our pharmacological and biochemical assays indicate that dopamine (DA) plays a modulatory role in auditory imprinting, we wanted to know whether and to what extent these areas receive dopaminergic input. In order to elicit the sources of dopaminergic input to the imprinting relevant forebrain areas retrograde fluorescent tracing was combined with DA immunohistochemistry. 4 to 10 day old chicks received single or multiple injections of fluorescent latex Microspheres (Molecular Probes) into the MNH, into the adjacent LPO and into the Nc. After an appropriate survival period chicks were perfused and immunohistochemistry was performed with an antybody against DA (Incstar), using several fluorescence detection systems. Retrogradly labelled neurons from the tracing experiments and immunolabelled fibers and somata were visualized under a confocal laser scanning microscope (Leica). The vast majority of dopaminergic fibers in the MNH emanate from neurons in the ventral tegmental area (VTA). The substantia nigra analogue Nc. tegmenti pedunculo pontinus (TPc) contained a large number of DA-positive somata which were double labelled after tracer injections into the LPO and few double labelled neurons after Nc injections. Results will be discussed with regard to the possible role of DA in imprinting-like learning processes and to forebrain organisation in birds in comparison with mamalian brain circuits.

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415.1

ACCURACY OF SPATIAL NAVIGATION: THE ROLE OF PLATFORM AND TANK SIZE. C.F. Mactutus* and R.M. Booze, College of Pharmacy, Tobacco & Health Research Institute, Graduate Center for Toxicology and College of Medicine, University of Kentucky, Lexington, KY 40546.

The Morris water maze task has great popularity for assessing both the cognitive processes and neurobiological mechanisms involved in rodent spatial learning. However, little systematic attention has been given to the role of several major parameters (e.g., tank and/or platform size) which may provide very different "instructions" to the rat. Young adult Sprague-Dawley male rats were trained at 90 days of age under one of three conditions: a small ($5cm^2$), medium ($10cm^2$), or large ($14cm^2$) escape platform in a standard 1.2m diameter tank. All animals (*ns*=18) received 8, 8, and 4 training trials on three consecutive days followed immediately by a probe test trial on the third day. After a 10-min interval, the animals received 4 additional training trials followed by a second probe test trial (the previous extramaze cues were absent). Platform size differentially and significantly affected acquisition--the most rapid decrease in latency was associated with the largest platform. All groups demonstrated a significant spatial bias during the test trial. In marked contrast to the training data, the annulus crossing measure, the sin qua non index of spatial accuracy, was enhanced inversely with platform size. Additional experiments demonstrated the generality of this relationship between spatial accuracy and relative platform size (1:60 - 1:450) was invariant to tank size (0.6m, 1.8m) or animal age (weanling, adult). In sum, the present data indicate that relative platform size is a major determinant of spatial learning, and more importantly, spatial accuracy. Collectively these data suggest that a relatively small platform size 1) maximizes the animal's reliance on extramaze cues and 2) ought to provide a more appropriate procedure to study spatial learning than one which supports the utilization of multiple and/or nonspatial strategies. (Supported by grants from NIH (AG05114, AG10747, & ES06259) and the Commonwealth of KY).

415.2

HIPPOCAMPAL LESIONS IMPAIR NEGATIVE PATTERNING, BUT NOT CONDITIONAL LEARNING, USING CONTEXTUAL CUES. D.M. Skinner* and D. van der Kooy. Dept. of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, M5S 1A8.

While performance on tasks employing spatial and contextual cues depends on the integrity of the hippocampus, we have previously shown that acquisition of conditional discriminations is not impaired by hippocampal lesions. Animals were trained on a conditional discrimination task that employed taste aversions. On some days animals were placed in a novel context (a distinct test box) prior to a flavor-LiCl pairing. On alternate days animals were exposed to a second distinct context prior to a flavor-saline pairing. Animals with aspiration lesions of the hippocampus acquired the task (learning to drink on safe context trials), but at a slightly slower rate than sham controls. We now report that these aspiration lesions of the hippocampus severely impair acquisition of a negative patterning task that employed the same cues. In a negative patterning task each of two cues is followed by an unconditioned stimulus when presented singly, but the unconditioned stimulus is withheld when the two elements are presented together. We used a novel 0.1 % saccharin solution and a distinct context as cues. Each of these cues alone signaled an injection of LiCl, but when presented together signaled the absence of LiCl. Both lesioned and sham animals acquired the conditional component of the task, consuming more saccharin in the novel context than in the home cage. However, the addition of context-LiCl trials impaired the performance of both groups. With further training, only the sham lesioned animals acquired the negative patterning task.

415.3

THE ROLE OF THE HIPPOCAMPAL SYSTEM ON TWO CONTEXT EFFECTS AFTER EXTINCTION. A. Wilson*, D.C. Brooks, & M.E. Bouton. Department of Psychology, University of Vermont, Burlington, VT 05405.

Contextual cues are important in determining performance in behavioral "interference" paradigms, such as extinction, in which the CS is associated with different outcomes in successive phases of the experiment. After extinction, contexts control performance by retrieving the current relation between the stimuli. This research examined the role of the hippocampus in mediating the effects of context after extinction. In *renewal*, conditioned responding to an extinguished CS recovers in the context in which it was conditioned when extinction occurs in another context. In a CER procedure, all animals were given CS-shock pairings in Context A. Half the animals from each group were given CS alone trials in Context A (Cont A, Forn A), half in Context B (Cont B, Forn B). Testing was conducted in Context A. None of the groups differed with respect to pre-CS baseline responding, rates of conditioning, or extinction. Rats with fornix lesions (Forn A) showed increased spontaneous recovery when compared to controls, but no attenuation of renewal. Suppression ratios [means (SEM)] on the first test trial were: Control A = .54 (.04), Control B = .08 (.03), Fornix A = .41 (.04), Fornix B = .16 (.04). In *reinstatement*, conditioned responding to an extinguished CS recovers when the animal is given unsignaled US presentations in the context of extinction. Animals were reassigned such that half received reinstating US presentations in the same context, half in a context different from extinction. Animals with fornix lesions (Forn Same) shocked in the extinction context did not show reinstated conditioned responding to the extinguished CS, whereas the comparable control group did (Cont Same). Suppression ratios [means (SEM)] on the first test trial were: Control Same = .16 (.06), Control Diff = .44 (.04), Fornix Same = .37 .03), Fornix Diff = .44 (.05). Results suggest that the hippocampal system plays a role in some, but not all, effects of context after extinction.

415.4

EVALUATION OF TIME OF DAY AS A CONDITIONAL CUE FOR RATS. A. Koerner*, R. J. Sutherland, G. M. Martin, R. J. McDonald, & S. Avery. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

We evaluate representational theories of conditioning which ascribe an important role to when and where an event occurs. We describe 4 discrimination experiments which show that rats' sensitivity to time of day should not be given a special role in theories of learning. Our data indicate that we should distinguish between two types of where: The place of training and the contextual cues present during training.

All experiments involve conditional discrimination training based upon place or time of day. The rats are trained that cue X, not cue Y, is correct at Time 1 or Place 1, and that cue Y, not cue X, is correct at Time 2, or Place 2. Experiment 1 shows that rats can use time of day as a conditional cue after extensive training if they are required to distinguish between a black and white cue in a Y-maze and that hippocampal damage abolishes the discrimination. Experiment 2 shows that rats do not learn to use time of day as a conditional cue when they are required to distinguish between a left and right turn. Comparison with a control group reveals that the rats adopt a win-stay/lose-shift strategy. Experiment 3 shows that rats after a single reversal readily use spatial location as a conditional cue when they are required to distinguish between a left and right turn. Experiment 4 eliminates spatially differential response requirements using operant chambers. Experiment 4 shows that rats are capable of using time of day and contextual cues (presence or absence of a clear plastic floor) as conditional cues after extensive training which does not involve changes in location of responding, when they are required to discriminate between a tone and light in an operant chamber. These results question the notion that time is a generally salient dimension in discrimination learning.

415.5

A VISUAL CUE ENHANCED BY SOUND IMPROVES SPATIAL LEARNING AND DECREASES RESPONSES TO DISTRACTION IN ANIMALS WITH HIPPOCAMPAL DAMAGE. E. Hebda-Bauer*, T. Briones, and B. Therrien, The University of Michigan, Ann Arbor, MI 48109

Spatial disorientation is caused by damage to the hippocampal formation (HPC). We previously reported that a single visual cue improves spatial learning of animals with HPC damage but the therapeutic effects are partially reversed by a simple environmental distracter. This study used a visual cue enhanced by sound to determine the effects of a multi-modal stimulus (cue) on cue navigation in the presence and absence of an environmental distracter. Adult male (n = 44) and female (n = 42) rats received unilateral or bilateral HPC lesions or sham surgery. Upon recovery, all animals were given four days of testing on the Morris water task to determine the amount of spatial disorientation. A visual cue, enhanced by pulsatile, amplified bursts of noise was then introduced. With the enhanced cue, males with unilateral or bilateral HPC damage and females with unilateral damage were not impaired. However, females with bilateral HPC lesions remained impaired on all measures ($p<.05$). After four days of testing with the cue, a distracter was introduced. In the presence of the enhanced cue, males with unilateral HPC damage were not distracted, performing as well as controls on all test days. In contrast, all females with HPC damage and males with bilateral lesions were more distractible than controls ($p<.05$). However, all animals were less distracted in the presence of the enhanced cue as compared to animals exposed to a single visual cue. We conclude that a visual cue enhanced by sound markedly improves spatial learning of disoriented animals. Importantly it eliminates or curtails the disruptive effect of a simple environmental distracter in animals with HPC damage.

415.6

RATS WITH HIPPOCAMPAL DAMAGE LEARN SPATIAL RELATIONSHIPS WHICH THEY CANNOT USE TO GUIDE NAVIGATION. R. J. Sutherland,* G. M. Martin, C. Edwards, & K. Williams. Depts. of Psychol., Univ. of New Mexico, Albuquerque, NM 87131 and Memorial Univ. of Newfoundland, St. John's, NFLD, A1B 3X9.

Many theories of hippocampal (HPC) function hold that its activity represents certain kinds of relational information. Medial temporal lobe amnesics demonstrate effects of prior learning about an episode if tested in indirect memory tasks, but not in direct tests of memory. We examined rats using two tests of spatial relational memory in the same environment; one direct (place navigation) and one indirect (dishabituation of exploration).

Twelve rats were trained in the Morris water task to locate a fixed, hidden platform. Half of the rats received multiple microinjections of a neurotoxin solution of kainate+colchicine. All rats were allowed to explore a dry pool in a new room in daily 5-min sessions for 37 days. Four objects were present in the pool in fixed locations. Two probe trials were conducted: 1. the pool and 4 objects were rotated 155° relative to the room and 2. two of the objects were transposed. Rats were tested for place navigation in the new and the old room.

The rats with HPC damage were profoundly impaired in the place navigation in the new and old rooms. In contrast, control rats and rats with damage detected the changes in spatial relationships in probe trials, as shown by reliable increases in exploration. Thus, learning the layout of an environment survives HPC damage, although this information is not used for accurate navigation.

415.7

HIPPOCAMPAL DAMAGE IN RATS CAUSES RETROGRADE AMNESIA FOR PLACE NAVIGATION BUT NOT FOR OBJECT DISCRIMINATIONS. R.S. Astur*, D.G. Mumby, M.P. Weisend, and R.J. Sutherland. Depts. of Psych. and Physiology, Univ. of New Mexico, Albuquerque, NM 87131.

In order to examine the effects of partial and complete hippocampal lesions on object and place memories, 29 rats were trained on a different object discrimination 13, 10, 7, 4, and 1 week(s) prior to surgery. To examine place memory, each rat was trained in the Morris water task in two different rooms, 14 and 2 weeks prior to surgery. Bilateral hippocampal lesions were made with multiple intrahippocampal microinjections of ibotenic acid. Ten rats received complete hippocampal lesions, 10 rats received partial hippocampal lesions, and 9 rats received sham lesions. Retrograde effects were assessed by testing all rats on the five object discriminations and the two pool problems that they had learned previously. Anterograde effects were assessed by teaching the rats two new object discriminations and one new pool problem. For the previously learned object discriminations, there were no differences between the groups in relearning the discriminations nor in their accuracy in the first five trials of testing. For the new object discriminations, there were no significant differences between the groups in learning either new object discrimination. For the previously learned pool problems, both HPC groups were impaired relative to controls in finding the platform and percent of time spent in the correct quadrant during probe trials. Only the complete HPC group did not learn the new pool problem. These data indicate that the HPC is important for navigating to places, but not for object discriminations.

415.8

RETROGRADE AMNESIA FOR PLACE AND CUE INFORMATION AFTER HIPPOCAMPAL DAMAGE IN RATS. M.P. Weisend,* & R.J. Sutherland, Depts of Physiology and Psychology, University of New Mexico, Albuquerque, NM 87131.

Retrograde amnesia after hippocampal damage may not share the same task specificity as anterograde amnesia (Weisend & Sutherland, 1993). We report here a replication and extension of our previous findings. Seventy-four Long-Evans hooded rats were trained in the same pool on two versions of the Morris water task: 1) fixed, hidden platform (place task) and 2) visible platform discrimination (cue task). Rats received 80 trials with the hidden platform and 160 trials with the visible platform discrimination. Hippocampal lesions were made using microinjections of kainate + colchicine either 1, 4, 12, or 36 weeks after training. During training, normal rats chose a visible platform based on the correct place learned rapidly in hidden platform trials. Rats rapidly mastered the visible platform discrimination after concurrent training stopped. Naive rats with hippocampal lesions given concurrent training did not show this pattern. Hippocampal damage produced retrograde amnesia for *both* place and cue tasks. Evidence for consolidation was clear only for place information in the group with 36 weeks between training and damage. Retrograde amnesia for cue task was equal across all training-damage intervals. Rats with hippocampal damage relearned the cue task. No rats with hippocampal damage relearned the place task. Interestingly, normal rats learned the cue task in a clearly discontinuous fashion while most of the same rats *re*learned the same discrimination in an incremental way *after* damage. Most naive rats with hippocampal damage also showed incremental learning of the cue task. We conclude that: 1) the intact hippocampus is used to solve some aspect of both place and cue tasks, 2) temporally graded retrograde amnesia is clear for place but not cue tasks after hippocampal damage, 3) the rapidly acquired place task can interfere with learning the cue task, and 4) after hippocampal damage rats learn the cue task in a fundamentally different way than normals. Funded by the RAC and SRAC of the University of New Mexico.

415.9

OBJECT DISCRIMINATION IN NORMAL AND HIPPOCAMPUS-DAMAGED RATS. D.G. Mumby*, D. Protz, R.S. Astur, R.L. Klein, R.J. Sutherland, and G.M. Martin. Depts. of Psych, University of New Mexico, Albuquerque, NM and Memorial University of St. John's, NF.

We examined whether rats can recognize objects independently of viewing perspective. Normal rats and rats with bilateral ibotenic acid lesions of the hippocampus (HPC) were trained to discriminate between three object pairs. Initial retraining with the objects in one orientation revealed that the original discriminations were retained (Mean errors to criterion, 4.0 & 5.1 for HPC and normals rats, respectively). Changing the orientation of the objects during training disrupted performance (Mean errors to criterion, 16 & 14 for HPC and normal rats, respectively). The objects were then coated with acrylic to increase the similarity of the objects' surface properties, including odor. Retraining the rats with the objects in the original orientation revealed that the acrylic did not affect performance (Mean errors to criterion, 2.1 & 3.3 for HPC and normal rats, respectively). The final test revealed that switching the orientations of the acrylic-coated objects interfered dramatically with acquisition of the discrimination (None of the rats reached criterion; Mean errors, 78 & 65 errors for HPC and normal rats, respectively). Further tests were carried out to assess the use of spatial information when the correct choice of an object depended upon its spatial location.

Object discrimination performance in our task depends upon object orientation. Moreover, it may be that rats solve object discriminations by distinguishing between object pairs on the basis of a single visual or surface property.

415.10

RECOVERY OF SPATIAL NAVIGATION IN THE MORRIS WATER MAZE FOLLOWING BILATERAL TRANSECTION OF THE FIMBRIA/FORNIX IN RATS. D.K. Hannesson* & R.W. Skelton. Psychology, University of Victoria, POB 3050, Victoria, B.C., Canada, V8W 3P5.

Fimbria/fornix (FF) lesions in animals are frequently used to model neurodegenerative and traumatic neuropathologies observed in humans, and have been used to evaluate the therapeutic efficacy of a variety of interventions, such as grafting or pharmacotherapy. However, recovery in the absence of interventions (i.e., spontaneous recovery) has rarely been investigated.

The present study was designed to assess spontaneous recovery of spatial learning and navigation in the Morris water maze (MWM) following bilateral knife cuts of the FF. Subjects were 32 male Long-Evans rats. They were either pretrained (6 days) or left naive and were given either FF or sham lesions. Post-lesion assessment in the MWM consisted of 13 days of testing with the platform in one location followed by 13 days with the platform in the opposite quadrant of the maze.

FF cuts produced a marked deficit in navigating to the first platform location. Pretraining attenuated the deficit. Considerable recovery was exhibited over the period of testing which probe tests indicated involved the use of a spatial strategy and not some other form of behavioral compensation. When the platform was moved, lesioned groups exhibited a marked deficit, which was not reduced in the pretrained group. Recovery was again observed which probe tests indicated entailed the use of a spatial strategy.

These results show that considerable spontaneous recovery does occur following bilateral transection of the FF but that recovery of spatial navigation may occur without recovery of spatial learning. Furthermore, these results suggest that a distinction may exist between interventions that enhance or speed spontaneous recovery and those that provide alternative recovery mechanisms. (Supported by NSERC Canada)

415.11

THE EFFECT OF SELECTIVE FIMBRIA-FORNIX CUTS ON STEREOTYPIC BEHAVIOR AND SPATIAL MEMORY. F. Jiang, R. Racine, J.Turnbull* Depts. of Medicine and Psychology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Fimbria-fornix lesions cause hyperactivity and learning difficulty in rats. During the course of other studies investigating memory deficit in fornical-lesioned rats we had occasion to observe pronounced stereotypic behaviour. Specifically a cannulated knife was used to produce selective lesion to the fornix in 12 Long Evans hooded rats; 9 animals undergoing sham operation served as controls. As expected the animals with fornical lesions demonstrated impaired acquisition, reversal, and working memory in the Morris water maze ($p<0.01$). However these animals also manifested maintained preference of swim direction, which could be clockwise or counterclockwise ($p<0.01$). Post mortem examination revealed that the cuts were complete and it is unlikely that these observations could be explained by technical factors. The stereotypic behavior may interfere with the testing of memory in this case, and since lesions to the FF disrupt cholinergic and gabaergic input to the hippocampus these results may also have some relevance to stereotopy in human disease states with similar deficits.

415.12

COMPLEMENTARY ROLES FOR THE HIPPOCAMPUS AND ENTORHINAL/PERIRHINAL CORTEX IN THE ACQUISITION OF SPATIAL AND NONSPATIAL TASKS IN THE RAT. L. Jarrard* and A. Hyko. Dept. of Psychol., Washington and Lee Univ., Lexington, VA 24450.

In order to study the involvement of the different components of the hippocampal formation in learning, rats with selective ibotenic acid lesions of the hippocampus, aspiration lesions of the entorhinal/perirhinal cortex (EC/PRC), and controls were trained in Exp. 1 on spatial and nonspatial versions of an 8-arm radial maze, and in Exp. 2 on concurrent visual discrimination and spatial rewarded alternation tasks. Rats with the hippocampus removed were especially impaired on the spatial tasks and did not differ from controls on the nonspatial tasks. In contrast, EC/PRC rats were like controls on the spatial tasks but were impaired on the nonspatial tasks. This pattern of results (e.g., a double dissociation) supports the view that the hippocampus and EC/PRC differ functionally. Specifically, in the rat the hippocampus seems to play an especially important role in the acquisition of spatial information, while the EC/PRC is more involved in the processing of complex, nonspatial information.

Supported by NSF Grant BNS-910955.

415.13

HIPPOCAMPAL AND NON-HIPPOCAMPAL BASED PLACE LEARNING IN THE RAT. Robert J. McDonald* and Norman M. White, Department of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

Performance on a variety of place learning tasks is impaired by damage to the hippocampal system in the rat (Olton, Walker, & Gage, 1978; Sutherland, Kolb, & Whishaw, 1982), but understanding of the neural substrates underlying place learning tasks that are not dependent on the hippocampal system is limited. In the present experiment the participation in place learning of hypothesized memory systems that include hippocampus, lateral nucleus of the amygdala or dorsal striatum was studied by requiring rats to discriminate between 2 arms of an 8-arm radial maze. Two behavioral variables were manipulated: 1) Movement - rats were either confined to the arms of the maze or allowed to run into the maze arms during training; 2) Cue Resolution - the use of distal room cues was assured by rotating the entire radial maze one arm position to the left at the start of each training day. Rats were required to discriminate 2 widely separated arms from each of which the could see a unique cue or set of cues, or between two adjacent arms from each of which they could see largely overlapping sets of cues. In the latter case the arms could be distinguished only by computing the differences in the spatial relationship of each arm to many of the same cues. A series of single and double lesions revealed the following about the roles of the movement and cue resolution variables in each of the three hypothesized memory systems. When the animals were confined to the arms of the maze only the amygdala system could process the information required to discriminate between the arms, and the discrimination was learned faster with unique than with overlapping cues. In both cases the passive discrimination was *facilitated* by hippocampal system lesions. When animals ran into the maze arms during training only the hippocampal system processed the information required to discriminate overlapping sets of cues, but both the hippocampal and dorsal striatal systems processed information that discriminated between unique cues. In the latter case the two systems appeared to process different kinds of information, in parallel, that led to the same solution.

415.14

LESIONS TO THE HIPPOCAMPAL SYSTEM DO NOT IMPAIR SELECTIVE ASSOCIATIONS IN RATS R.A. Murphy, R.J. McDonald, F.A. Guarraci & A.G. Baker*, Department of Psychology, McGill University, Montréal, Quebec, Canada, H3A 1B1.

There is some evidence that lesions to the hippocampus impair performance on Kamin's blocking, on overshadowing and on relative validity procedures. These selective association procedures require that subjects ignore, to some extent, the relationship between a redundant CS and a US. It has been suggested that the hippocampus may be required to filter this redundant information. We trained rats with either electrolytic lesions of the fornix, or colchicine lesions of the dentate gyrus of the hippocampus, and controls on a appetitive operant version of Wagner, Logan, Haberlandt and Price's (J. Exp. Psych., 76: 171-180, 1968) relative validity procedure. We used a variable interval discrete-trial bar press procedure with two stimulus compounds containing a common element (AX, BX). Half of each group were trained with a True-discrimination (TD; AX+, BX-) in which AX trials were reinforced and BX trials were not while the other half were trained with a Pseudo-discrimination (PD; $AX^{+/-}$, $BX^{+/-}$) in which both AX and BX trials were reinforced 50% of the time. In spite of the fact that X is reinforced equally often in both groups, previous research has demonstrated that in comparison to PD trained animals, TD trained normals show less responding to the partially reinforced stimulus (X) because the other more valid predictors of reinforcement (A and B) overshadow conditioning to X. During our tests of X, all three groups showed overshadowing of X following true-discrimination training. Consistent with previous experiments in our lab the lesions resulted in increased rates of lever pressing during reinforced and nonreinforced trials and during the test of X. These results suggest that the hippocampus is not required for this selective association phenomenon.

415.15

RATS WITH ANGULAR BUNDLE TRANSECTIONS SHOW NORMAL ACQUISITION BUT IMPAIRED LONG-TERM RETENTION OF OBJECT DISCRIMINATIONS. N. Vnek[1*], T.C. Gleason[1], L.F. Kromer[2] and L.A. Rothblat[1]. [1]Dept. of Psychology, George Washington University, Washington, DC 20052, and [2]Dept. of Cell Biology, Georgetown University, Washington, DC 20007.

Investigations of the neurobiology of memory using experimental animals have succeeded in modeling many of the characteristic features of amnesia seen in human clinical populations. To examine long-term memory, however, animal models of amnesia often employ extended measures of acquisition, which stand in contrast to the retention measures used with humans.

To determine the role of hippocampal/parahippocampal circuitry in both information acquisition *and* long-term retention, rats with bilateral transections of the angular bundle were trained on three object discrimination problems and then retrained two weeks later to measure retention. Rats with discrete lesions of the angular bundle, which disrupt perforant path connections from entorhinal cortex and efferent hippocampal-cortical projections, acquired the discrimination problems normally but showed a marked deficit in retention. Thus, the role of this circuitry may be limited to maintaining some types of information (e.g., single object discriminations) for retention. Behavioral paradigms that include a measure of retention may be particularly important for characterizing mnemonic deficits in animal models of amnesia. Supported by ONR and GWU Facilitating Fund.

415.16

MOVEMENT-PRODUCED HIPPOCAMPAL AND PASSIVE NON-HIPPOCAMPAL LEARNING IMPEDE AMYGDALA-BASED STIMULUS-REWARD LEARNING. Norman M. White* and Robert J. McDonald, Psychology, McGill University, 1205 Dr. Penfield Ave, Montreal, Quebec, H3A 1B1.

We previously reported that the conditioned cue preference (CCP) learned by rats on the 8-arm radial maze using only distal cues to distinguish between food and no-food arms is eliminated by lateral nucleus of the amygdala lesions and potentiated by fornix lesions, suggesting that this form of stimulus-reward learning is mediated by a neural system that includes the amygdala but not the hippocampus, and that the hippocampal system interferes with amygdala-based learning of the CCP. 1) We tested the hypothesis that the cause of this interference is the acquisition of a spatial map of the maze room by the hippocampal system during 10 min of free exploration pre-exposure to the maze, given to all animals before CCP training. Rats not pre-exposed to the maze, or pre-exposed to a maze in a different room exhibited potentiated CCPs, comparable to those of animals with fornix lesions, suggesting that the CCP in normal animals is inhibited due to learning about spatial cues in the maze room. 2) Rats with fornix lesions made after pre-exposure (in the same room), or after CCP training but before testing, exhibited CCPs comparable to those observed in normal animals, suggesting that acquisition of spatial information by the hippocampal system during pre-exposure requires an intact fornix; however, the fornix is not required for subsequent inhibition of stimulus-reward learning in the amygdala system. This inhibition may be mediated via entorhinal cortex. 3) When rats were pre-exposed to the maze by confining them in the arms (instead of exploring freely), the subsequently acquired CCP was comparable to that in normal animals, but was *not* potentiated by fornix lesions, suggesting that passive pre-exposure may produce non-hippocampal learning, possibly mediated in the amygdala system, that impedes stimulus-reward acquisition by the same system. There may be two different latent-inhibition-like effects, involving two different kinds of learning and mediated by two different memory systems.

415.17

DISSOCIATION OF HIPPOCAMPAL AND STRIATAL CONTRIBUTIONS TO PLACE NAVIGATION IN THE WATER MAZE. B.D. Devan*, E.H. Goad, H.L. Petri. Dept. of Psychology, Towson State University, Towson, MD 21204.

Previous studies have shown that damage to either the hippocampus or striatum of the rat results in severe spatial impairments. The purpose of the present study was to compare the effects of hippocampal (fornix/fimbria) and striatal (caudate-putamen) lesions on acquisition of the standard place version of the Morris water task and a modified cue version that attenuated simultaneous acquisition of a place response. Rats with fornix/fimbria lesions were impaired during a late stage of place task acquisition but were not impaired on the cue task. Caudate-putamen lesions resulted in a severe place task impairment and a transient cue task impairment, both of which were characterized by an early phase of thigmotaxic navigation. The escape latency measure was particularly sensitive to thigmotaxic behavior and may not accurately reflect allocentric spatial learning. Post-hoc analyses of start point heading angles suggested that rats with fornix/fimbria lesions used position response and guidance strategies. These rats also demonstrated a significantly weakened spatial bias for the former training quadrant on the platform removal probe and reduced flexibility in navigating to a novel platform location on the platform relocation test. In contrast, rats with caudate-putamen lesions spent a greater amount time swimming in the former training quadrant on the platform removal probe, and despite poor place acquisition latencies showed greater improvement when required to learn a new platform position on the relocation test.

The results revealed dissociable aspects of water maze performance following damage to the fornix/fimbria or caudate-putamen. It is suggested that the hippocampus mediates the allocentric spatial aspects of the water maze place task while the striatum may mediate acquisition of the non-allocentric procedural components of both place and cue versions. The results support a multiple neural-systems view of learning/memory and further suggest that in the intact mammalian brain multiple systems may acquire different forms of information simultaneously.

415.18

"PLACE" VERSUS "RESPONSE" LEARNING DEBATE REVISITED IN THE BRAIN. M. G. Packard* Dept. Psychology, Univ. New Orleans, N.O., LA 70148.

The historical debate between psychologists who favored "cognitive" learning theories and those who favored "stimulus-repsonse" learning theories can be characterized by the tendency of rats to display place or response learning in the cross-maze paradigm, respectively. Findings indicating that normal rats can employ either mechansim in learning to approach the consistently baited arm of a cross-maze (cf. Restle, 1957), raises the possibility that these two learning mechansims are mediated by distinct neural systems. Consistent with this possibility is evidence indicating that the mammalian brain contains multiple memory systems which differ in the "type" of memory they mediate. The present study was designed to examine the hypothesis that place and response learning are mediated by independent hippocampal and caudate nucleus memory systems, respectively.

Rats were trained to approach a consistently baited arm in a cross-maze from the same start box (4 trials/day/14 days). On days 8 and 16 a single probe trial was given, in which rats were placed in the start box of the cross-maze opposite that used in training, and allowed to approach a maze arm. On the probe trials, rats which selected the baited maze arm were designated place learners, and rats which selected the unbaited maze arm were designated response learners. 3 minutes prior to the probe trials, rats received bilateral injections of either saline or a 2% lidocaine solution (in order to produce neural inactivation) into either dorsal hippocampus or dorsolateral caudate nucleus. Saline treated rats displayed place learning on the day 8 probe trial, and response learning on the day 16 probe trial, indicating that with extended training, there is a shift in learning mechanisms controlling behavior. Rats receiving lidocaine injections into hippocampus showed no preference for place or response learning on the day 8 probe trial, and displayed response learning on the day 16 probe trial, indicating a blockade of place learning following inactivation of the hippocampus. Rats receiving lidocaine injections into the caudate nucleus displayed place learning on both the day 8 probe trial and the day 16 probe trial, indicating a blockade of response learning following inactivation of the caudate nucleus. The results indicate that the hippocampus and caudate nucleus selectively mediate place and response learning tendencies, respectively, and offer a "neurobiological" solution to the place versus response learning debate.

415.19

EFFECTS OF ELECTROLYTIC AND IBOTENATE LESIONS OF THE ROSTRAL GLOBUS PALLIDUS ON WATER MAZE AND CHEESEBOARD ALLOCENTRIC SPATIAL TASKS. G.D. Coover*, M.E. Oakes, R.C. Meyer and J.V. Corwin. Psychology Dept., Northern Illinois Univ., DeKalb, IL 60115.

A very extreme deficit in performance of the Morris Milk Maze (MM) task was produced by small electrolytic lesions in rostral globus pallidus (rGP), but not by lesions in the center of GP (Meyer & Coover, Soc. Neurosci. Abstr., 18:1565). The present study examined the nature of this deficit by testing performance in both negatively (MM) and positively (cheeseboard) reinforced tasks, and whether comparable deficits would be produced by ibotenate lesions of rGP.

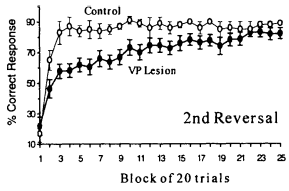
Testing in the MM began 4 weeks following surgery with eight 4-trial sessions of escape to a platform in the center of the same quadrant of a 1.6-m diam tank. The platform was submerged after the first session, and further hidden by adding milk after the 3rd session. Two weeks later, the rats were tested during six 4-trial sessions to find half a Froot Loop in the same spatial location amongst 177 holes. Rats with electrolytic lesions of the rGP failed to escape in the MM within 120 sec in most cases (5 out of 8 rats), and showed almost no reduction in their 60+ sec latency to find the food reward. By contrast, sham surgery and vehicle injection control groups, and also a group receiving 3 μg ibotenate under ketamine anesthesia, achieved MM escape within 20 sec by the third session, and reduced their food finding latency from 60 sec the first session to less than 20 sec by the fifth session. The experimental group receiving ibotenate lesions of rGP under Nembutal anesthesia did exhibit deficient MM performance over the first four sessions, which might reflect a spatial navigation deficit. However, the more extreme deficits produced by electrolytic lesions in rGP suggest a broad disturbance of instrumental performance.

415.20

LESIONS OF THE VENTRAL PALLIDUM INTERFERE WITH REVERSAL OF ODOR DISCRIMINATION IN RATS. X.-C. M. Lu* and J. L. Price. Dept. of Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Previous studies have shown that interruption of the projection from the globus pallidus to the ventroanterior and ventrolateral thalamic nuclei interferes with the inhibition of conflicting motor programs. Lesions of the mediodorsal thalamic nucleus, which receives input from the ventral pallidum (VP), interfere with reversal learning. A common feature of these deficits is the animals' inability to inhibit previously learned behavior in order to switch to a new behavioral or cognitive strategy.

This study examined the role of VP in inhibition of responding to a previously rewarding odor stimulus. In the first, or "inhibition", task, two odors which rats had learned as positive (rewarding) stimuli in previous separate tasks were paired together for discrimination. One odor remained positive and the other became a negative (nonrewarding) stimulus. In each of two subsequent "reversal" tasks, 2 novel odors were paired for initial discrimination learning. On the next day, the significance of these odors were reversed. Experimental rats with bilateral VP lesions, and sham controls rats were compared with these tasks. Histological verification of the lesions was performed in experimental rats. In all three tasks the lesioned rats made substantially more errors than the control rats, and some rats failed to reach criterion in 500 trials. With the two reversal tasks these differences were significant, although with the inhibition task the difference did not reach significance. Supported by NIH grant DC00093.



415.21

THE VENTRAL STRIATAL - VENTRAL PALLIDAL AXIS AND ASSESSMENT OF EFFORT REQUIRED FOR A REWARD. **V.J. Brown,* G. Symons, P. Winn and M.P. Latimer** School of Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU

The ventral striatum has been implicated in reward-related processing, but its separate contributions to the perception of reward value and reward cost is not known. The present study sought to determine the involvement of the ventral striatal-ventral pallidal (VS-VP) system in assessment of reward cost. We measured progressive ratio (PR) responding, in which the cost in lever presses of a single food pellet increases incrementally with each pellet. Bilateral ibotenate (IBO) lesions were made in VS (0.06M, 0.8µl injections/hemisphere; sham lesions 0.8µl PBS) or VP (0.06M, 0.5µl injections/hemisphere; sham lesions 0.5µl PBS) of rats after 21 days training and at least 30 days before testing started. The point when normal rats and shams stopped responding (breaking point) under a PR schedule was stable over days, as were latencies to collect pellets; the post-reinforcement pause increased as a function of schedule progression. These effects are consistent with previous reports. Rats with VS IBO lesions showed significant increases in breaking point, indicating a willingness to work at greater cost for pellets. Rats with VP IBO lesions also showed increases in breaking point, and a greater range of breaking points than controls. Rats with VP lesions did not show an increase in post-reinforcement pause as a function of the PR increments. As this increase reflects a reluctance to resume work in the light of perceived greater effort required for reward this result is interpreted in terms of a deficit in perception of increasing reward cost. Overall these data demonstrate that the effect of lesions in the VS-VP axis is not simply to decrease motivation to work for food reward. Rather, these data suggest that lesioned rats may have lost the ability accurately to perceive the increasing cost of reward. Thus, rather than a change in motivation *per se* there appears to be alteration in the perception of the increasing cost of reward.

LEARNING AND MEMORY: PHARMACOLOGY—EXCITATORY AMINO ACIDS

416.1

CHANGES IN GLUTAMATE RECEPTOR PROPERTIES IN THE CEREBELLAR CORTEX OF CLASSICALLY CONDITIONED RATS. Sarah Pollock, Dave Lavond*, Richard F. Thompson, Matti Mintz, & Georges Tocco. Psychobiology Unit, Psychology Department, Tel Aviv University, Israel & University of Southern California, Neurosciences Program, HEDCO Neurosciences Building, Los Angeles, CA, 90089-2520.

The cerebellar cortex is believed to be involved in the neural plasticity associated with the conditioned eyelid response. Because glutamate receptors have been shown to play a pivotal role in various forms of synaptic plasticity, we planned to determine their involvement in this learning model using quantitative ligand binding autoradiography.

Male Wistar rats were trained on a delay paradigm with paired presentations of conditioned (tone) and unconditioned (electric shock to one eye) stimuli. Naive and unpaired animals were used as controls. Quantitative ligand binding autoradiography using ligands specific for the AMPA subclass of glutamate receptors revealed an increase in tritiated AMPA and CNQX binding on the contralateral side of the trained animals (compared to the ipsilateral) with little or no side difference on the unpaired and naive animals. Conditioning did not result in any change on tritiated TCP binding (NMDA specific).

These results support molecular changes of glutamate receptors in cerebellar cortex correlated with learning.

416.2

PICROTOXIN INFUSIONS INTO A CRITICAL REGION OF CEREBELLUM PREVENT BOTH ACQUISITION AND RETENTION OF CLASSICALLY CONDITIONED EYEBLINK RESPONSE. S. Bao, L. Chen, J. J. Kim, J. K. Thompson*, and R. F. Thompson, Neurosciences Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520.

Microinfusions of a $GABA_A$ antagonist picrotoxin (PTX) in the cerebellum have been shown to block the expression of classically conditioned eyeblink response. In the present study, we examined the effect of PTX on the acquisition of conditioned eyeblink response. New Zealand white rabbits, implanted with cannulae aimed at the ipsilateral interpositus (IP) nucleus, underwent five days of standard delay tone-airpuff training while receiving continuous infusions (0.13 µl/min) of PTX (10 nmol/µl) into the IP. Then, they were given 3 days of training with artificial cerebrospinal fluid (ACSF) infusions. During the 5 days with PTX, animals showed no signs of CRs while exhibiting normal unconditioned responses to the airpuff. When switched to the ACSF, these animals learned eyeblink CRs as if they were naive. When PTX was infused again, CRs were completely abolished. These results support earlier reversible inactivation studies that demonstrated that the cerebellum is critical for acquisition and expression of eyeblink conditioning. Supported by grants from NRSA 1F32MN10521-01 BNR to JJK and NSF BNS-8718300 & NIH (NIA) AG05142 to RFT.

416.3

INVOLVEMENT OF METABOTROPIC GLUTAMATE RECEPTORS IN LONG-TERM POTENTIATION AND LEARNING AND MEMORY. G. Riedel*, W. Wetzel, S. Vieweg and K. G. Reymann. Federal Inst. for Neurobiology, P.O. Box 1860, 39008 Magdeburg, Germany.

Metabotropic glutamate receptors (mGluRs) are known to play a crucial role in both the induction and maintenance of hippocampal long-term potentiation (LTP). It is conclusive, if LTP is of physiological relevance, that mGluRs should play an important role in learning and memory as well. We investigated this hypothesis applying electrophysiological and behavioral standard procedures.

Electrodes were implanted into the perforant path for stimulation and in the dentate gyrus for recording in the rat. (R,S)-α-methyl-4-carboxyphenylglycine (MCPG), a selective mGluR antagonist, was injected in two concentrations (A=20.8 μg; B=104.2 μg i.c.v.) 30 min prior to tetanus-induced LTP or learning, respectively. For comparison, a novel form of shock-reinforced Y-maze spatial alternation and a brightness discrimination task were performed. MCPG blocked different phases of LTP in a concentration dependent manner. Dosis B abolished a potentiation completely and also inhibited learning in the spatial Y-maze. Although these animals showed an acquisition deficit in the discrimination task, no difference to controls occurred during retention test 24 hours after training. Application of A resulted in a considerable 2.5 hours potentiation, which resembled the time course of short-term potentiation (STP). The same concentration had no effect in spatial alternation learning or the brightness discrimination task. These data further substantiate that mGluRs are involved in the expression of LTP in the dentate gyrus and consolidation of spatial alternation memory. The effects on both were dose-dependent, i.e. only the high concentration of MCPG induced a complete block, whereas no learning impairment occurred in the low dose group where the potentiation lasted up to 2,5 hours. We therefore assume, that STP in the hippocampus is the crucial factor for the learning of spatial tasks in the long term. Block of STP, however, results in amnesia.

416.4

Intra-Amygdala CNQX does not block retention of Escape Training. M.H. Mesches*, M. Bianchin and J.L. McGaugh, Center for the Neurobio. of Learning & Memory & Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717-3800.

It is well established that manipulations of the amygdala alter memory. Recent experiments find that the memory impairment induced by lesions of the amygdala may be attenuated by increased degree of training, suggesting that the amygdala is not the critical site of memory storage. However, it can be argued that compensatory mechanisms or incomplete lesions may account for the intact memory. Moreover, if the amygdala is a site of memory storage, a possible mechanism mediating this plasticity would be long term potentiation (LTP). Several studies support the hypothesis that the activation of amygdaloid NMDA receptors is critical for memory storage. To test whether AMPA receptor-dependent expression of LTP within the amygdala is involved in the expression of retention, several recent studies have examined the effects of the AMPA-receptor blocker, CNQX on performance of aversively motivated memory tasks. In these studies CNQX blocks or impairs retention when administered prior to testing.

The present study further examined the effects of intra-amygdala infusion of CNQX on the retention of animals trained with different degrees of escape training. In addition, the effects of CNQX on locomotor activity and nociceptive thresholds were also measured. Male rats were implanted bilaterally with cannula aimed at the amygdala. One week later they were trained to escape from the dark, shock compartment of a straight alley maze, receiving either No Shock, 1 trial, or 10 trials of 0.75 mA footshock. Eight days later CNQX (0.5 μg/0.5μl) was infused into the amygdala, 10 min later the rats were placed in the lighted compartment and the initial latency to enter the dark compartment was measured. Each time the rat entered the dark compartment is was shocked with a 0.3 mA footshock until it escaped to the lighted compartment. The number of trials required until the rat stayed out of the dark compartment for 100 sec was used as a measure of retention. CNQX blocked the training dependent increase in the latency to enter the dark compartment ($p<0.05$) and increased the number of trials required to reach criterion ($p<0.05$). However, CNQX did not block the effects of previous training. Animals under the influence of CNQX required a lower number of trials to reach criterion durign training if they had previously received shock ($p<0.05$). Rats which received CNQX 10 min before testing showed increased activity in an open field and decreased sensitivity to footshock. Taken together the data presented above suggest that the amygdala AMPA receptors are not critically involved in the retention of escape training. Furthermore, the data does suggest that other performance variables such as locomotor activity and nociceptive threshold are affected by blockade of the amygdala AMPA receptors.

Supported by USPHS MH12526, NIMH & NIDA (JLM).

416.5

THE EFFECTS OF MUSCIMOL INFUSIONS INTO THE AMYGDALA AND MEDIAL SEPTAL AREA IN MEMORY FOR MAGNITUDE OF FOOD REWARD. J.M. Williams, R.J. Nelson* and D.S. Olton. Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Rats were trained on a go, no-go task measuring memory for magnitude of food reward. In the study phase of the task, the rats were given one of two cereals. One cereal contained 25% sugar, the other 50% sugar. One of the two cereals was designated as the positive stimulus and the other as the negative stimulus. If the rat received the positive stimulus in the study phase, in the subsequent test phase the rat received another food reward which was placed beneath an object in a foodwell. If the rat received the negative food stimulus during the study phase, no food was placed beneath the object in the foodwell. Performance was measured as latency to uncover the foodwell in the test phase. A previous study using the aforementioned procedure found that lesions to the amygdala, but not the hippocampus significantly impair performance in this memory task.

The present study seeks to further clarify the role of the amygdala in memory for magnitude of food reward. After reaching criterion level (significant difference in latency to respond to the positive vs. negative stimulus), rats received cannula implantations into both the medial septal area and amygdala. All rats received infusions of muscimol (15ng and 30ng) into the amygdala and the medial septal area. The effects of muscimol into each of these areas were compared to saline infusion trials and trials in which no infusions were given. The effects of muscimol on memory for magnitude of reinforcement will be discussed.

416.6

INTRA-SEPTAL INFUSIONS OF MUSCIMOL IMPAIR SPONTANEOUS ALTERNATION PERFORMANCE: FAILURE TO REVERSE THE DEFICIT WITH GLUCOSE M.B. Parent* and P.E. Gold. Department of Psychology, University of Virginia, Charlottesville, Virginia 22903.

Intra-septal infusions of drugs that act at cholinergic, noradrenergic, opioid peptidergic, and glutamatergic receptors influence spontaneous alternation performance. The present experiment examined the role of the medial septal GABAergic system in spontaneous alternation performance by infusing the GABA agonist muscimol 15 min prior to training in a spontaneous alternation task. With evidence that systemic and intra-septal infusions of glucose reverse the impairing effects of intra-septal infusions of opioid agonists (Ragozzino, Parker, & Gold, Brain Research, 1992), the efficacy of intra-septal infusions of glucose in reversing muscimol-induced deficits was also examined. Intra-septal infusions of muscimol (1 or 3 nm/0.5 μl/1 min) significantly impaired spontaneous alternation performance. Glucose (8.3, 16.7, or 33.3 nm/0.5 μl/1 min) did not affect spontaneous alternation performance or reverse the deficit produced by intra-septal infusions of muscimol (1 nm). These results indicate that activation of GABAergic receptors in the medial septum impairs spontaneous alternation performance. This finding is similar to results obtained with morphine infusions. However, in contrast to the results with morphine, glucose did not reverse the muscimol-induced impairment. This pharmacological distinction suggests that glucose may interact with specific neurotransmitter systems in the medial septum to influence spontaneous alternation performance. [Supported by NIA (AG 07648), NSF (BNS-9012239), and NIH (HD07323)].

416.7

Microinjections of NMDA and non-NMDA receptor antagonists into the "core" and "shell" subregions of the nucleus accumbens impair acquisition and performance in a spatial learning task. C.S. Maldonado-Irizarry*[1] and A.E. Kelley [2]. [1] Northeastern University, Dept. Psychology, Boston, MA 02115; [2] University of Wisconsin-Medical School, Dept. of Psychiatry, Madison, WI 53706.

To further investigate the hypothesized functional dissociation between the core and shell subregions of the nucleus accumbens, the following experiments were conducted. The effects of microinfusions of AP-5, a NMDA antagonist, and DNQX, a non-NMDA antagonist into the core and shell subregions of rats on the acquisition and performance phases of a food search task were tested. In this spatial learning paradigm, hungry rats were required to learn a specific pattern of food-baited holes in a large open field. Parameters measured were total time spent searching for food and visits to empty and baited holes (errors). For both experiments, separate groups of rats were used for the acquisition and performance phases of this paradigm. In the acquisition phase, rats were infused with vehicle or DNQX (0.75 μg/0.5 μl) into either the core or shell subregions. DNQX treatment in the core group resulted in an increase in both time to complete the task and errors. DNQX in the shell group did not affect time but slightly increased errors. In the performance phase, microinjections of DNQX (0, 0.075, 0.75 μg/0.5 μl) were given on test days. Treatment significantly impaired both behavioral measures in the core and shell groups; however, the core group was significantly more impaired than the shell group. In Experiment 2, the effects of microinfusions of AP-5 (0, 0.2, 1.0 μg/0.5μl) in the performance phase were tested. AP-5 significantly increased only the time to complete the trial in both the core and shell groups. However, AP-5 caused significantly more errors in the core than in the shell group. At this time, experiments aimed at investigating the role of NMDA receptors in the acquisition phase of this task are in progress. The present results suggest that blockade of EAA receptors in the accumbens disrupts the acquisition and performance of rats in a food search task; moreover, the accumbens core subregion is more sensitive to the effects of these antagonists on spatial learning and performance than the shell subregion.

416.8

EFFECTS OF D-CYCLOSERINE, AN AGONIST AT THE GLYCINE SITE OF THE NMDA COMPLEX, ON DISCRIMINATION LEARNING IN YOUNG RATS. M.W. Lilliquist*, K. Nixon, and A. Amsel Dept. Psychology and Inst. for Neuroscience, Univ. of Texas at Austin, Austin, TX 78712.

The *N*-methyl-D-aspartate receptor complex, a subclass of excitatory amino acid receptors, has been implicated in the processes of learning and memory. Long-term potentiation, a putative mechanism for the cellular basis of learning and memory, has been shown to be modulated by ligand activity at both the glycine and NMDA sites of the receptor complex. Previous work has shown that D-cycloserine (DCS), an anti-mycobacterial drug, binds with high affinity to the glycine site, and in separate studies has been shown to enhance performance on several types of learning and memory tasks. In the present study, young rats (16-17 d.o.) were trained on patterned single alternation (PSA), a memory-based discrimination learning task, following systemic injection with DCS (1.0, 3.0, or 10.0 mg/Kg). Additional subjects were prenatally exposed to ethanol via maternal intubation during the second half of gestation. DCS failed to facilitate PSA learning in either normal animals or in animals prenatally exposed to ethanol, even though prenatal ethanol exposure has been shown to reduce NMDA-mediated cellular response and glutamate binding. These data contribute to an emerging body of research which indicate inconsistent effects of the glycine agonist DCS on learning in both human and animal studies, and they contrast with consistent effects observed with agents which act upon other loci within the NMDA complex. (Supported by NIAAA grant AA07052)

416.9

DIFFERENTIAL EFFECTS OF MK-801 AND SCOPOLAMINE ON WORKING AND REFERENCE MEMORY IN RATS. P. Barnéoud*, M. Mazadier, T. Rouyer, M. Reibaud and J.-C. Blanchard. Rhône-Poulenc Rorer S.A., CRVA, 94403 Vitry sur Seine Cedex, France.

Evidence for different types of memory in rats may lead to development of animal models for human memory disorders and provides informations on neurobiological systems underlying these processes. The effects of the noncompetitive NMDA antagonist MK-801 were compared to those of the muscarinic antagonist scopolamine hydrobromide on working and reference memory in the radial maze. In order to discriminate between the working memory and the reference memory, the radial maze was partially baited with only 4 to the 8 arms baited. Moreover, we studied the consequence of angles between the baited arms ("baiting" pattern) on the memory task. The behavioral acquisition of food-deprived male Long-Evans rats was slightly delayed when the angle differed from 90° (45° or 135°), indicating that the pattern corresponding to the 90° was easier to learn. The pharmacological study was performed on the trained animals. MK-801 (0.12 mg/kg, IP, 60 min before a session) significantly increased the number of working memory errors (re-entries into baited arms) and reference memory errors (entries into never baited arms) whereas the running time was barely lengthened. In details, the working memory errors were dramatically increased in the case of "difficult" patterns (45°, 135°) but not in the "easy" one. Scopolamine (0.5 mg/kg, IP, 30 min before a session) increased the running time as much as threefold whereas it slightly increased the working memory errors and had no effect on reference memory errors, whatever the pattern may be.

These findings indicate that MK-801 interacts with memory processes more selectively than does scopolamine hydrobromide. Moreover, the use of "difficult" and "easy" baiting patterns may be a sensitive method to analyze amnesic compounds.

416.10

EFFECTS OF BACLOFEN ON LEARNING AND MEMORY IN RATS AS ASSESSED USING A WATER MAZE. R.M. Wylie*, G.J. Kant and N.G. Bowery[1]. Dept. Med. Neurosci., Walter Reed Army Inst. of Res., Washington DC 20307-5100 & [1]Dept. Pharmacol., School of Pharmacy, Univ. of London, London WC1N 1AX.

We evaluated the effects of the $GABA_B$ agonist baclofen on learning and performance of rats in a water maze. Once the rats achieved criterion, (-)baclofen (3mg/kg) was injected i.p. in half of the rats 15 minutes prior to testing on each of 3 consecutive days. The treated rats took significantly longer to complete the maze. In two additional studies, we gave the rats daily injections of (±)baclofen (6mg/kg) prior to training them on new configurations of the maze. We found no significant difference between the treated and control rats on the number of trials required to reach criterion but did find that after reaching criterion the baclofen-treated rats required significantly longer times to complete the maze. When no drug was administered, the performance of the two groups was not significantly different. These results suggest that baclofen impairs performance but does not affect learning.

416.11

THE EFFECTS OF FG 7142 ON PREFRONTAL CORTICAL DOPAMINE AND SPATIAL WORKING MEMORY IN RAT AND MONKEY. BL Murphy*, RH Roth, and AFT Arnsten. Yale Med.Sch, Depts. Pharmacol. & Neurobiol., New Haven, CT 06510

The benzodiazepine inverse agonist FG 7142 (20 mg/kg; i.p.) has been shown to selectively increase the DOPAC/DA ratio in the prefrontal cortex (PFC), but the consequences of this treatment on the working memory functions of the PFC have not been examined. The present study tested the effects of FG on spatial working memory in the rat (delayed alternation in a T-maze) and in the monkey (delayed response in a WGTA). In rats, FG (20 mg/kg; i.p.) significantly impaired delayed alternation performance. This impairment was reversed by pretreatment with the benzodiazepine antagonist RO15-1788 (20 mg/kg; i.p.). Consistent with the hypothesis that FG impairs delayed alternation by increasing DA release, low doses of the DA antagonist, haloperidol (0.1-0.2 mg/kg; i.p.) were able to ameliorate the FG-induced delayed alternation deficit. The glycine partial agonist, (+) HA-966 can act as an antagonist at the glycine site of the NMDA receptor. Pretreatment with (+) HA (20 mg/kg; i.p.), attenuates the effect of FG on measures of DA turnover and on delayed alternation. Similar responses were observed in monkeys: FG (0.2 mg/kg; i.m.) impaired delayed response performance, and this impairment could be reversed by haloperidol (0.005mg/kg; i.m.) or the D1 antagonist SCH 23390 (0.0065 mg/kg; i.m.). Preliminary results indicate that pretreatment with (+)HA may attenuate FG-induced impairment. These results suggest that excessive DA release in the PFC impairs spatial working memory performance. (Supported by grants NSF:GER-9253954; MH 44866: and MH 14092).

416.12

SPERMIDINE POTENTIATES DIZOCILPINE-INDUCED LEARNING IMPAIRMENT OF RATS IN A 14-UNIT T-MAZE TO DEMONSTRATE POLYAMINE MODULATION OF NMDA RECEPTOR. A. Shimada*, E. L. Spangler, E. D. London, and D. K. Ingram. Gerontology Research Center, NIA, and Addiction Research Center, NIDA, NIH, Baltimore, MD 21224

The NMDA receptor, a ligand-gated ion channel complex has been reported to be involved in memory processes. Learning is impaired following administration of dizocilpine (DIZO), a non-competitive antagonist of the NMDA receptor. Polyamines, such as spermine and spermidine, interact with the NMDA receptor to enhance binding of DIZO, which blocks the ion channel. The present study assessed action of polyamines as modulators of learning via NMDA receptor activation. DIZO (0.05 mg/kg) was given i.p. before maze learning, at a dose that produced a slight, nonsignificant impairment of maze learning. Pretreatment with 80 mg/kg but not 15 or 40 mg/kg spermidine (SPD) (i.p.) before DIZO impaired maze learning compared to saline controls. Administration of 80 mg/kg SPD without DIZO did not impair maze learning. The results are consistent with the view that systemic injection of a polyamine can modulate learning processes involving the NMDA receptor. Studies are ongoing under the hypothesis that systemically injected SPD may ameliorate impaired maze learning in aged rats by activating the NMDA receptor.

416.13

ANTEROGRADE AMNESTIC AND ANTICONVULSANT EFFECTS OF TWO TYPES OF NMDA RECEPTOR ANTAGONISTS: MK-801 AND HA-966. X. Xu*, P. Klinger, and R. Davis. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48104-1687.

The anterograde amnestic effects of noncompetitive NMDA antagonists MK-801 and HA-966 on classic fear conditioning in goldfish (*Carassius auratus*) were examined in a series of experiments. Experiments 1 and 2 contrasted the anterograde amnestic effects of MK-801, (+)HA-966, and (-)HA-966. Experiments 3 and 4 investigated whether the potency of MK-801, (+)HA-966 or (-)HA-966 in blocking NMDA induced convulsions paralleled their potency in producing amnesia. The results showed that MK-801 was more potent than (+)HA-966 in producing anterograde amnesia, while (-)HA-966 did not produce anterograde amnesia. The anticonvulsant potency of MK-801, (+)HA-966, and (-)HA-966 paralleled their amnestic potency. These findings suggested that MK-801 and (+)HA-966 produced anterograde amnesia by their antagonism of NMDA receptor complex specifically.

416.14

SELECTIVE ENHANCEMENT OF AMPA RECEPTORS IMPROVES NEOCORTICAL LEARNING. R. Granger*, C. Mangione, D. Anghesom, A. Lopez, M. Davis, B. Tran, G. Rogers & G. Lynch. CNLM, Univ. of Calif., Irvine, CA 92717.

In a novel neocortically dependent auditory task, the first pharmacological agents to specifically enhance glutamatergic receptor action in the brain ("AMPAKINEs") were tested for their effects on learning. AMPAKINEs selectively enhance the glutamate receptor, which mediates all normal fast excitatory transmission, and is the likely site of synaptic long-term potentiation (LTP), the leading candidate substrate for learning. In the first reported tests of behavioral effects of these drugs, Staubli et al., *PNAS*, 91: 777 (1994) and Granger et al., *Synapse*, 15: 326 (1993) showed that they enhanced learning in a radial arm maze and an olfactory discrimination task, which are hippocampally and paleocortically dependent, respectively. We report here on a novel auditory discrimination task, and show that performance on this task is improved by AMPAKINEs. The task consists of a maze in which each arm has a high-frequency speaker. Only two speakers are active on any given trial. Each speaker continuously plays a complex natural sound, recorded and then shifted up into the rat's hearing range (10KHz and above). On each trial, the rat receives a water reward for approaching the "correct" one of the two sounds, whose spatial location changes with each trial. Rats learn many such sounds rapidly, and exhibit weeks-long retention of learned sounds. Selective lesions of primary auditory neocortex severely impairs learning of new sounds. In an experiment counterbalanced for sound preference, learning and retention, and in which animals serve as their own controls for drug effects, rats were tested on very difficult sound discriminations, and given a small number of training trials. On days when they were not given the drug, animals were unable to learn the sounds significantly above chance approach levels. On days when they received the drug five minutes before entering the maze, they exhibited a 41% improvement over their non drug performance. This is the first demonstration of enhancement of learning in a neocortically dependent animal model. (Supported by AFOSR F49620-92-J-0307 and ONR N00014-89-J-1255).

416.15

A CRITICAL ROLE FOR THE NMDA RECEPTOR SITE IN COCAINE DRUG CONDITIONING PROCESSES. Ernest N. Damianopoulos* and Robert J. Carey. Psychiatry, SUNY Health Science Center and Research and Development Service-151, VA Medical Center, Syracuse, NY 13210

The role of the NMDA receptors in cocaine conditioning and sensitization was studied in four groups of matched Sprague-Dawley rats. A sub-motoric dose of the NMDA antagonist MK-801 (0.1 mg/kg ip) was employed using a novel dual-compartment Pavlovian drug conditioning paradigm. The animals were placed sequentially in two similar but distinct test environments. In the first compartment, the animals always received a non-drug test (20 min) Upon removal, the animals received either saline, cocaine (10 mg/kg ip), MK-801 or MK-801 plus cocaine depending on group assignment and were then placed immediately into the second compartment (20 min). Every other day over a 12-day period, the animals of each group (n = 6) received 1 non-drug test followed by 1 saline/drug test. Across all drug treatment days and subsequent tests for conditioning, there were no statistical differences between the saline and drug treatment groups in the non-drug test environment on locomotor distance. Thus, non-specific, non-associative response sensitization effects were not observed. Cocaine, however, had a consistent stimulant effect on locomotor behavior in the drug test environment either when administered alone or in combination with MK-801. Following a 1-day and again after a 21-day withdrawal, all animals were administered a non-drug test for conditioning. The results showed that the cocaine induced enhancement of locomotion was conditioned to the exteroceptive cues of the drug-associated environment but this effect was not present in the animals treated with cocaine plus MK-801. While MK-801 blocked cocaine conditioning, MK-801 had no effect on the cocaine locomotor stimulant response.

416.16

PREGNENOLONE SULFATE INFUSION ANTAGONIZES DIZOCILPINE AMNESIA: MEDIATION BY ALLOPREGNANOLONE. D.L. Cheney*, D. Uzunov, E. Costa and A. Guidotti; FGIN, Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, D.C.

Dizocilpine, a non-competitive N-methyl-D-aspartate (NMDA) antagonist (0.1mg/kg,i.p.), disrupted the passive avoidance response in control and adrenalectomized/castrated (ADX/CX) male rats. Infusion of pregnenolone sulfate (20mg/kg,i.v., 5min) antagonized dizocilpine induced amnesia and increased the brain content of pregnenolone, progesterone and allopregnanolone as measured by gas chromatography-mass fragmentography. Similar effects were observed with CPPene, a competitive NMDA antagonist (2.5mg/kg,i.p.). Five to ten fold increases in allopregnanolone were observed in olfactory bulb, striatum, hippocampus and cortex following infusion of pregnenolone sulfate. Pretreatment with the 5α-reductase inhibitor SKF105111 (5mg/kg,i.v.) blocked the pregnenolone sulfate antagonism of dizocilpine amnesia and prevented the increase of allopregnanolone in olfactory bulb, striatum and cortex. These results support the hypothesis that the amnesia elicited by the NMDA antagonist dizocilpine is counteracted by pregnenolone sulfate via its ability to increase the brain content of allopregnanolone.

416.17

EFFECTS OF PHENYTOIN ON LEARNED APPETITIVE RESPONSES AND THE SUBSEQUENT ACQUISITION OF ESCAPE AND AVOIDANCE RESPONSES. N.L. Mohr[1], J.E. Steinmetz[1,2*], and P.E. Garraghty[1,2]. [1]Dept. of Psych. and [2]Prog. in Neural Sci., Indiana Univ., Bloomington, IN 47405.

Phenytoin (Dilantin) has been used for the control of epileptic seizures for more than 55 years. This drug has proven to be an effective antileptic, but it may also be associated with deficits in behavior and cognition. Here, we have evaluated the effects of instituting chronic phenytoin maintenance in rats that had undergone initial training in an appetitive instrumental task with a subsequent switch to an aversive (escape/avoidance) task. We intially trained deprived rats to produce a bar press in the presence of a tone stimulus for a food reward. We then began treating the rats daily with phenytoin (solution, Parke-Davis). Our goals were to determine the extent to which the animals' behavior in the appetitive task was affected by the intstitution of phenytoin treatment, and how the acquistion of the new escape/avoidance responses were affected. Our results suggest three findings relative to control observations from drug-free rats: 1) a reduction in response latency in the appetitive phase of the experiment after the initiation of drug treatment; 2) a prolonged latency (over session days) in the acquisition of the initial escape response after the animals are switched to the aversive task; and 3) a failure to acquire the avoidance response. These results, in sum, suggest that phenytoin has substantial, and negative (at least for findings 2 and 3) consequences for learning in subjects chronically maintained on the drug, perhaps because of its antagonistic effects on NMDA receptors.

LEARNING AND MEMORY: PHARMACOLOGY—OTHERS I

417.1

ACUTE DIAZEPAM ADMINISTRATION PRODUCES MEMORY DEFICITS SIMILAR TO THOSE DUE TO CHRONIC ALCOHOL CONSUMPTION. N. Borde, R. Jaffard and D. Béracochéa*. Lab. Neurosciences Comportementales et Cognitives, CNRS URA 339, Univ. Bordeaux I, avenue des Facultés 33405 Talence Cedex France.

The effects of the benzodiazepine agonist (Diazepam) on delayed alternation in a T-maze in mice were studied. Delay intervals (DI) separating the acquisition trials from the retention trial were either 30 sec or 6 hours. The 6 hours trial was further used as an acquisition trial for a subsequent alternation trial (DI: 30 sec) aimed at measuring short-term memory as well as the eventual sedative effects of the drug. Diazepam (1.0 or 1.5 mg/kg) was administered i.p. 30 mn before the 6 hr retention trial. Results showed that the 1.5 but not the 1.0 mg/kg dose induced a memory deficit, as compared to saline treated subjects. No impairments were observed on the short-term trial. Furthermore, the Diazepam-induced memory impairment was reversed by a context-change occuring before the retention trial even though this finding requqires further experiments to be firmly established. The effects of Diazepam on memory appear to be similar to those resulting from a long-term ethanol administration and which appear to result from an impairment of the retrieval phase of memory processes.

Supported by CNRS URA 339.

417.2

ANATOMICAL AND TEMPORAL SPECIFICITY OF CHLORDIAZEPOXIDE- AND LIDOCAINE-INDUCED SPATIAL MEMORY IMPAIRMENTS. R.W. Stackman* and T.J. Walsh. Dept. Psychology, Rutgers Univer., New Brunswick, NJ 08903.

The functional activity of cholinergic neurons in the medial septum (MS) that project to the hippocampus is modulated by an intrinsic GABA-benzodiazepine (BDZ) mechanism. Intraseptal injection of the BDZ, chlordiazepoxide (CDP) to rats (i) impairs working memory (WM), (ii) decreases hippocampal high-affinity choline transport, and (iii) decreases dentate gyrus extracellular responses. Further, intraseptal flumazenil, a BDZ antagonist, reverses systemic CDP-dependent amnesia (Stackman & Walsh, 1992; Walsh et al., 1993; Stackman et al., 1993). These data indicate a septal BDZ substrate capable of impairing WM and modulating cholinergic activity in the HPC, is likely the site of action of the amnestic action of CDP. Experiment 1 examined the contribution of neural regions proximal to the septohippocampal network, to the expression of CDP-induced amnesia. Infusion of 30 nmoles of CDP into the MS impaired WM in a delayed non-match-to-sample (DNMTS) radial-arm maze (RAM) task. This dose of CDP failed to alter RAM performance when infused unilaterally into the cingulate gyrus, or bilaterally into the lateral septum, or the nucleus basalis. Infusion of the local anesthetic, lidocaine (147 nmoles) into these sites produced similar behavioral effects. Experiment 2 examined the time-dependent nature of intraseptal CDP-induced amnesia. Rats were trained in the DNMTS RAM task and then injected with 30 nmoles CDP into the MS at one of the following posttraining times: 0, 15, 30, 45, or 60 mins. Rats infused with CDP immediately posttraining exhibited WM impairment. Infusion of CDP at the other posttraining times failed to significantly impair performance. These data indicate that MS is the site of action for CDP-induced amnesia, and this structure is critically involved in an early encoding or maintenance phase of spatial WM. Supported by NSF Grant BNS 9222097 to TJW.

417.3

CHRONIC BENZODIAZEPINE (BZ) TREATMENT FACILITATES SPATIAL LEARNING IN THE MORRIS WATER MAZE (MWM).
M.S. Mohamed[†*], W.S. Messer[‡] and E.I. Tietz[†]. [‡]Dept. of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, 43606, and [†]Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699.

Acute BZ treatment, which potentiates GABA inhibition interferes with long-term potentiation (LTP) and impairs spatial learning. Reducing GABA inhibition in hippocampal CA1 region facilitates LTP. Chronic flurazepam (FZP) treatment reduces GABA inhibition in the CA1 region *in vitro*. We hypothesized facilitation of LTP in hippocampal slices from chronic FZP-treated rats. However, in a previous study using 10 theta bursts, LTP was induced to a similar degree in FZP-treated and control slices but was not maintained in FZP-treated slices. Since LTP mediates spatial learning we evaluated the effect of 1 week FZP treatment (100 mg/kg x 3 days; 150 mg/kg x 4 days, p.o in .02% saccharine) in the MWM. Treated rats were offered saccharine H_2O for 2 days before and after FZP treatment. Control rats received saccharine H_2O. Rats were adapted to the maze by allowing them to swim freely for 120 s over 2 days. Two days after FZP-treatment, we tested each rat's ability to find a hidden platform. Rats were trained 4 trials/day/4 days and tracked with a video camera connected to a computer. Latency to escape(s), path length (cm), and speed (cm/s) were averaged. Speed was constant for each group ($p=.77$) indicating no motor effect due to FZP treatment. The latency of FZP-treated rats to find the platform was less than controls (treatment effect, $p=.043$) suggesting that reduced GABA inhibition associated with chronic BZ treatment facilitates spatial learning, consistent with our previous hypothesis. Future studies will explore LTP in chronic FZP-treated rats using a 5 theta burst paradigm. Supported by NS01493 to W.S.M. and NIDA grants RO1-DA04075 and RSDA K02-DA00180 to E.I.T.

417.4

THE BENZODIAZEPINE DIAZEPAM DISRUPTS THE RECENCY EFFECT BUT NOT THE PRIMACY EFFECT IN RHESUS MONKEYS TRAINED ON A SERIAL PROBE RECOGNITION TASK.
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The effects of diazepam (0.2, 0.8, 1.6 and 3.2 mg/kg) on the primacy and recency memory effects were evaluated in four rhesus monkeys using a serial probe recognition (SPR) task. Only the highest dose of diazepam (3.2 mg/kg) significantly disrupted the monkeys' performance on the SPR task, causing an increase in the number of errors and an increase in the response latencies on the probe trials. Analyses of the serial position curves for each monkey further revealed that the highest dose (3.2 mg/kg) of diazepam disrupted both the recency effect and the middle portion of the serial position curves in all the monkeys tested. In contrast, the primacy effect was relatively unaffected by any of the doses of diazepam tested. Previous reports have suggested that the primacy effect is mediated by the establishment of contextual or configural associations and the recency effect is mediated by the establishment of simple associations. Therefore, we have proposed that diazepam interferes with the simple associative processes involved in the recency effect to a much greater extent than it does with the contextual or configural associative processes involved in the primacy effect. This is the first demonstration showing that diazepam can produce such selective memory impairments in nonhuman primates.

417.5

EFFECTS OF CONVULSANT AND ANTICONVULSANT AGENTS ON MEMORY IN SQUIRREL MONKEYS.
J.M. Moerschbaecher* and E.D. Pakarinen, Department of Pharmacology, LSU Medical Center, New Orleans, LA 70112

It has been reported that subconvulsive doses of convulsant agents such as strychnine and pentylentetrazole may enhance memory in rodents studied under various behavioral procedures. The present study was designed to determine if similar results might obtain in monkeys. Responding by squirrel monkeys was maintained by food presentation under a repeated acquisition of behavioral chains procedure. Monkeys acquired a different three-response chain each session. Sequence completions were reinforced under a fixed-ratio 5 schedule (FR 5) and errors produced a brief timeout. After the monkey reached a predetermined acquisition criterion, the session was stopped and either a 90 min or 24 hr delay was interposed. Following the delay the subject was retested on the same discrimination and retention quantified as percent savings. When administered immediately after the monkey reached the acquisition criterion strychnine and pentylenetetrazole had no effect on percent savings under the 24 hr delay. Similarly, the delta opioid agonist, BW373U86[(±)-4-((R)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-N,N-diethylbenzamidedihydrochloride], had little or no effect on percent savings following either 90 min or 24 hr delays. This was true even at doses of BW373U86 which produced convulsions. In contrast, at high doses triazolam decreased percent savings following a 24 hour delay. These results suggest that at subconvulsive doses convulsant agents have little or no effect while anticonvulsant agents such as triazolam can disrupt memory processes in squirrel monkeys. (Supported by USPHS Grants DA03573 and DA04775.)

417.6

LATENT INHIBITION AND PARTIAL REINFORCEMENT DO NOT RETARD THE DEVELOPMENT OF CONTINGENT TOLERANCE TO THE ANTICONVULSANT EFFECTS OF DIAZEPAM.
T.E. Kippin*, L.E. Kalynchuk, T.J. Kornecook, & J.P.J. Pinel. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C. (Canada) V6T 1Z4.

Contingent drug tolerance is a widely reported phenomena. However, the principles by which contingent tolerance develops are not well understood. Unlike other forms of drug tolerance, contingent tolerance does not appear to be under the influence of Pavlovian conditioning. The present study investigates the role that latent inhibition and partial reinforcement play in the development of contingent tolerance to the anticonvulsant effects of diazepam (DZ). In experiment 1, kindled rats were injected with either 5mg/kg of DZ or vehicle without convulsive stimulation, three times daily, for ten days. Forty-eight hours following the last injection, both groups were switched to a DZ before stimulation schedule to assess the development of contingent tolerance to the anticonvulsant effects of 2mg/kg of DZ. Neither group displayed significant tolerance on the first trial, however, both groups developed contingent tolerance within 15 trials. Prior DZ exposure alone, not only, failed to retard, but facilitated the development of contingent tolerance. In the second experiment, kindled rats were exposed to a schedule which alternated between DZ injection followed by convulsive stimulation and DZ or vehicle injection followed by handling. Partial pairing of DZ injections with convulsive stimulation (reinforcement) failed to retard the development of contingent tolerance to the anticonvulsant effects of 2.5 mg/kg of DZ. Further, the extinction of tolerance in the two groups did not differ significantly. The results of these two experiments suggests that contingent tolerance to the anticonvulsant effects of DZ is not under the influence of latent inhibition or partial reinforcement.

417.7

EFFECTS OF INFUSIONS OF CHLORDIAZEPOXIDE AND ß-CCM INTO THE BASAL FOREBRAIN ON SUSTAINED ATTENTION.
L.A. Holley*, C. Apple, J. Turchi and M. Sarter. Dept. Psychology and Neuroscience Program, Ohio State Univ., Columbus, OH 43210.

Using a recently developed, valid task for the measurement of sustained attention in rats (Bushnell et al. 1994; McGaughy & Sarter 1994), the systemic administration of the benzodiazepine receptor (BZR) agonist chlordiazepoxide (CDP) was found to potently and dose-dependently decrease the relative number of hits but not of correct rejections. Furthermore, the effects of CDP interacted with signal salience. As expected in intact animals, BZR inverse agonists failed to facilitate performance. Here we show that intracranial infusions of CDP (20, 40 µg/0.5µl/hemisphere) or ß-CCM (1.5, 3.0 µg/0.5 µl/hemisphere) into the substantia innominata of the basal forebrain reproduce the effects of the systemic administration of these compounds. Moreover, the effects of intrabasalis CDP were more efficacious in decreasing the relative number of hits than following systemic administration. In contrast to the effects of systemic administration, these potent effects of intrabasalis CDP on vigilance performance were not associated with equally potent effects on the number of errors of omission. These data support the hypotheses that the basal forebrain represents a sufficient anatomical substrate mediating the attentional effects of BZR agonists. Also, these findings parallel the effects of systemic and intrabasalis administrations of BZR ligands on cortical acetylcholine efflux (Moore, Sarter & Bruno 1993, 1994).

417.8

A NOVEL BENZODIAZEPINE INVERSE AGONIST, S-8510, AS A COGNITIVE ENHANCER.
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Benzodiazepine (BDZ) inverse agonists can be therapeutic drugs for dementia including Alzheimer's disease (TINS 11 13, 1988). We showed that some BDZ inverse agonists ameliorate amnesia produced by several procedures in animal models (Prog. Neuro-Psychopharmacol.Biol.Psychiat. 12 951, 1988). At high doses, however, the inverse agonists induce adverse actions such as proconvulsant and anxiogenic effects. Here we report that a novel BDZ inverse agonist, S-8510, promotes anti-amnesic effects without producing adverse actions. S-8510 ameliorated amnesia produced by scopolamine or BDZ in the rat water maze and passive avoidance paradigms. S-8510 did not induce any sign of anxiety and convulsion by itself even at doses 10-100 times higher than those effective for anti-amnesic tests. S-8510 increased extracellular levels of acetylcholine and noradrenaline in the hippocampus and cerebral cortex. It also augumented long-term potentiation induced by tetanic stimulation of Schaffer-collateral /commisural fiber-CA1 synapses. Dose-response relationships of these actions are finely graded, their slope being much less steep than that of a convulsant, pentylenetetrazol. Moreover, S-8510 shared some pharmacological feature with antidepressants. It is concluded that S-8510 is a nootropic drug with different mechanism of action from other previouly reported nootropics.

417.9

MEMORY ENHANCEMENT WITH THE DELTA OPIOID RECEPTOR ANTAGONIST NALTRINDOLE IS DEPENDENT ON TRAINING PARAMETERS IN THE CHICK. P.J. Colombo*, S.E. Moran, J.L. Martinez, Jr., E.L. Bennett & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Administration of enkephalins or delta opioid receptor agonists either enhance or impair memory formation and these effects are dependent on treatment and training conditions. We examined whether memory effects can be produced by administration of the delta receptor antagonist naltrindole (NTI). Two-day-old chicks were injected with saline or NTI in the medial hyperstriatum ventrale 5 min before one-trial peck-avoidance training. To give relatively weak training so that either impairment or enhancement could appear, chicks received either of 2 training conditions: (1) The Latent Inhibition (LI) condition involved 4 pretraining exposures to a dry chrome bead followed by one exposure to a bead coated with the chemical aversant methylanthranilate (MeA). (2) No-LI chicks were given one exposure to a bead coated with 1% MeA. Approximately 40-50% of saline injected chicks in each training condition avoid pecking a dry bead at 24 h test. In LI trained chicks, NTI caused a dose dependent increase in avoidance at 24 h test, the highest dose producing significant memory enhancement. In contrast, NTI had no significant effect in the No-LI trained chicks. These findings suggest that memory enhancement resulting from opioid antagonism is dependent on training conditions. Specifically, endogenous opioids may be selectively involved in memory formation for training that involves discrepant information about conditioned stimuli.

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417.10

[LEU]ENKEPHALIN CAUSES MEMORY ENHANCEMENT FOR AVERSIVELY AND APPETITIVELY MOTIVATED TRAINING IN FOUR-DAY-OLD CHICKS. E.L. Bennett*, P.J. Colombo, J.L. Martinez, Jr. & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

We reported previously that administration of [Leu]enkephalin (LEU) in two-day-old chicks impairs memory formation for peck-avoidance (PA) training. The current study examined the effects of LEU administration on memory for both appetitive discrimination (AD) and PA training. Food motivation cannot be used effectively in chicks younger than three days of age, because their behavior is not influenced by ingestion of food. Therefore, the current study examined the effects of LEU on both PA and AD training in four-day-old chicks for the following reasons: (1) to determine if LEU produced similar effects at days 2 and 4 post-hatch; (2) to insure that comparison of the effects of LEU on appetitively and aversively motivated learning used chicks of the same age. Four-day-old chicks were injected with either saline, 0.03, 0.1, or 0.3 mM LEU in the medial hyperstriatum ventrale 5 min before either PA or AD training and tested 24 h later. Administration of each of the doses of LEU significantly enhanced memory for PA training when compared to saline-injected chicks at 24 h test. Similarly, chicks injected with 0.3 mM LEU showed significant memory enhancement for AD training when compared to saline-injected chicks at 24 h test. These results suggest the following: (1) LEU causes similar effects on both aversively and appetitively motivated learning in 4-day-old chicks, and (2), LEU produces different effects on memory formation in 2- and 4-day-old chicks. Supported by DA05492 (PJC), DA04795 (MRR), & DA04195 (JLM).

417.11

MODULATION OF MEMORY FORMATION FOR A ONE-TRIAL PECK AVOIDANCE TASK IN CHICKS GIVEN CENTRAL INJECTIONS OF MU, DELTA, AND KAPPA SELECTIVE OPIOID ANTAGONISTS. D.W. Lee*, E.L. Bennett, & M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA, 94720.

To investigate opioid-mediated memory formation, chicks were injected with opioid antagonists or saline 5 min before training on a one-trial peck avoidance task, then tested 24 h later for retention. A weak concentration (10%) of the aversant liquid methylanthranilate (MeA) was used as the aversive stimulus; 10% MeA results in intermediate retention levels (≈40%) allowing the investigation of both memory impairment and/or enhancement within identical experimental protocols. Several experiments were conducted administering i.c. bilateral injections of either mu, delta, or kappa selective antagonists (CTOP, ICI174-864, norBNI, respectively) or saline directed into the region of the intermediate medial hyperstriatum ventrale (IMHV; the avian homolog of mammalian visual association cortex) or lobus parolfactorius (LPO; homolog of basal ganglia).

Mu antagonist CTOP (.01 nmoles/hemisphere) impaired memory when injected into the LPO only. Delta antagonist ICI enhanced memory in both the IMHV (3.0 nmoles/hemisphere) and LPO (10.0 nmoles/hemisphere) but effect in LPO only approached significance ($p < .10$). Kappa antagonist norBNI impaired and enhanced memory in a dose dependent way when injected into the IMHV (.10 nmoles/hemisphere impaired; 10.0 nmoles/hemisphere enhanced). The same pattern was observed when norBNI was injected into LPO but only impairment by .30 nmoles/hemisphere was found to approach significance ($p < .10$).

Although opioids clearly modulate memory formation in the chick, the direction of their effects (impairment or enhancement) is highly dependent upon 1) dose; 2) receptor type (mu, delta, or kappa); and 3) brain location (IMHV or LPO).

Supported by NIDA grants DA05396 (DWL) and DA04795 (MRR).

417.12

THE MU ANTAGONIST CTOP ENHANCES MEMORY FORMATION IN THE 2-DAY-OLD CHICK. M.A. Leissring[1,2], J.C. Huang[1], A.M. Anderson, M.R Rosenzweig[1], E.L. Bennett[1] and J.S. Williston*[2]. [1]Department of Psychology University of California, Berkeley, CA 94720. [2]Department of Biology, San Francisc State University, San Francisco, CA 94132.

Leissring *et al.* (1993) reported that the mu agonist DAGO (0.01 mM) inhibits learning and/or memory formation in 2-day-old chicks. To extend thesefindings, we investigated the effects of the mu antagonist CTOP in two successive experiments, the first, designed to determine a dose-response function for CTOP; and the second, to evaluate if the inhibitory effect of 0.01 mM DAGO found previously could be attenuated when administered in a cocktail with various doses of CTOP. Both experiments employ the 1-trial peck-avoidance task developed by Cherkin (1969). In this task, chicks are presented for 10s with a stainless steel bead dipped in methylanthranilate, a liquid aversant. At test, animals are presented with a similar but dry bead. Chicks avoiding the test bead are recorded as having remembered the aversive stimulus; those pecking at it, as amnesic. All experimental agents were diluted in chick Ringer's solution (RING) and administered 5 min before training, via bilateral, i.c. injections (10 μL/hemisphere) into the region of the intermediate medial hyperstriatum ventrale. Animals were tested 24h posttraining.

In the dose-response function, groups of chicks were administered either RING or various concentrations of CTOP (0.001, 0.01, or 0.1 mM). Groups injected with either 0.01 mM or 0.1 mM CTOP demonstrated significant enhancement relative to RING-injected controls (N = 59-64 total chicks per group across 3 replications).

In the second experiment, groups of chicks were injected with either RING alone, DAGO(0.01mM), CTOP(0.01mM), CTOP(0.1mM), or cocktails of DAGO(0.01mM) in combination with either CTOP(0.01mM) or CTOP(0.1mM). This experiment replicated the results of the first experiment for groups given 0.1 mM CTOP, which exhibited significantly enhanced retention relative to controls; however, this experiment did not replicate those for 0.01mM CTOP. Animals administered 0.01mM DAGO exhibited significantly impaired retention relative to controls, replicating the findings obtained by Leissring *et al.*(1993). In contrast, animals administered 0.01 DAGO combined in a cocktail with 0.1 mM CTOP exhibited retention levels which did not differ significantly from RING-injected controls, i.e., co-administration of the two agents cancelled the effects of each agent administered alone. These results provide evidence that the effects of DAGO and CTOP on memory formation are mediated via their actions on the mu-opioid receptor. (N = 57-64 total animals per group across three replications.) (Supported by NIDA grant DA04795)

417.13

NALOXONE INDUCED OPIOID WITHDRAWAL SIGNS IN RATS FOLLOWING EXPOSURE TO A CONTEXT ASSOCIATED WITH SHOCK. G.C. Abrahamsen, B.J. Caldarone, D.L. Mongeluzi, H.S. Stock, and R.A. Rosellini*, State University of New York: University at Albany.

Work in our laboratory has recently shown that acute opioid withdrawal elicited by naloxone is enhanced when morphine is administered in the presence of shock associated cues. To further explore the nature of these effects two experiments were conducted to examine whether naloxone could elicit signs of opioid withdrawal in animals exposed to a context associated with shock even in the absence of morphine. Experiment 1, utilizing a high (10 mg/kg) dose of naloxone, revealed that forepaw tremors but no other classic opioid withdrawal sign could be induced by naloxone following exposure to a context associated with shock. Experiment 2 further examined this effect across a range of naloxone doses (0, 1, 5, 10 mg/kg). This experiment revealed that the emergence of forepaw tremors following exposure to a shock associated context is dependent upon a high (10 mg/kg) dose of naloxone. Furthermore, other signs of withdrawal such as mastication and teeth chattering were most evident at lower doses of naloxone. These findings are consistent with other studies that have shown that naloxone can induce signs of opioid withdrawal following the exposure to physical stressors.

417.14

NALOXONE AUGMENTS PAVLOVIAN CONDITIONING OF CONCOMITANT HEART RATE AND MEDIAL PREFRONTAL CORTEX NEURONAL RESPONSES. L.L. Hernández*, K.L. Watson and C.M. Gibbs. VA Medical Center and U South Carolina, Columbia, SC 29201.

Low doses of naloxone-HCl (NX) augment Pavlovian cardiac conditioning and delay its extinction in rabbits. Since the medial prefrontal cortex (PFCm) participates in cardiac conditioning, we assessed whether NX (0.1 or 0.5 mg/kg, i.v.) has parallel influences on conditioned heart rate responses (HR CRs) and concomitant, prefrontal multi-unit activity (MUA) during differential Pavlovian training (one tone [CS+] paired with shock, another [CS-] presented alone). Prior to training, each NX dose reduced the magnitude of HR orienting responses to the tones but did not alter tone-evoked increases in PFCm MUA, compared to saline. During conditioning, 0.5 mg/kg NX increased the overall magnitude of HR CRs, while 0.1 mg/kg NX reduced HR CR magnitude; nonetheless, each dose enhanced HR discrimination during training and extinction. Unit CRs consisted of evoked activity increases that grew larger during training and, in saline-treated animals, were of greater magnitude to the CS+ vs. CS-. NX augmented MUA discrimination during training and extinction, but the higher dose increased, while the lower decreased, the size of the MUA CR to the CS+. These data show that NX has similar effects on CS-evoked changes in HR and PFCm MUA during Pavlovian training and extinction, and suggest that endogenous opioid systems in or afferent to the PFCm modulate Pavlovian discrimination.

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417.15

GLUCOSE INFUSIONS INTO THE SEPTUM BLOCK THE EFFECTS OF INTRASEPTAL MORPHINE INJECTIONS ON HIPPOCAMPAL ACETYLCHOLINE OUTPUT AND MEMORY. M.E. Ragozzino* & P.E. Gold. Dept. of Psychology, U. of Virginia, Charlottesville, VA 22903.

Recent findings suggest that a modest increase in circulating glucose levels directly modulates brain mechanisms involved in mnemonic processing. Glucose infusions into the septal area reverse amnesia induced by intraseptal morphine infusions. Amnesia following intraseptal morphine infusions may be due, in part, to opioid inhibition of cholinergic neurons with glucose reducing this effect. The present experiment determined whether glucose reverses the effects of intraseptal morphine injections on acetylcholine (ACh) release in the hippocampal formation and on memory. Samples of extracellular ACh were assessed at 12-min intervals using *in vivo* microdialysis with HPLC-EC. From 20-40 min after intraseptal morphine injections (4.0 nmol), ACh output was reduced by 25% compared to baseline levels. Concomitant treatment with glucose (18.3 nmol) blocked this effect, with ACh output similar to baseline rates. Glucose and CSF injections alone did not change ACh output compared to baseline. Two days after microdialysis testing, rats received septal infusions 25 min prior to spontaneous alternation testing. Intraseptal morphine infusions reduced alternation scores which were reversed by concurrent glucose infusions. The effects of intraseptal infusions on alternation performance and ACh output were significantly correlated. These findings suggest that glucose may ameliorate memory deficits produced by intraseptal morphine infusions by directly or indirectly blocking the opioid inhibition of cholinergic neurons. (Supported by NSF (BNS-9012239), NIA (AG 07648) and ONR (NOOO1489-J-1216).

417.16

MEMORY FACILITATION INDUCED BY SYSTEMIC AND INTRA-AMYGDALA APPLICATION OF CCK-FRAGMENTS

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Cholecystokinin (CCK) exists in several molecular forms in the CNS: while CCK-8S has a high affinity to A-receptors (in CNS and periphery) and central B-receptors, CCK-4 binds to the B-receptor. Involvement of CCK in memory processes has been suggested, while the critical receptor subtype has not yet been determined. We investigated memory modulating effects of CCK-8S and Boc-CCK-4 after post-trial systemic (IP) injection using habituation (rearing behavior in an open field; nose-poke in a hole-board) as a learning task. An inhibitory avoidance task was used to test the influence of both fragments after post-trial, unilateral injection into the amygdala. IP injection of both CCK-fragments immediately after training reduced exploratory activity, indicating enhanced habituation, and thus a facilitation of memory. Immediately post-trial injected CCK-8S or Boc-CCK-4 into the amygdala facilitated avoidance learning. Application with a delay of 2.5 or 5h post-trial was not effective, ruling out proactive effects of the treatments on test performance. Using a conditioned place preference task, CCK-8S and Boc-CCK-4 had neither aversive nor reinforcing properties after IP or intra-amygdala injections. The results point to an involvement of the B-receptor in memory processes and show first evidence for post-trial effects of CCK on habituation learning in rats.

417.17

ENHANCEMENT OF MEMORY PROCESSING IN AN INHIBITORY AVOIDANCE AND RADIAL MAZE TASK BY POSTTRAINING INFUSION OF BOMBESIN INTO THE NTS. C.L. Williams* & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717-3800.

Bombesin is a peptide known to modulate memory storage when given either systemically or intraventricularly immediately after training. Two experiments were conducted to determine whether the nucleus of the solitary tract (NTS) mediates the effects of bombesin on memory. In the first experiment male Sprague Dawley rats were trained in an inhibitory avoidance task (0.35 mA, 0.5 s footshock) and bombesin or vehicle was infused unilaterally into the NTS through implanted cannulae immediately after training. Retention was assessed either two or seven days later. Doses of 25 ng or 50 ng of bombesin significantly enhanced retention on the two day test ($p < .05$ and .01 compared with vehicle controls respectively). There were no differences between the drug and control groups on the seven day retention test. In the second experiment, bombesin (25, 50, or 250 ng) or vehicle was infused unilaterally into the NTS immediately after the animals were trained in a win-shift radial arm maze task. On retention tests given 18 hours later, groups that received 25 ng or 50 ng of bombesin made a significantly greater percentage of correct choices on the retention test than did the vehicle-treated controls ($p < .02$ and $p < .05$ respectively). The findings indicating that bombesin influences retention by activating the NTS is consistent with recent evidence suggesting that the NTS is involved in regulating memory storage.

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417.18

COMPARISON BETWEEN TWO RAT STRAINS IN AN OBJECT EXPLORATION TEST: EFFECT OF A TRH ANALOGUE, RGH2202.

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Thyrotrophin-releasing hormone (TRH) produces a positive effect on cognition in Alzheimer's patients (Molchan et al, 1992) and a TRH analogue, RX77368, has been shown to improve performance in senescent rats in a passive avoidance task (Watson et al, 1993), which involves an aversive procedure. Object Exploration Tests (OET) for memory are based on knowledge that a rat will explore spontaneously a new environment and precludes artificial motivation of the animal.

In the present study, we used an OET protocol which has been suggested to produce delay-dependent memory disruption (Ennaceur & Delacour, 1989). Two consecutive exploration trials, T1 and T2 (t=3min) were separated by an inter-trial interval (ITI) of either 1min, 1h or 24h. During T1, two identical objects were introduced; one was replaced by a novel object in T2. There is a significant decrease in total exploration time (ET) of male Wistar (WR) rats (250-300g) from T1 to T2 following an ITI of 1min ($p<0.01$, Mann Whitney U-test), 1h ($p<0.05$) and 24h ($p<0.05$). Male Lister Hooded (LH) rats (250-300g) show no difference in ET from T1 to T2. WR have a lower ET than LH during T2, which is significant following an ITI of 1min ($p<0.01$, Mann Whitney U-test) and 24h ($p<0.01$), and is close to significance following 1h.

LH significantly explored a novel object more than a familiar object in T2, following an ITI of 1min ($p<0.01$, Wilcoxon Rank Sum Test) and 1h ($p<0.01$). There was no significant difference in exploration of objects following an ITI of 24h. The effect of a TRH analogue, RGH2202 (5.0mgkg^{-1} i.p.) administered 30min prior to testing, at a dose shown to produce positive cognitive effects in other tests (Drago et al, 1991), did not produce a significant difference in time exploring objects following an ITI of 24h. There was no difference in exploration of objects following any ITI by WR, so we were unable to determine the effect of RGH2202 in this strain. Therefore, RGH2202, at this dose, does not produce cognitive-enhancing effects in LH in this test. Further work is required to determine a dose-related effect of RGH2202 to confirm these results.

417.19

NEUROTENSIN RECEPTORS DECREASE AS A FUNCTION OF AGING AND COGNITIVE PERFORMANCE IN THE RAT BRAIN. W. Rowe*, S. Kar, M.J. Meaney & R. Quirion, Depts. of Psychiatry, Neurology and Neurosurgery, Douglas Hospital. Res. Ctr, McGill Univ., Montreal, Canada, H4H 1R3

Cognitive deficits are often associated with an impairment of cholinergic function. However, functional changes in the activity of other neurotransmitter systems such as neurotensin (NT) may also underlie certain cognitive behaviors. High concentrations of NT-like immunoreactivity and receptors are often found associated with areas known to be involved in mnemonic processing as well as in cholinergic neurons. Further, alterations in NT receptor density have been reported to be associated with the amnesia produced by lesions of basal forebrain neurons (Wenk et al., 1989, Behav. Neurosci., *103*: 765-9). Thus, the objectives of the present study were to evaluate if NT binding sites are altered in the aged rat brain and if these alterations are related to cognitive status. Aged (24-25 month old) Long-Evans rats were behaviorally screened using the Morris Water Maze task (3 trials/day for 5 days). Animals were classified as either aged, cognitively impaired (AI) or cognitively unimpaired (AU) based on their relative performances in the task compared to young control (CTL) animals. Decreases in [^{125}I]NT binding were observed in the hippocampal formation (dentate gyrus), entorhinal cortex, septum and hypothalamus as a function of age. Both aged groups also showed significant ($p<0.05$) reductions in NT binding sites compared to CTL in the hippocampal CA3 sub-field, with the AI animals exhibiting the lowest levels. Thus, the alterations in [^{125}I]NT binding sites in the AI animals suggest the involvement NT-ergic systems in age-related cognitive deficits.

In the nucleus accumbens and ventral tegmental area [^{125}I] NT binding sites were decreased as a function of age while binding in the medial forebrain bundle was decreased as a function of age and cognitive status. These decreases in [^{125}I] NT binding may be of particular interest given the purported relationship between NT and dopamine (DA) and considering the role of DA in cognitive processing in aged animals (see Hersi et al., 1994, this meeting).

417.20

DOPAMINE D1 DRUGS MODULATE LEARNING ABILITIES OF AGED MEMORY IMPAIRED RATS. A. I. Hersi[1]*, W. Rowe[1], R. Quirion[1] and P. Gaudreau[2]. [1]Douglas Hospital Res. Ctr. and Dept. of Neurology/Neurosurgery, McGill Univ., Verdun, Que. Canada H4H 1R3, [2]Hopital Notre Dame, Dept. of Neuroend. Université de Montréal, Que. Canada H2L 4K8.

Lately, it has become apparent that besides acetylcholine, a number of other neurotransmitters contribute to learning and memory either directly or via their interaction with the central cholinergic system. For example, spatial mnemonic deficits brought on by pharmacological manipulations such as hippocampal denervation or the blockade of cholinergic receptors are reportedly attenuated by the administration of dopaminergic (DA) drugs. It is also known that DA drugs can facilitate learning in various paradigms in young animals. In the present study, we examined if DA D1 drugs can modulate learning in aged animals suffering from cognitive deficits. 24-25 mo. old Long Evans male rats that were classified as memory impaired on the basis of the Morris Swim Maze task were used. The DA D1 agonist SKF38393 (3.0 mg/kg i.p.) administered 15 min before trial significantly reduced the latency to find the platform in this task. On the other hand, the D1 antagonist SCH23390 (0.05 mg/kg i.p) slightly, but nonsignificantly, exacerbated the mnemonic deficits in these aged animals. Interestingly, receptor autoradiography failed to reveal any differences in [^{3}H] SCH23390 binding (D1 receptors) between young (6mo), aged-memory impaired and aged-memory unimpaired rats in any of the brain areas examined (hippocampus, cortex and striatum). In summary, D1 drugs can modulate spatial memory impairements in aged rats but these deficits do not appear to be directly related to changes in DA D1 receptor levels.

417.21

CHANGES IN BRAIN SOMATOSTATIN CONTENTS IN MEMORY DEFICIENT RATS: COMPARISON WITH CHOLINERGIC MARKER.
N. Matsuoka*, N. Maeda, M. Yamazaki and I. Yamaguchi. Basic Research Group, Tsukuba Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3, Tokodai, Tsukuba, Ibaraki, 300-26 Japan.

To clarify the role of the brain somatostatinergic system in the cognitive function of rats, changes in the mnemonic performance of rats in passive avoidance and water maze tasks and in brain somatostatin (SST) contents were comparatively investigated in young rats subjected to brain cholinergic, somatostatinergic and serotonergic depletion and in aged rats. Lesion of the nucleus basalis magnocelluralis (NBM) and treatment of cysteamine, a SST depletor, resulted in significant memory deficits in passive avoidance task, whereas neither transection of the fimbria-fornix (FF) nor raphe lesion by local injections of 5,7-dihydroxytryptamine affected the performance in the task. When their cognitive performance were assessed in the water maze, NBM-, FF-lesion and cysteamine but not raphe lesion significantly impaired the acquisition of spatial memories. Aged rats showed severe memory impairment in both tasks. Comparisons of the brain neurochemistry showed that NBM lesion produced a selective reduction in the cortical cholinergic marker, choline acetyltransferase (ChAT), and striatal SST level, while FF-lesion caused marked loss of ChAT in the hippocampus and posterior cortex and a significant reduction in hippocampal SST. On the other hand, cysteamine significantly reduced the SST contents in all the brain regions examined but minimally affected ChAT activities. Raphe lesioning hardly affected either somatostatinergic or cholinergic markers in the brain areas. Significant reduction in the striatal ChAT and elevation in SST contents in the frontal cortex were found in aged rats compared with young rats. These results suggest that changes in the brain somatostatinergic and cholinergic transmission are differently involved in the cognitive deficits in experimental animal models of dementia employed.

NEUROETHOLOGY: INVERTEBRATES

418.1

NON-NOXIOUS STIMULATION ELICITS GRADED INKING IN *APLYSIA*.
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Ink provides *Aplysia* with a chemical defense against predators such as sea anemones (Nolen et al, submitted). In interactions with anemones, *Aplysia* release small amounts of ink following contact with a single anemone tentacle and larger amounts if they are picked up by numerous tentacles. Thus, a critical component of the RELEASER for inking may be the area of skin stimulated by the predator.

We used a mechanical stimulus array to simulate the action of anemone tentacles as they snare a snail and pull it off the substratum. An array (1 to 9 1cc syringes) was placed on the parapodium of a small adult *A. californica* (8 - 12cm) and weak suction applied as the animal was lifted off the bottom of its aquarium. The probability of inking increased as a function of the size of the array: Four of 14 animals (28.6%) inked in response to a single stimulator (12.6mm^2); whereas 11 of 16 (68.8%) inked in response to a 3x3 array distributed over 4cm^2 of skin ($p=0.0281$). Ink was released in a graded fashion: Ink scores increased 2.8 times as the stimulus array was increased from 1 to 9 stimulators ($p=0.025$).

Substantial amounts of ink could be released on 4 successive trials from animals with full glands (using anemone tentacles or tail shocks). Animals with low ink stores had high thresholds and long latencies (latency was negatively correlated with the amount of ink released, $r = -0.9841$, $p<0.001$). Still, these animals released only about half (or less) of their gland's stores of ink on trial one [e.g., even with a noxious head shock, only 49.7±13.8% (mean±sem) of the gland's ink was released on the first trial; 6 to 12% was released on successive trials.]

Stereomicroscopic analysis of the gland during successive inking trials suggests that individual ink vesicles are stochastically activated and that ink secretion from vesicles is not all-or-none. The stochasticity of ink vesicle activation can be explained by branch-point conduction failures in the fine peripheral projections of ink motor neurons where they innervate vesicles in the ink gland.

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418.2

NOXIOUS STIMULI INDUCE LONG-TERM CHANGES IN CARDIOVASCULAR FUNCTION IN APLYSIA CALIFORNICA
J.K. Krontiris-Litowitz*. Dept. of Biological Sciences, Youngstown State University, Youngstown, OH 44555.

Cardiovascular function in Aplysia can be altered by environmental factors. Previous studies have shown that temperature, oxygen content, and noxious stimuli can induce changes in heart rate (Dieringer et al., 1978). This study investigates whether or not environmental stimuli, particularly noxious stimuli, can induce long-term changes in cardiovascular function.

Cardiovascular function was monitored in an in vitro preparation via a flow-through pressure cannula implanted in the anterior aorta. Noxious, electric shocks were delivered to the lateral tail via implanted electrodes (3 trains/10 sec duration/60 Hz/15 min intervals).

Tachycardia was observed in all animals 30 min after presentation of the noxious stimulus (n=8). Heart rate was significantly increased when compared to controls that had not received noxious stimuli ($p < .05$). Tachycardia lasted for at least 90 min in 6 animals and for 6 hours in 2 animals in which observation time had been extended. These studies indicate that noxious stimuli can induce long-term changes in cardiovascular function.

418.3

COUPLED NEURONAL OSCILLATORS IN THE MEDICINAL LEECH.
C.G. Hocker* and W.O. Friesen. Center for Biological Timing and Department of Biology, University of Virginia, Charlottesville, VA 22903-2477

A leech usually has five to fifty undulations during one swimming episode though longer swims can be observed. Fictive swims observed in isolated chains of midbody ganglia are typically ten to twenty cycles long in accordance with this episodic behavior. Unlike the isolated lamprey nerve cord which displays continuous fictive swimming in the presence of N-methyl-D-aspartate, no chemical has yet been found (*eg.* serotonin) that can remove the episodic nature of the fictive swims. However when the tail ganglion is included, spontaneous fictive swims occur with episodes an order of magnitude greater in length. The period variability of these long episodes contains a frequency modulation (FM) whose period is around ten cycles.

Since one ganglion in serotonin has a fictive swim oscillation that does not have the FM, we expect that the FM is due either to an oscillation in the excitation from the tail ganglion or to an intrinsic oscillation in the coupling between the swim oscillators. We proceeded to test this in four ways. First, recording in the anterior section from the median connective which carries much of the excitatory input from the tail ganglion does not reveal any periodic spiking of the appropriate period during fictive swimming. Second, warming or cooling the tail relative to the midbody ganglia does not cause a significant shift in the period of the FM. Third, examination of occasional long fictive swims just after removing the tail ganglion from the rest of the cord revealed that the FM still occurs without the tail. Fourth, uncoupling the anterior swim oscillators from the posterior oscillators by severing both lateral connectives between two ganglia causes long episodes to be broken apart into shorter episodes lasting around 10 cycles. We conclude that the FM is an intrinsic part of the intersegmental coupling and is not from the tail ganglion.

418.4

MECHANISMS OF INTERSEGMENTAL COORDINATION IN THE MEDICINAL LEECH.
W.O. Friesen* and C.G. Hocker. Dept. of Biology, NSF Ctr. for Biol. Timing, Univ. of Virginia, Charlottesville, VA 2293-24771.

Our current understanding of the neuronal system that controls swimming in the leech includes three sets of oscillators: 1) a chain of serially homologous segmental oscillators in midbody ganglia that generates and coordinates the swimming rhythm; 2) an unidentified oscillator associated with the head ganglia, which may interfere with swimming activity via reciprocal inhibition; and 3) an unidentified oscillator in the tail ganglion that modulates the cycle period of swimming via slow, excitatory, phasic output to the swim oscillator.

We investigated the nature of the segmental oscillators to determine whether 1) midbody ganglia comprise one or two competent oscillators, 2) ascending or descending connections are strongest, and 3) termination of swimming activity might arise from destructive interference between segmental oscillators with differing cycle periods. Experiments were performed on the leech isolated nerve cord extending from midbody ganglion 2 to the tail ganglion. To separate ascending from descending inputs, we severed one lateral connective anterior to, and the contralateral lateral connective posterior to a midbody ganglion (Z-semi-isolated). We observed relative coordination in this preparation with either end of the chain exhibiting the shorter cycle period. During relative coordination, oscillations in motor neurons on the left and right sides of the Z-semi-isolated ganglion were phase-locked to impulse bursts either in the anterior or posterior chain; in no case did we see independent oscillations on the two sides of this ganglion. We conclude that midbody ganglia contain a single swim oscillator, that ascending and descending intersegmental interactions are nearly equal in strength and that swimming activity in the Z-preparation can be terminated prematurely when anterior and posterior chains have differing cycle periods.

418.5

DISHABITUATION OF ACOUSTIC STARTLE SHOWS FREQUENCY CATEGORIZATION BY CRICKETS. R.A. Wyttenbach* and R.R. Hoy. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Flying Polynesian field crickets (*Teleogryllus oceanicus*) have an acoustic startle response to pulses of ultrasound. This consists of several movements that steer the cricket away from the sound source. One aspect of the response, metathoracic leg swing, is of short latency and duration.

Ultrasound startle decrements with repetition in a way consistent with standard criteria for habituation. It declines exponentially, recovers spontaneously, declines more rapidly with higher repetition rates or lower intensities, and dishabituates after presentation of a novel stimulus.

Dishabituation of acoustic startle can be used as a "same-different" test for crickets. A stimulus that is novel relative to the habituating stimulus will dishabituate, while a stimulus that is not considered different from the habituating stimulus will not dishabituate.

We have used this test to determine which frequencies of sound are considered different from ultrasound. Crickets were habituated with 5 pulses of 20 kHz at 10 dB over threshold (reducing the response to 35% of the initial response) and then presented with a dishabituating pulse followed by a test pulse of 20 kHz as before. All pulses were 750 ms apart. The dishabituating pulse was 10 dB greater than the habituating pulse, ranged from 5 to 25 kHz, and came either from the same side as the habituating pulses or from the opposite side.

Pulses of any frequency dishabituate when presented from the direction opposite the habituating train, but only pulses below 17 kHz dishabituate when presented from the same direction as the habituating train. Thus pulses below 17 kHz are categorized as different from 20 kHz, while those 17 kHz or greater are not distinguished from 20 kHz.

418.6

NEGATIVE THERMOKINESIS DURING FLIGHT IN THE LOCUST. R.M. Robertson* and C.T. Kuhnert. Dept. of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Locusts aggregate in regions with an optimal environmental temperature, yet there is currently no evidence for locomotor thermokinesis in locusts. We have investigated whether tethered flying locusts will steer relative to a radiant heat source.

Adult male *Locusta migratoria* were tethered and flown in front of a wind tunnel. Wing movements and postural adjustments of the abdomen and hindlegs were monitored with high-speed cinematography (250 frames s^{-1}) and with videography (60 frames s^{-1}). In separate experiments, abdominal movements were monitored using a capacitative position transducer. Brooder lamps (250 watt) on the right and left of the animal provided directional heat sources.

Within 1s of turning on a lamp, locusts made characteristic steering manoeuvres away from the direction of the source. Interposing a transparent acrylic barrier (1.2 cm thick) markedly reduced the steering responses. Neither covering the eyes and ocelli with thick nail polish, nor interposing infra-red filters (Edmund Scientific, P60 033) between the locust and the lamp, had an effect on thermokinesis.

We conclude that flying locusts steer away from heat sources by detecting infra-red radiation. We are currently investigating which structures are involved in mediating directional infra-red sensitivity.

418.7

SYNCHRONIES IN THE NORTH AMERICAN FIREFLY PHOTINUS CAROLINUS. J. Copeland and A. Moiseff*. Department of Biology, Georgia Southern University, Statesboro, GA 30460 and Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269-3042.

Photinus carolinus shows discontinuous synchrony. Bursts of 5 - 8 flashes at 2 Hz occur concurrently every 14 - 19 sec. When the terrain is flat and forested, the synchrony is a unison synchrony. When the terrain is a steep forested hill, the synchrony is a wave synchrony. Thus, the same species of firefly is capable of showing unison or wave synchrony depending on the topographical conditions.

We studied unison synchrony by employing low light level videophotography and frame-by-frame playback analysis as well as photometric recordings. The latter was combined with computer acquision and analysis in the field. Data obtained from groups of individually caged males revealed that group flashing began concurrently, ended concurrently, and the flashes within the burst occurred concurrently, resulting in unison synchrony. To investigate the mechanisms responsible for this synchronic behavior, simulated flashes were presented to individual fireflies at various time during their flash pattern. Stimulus flashes presented during the interburst interval enhanced or inhibited the next flash depending on when the stimulus was presented. Stimulus flashes presented during the interflash interval, however, had no noticeable effect.

We suspect that the interburst oscillator can be modulated through anticipatory (unison) or triggered (wave) mechanisms. We also suspect that the interflash oscillator, once released (in the ethological sense) is not suceptible to feedback.

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418.8

VISUAL CUES MAY INFLUENCE THE ESCAPE RESPONSE OF COCKROACHES. S.Ye* and C.M. Comer. Neurosci. Group, Dept. Biol. Sci., Univ. of Illinois, Chicago, IL 60680

Periplaneta americana responds to abrupt touch of an antenna by turning away from the stimulus and running. This response occurs at very short latency, and the angle of initial turn is typically contraversive to the antenna touched. We have noted that vision is not essential for production of escape turns (Comer et al., 1994; J Comp Physiol 174:13). However, visual cues are usually present as touch of antennae occurs. We were therefore interested in knowing if aspects of escape are influenced by vision.

We report here behavior studies on intact animals tethered in a high-speed locomotion tracker, and electrophysiological studies of descending interneurons. When lateral eyes and ocelli were covered with opaque paint, animals turned reliably following antennal touch. But the duration of subsequent running was decreased. In another experiment, sighted animals were tested for response to a stimulus probe placed near an antenna. Animals responded by orienting an antenna toward the probe and touching it if the probe had high visual contrast with background. If the probe had low contrast, contact with the probe was at chance levels. These data indicate that cockroaches visually detect stimuli applied to the antennae, and that vision (while non-essential) may influence escape performance.

Intracellular recording and dye-injection revealed an interneuron, with soma in the brain, that responds to visual stimuli of the type used in behavior tests. In recordings from neck connective, the neuron typically responded by firing a train of spikes as a probe moved across the visual field of the contralateral eye. When antennal touch occurred, the firing of this neuron preceded that of descending mechano sensory interneurons. The cell's axon (15-20 μm in diameter) extends to the thoracic ganglia.

We suggest that while visual cues are not essential for touch-evoked escape, they may influence some elements of the escape response. The role of descending interneurons in such an influence is under investigation.

This research supported by NSF grant IBN-9222619.

418.9

TESTING FOR A POPULATION VECTOR CODE FOR WIND DIRECTION IN THE COCKROACH GIANT INTERNEURONS. R. Levi, J. M. Camhi* Dept. Cell and Animal Biology, Hebrew University, Jerusalem, Israel.

One proposed mechanism by which a neural assembly codes stimulus direction is the population vector code (PVC). Originally observed in the monkey cortex, this is tested here in the much simpler cockroach escape system. Specifically, we test 3 bilateral pairs of giant interneurons (GIs) that respond directionally to wind stimuli and evoke an evasive turn. GI 3 responds most strongly to wind from the ipsilateral front. Such wind evokes large turns away. GI 2 to responds most strongly to wind from the ipsilateral rear. Such winds evoke small turns away. GI 1 responds equally to both these directions (Kolton and Camhi, Soc. Neurosci. Abst., 1993). By a PVC, one represents each cell's response as a vector arrow, whose direction is the cell's best excitatory direction, and whose length is the number of spikes evoked by the given wind stimulus. Vector summation of GIs 1, 2 and 3 gives good agreement (± 20°) between the actual wind direction and that calculated using the PVC.

To help determine whether the cockroach actually uses a PVC, we modified the spike trains of individual GIs during wind-evoked turning behavior and determined the effects on the turn. Wind puffs from 90° right evoke, in a cockroach tethered on a slick surface, turning movements to the left. We analyzed the initial leg movements and body's turning tendency using a high speed video (250 frames/s). During another puff from the same angle, we now added spikes, by intracellular current pulses, to either right GI 3 or 2. Roughly doubling the number of spikes in GI 3 (from ~ 6 to 12) has produced, to date, responses corresponding to larger turns to the left in 7 out of 9 cockroaches, and no change in one other. Adding spikes instead to GI 2 has produced, to date, responses corresponding to smaller turns in 4 out of five cockroaches. These results are consistent with the predictions of a PVC, since increasing the number of spikes lengthens a GI's vector arrow and thus draws the direction of the wind specified by the PVC toward that GI's best excitatory direction.

418.10

SPONTANEOUS SELF-GENERATED COUNTERTURNS DURING TETHERED FLIGHT OF MALE MOTHS, *Manduca sexta*. K Baker[1], MA Willis[2], EA Arbas[2]*. [1]Center for Neurogenic Communication Disorders and [2]ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721

Flight behavior of moths, *Manduca sexta*, is known to be modulated by odors and visual patterns. In plumes of female pheromones, for example, flight tracks of males assume a characteristic upwind zigzagging pattern. Our laboratory is researching the sensori-motor basis of this pattern. We have recorded spontaneous activity patterns of tethered moths in the 1st hour of their circadian activity cycle, without specific olfactory or visual stimulation. Movement patterns were logged visually and videotaped. Synchronous EMG recordings from various wing muscles revealed underlying motor patterns.

Moths typically activated wing fanning (pre-flight warmup), followed by large amplitude wing beating indicative of tethered "flight", within the first 10 min. of scotophase. Flight continued in bouts from a few sec. to 14 min. duration. During flight bouts, moths mostly maintained postures which were consistent with stable forward orientation. Often, however, they showed spontaneous changes in posture during wing beating that we interpret as attempts to turn, including: (1) turning of the head and, (2) curling the abdomen in the direction of the intended turn, as well as (3) retraction of the wings on the inside of the turn, and protraction of those outside the turn. Attempted turns were sometimes executed in rapid alternating succession, reminiscent of counterturning during unrestrained zigzagging flight. These observations suggest a capability to organize counterturning intrinsically, rather than only in response to afferent inputs. Timing of movements and motor outflow patterns underlying these spontaneous "open-loop" maneuvers are being analyzed for comparison with similar patterns activated and modulated by visual and pheromonal stimuli. [Supported by NSF IBN 9216532].

418.11

MODULATION OF MOTOR PATTERNS IN A TETHERED MOTH DURING MULTI-MODAL STIMULATION. DE Wood* and EA Arbas, ARL Div. of Neurobiology, Univ. of Az., Tucson, Az. 85721

We are studying the flight behavior of the male sphinx moth, *Manduca sexta*, as a model for the generation and control of oriented locomotion. Of interest is control of pheromone-evoked 'zig-zagging' flight of males searching for mates. This pattern of flight exhibits a consistent interturn interval as the male progresses upwind. Previous results describing flight activity in this animal used both free flying animals and reduced preparations from which EMGs of flight muscles could be monitored (Willis and Arbas, 1991; Kanzaki and Arbas, 1990). This investigation describes aspects of the control of flight under various multi-modal stimulus arrangements while recording *en passant* neurograms of particular motor nerves. Study of flight initiation shows that short bouts of stereotyped flight are evoked by short (< 1 s) exposures to light. Short bouts of flight do not include temporally regular turns as seen in zig-zagging or casting (flight after odor loss). These short bouts are useful for comparison to more complex flight patterns. Chlordimeform (CDM) induces motor outflows to flight muscles (Kanzaki and Arbas, 1990). Flight patterns evoked by this drug are less stereotyped than flight evoked by brief light stimuli and include patterns indicative of less consistent turning than in unrestrained casting or zig-zagging flight. CDM applied to flying animals accelerated bursting of indirect wing depressor muscles. Visual stimuli simulating left and right turns can initiate flight with regular turning intervals. Visual stimuli combined with pheromonal stimulation elicited frequency modulation of the flight rhythm reminiscent of that observed during free flight. We are continuing this study using more complex computer driven stimulus protocols accompanied by recordings of motor rhythms in the thoracic ganglia. (Supported by NIH NRSA 1 F32 DC 00097-01 and NSF IBN 9216532).

418.12

CAN NEUROGENESIS EXPLAIN MUSHROOM BODY REORGANIZATION IN THE BRAIN OF THE ADULT HONEY BEE? J.L. Strande, S.E. Fahrbach and G.E. Robinson*. Department of Entomology and Neuroscience Program, University of Illinois, Urbana, IL 61801.

A reorganization of the mushroom bodies in the brain of the adult worker honey bee (*Apis mellifera*) is associated with behavioral development and colony division of labor (Withers et al., 1994, Nature 364, 238-240). To probe the proximate mechanisms of this neuroanatomical plasticity, we tested the hypothesis that neurogenesis occurs in the adult bee brain. Bees were exposed to the proliferation marker bromodeoxyuridine (BrdU) at 3 stages of behavioral development: one-day-old, nursing, and foraging. BrdU was administered in 3 ways: *in vivo*, either orally (in sugar syrup) or via injection into the abdomen, or *in vitro*, with brains incubated in bee culture medium containing BrdU. Immunohistochemical experiments completed to date revealed only one BrdU-labeled nucleus of a single mushroom body neuron in 2140 sections of tissue examined. Several controls were processed simultaneously with adult bee brain tissue. Nervous tissue from *Manduca sexta* larvae, in which neurogenesis is known to occur, revealed extensive BrdU labeling. Neurogenesis was also readily detected in nervous system tissue from honey bee larvae and pupae. These results indicate that neurogenesis occurs very infrequently or not all in the adult bee brain. It does not appear that the previously observed mushroom body reorganization can be explained by neurogenesis. *Supported by NSF grant IBN 9211386 and an NSF REU Award (SEF and GER) and NIMH grant MH42274-01 (GER).*

DRUGS OF ABUSE: CNS STIMULANTS—TOXICITY

419.1

MDMA INCREASES THE EXTRACELLULAR CONCENTRATION OF 2,3-DIHYDROXYBENZOIC ACID IN THE STRIATUM: EVIDENCE FOR INCREASED HYDROXYL RADICAL FORMATION. G.A. Gudelsky* and B.K. Yamamoto, Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106

It is well recognized that the administration of 3,4-methylenedioxymethamphetamine (MDMA) results in a long-lasting depletion of brain 5-HT. Evidence is supportive of the view that MDMA-induced depletion of 5-HT is due, in part, to the acute and sustained release of dopamine (DA). The purpose of the present study was to examine whether the MDMA-induced increase in the extracellular concentration of DA in the striatum is accompanied by the formation of hydroxyl free radicals that result from DA autoxidation. Hydroxyl radical formation was assessed in dialysis samples from the striatum by quantifying the extracellular concentration of 2,3-dihydroxybenzoic acid following its conversion from salicylic acid. The dialysis probes were perfused with dialysis buffer containing 5 mM salicylate for 3 hrs at vaious times following drug treatment. The ip injection of MDMA (20 mg/kg) resulted in an immediate increase in the concentration of 2,3-DHBA which was 450% of that in vehicle-treated controls. An MDMA-induced increase in 2,3-DHBA formation was observed for at least 6 hrs. A 30 min application of MDMA (10 uM) through the dialysis probe also increased 2,3-DHBA concentrations which were similar in magnitude to those produced by systemic administration. In rats in which the MDMA-induced increase in DA release was attenuated by treatment with mazindol, the MDMA-induced increase in 2,3-DHBA formation also was significantly attenuated. These findings are consistent with the view that MDMA produces a DA-dependent increase in hydroxyl radical formation.

419.2

POTENTIATION OF MDMA-INDUCED DOPAMINE RELEASE AND SEROTONIN NEUROTOXICITY BY SEROTONIN$_2$ AGONISTS. J.F. Nash*, B.K. Yamamoto and G.A. Gudelsky, Depts. of Psychiatry and Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106

The effect of serotonin (5-HT$_2$) agonists, DOI and 5MeODMT (5-methoxy-N,N-dimethyltryptamine), on MDMA (3,4-methylenedioxy-methamphetamine)-induced release of dopamine (DA) in the striatum was studied in rats by the use of in vivo microdialysis. A concentric dialysis probe was inserted into the striatum and dialysis samples were collected every 30 min for 240 min. MDMA (10 mg/kg, sc) administration increased the release of DA in the striatum. The MDMA-induced increase in the extracellular concentration of DA was enhanced significantly in rats treated with either DOI (2 mg/kg, ip) or 5MeODMT (15 mg/kg, ip). Neither DOI nor 5MeODMT alone affected the extracellular concentration of DA in the striatum. The effect of DOI on the long term depletion of striatal 5-HT produced by MDMA also was investigated. The striatal concentration of 5-HT was reduced slightly, but not significantly, 7 days following the administration of MDMA (10 mg/kg, sc). However, 7 days following the concomitant treatment with DOI and MDMA the striatal concentration of 5-HT was significantly less than that in rats treated with MDMA alone or the vehicle-treated controls. It is concluded that activation of 5-HT$_2$ receptors is an important determinant of the acute increase in extracellular DA and, consequently, the long-term depletion of brain 5-HT produced by high dose administration of MDMA.

419.3

THE STRIATAL NEUROTOXICITY INDUCED IN THE C57BL/6J MOUSE BY D-AMPHETAMINE IS BLOCKED BY RESTRAINT STRESS BUT NOT BY SUPPLEMENTAL CORTICOSTERONE. D.B. Miller* and J. P. O'Callaghan. U.S. EPA, RTP, NC 27711

Amphetamines (AMPs) are potent stimulants and striatal (ST) dopaminergic neurotoxicants. Stress affects AMP stimulation but its effects on AMP neurotoxicity are not well examined. Restraint stress activates the adrenal axis and elevates corticosterone (CORT) levels. Here, we determined if restraint or supplementation with CORT affected d-AMP neurotoxicity. Astrogliosis, quantified by immunoassay of GFA, was taken as an index of neural damage. Body temperature (BT) was also monitored as restraint can cause hypothermia and BT is a factor in AMP neurotoxicity. Female mice (6/group/cage) were given SAL or d-AMP at a dosage (s.c. as the base) of 0 or 5 mg/kg, respectively,every 2 hrs beginning at 11:30 AM for a total of 4 inj. Mice were restrained in 50 ml centrifuge tubes, beginning at 0.5 hr prior to the 1st inj and until 1 hr following the 4th inj. CORT was placed in the drinking water (20μg/ml; ~ 3.2 mg/kg) 1 week prior to d-AMP inj and continued until the ST was obtained at 72 hrs post the last inj. BT was recorded prior to dosing and at 2 hrs following each inj. d-AMP caused hyperthermia (1 - 2 °C) and neural damage as indicated by a large (300%) increases in ST GFA. Restraint totally blocked the d-AMP hyperthermia as well as the GFA elevation. CORT had no effects. The data suggest an elevation in BT is important in d-AMP neurotoxicity and that adrenocortical status may not play a role in AMP neurotoxicity. However, the dosage of CORT utilized was low and did not cause thymic involution. Higher dosages, as well as the elimination of circulating CORT, such as by adrenalectomy, should be investigated.

419.4

D-Amphetamine (AMPH) Levels in Caudate-Putamen (CPU) Microdialysates After Doses that Produce Either Behavioral or Neurotoxic Effects. P. Clausing*, R.R. Holson, W. Slikker Jr., B. Gough and J.F. Bowyer, NCTR/FDA, Jefferson, AR 72079-9502.

Six mo. old ♂ Sprague-Dawley rats (4/group) were dosed with either 1 or 2.5 mg/kg AMPH sc to produce increased motor activity and/or stereotypy. Forty to 60 min. after 1 mg/kg, when motor activity was maximum, AMPH levels in the microdialysate were maximal at (mean ± SEM) 0.28 ± 0.04 μM. Forty to 60 min after 2.5 mg/kg the microdialysate levels rose to a max. of 0.51 ± 0.09 μM. Stereotypic behavior started at this time and lasted for 1-2 hrs. In a second experiment, 6 and 12 mo. old rats (5/group) were given AMPH sc either 4x5 mg/kg in a 24°C environment (known to produce neurotoxicity) or 4x15 mg/kg in a 10°C environment (not neurotoxic) at 2 hr intervals. It was hypothesized that in the cold environment the higher doses would produce higher CPU-extracellular AMPH levels but no long-term neurotoxicity. Peak levels were: 1.5 ± 0.3 μM (6 mo. old, 4x5 mg/kg, 24°C), 2.6 ± 0.7 μM (12 mo. old, 4x5 mg/kg, 24°C), 3.0 ± 0.4 μM (6 mo. old, 4x15 mg/kg, 10°C), 4.1 ± 0.5 μM (12 mo. old, 4x15 mg/kg, 10°C). Thus, 12 mo. old rats had significantly higher AMPH levels than 6 mo. old. Cold room rats dosed with triple the dose had 1.4-2.0 times the AMPH levels seen in rats kept at room temperature. *In vitro* recovery efficiency of the microdialysis probes was estimated to be 15-20%. Thus, approximately 2 μM extracellular AMPH is necessary to produce behavioral effects (assuming levels in the nucleus accumbens are similar to those in the CPU) while levels after neurotoxic doses are between 10 and 30 μM.

419.5

Using Orthophthaldialdehyde (OPA) and 3-Mercaptopropionic Acid (MERA) Derivatization to Quantify D-Amphetamine (AMPH) Levels in Striatal Microdialysates by High Performance Liquid Chromatography (HPLC) J.F. Bowyer* and P. Clausing. NCTR/FDA, Jefferson, AR 72079-9502.

Rats implanted for microdialysis in the striatum were dosed with AMPH (1 to 15 mg/kg, sc). The microdialysate buffer consisted of 140 mM NaCl, 1.5 mM K_2HPO_4, 1.5 mM KCl, 1.5 mM $MgCl_2$, 1.25 mM $CaCl_2$ and 10 mM glucose, pH 7.4. Dialysate was collected continually at 20 min intervals (1 μl/min.) for up to 10 hrs starting at 2 hrs. prior to dosing. The samples were either stored at -70°C or analyzed immediately by HPLC. AMPH levels were determined by derivatizing 10 to 20 μl microdialysate aliquots for 2 min with 20 μl of 0.1 M borate buffer, pH 9.4, containing 5 mg OPA and 40 μl MERA per ml and 70 to 80 μl H_2O. Then 75 μl of the derivatized sample was separated on a C18 column using a 50 mM potassium phosphate buffer, pH 5.5, and a gradient of 35% to 65% methanol with 1.5 ml/min flow rate. Fluorescent detection (340 nm λ excitation, 440 nm λ emission) was used. The AMPH derivative had a retention time of 14.4 min which was 2 min more than all the amino acid derivatives and there were no peaks within ±1.5 min of the AMPH derivative. Very little p-hydroxy-amphetamine was detected in the microdialysate after AMPH sc. Microdialysate buffer spiked with AMPH to obtain 0.1 to 10 μM AMPH concentrations was used to quantify microdialysate AMPH levels. The detection limit was 0.05 μM AMPH in 20 μl of microdialysate, equivalent to 135 picograms. From 0.27 to over 27 nanograms AMPH can be quantified in microdialysate using these methods.

419.6

Nitric Oxide May be a Prominent Factor Involved in the Release of Dopamine (DA) During Methamphetamine (METH) Exposure *In Vivo*. [1]B. Gough*, [1]R.R. Holson, [2]W. Slikker Jr. and [2]J.F. Bowyer. [1]Div. of Repro. & Develop. Toxicol. and [2]Div. of Neurotoxicol., NCTR/FDA, Jefferson, AR 72079-9502

We have previously shown that inclusion of the nitric oxide synthetase (NOS) inhibitor nitro-arginine (NARG) in the microdialysis buffer greatly reduced caudate/putamen (CPU) extracellular DA during exposure to neurotoxic doses of METH (Neurosci. Abs.# 686.10, 1993). We now show that, in rat, inclusion of N^G-Nitro-L-Arginine Methyl (L-NAME, a NOS inhibitor) or D-NAME (less potent NOS inhibitor) in the microdialysis buffer affects CPU extracellular DA levels in a manner suggesting that NO may play a prominent role in the *in vivo* DA release from neurotoxic doses of METH. Neither D- nor L-NAME in microdialysis buffer prior to METH affected the relative CPU extracellular levels of DA, DOPAC or HVA. However, 100 μM L-NAME produced a **64%** decrease in the relative extracellular CPU DA levels during METH exposure to 4 doses of METH (5 mg/kg i.p./2 hrs) while 100 μM D-NAME minimally affected (**18%** decrease) DA levels. At 20 μM L-NAME the relative extracellular DA levels declined **38%** during METH exposure. L-NAME did not affect DOPAC and HVA levels during METH exposure. Paradoxically, 20 μM of either Na^+ nitroprusside (SNP) or isosorbide (ISB) in the microdialysis buffer dramatically decreased the relative extracellular DA, DOPAC and HVA levels during METH exposure. It is possible that in the CPU the NO generated by SSP and/or NO_3 by ISB is much greater than NO generated by METH alone, and the excessive levels of NO_x generated by SSP and ISB are producing neurotoxic effects or are inhibiting DA synthesis (thus the dramatic decrease in DOPAC and HVA). Alternatively, L-NAME and NARG are potent inhibitors of another enzyme/receptor other than NOS that affects DA release.

419.7

PRENATAL METHAMPHETAMINE CAUSES SPECIFIC METABOLIC CHANGES IN THE BASAL GANGLIA SEEN AT MATURATION. A.D. Weissman* and S. Caldecott-Hazard. Department of Anesth., Seton Hall University, School of Graduate Medical Education, South Orange, NJ 07079

Methamphetamine exposure *in utero* has been shown to produce profound alterations in the adult brain monoamine system. These effects are also reflected in subtle changes in adult performance of specific behavioral tasks. Human maternal methamphetamine abuse may have a similar impact on the fetal brain that may persist into adulthood. We sought to model the drug's developmental effects by chronically exposing fetal rats to it. The ^{14}C-deoxyglucose technique (DG) was used to measure adult brain metabolism and as a sensitive indicator of long term changes in neuronal function. *In utero* methamphetamine (10 mg/kg/bid) was administered throughout gestation. Drug related maternal anorexia, malnutrition, altered blood pressure/temperature and postnatal maternal care were controlled by using rats made tolerant to the drug's side effects and by cross fostering. At birth, the drug exposure ended and the brain metabolism of the drug-free adult offspring were behaviorally assessed and mapped with DG. An increased rate of glucose utilization was seen in the globus pallidus of the prenatally treated animals. No changes were seen in other areas of the basal ganglia, lateral habenula or medial dorsal prefrontal cortex. These results are in contrast to our previous observations in these areas following amphetamine withdrawal in adults. However, both paradigms produced a similar decrease in open field activity.

In utero drug treatment produces specific alterations in brain metabolism in the adult offspring at 30 days of age. These changes were accompanied by both neurochemical and performance deficits and point to the production of an altered adult neurochemical and behavioral state that can be attributed to the prenatal neurotoxic effects of methamphetamine on specific brain sites.

419.8

METHAMPHETAMINE NEUROTOXICITY PRODUCES ROBUST CHANGES IN OPEN FIELD BEHAVIOR IN RATS. K.A. Trujillo*, D.T. Chalmers, K. Boateng, S.J.Watson and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

Treatment with high doses of methamphetamine (METH) leads to well-described long-term decreases in serotonin and dopamine in certain regions of the brain. Despite quite dramatic reductions in these neurotransmitters, very few baseline behavioral changes have been observed in rats following neurotoxic regimens of METH. The present study was undertaken to examine behavioral and neurochemical consequences of METH neurotoxicity.

Adult, male Sprague-Dawley rats received injections of saline or METH (6.5 mg/kg s.c.) four times daily, at two hour intervals, for four days. Ten days following treatment behavior was assessed in a large (4 ft x 4 ft) plexiglass open field apparatus. Animals were placed in the center of the open field and behavior was observed for ten minutes. Four days following behavioral assessment, animals were sacrificed, and the brains examined by immunocytochemistry, receptor autoradiography and in situ hybridization for changes in serotonergic function.

As has been previously described, METH produced significant reductions in serotonergic indices in several brain regions, including neocortex, hippocampus and caudate-putamen. Other areas, such as substantia nigra appeared to be spared. Animals that had received METH showed a significant increases in locomotor activity and rearing, relative to saline controls. The increases in behavior were seen primarily toward the end of the ten minute session, and occurred despite the fact that animals had been drug-free for ten days. The results suggest that METH neurotoxicity produces significant changes in locomotion and exploration. These changes may be due to decreases in serotonergic and/or dopaminergic function. (Supported by NIDA DA02265 and NIMH MH42251. KAT and DTC are recipients of NARSAD Young Investigator Awards).

419.9

EFFECTS OF METHAMPHETAMINE-INDUCED NEUROTOXICITY ON NEUROTROPHIC FACTOR EXPRESSION IN ADULT MICE. D.S. Albers[1]*, M.S. Saporito[2], W.J. Friedman[3], P.K. Sonsalla[1]. [1]Neurology Dept., UMDNJ-RWJMS, Piscataway, NJ 08854; [2] Cephalon, Inc., West Chester, PA 19380; [3] Dept. of Neurosci. and Cell Biol., UMDNJ-RWJMS, Piscataway, NJ 08854.

In experimental animals, methamphetamine (METH) causes pronounced neurotoxic effects to monoaminergic neurons. Studies have demonstrated that various pharmacological agents prevent METH-induced neuropathology indicating that several processes may be involved in mediating the toxicity of METH. Several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), have significant roles in development, maintanence and survival of dopaminergic neurons. Roles for neurotrophic factors in supporting the survival of dopaminergic neurons in the basal ganglia remain to be determined, especially under conditions of stress or injury. Therefore, our aim was to characterize the dynamics of BDNF expression during and after an insult to nigrostriatal dopmainergic neurons by METH in mice. Our results indicate that expression levels of BDNF increase approximately 2-4 fold in the mesencephalon and cortex 24 h after initiation of a neurotoxic regimen of METH. These results demonstrate that stress or injury to dopaminergic neurons in the basal ganglia by METH can enhance the regional expression of neurotrophic factors (i.e. BDNF) in an adult mouse brain.

419.10

METHAMPHETAMINE SENSITIZATION INCREASES VULNERABILITY OF THE PREFRONTAL CORTEX TO METHAMPHETAMINE TOXICITY B.K. Yamamoto* and S.E. Stephans. Depts. of Psychiatry and Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106.

Sensitization to the behavioral and neurochemical effects of stimulants occurs following a low dose intermittent pretreatment regimen. This is manifested as an increase in behavioral stereotypy and mesostriatal dopamine (DA) function subsequent to the administration of an acute low dose challenge. These effects occur without evidence of dopamine (DA) depletion. The purpose of this study was to examine if a sensitization regimen of methamphetamine (METH) enhances DA release and the subsequent depletion following neurotoxic doses of METH. Male rats were injected once daily for 7 days with METH (2mg/kg, i.p.) or saline. Following a 7 day withdrawal, the rats were challenged with 3 subcutaneous injections of METH (7.5mg/kg), each injection given 2 hours apart. Microdialysis was used to measure extracellular DA levels in striatum (str) and prefrontal cortex (pfc) of awake rats. Rats were sacrificed 4 days later and DA tissue content was assayed. In METH pretreated rats, DA release in pfc but not in str was significantly increased during the METH challenge as compared to rats pretreated with saline ($p<0.02$). One week later, this group of rats also exhibited a greater depletion of DA in pfc ($p<0.02$) compared to the saline preteated group. Saline and METH pretreated rats given a saline challenge were not different from controls. Conversely, striatal DA was significantly less depleted by a METH challenge in METH pretreated compared to saline pretreated rats ($p<0.02$). These data provide evidence that pfc DA neurons are preferentially susceptible to the neurotoxic effects of METH in rats previously sensitized to METH. Augmented DA release in the pfc may be partially responsible for the dopamine depletion following a METH challenge.

419.11

DOPAMINE AND GLUTAMATE EFFLUX ARE INCREASED IN PREFRONTAL CORTEX OF RATS SENSITIZED TO METHAMPHETAMINE
S.E. Stephans* and B.K. Yamamoto. Depts. of Psychiatry and Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106.

Behavioral and neurochemical sensitization occurs following intermittent, low doses of psychostimulants such as methamphetamine (METH). This is manifested as behavioral stereotypy and increased mesostriatal dopaminergic function following administration of a challenge dose. No direct comparisons have been made with regard to whether the increased dopamine (DA) response *in vivo* is mediated by an impulse-dependent or independent mechanism. Furthermore, it is not known if release of other neurotransmitters is affected by this pretreatment regimen of METH. The purpose of this study was to investigate if DA and glutamate (glu) release in the prefrontal cortex (pfc) is augmented in METH sensitized rats upon a low challenge dose of METH or potassium (K^+) stimulation. Male rats were pretreated once a day for 5 days with METH (2mg/kg, i.p.) or saline. Following a 7 day washout period the rats were challenged with either a low dose METH injection (2mg/kg, i.p.) or K^+ (80mM) infused into pfc. Microdialysis was used to measure extracellular DA and glu in pfc. K^+ infusion into the pfc of METH pretreated rats increased extracellular DA and glu levels significantly from baseline values ($p<.02$). These increases were greater than in saline pretreated rats ($p<.05$). The low dose METH challenge significantly increased extracellular DA but not glu concentrations to a greater extent in METH pretreated compared to saline-pretreated rats ($p<.02$). These data provide evidence that a low dose pretreatment regimen of METH enhances DA transmission in the pfc through both transporter and impulse-dependent processes. In addition, enhanced glu release in pfc may play an important role in the behavioral effects associated with METH sensitization.

419.12

INFLUENCE OF PRIOR BEHAVIORAL SENSITIZATION ON THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE.
M. Sparago, J. Yuan and G. Ricaurte*. Department of Neurology, Johns Hopkins Medical Institutions, Baltimore, MD 21224.

Sensitization to psychomotor stimulant drugs is associated with alterations in brain dopamine (DA) neurotransmission. DA release has been implicated in the neurotoxic action of methamphetamine (METH). The purpose of the present study was to determine whether prior sensitization influenced the neurotoxic effects of METH on brain DA and serotonin (5-HT) neurons.

To produce sensitization, mice were treated with METH (15 mg/kg, i.p.) once daily for 15 consecutive days. Control animals were treated identically with saline. One week later, the development of behavioral sensitization was documented by demonstrating a leftward shift in the dose-response curve for stereotypy in METH-treated mice. After an additional one-week drug-free period, sensitized animals, along with controls, were treated with neurotoxic doses of METH. Seven days later, the animals were sacrificed and indexes of dopaminergic and serotonergic neuronal integrity were measured. Sensitization did not augment either the dopaminergic or serotonergic neurotoxic effects of METH in the striatum. To the contrary, the sensitizing regimen of METH appeared to render the animals partially tolerant to the drug's DA neurotoxic effects.

These results indicate that a chronic regimen of METH that induces behavioral sensitization does not render brain dopamine and serotonin neurons more vulnerable to METH's neurotoxic actions. Further, the partial tolerance observed suggests that the neurochemical mechanisms underlying the expression of behavioral sensitization may at least partially overlap those mediating METH's neurotoxic actions.

419.13

METHAMPHETAMINE(METH)-INDUCED TOXICITY IN VITRO INVOLVES THE NITRIC OXIDE TOXIC PATHWAY.
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Methamphetamine (METH) is a drug of abuse which causes deleterious effects on both the serotonin (5-HT) and dopamine (DA) in the mammalian brain. Although these effects of methamphetamine have been known for many years, the basic mechanisms involved in the manifestations of these toxic changes have remained elusive. Recent evidence suggests that the superoxide radical might play an important role in these changes (Cadet et al., 1994). In order to further evaluate the role of oxidative mechanisms in METH-induced neurotoxicity, we examined the effects of methamphetamine in a *in vitro* model of rat fetal mesencephalic cells. METH causes loss of TH-positive cells and process degeneration. In addition, the drug causes an increase in reactive gliosis as shown by the number of cells that stain for and by the intensity of staining with glial fibrillary acidic protein (GFAP) antibodies. Co-incubation of METH-treated cells with either the NOS blocker, nitroarginine, or with the ADP-ribosylation inhibitor, benzamide, attenuated METH-induced toxic effects. Both benzamide and nitroarginine also attenuated the glial response although the effects of benzamide on glial cells were somewhat more potent. These results further support a role for free radicals in METH-induced neurotoxicity and reactive gliosis.

419.14

EFFECTS OF d-FENFLURAMINE AND m-CHLOROPHENYLPIPERAZINE ON ACUTE 5-HT RELEASE AND LONG-TERM 5-HT DEPLETION IN RAT BRAIN.
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The serotonin (5-HT) agonists d-fenfluramine (FEN) and m-chlorophenylpiperazine (mCPP) are reported to release neuronal 5-HT by a Ca^{++}-independent, fluoxetine-reversible mechanism. In the present work, we compared the acute effects of FEN and mCPP on extracellular 5-HT and dopamine (DA) using in vivo microdialysis methods in rats. The long-term consequences of repeated injections of FEN or mCPP on 5-HT neurotransmission were also examined. In the acute study, FEN or mCPP (1, 10, 100 μM) was infused into the nucleus accumbens of conscious rats via microdialysis probes, and monoamines were assayed in dialysate samples by microbore HPLC-EC. FEN and mCPP caused equivalent, dose-dependent increases in 5-HT, but not DA, at 1 and 10 μM doses. At the 100 μM dose, both drugs elevated dialysate DA as well as 5-HT. In the long-term study, rats were treated with FEN or mCPP (0, 3, 10 mg/kg, ip), one injection every 2 hr for 8 hr. Two weeks after the repeated dosing regimen, FEN-treated rats exhibited marked depletions in tissue levels of 5-HT (78%) and 5-HIAA (72%) in frontal cortex, whereas mCPP-treated rats showed no signs of 5-HT depletion. Thus, mCPP appears to enhance acute 5-HT release, in a manner similar to FEN, without causing damage to 5-HT nerve terminals. The present data suggest the possiblility that 5-HT release per se may not be the causative factor in the serotonergic neurotoxicity associated with FEN and other substituted amphetamine derivatives.

419.15

BEHAVIORAL AND NEUROTOXIC EFFECTS OF METHCATHINONE.
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Cathinone is a naturally-occurring amphetamine analog found in the Khat plant. Like amphetamine, cathinone has been abused and, in high doses, is selectively toxic to brain dopamine (DA) neurons. Methcathinone ("Cat") is a synthetic cathinone derivative that has recently surfaced in the illicit drug market. Because it contains an asymmetric carbon, methcathinone is available in D and L forms, and it is the L form that is used recreationally. The present studies evaluated the pharmacologic and neurotoxic properties of methcathinone stereoisomers in laboratory animals. Pharmacologic studies in mice indicated that L-methcathinone was approximately five times more potent as a locomotor stimulant than D-methcathinone. By contrast, in neurotoxicity studies, the D isomer proved more toxic to DA neurons in mice. To evaluate methcathinone's serotonin (5-HT) neurotoxic potential, additional studies were performed using rats, since mice are often refractory to the 5-HT neurotoxic effects of amphetamine derivatives. In the rat, L-methcathinone produced toxic effects on brain 5-HT (as well as DA) neurons, and its effects were more pronounced than those of D-methcathinone. These results indicate that: 1) Under certain conditions, it is possible to dissociate the pharmacologic from the neurotoxic effects of amphetamine derivatives; 2) The mechanisms mediating the behavioral effects of amphetamine derivatives may be separable from those underlying their neurotoxic actions; 3) Stereochemical and species effects may influence not only the degree but also the type of neurotoxicity produced by an amphetamine derivative and 4) N-methylation confers 5-HT neurotoxic activity onto the cathinone molecule.

420.1

MEDIAL SEPTAL LESIONS ENHANCE LOCOMOTOR SENSITIZATION TO AMPHETAMINE. J. E. Kelsey* and J. A. Grabarek. Dept. Psychology, Bates College, Lewiston, ME 04240.

Sensitization to the locomotor-enhancing effects of several drugs appears to critically involve the dopaminergic mesolimbic pathway from the VTA to the n. accumbens. Such locomotor sensitization appears to be modulated by projections from the hippocampus to the n. accumbens. In this experiment, we examined sensitization to the locomotor-enhancing effects of amphetamine in rats with lesions of the medial septum, which supplies the major cholinergic projection to the hippocampus. During acquisition, the rats were injected i.p.with 1.0 mg/kg amphetamine sulfate or isotonic saline and immediately placed in a 58 cm^2 black open field where distance travelled was measured during six 1-hr sessions spaced 2 days apart. All rats were then injected i.p. with 0.4 mg/kg amphetamine and isotonic saline 2 days apart. Although there were no differences between the sham-operated control and lesioned rats in the acute locomotor response to either 0.4 or 1.0 mg/kg amphetamine, enhanced locomotor sensitization and conditioned sensitization were observed in the rats with medial septal lesions during acquisition and testing. These data suggest that the input from the septum to the hippocampus normally serves to inhibit sensitization to the locomotor-enhancing effects of amphetamine. Perhaps this projection also acts to inhibit sensitization to the rewarding effects of amphetamine.

420.2

LESIONS OF THE PEDUNCULOPONTINE NUCLEUS SPARE SENSITIZATION AND CONDITIONING OF AMPHETAMINE-STIMULATED LOCOMOTOR ACTIVITY. F.A. Guarraci*, N. Melanson, M.C. Olmstead and K.B.J. Franklin. Dept. Psychology, Mcgill University, 1205 Dr Penfield Ave, Montreal, Quebec, Canada H3A 1B1.

The locomotor stimulant effects of amphetamine and other psychostimulants have been linked to their reinforcing effects in that the phenomena seem to have a common neural substrate in the modulatory effect of dopamine on the outflow from the ventral striatum. One component of the ventral striatal outflow passes via the ventral pallidum to the region of the pedunculopontine tegmental nucleus (PPTg) which lies within the midbrain locomotor region. Recently it has been shown that excitotoxin lesions of the PPTg block the reinforcing effects of amphetamine, morphine and brain stimulation but do not appear to block the acute locomotor stimulant effects of amphetamine (Olmstead and Franklin, 1994). However it remains possible that the PPTg plays a role in the long term effects of amphetamine reflected in conditioning or sensitization of stimulant effects. We now report that NMDA-induced lesions of the PPTg do not alter the augmentation of the locomotor response with repeated injections of 1.5 mg/kg amphetamine, or the subsequent hyperactivity exhibited by animals tested drug free. The reinforcing and locomotor stimulant effects of amphetamine seem to be dissociable at the level of the PPTg.

420.3

Effect of chronic amphetamine on the behavioral dose-response curve. P.K. Randall*, P. Hodge, J.S. Randall College of Pharmacy and Inst. for Neuroscience, Univ of Texas, Austin, Texas 78712.

The response to amphetamine (AMPH) increases with repeated administration. With drugs such as AMPH producing curvilinear dose-response curves and multiphasic time courses, sensitization-induced changes may be difficult to interpret.

The purpose of this study was to determine whether sensitization could be more simply described as a horizontal shift in the dose response curve rather than as an alteration in the magnitude of response. Sprague-Dawley rats were injected in the home cage with 5 mg/kg AMPH (IP) every 4 days for a total of 5 treatments. Four days after the last treatment the response to 0,.5,1,2,4,8, and 16 mg/kg AMPH was determined. Locomotion and rearing were assessed via computer and stereotypic ratings assigned every six min for two hours prior to, and 5 hours following, injection.

Locomotion and rearing showed typical multiphasic patterns and chronic treatment elevated or diminished these depending upon the dose and time (GroupXDoseXTime interaction ,$p<.05$ for both). The rating scale data were described by a family of quantal curves in which individual curves represented the probability that the rating would meet or exceed each rating from 1 to 9. The ED50's and the horizontal deviation of each were determined by non-linear regression using a maximum liklihood criterion. At 6 and 12 min post-injection there was a 3.4 fold parallel shift to the left in the overall dose-response curve. This shift systematically decreased with time until at 60-90 min there were no differences between the groups. The reciprocal of the ED_{50}'s in both groups fell exponentially following 1 hr. The sensitized animals were marked by a more rapid decrease in this measure than the control animals.

420.4

AN INNOVATIVE TECHNIQUE FOR MEASURING AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION: HYPERACTIVE RATS RANKED HIGHER THAN CONTROLS. **J.G. Kohlert* and G.J. Bloch. Dep't of Psychology, Brigham Young University, Provo, UT, 84602.**

General locomotor activity was used to assess the degree of "sensitization" induced by moderate doses of amphetamine. Adult male rats were given an injection of saline or amphetamine (.75 1.0, 2.0, or 3.0 mg/kg) once per day for 4 days. The pattern of activity for each rat was scored by assigning a rank based on performance in all 4 sessions. Ranks progressed on a scale from 1 (activity seen in saline rats) to 12 (activity seen in rats spending considerable time in stereotypic behavior). Ranks differed significantly among the doses ($p<0.05$, Mann-Whitney U); these increased in a dose-response fashion. Behavioral analysis revealed an inverse relationship between locomotor activity and stereotypy ($p<0.0001$, Least Squares Regression). Interestingly, although total activity decreased with increased stereotypy, locomotor activity actually increased during those times when animals were not engaged in stereotypical behavior. Importantly, hyperactive rats had higher sensitization rankings than dose-matched controls ($p<0.05$, Mann-Whitney U).

420.5

INTRAVENOUS AMPHETAMINE PRODUCES CONDITIONED PLACE PREFERENCE USING A SINGLE TRIAL IN RATS. J. M. Valone*, T. R. Gibson, R. A. Bevins and M. T. Bardo. Department of Psychology, University of Kentucky, Lexington, KY 40506.

Previous research using intravenous (IV) morphine (MORP) has shown that conditioned place preference (CPP) can be obtained after one trial. The present study assessed the rewarding effects of IV amphetamine (AMPH) using a single trial CPP procedure. Rats were surgically implanted with an IV jugular catheter for drug delivery. Conditioning consisted of injecting each rat with AMPH (0.1, 0.3, 1 or 3 mg/kg) at the beginning of a 30-min placement into one compartment of the CPP apparatus. On a separate day, animals also received a 30-min exposure to the other compartment following saline. Sham controls were exposed to both compartments without drug. On Day 3, rats received a 15-min preference test. In Experiment 1, there was a dose-dependent increase in locomotor activity on the conditioning day. Further, CPP analysis revealed that 1 and 3 mg/kg AMPH, but not 0.1 or 0.3 mg/kg AMPH, produced a significant preference for the drug-paired compartment relative to sham controls. This preference indicated that AMPH has a rewarding effect after a single injection. Experiment 2 examined whether the AMPH-induced increase in activity could be dissociated from its rewarding property. IV MORP (10 mg/kg) was used as previously described. Consistent with previous research, MORP conditioned rats preferred the drug-paired compartment compared to shams, while showing a significant decrease in activity. Combined, these data suggest that both AMPH and MORP have an acute rewarding effect that is independent of increased activity.
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420.6

GENETIC ANALYSIS OF METHAMPHETAMINE'S EFFECT ON FEEDING BEHAVIOR IN BXD RECOMBINANT INBRED MICE. S. Angeli-Gade*, and J.K. Belknap. Dept. of Medical Psychology, Oregon Health Sciences University, Portland, OR 97201.

Amphetamines at low doses have been shown to produce anorexia in both humans and animals. To identify the genes mediating this effect, BXD recombinant inbred mice were tested on a five day food restriction paradigm which resulted in stable food intake. Mice from each strain were then divided into two equal groups. On Day 6, Group 1 was given 1.0 mg/kg methamphetamine (MA), s.c., whereas Group 2 was given the saline vehicle. On the next day (Day 7), both groups were given MA. Consumption was measured for the first 30 minutes. Significant drug and strain effects for the 24 strains tested were seen. Food consumption was decreased on average by 54%. In the extreme strains, food consumption was decreased by as much as 86% (BXD 1) or as little as 15% (BXD 11). Following behavioral testing, QTL analysis ($p<.01$) identified distinct candidate genes on chromosomes 4 (*Hcf-3*), 9 (*Fv-2*), 11 (*D11Mit2*), 12 (*D12Nyu1*), 13 (*Tpmt*), 15 (*D15Mit3*), 18 (*D18Mit15*) and 19 (*D19Byu1*). Verification of these loci using PCR in an independent F2 population is ongoing. Supported by NIDA DA07262 and Contract DA277-90-7405.

420.7

GENETIC RECOMBINATION OF AMPHETAMINE-INDUCED MOTOR BEHAVIORS. A. Fleischer,[1] C. Vadasz*[1,2] [1]Laboratory of Neurobehavioral Genetics, Nathan Kline Institute, Orangeburg, NY 10962 and [2]New York University Medical Center, NY 10016; USA

Quantitative Trait Loci (QTLs) responsible for high and low expression of murine mesencephalic tyrosine hydroxylase activity (TH/MES) were transferred onto C57BL/6ByJ (B6) inbred mouse strain background by successive backcrosses with concomitant selection for the extreme expressions of TH/MES (Vadasz, 1990). Subsequently, Artificially Selected Congenic Recombinant Inbred (ASCRI) strains were derived from the selection lines. To investigate the effects of the gene transfer, locomotor activity (distance covered; D) and frequency of occurrence of rearing on the hind legs (R) were recorded for 30 min after i.p. injections of 0, 2.0, and 4.0 mg/kg D-amphetamine in the progenitor strains (B6 and BALB/cJ) and in two B6.C animal model lines (N=10). Analysis of the data indicates that the D/R ratio was significantly higher in the BALB/cJ strain than in the B6 strain. Similar D/R values were obtained for the B6 and the B6.C lines at 0 and 2.0 mg/kg D-amphetamine. After administration of 4.0 mg/kg D-amphetamine the four strains fell into two classes, each class consisting of a progenitor and a B6.C line. The results are consistent with the hypothesis that both TH/MES and amphetamine-induced behaviors are affected by the same QTLs.

420.8

NEUROETHOPHARMACHOLOGY OF AMPHETAMINE AND ANTIPSYCHOTIC DRUGS IN NUCLEUS ACCUMBENS AND AMYGDALA OF SOCIALLY INTERACTING RATS. Z. R. Wang*, M. Bonta, and G. V. Rebec. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

A neuroethological approach has been used to compare the effects of haloperidol and clozapine on motor, social, and motivational behavior of amphetamine-treated rats. Adult, male rats -- tested simultaneously in groups of three -- were exposed to a relatively enriched environment. In each tested group, one rat received amphetamine in nucleus accumbens (NAC) or amygdala (AMG) followed within 15-20 min by either haloperidol or clozapine. The other two rats were used as companions (no infusion) and controls (saline). Behavior patterns were classified into three main elements: stereotyped motor behavior, social contacts, and motivational behavior. Each element consisted of several behavioral subcategories. Behavior was recorded on video tape and rated by a trained observer. The results indicate: 1) amphetamine induces more stereotyped motor behavior in NAC rats than in AMG rats; 2) clozapine effectively reduced all amphetamine-activated stereotyped motor, social and motivational behavior in both NAC and AMG rats, while haloperidol was less effective in AMG rats; and 3) the behavioral effects of both amphetamine and the antipsychotic drugs showed marked individual differences. A neuroethological analysis can provide important new information on the mechanisms underlying the complex behavioral effects of these and other drugs known to alter dopamine transmission.

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420.9

D-AMPHETAMINE (AMPH) DECREASES SYMPATHETIC NERVE DISCHARGE (SND) IN ANESTHETIZED RATS. W. Liu, M.C. Cuntapay and K.J. Varner*. Dept. of Pharmacology and Experimental Therapeutics & Alcohol and Drug Abuse Center, LSU Medical Center, New Orleans, LA 70112.

Studies in this and other laboratories have shown that cocaine, a well known sympathomimetic, decreases rather than increases SND in anesthetized and conscious animals. The purpose of this study was to determine whether AMPH, another sympathomimetic, would also decrease SND in pentobarbital-anesthetized rats. The studies were performed in male Sprague-Dawley rats (275-325 g). Cannulae were placed in the femoral artery and vein. The trachea was cannulated and the rats mechanically ventilated. Splanchnic SND was recorded using bipolar platinum electrodes (0.30-3 KHz bandpass). Two groups of rats (n=5 each) were used. One group received 0.01 and 0.5 mg/kg AMPH, and the other 0.1 mg/kg AMPH intravenously. AMPH dose-dependently decreased SND (-4±2 to -85±5%). The duration of the SND responses ranged from 1.4±0.6 to 66±8 min. AMPH also dose-dependently increased heart rate (39±19 bpm max, 1.4±0.6 to 18±7 min duration). Doses of 0.01 and 0.1 mg/kg, AMPH increased mean arterial pressure (MAP; 18±2 mmHg max.). Doses of 0.5 mg/kg AMPH increased (32±3 mmHg, 3±0.5 min duration) and then decreased (-36±7 mmHg, 48.5±11 min duration) MAP. These data show that AMPH, like cocaine, decreases SND in anesthetized rats. These also findings suggest that an increase in sympathetic outflow is not responsible for the HR and pressor responses elicited by AMPH. (supported by NIDA DA08255).

420.10

GENE EXPRESSION ASSOCIATED WITH AMPHETAMINE SELF-ADMINISTRATION BY RATS INTO THE NUCLEUS ACCUMBENS VIA MICRODIALYSIS PROBES. O.R. Javid, P.H. Janak,* S.A. Brooks, R.R. Rule, E.J. Barea-Rodriguez, W. Meilandt, J. Rigter and J.L. Martinez, Jr. Department of Psychology, University of California, Berkeley CA 94720.

We investigated whether self-administration of amphetamine could be supported by local administration of the drug into the n. accumbens via a microdialysis probe, and whether amphetamine self-administration induces alterations in gene expression. Male Sprague Dawley rats previously autoshaped to respond on a lever for food were implanted with cannulae in the n. accumbens. After surgical recovery, subjects received 60 lever presentations on a 5 min fixed-interval schedule; lever responses were reinforced by 1 min intra-accumbens infusion of either amphetamine or Ringers's solution. Lever-touch responding was maintained by delivery of 12 µg/2µl amphetamine, but not Ringer's solution, into the right n. accumbens via a microdialysis probe. Videotape analysis indicated that rats reinforced with amphetamine were more active than rats reinforced with Ringer's solution. Drug-induced changes in gene expression were examined by isolating mRNAs from either the drug-infused right forebrain (minus the caudate) of a single rat or the left cannula-implanted control forebrain (minus the caudate), immediately after a 5-hr self-administration session. A cDNA library generated from the right drug-infused forebrain was subtracted from cDNAs amplified *in vitro* from the contralateral control forebrain. This subtraction yielded numerous recombinant plaques that are being characterized. These results suggest that amphetamine self-administered via microdialysis probes into the n. accumbens maintains conditioned lever-touch responding, and that a single 5-hr self-administration session may induce new gene expression.

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420.11

DAILY POST-SESSION D-AMPHETAMINE OR QUINPIROLE ADMINISTRATION IMPAIRS ACQUISITION OF A POSITIVELY MOTIVATED AUTOSHAPED LEVER-TOUCH RESPONSE IN RATS. E.A. Plumley*, R.R. Rule, P.H. Janak and J.L. Martinez, Jr.
Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

The effects of injections of either d-amphetamine or quinpirole, the D2 dopamine receptor selective agonist, on the development of a lever-touch response in an autoshaping paradigm were investigated. Male Sprague-Dawley rats received daily, post-session, intraperitoneally administered injections after 10 pairings of the insertion of a retractable lever (CS) and the delivery of a food pellet (US). Reinforcement delivery was immediate if the subject contacted the lever, otherwise, the pellet was delivered upon lever retraction. Amphetamine, at a dose of .3 mg/kg [$F(1,35) = 26.5$, $p < 0.0001$] impaired the development of the lever-touch response, as compared to saline treated control rats, and quinpirole at a dose of .3 mg/kg [$F(1,34) = 30.7$, $p < 0.0001$] completely abolished the acquisition of the response. Neither drug affected acquisition at a lower dose (.03 mg/kg). Administration of the higher doses of amphetamine (1 mg/kg) and quinpirole (2 mg/kg) also impaired responding, although the impairment likely was due to toxic effects of the drugs. Initiation of either quinpirole or amphetamine treatment after complete acquisition of the autoshaping task did not affect responding. Taken together these results indicate that post-session administration of amphetamine impairs and quinpirole blocks the acquisition of an autoshaped lever-touch task, and that the impairment is not due to an aversive action of the drug on performance (Supported by DA 06192 to JLM and MH 15860 to EAP).

420.12

DAILY PRETRAINING ADMINISTRATION OF D-AMPHETAMINE IMPAIRS ACQUISITION OF A CLASSICALLY CONDITIONED LEVER-TOUCH RESPONSE IN RATS. R.R. Rule*, P.H. Janak and J.L. Martinez, Jr.
Department of Psychology, University of California, Berkeley, CA 94720.

We previously found that posttraining administration of amphetamine does not affect performance of a classically conditioned lever-touch response. We now report that pretraining administration of d-amphetamine impairs acquisition of this same response. Sprague-Dawley rats were classically conditioned to touch a retractable lever (CS) which immediately precedes the delivery of a reward pellet. A retractable lever was presented to subjects for ten seconds. Upon retraction of this lever, rats received a pellet of food. There was no contingency between food presentation and lever-touch responding. This procedure was repeated ten times daily with an intertrial interval of 45 sec. Animals were run for ten consecutive days. Rats received a daily i.p. injection five minutes prior to each training session. Animals receiving .3 mg/kg amphetamine performed significantly fewer lever-touches than animals receiving pretrial injections of saline. The performance of the amphetamine animals increased from an average of 1.4 lever touches on Day 1 to 2.8 lever-touches on Day 10. This increase is less than that of saline-treated animals who increased from an average score of 0.9 on Day 1 to 5.9 on Day 10. The acquisition rate in the control rats is less than that seen in instrumental conditioning or combined classical and instrumental conditioning (autoshaping). This research is supported by a Ford predoctoral fellowship (RRR)and DA06192 (JLM).

420.13

DELAYED NON-MATCH TO POSITION PERFORMANCE IS IMPAIRED IN RATS GIVEN HIGH DOSES OF METHAMPHETAMINE. **S.D. Friedman, A.E. Butt, B.G. Cooper, M. Hagen, M.R. Markham, and G.K. Hodge*. Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131.**

Methamphetamine (MA) administered to rats in high doses causes neurotoxic injury to dopaminergic and serotonergic neurons (De Vito & Wagner, 1989; Wagner, Woertwein, & Forman, 1990) and disrupts performance in certain behavioral paradigms (Walsh & Wagner, 1990). Previously, we have shown that MA-treated animals are impaired in operant discrimination reversal learning (Cooper et al., 1991), and in delayed alternation T-maze performance (Cooper et al., 1992; 1993). In the current study, we examine MA-induced impairments in a delayed non-match to position task using a novel, computerized, touch-screen equipped operant conditioning apparatus (Markham, Butt, & Dougher, in preparation). Rats received a total of four subcutaneous injections of either MA (12.5 mg/kg) or saline, with 2 hr intervals between injections. Upon recovery, animals were reduced to 85% of their free-feeding weight and were shaped to activate a computer touch-screen for food reinforcement. An automated version of the T-maze alternation paradigm (see Cooper et al., 1992) was then implemented in two phases. In the first phase, animals were reinforced for pressing a rectangular image located on either the left or right side (sample position) of a touch-screen equipped monitor. Following a delay of 1 s, a pair of rectangular stimuli appeared on the monitor and animals were reinforced only for selecting the stimulus located on the opposite side relative to the sample. Upon reaching a criterion of 80% correct, animals began the second phase of training where delays of 1, 10, 20, 40, and 80 s intervened between the presentation of the sample and choice stimuli. Compared to controls, acquisition performance in MA-treated animals was impaired at delay intervals of 1, 10, and 20 s (MANOVA; $p < .05$). Results suggest that high doses of MA cause lasting impairments in reinforced alternation performance and that these impairments are exacerbated by temporal delays. (GKH supported by UNM RAC #93-33).

420.14

ENVIRONMENT MODIFIES THE PATTERN AND CHARACTER OF BEHAVIORAL SENSITIZATION PRODUCED BY METHAMPHETAMINE. T.Ohmori*, T.Abekawa and T.Koyama. Dept. Psychiatry, Hokkaido Univ. Sapporo 060 Japan

Effects of environmental inhibition of the methamphetamine (MA)-induced behavior on the establishment and mode of expression of behavioral sensitization were examined. Rats received daily injection of MA for 10 days (1mg/kg, sc). Half of them were immediately returned to the home cage (normal cage group) and the other half were individually confined in a cylinder with a diameter of 13 cm (narrow cage group) for 3 hrs after each injection. The third group received saline in the home cage (saline group). After a 7-8 day or 17-18 day withdrawal period, they were readministered with MA (1mg/kg or 0.5 mg/kg). The normal cage group showed significant enhancement in horizontal motor activity compared with the saline group, indicating the development of behavioral sensitization. In contrast, the narrow cage group showed no or slight enhancement in the motor activity. However, they showed significantly intensified stereotyped behavior compared with the saline group. Microdialysis studies revealed no enhancement in the ability of MA to increase dopamine release from the nucleus accumbens in the normal as well as narrow cage group compared with the saline group, when examined after either a 7-8 day or 17-18 day withdrawal period. These results suggest that environmental inhibition of actual movement under the drug effect modifies the pattern and character of behavioral sensitization. The neurochemical basis of the development of behavioral sensitization and the environmental modification of its expression remains unknown.

420.15

EFFECT OF REPEATED METHAMPHETAMINE PRETREATMENT ON FREEZING BEHAVIOR INDUCED BY CONDITIONED FEAR STRESS. K.Tsuchiya, T.Inoue and T.Koyama*. Dept. of Psychiatry., Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

We examined the effect of methamphetamine pretreatment on conditioned fear stress in male Wistar-King rats. Rats received methamphetamine or the vehicle according to the repeated escalating dose schedule (1.25, 2.5, 3.75, 5 mg/kg s.c x2/every other day for a week). After a 5 day drug abstinent period, the rats were exposed to electric foot-shock (2.5 mA for 30 min) for two days. Twenty-four hours after the last foot-shock session, the rats were again placed in the shock chamber without shocks and observed for 5 min. Methamphetamine pretreatment significantly increased conditioned freezing behavior, suggesting that rats previously exposed to chronic methamphetamine are more sensitive to subsequent psychological stress than control rats. Repeated methamphetamine treatment did not decrease basal dopamine and serotonin concentrations in the brain. Furthermore, co-administration of MK-801 (non-competitive NMDA antagonist), amfonelic acid (dopamine reuptake inhibitor) or fluoxetine (serotonin reuptake inhibitor) with methamphetamine did not alter the enhanced freezing behavior. Taken together, it seems that methamphetamine-induced hypersensitivity to anxiety is not due to the toxic effect of methamphetamine. While co-administration of SCH23390 (D1/5 antagonist) or raclopride (D2/3 antagonist) had no effect on the methamphetamine-induced increase in freezing, co-administration of YM-09151-2 (D2/3/4 antagonist) prevented this increase. These results suggest that methamphetamine-induced enhancement of anxiety is mediated by D2-like receptors.

420.16

EVIDENCE THAT HIGH AND LOW RESPONDERS TO NOVELTY SHOW DIFFERENCES IN BEHAVIORAL RESPONSES TO DEXAMPHETAMINE AND ETHANOL. M.A. Gingras* and A.R. Cools. Psychoneuropharmacology University of Nijmegen, P.O. 9101, 6500HB Nijmegen, The Netherlands. ENA

The aminergic make-up of the ventral and dorsal striatum differs between high (HR) and low (LR) responders to novelty (Can.J.Phys.Pharmacol.71,335,1993). These two types of individuals normally co-occur in unselected outbred populations of Wistar rats. These rats are known to react differentially to self-administration of dexamphetamine; they also show distinct locomotor responses to dexamphetamine (Science 245, 1511, 1989). The present study had two purposes. First, it was investigated whether dexamphetamine-induced stereotyped behavior also differs between these rats; for the sake of comparison, a study of the locomotor effects of dexamphetamine was included. Second, we investigated to what extent these rats differentially react to ethanol, since this drug is also known to influence the aminergic activity in the striatum. Male HR and LR rats were selected on an open-field (Brain Res. Bull. 24, 49, 1990). Stereotyped tracking behavior (STB; dexamphetamine: 0.5-2.0 mg/kg/sc) was studied on the same open field, using an video-computer program: HR showed STB at 1.0 mg/kg, whereas LR needed a much higher dose (2.0 mg). HR showed a significant greater locomotor activity, measured in boxes (50x30 cm) than LR following administration of dexamphetamine at doses (0.5-2.0 mg/kg/ip). Second, ethanol consumption was assessed. Animals were maintained on alternate day presentation of ethanol and water; ethanol solutions were given in increasing steps of 1%. LR rats show-ed significantly higher ethanol intake and preference than did HR rats. Animals were maintained on 10% ethanol to determine preference stability; line differences remained stable throughout the entire period. In conclusion HR rats differ from LR rats both in stereotyped and locomotor responses to dexamphetamine and ethanol intake. It remains to be investigated to what extent these dif-ferences are causally coupled to differences in the aminergic make-up of the striatum.

420.17

EFFECTS OF *d*-FENFLURAMINE ON THE STIMULUS PROPERTIES OF MDA STEREOISOMERS L.E. Baker*, C. Sullivan, and M. Taylor Department of Psychology, Western Michigan University, Kalamazoo, MI 49008.

The stereoisomers of the designer drug 3,4-methylenedioxyamphetamine (MDA) have been reported to have distinct stimulus properties: (+) MDA is presumably more similar to *d*-amphetamine while (-) MDA is more closely related to hallucinogens, such as DOM. While MDA is structurally similar to amphetamine, its behavioral effects appear to be mediated by both dopamine and serotonin. We have recently demonstrated evidence which suggests serotonergic depletion (with a neurotoxic regimen of fenfluramine) may enhance the amphetamine-like effects of a related compound, MDMA.

In the present study, we examined the effects of *d*-fenfluramine administration (6.0 mg/kg b.i.d. for four days) on the discriminative stimulus properties of the stereoisomers of MDA. Male sprague-dawley rats were trained to discriminate either (+) MDA (1.5 mg/kg; n=8) or (-) MDA (1.5 mg/kg; n=8) from saline in a two-lever operant task. Several doses of d-amphetamine (0.25 -1.0 mg/kg) and the training drugs (0.19-0.75 mg/kg) were administered during independent test sessions prior and subsequent to the *d*-fenfluramine regimen.

In contrast to previous suggestions that (+) MDA is similar to amphetamine, the present results indicated that *d*-amphetamine did not substitute for either isomer of MDA. In addition, while (+) MDA substituted completely for (-) MDA, (-) MDA only engendered partial substitution for (+) MDA. In test sessions following *d*-fenfluramine treatment, however, (-) MDA substituted completely for (+) MDA. These results suggest that *d*-fenfluramine induced neurotoxicity may influence the cue properties of the stereoisomers of MDA, making them more similar. Although there were noted increases in drug appropriate responding during amphetamine post-tests, such increases could not be attributed to the effects of *d*-fenfluramine treatment.

420.18

DEXFENFLURAMINE LACKS AMPHETAMINE-LIKE ABUSE POTENTIAL. K.W. Locke[1]*, T.R. Levesque[1], K.L. Nicholson[2] and R.L. Balster[2]. [1]Interneuron Pharmaceuticals Inc., Lexington, MA 02173 and [2]Dept. of Pharmacol. and Toxicol., Medical College of Virginia, Richmond, VA 23298.

The amphetamine-like abuse potential of dexfenfluramine (dFEN) was evaluated using drug discrimination and self-administration procedures. Male Fischer rats were trained to discriminate either dFEN (1.0 mg/kg) or d-amphetamine (dAMP; 1.0 mg/kg) from saline in a two-choice discrete-trial avoidance paradigm. In dAMP-trained rats, dFEN (0.5-4.0 mg/kg) engendered almost exclusively saline-appropriate responding. In dFEN-trained rats, dAMP (1.0-4.0 mg/kg) engendered entirely saline-appropriate responding in 3 of 6 rats and intermediate levels of dFEN-appropriate responding in the remaining animals. Potential reinforcing effects of dFEN were also evaluated in 3 male rhesus monkeys trained to self-administer cocaine (iv) during daily 60 min sessions under a fixed-ratio 10 schedule. Various doses of dFEN (30-1000 ug/kg/infusion) and dAMP (10 ug/kg/infusion) were substituted for cocaine in 4 consecutive daily sessions. In all subjects, dFEN maintained rates of self-administration within the range of rates maintained by saline and considerably below those maintained by cocaine and dAMP. Furthermore, the within-session distribution of responding with dFEN resembled that produced by saline. Taken together, these results strongly suggest that dFEN will not have amphetamine-like abuse potential in man.

420.19

FACTORS AFFECTING THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO APOMORPHINE. B.A. Mattingly*, K. Koch, F.H. Osborne, & J.E. Gotsick. Department of Psychology, Morehead State University, Morehead, KY 40351.

Research suggests that behavioral sensitization to the direct dopamine agonist, apomorphine (APO) develops through both associative and non-associative mechanisms. The present study further assessed the role of environmental factors in APO-induced behavioral sensitization. In Exp. 1, rats were treated daily with either APO (5 mg/kg) or vehicle (VEH) and tested for activity in either an open-field or a running-wheel. On Day 9, one-half the rats in each drug/apparatus group were tested in the alternate activity test apparatus. APO-treated rats displayed progressively greater activity over days in both the open-field and running-wheel (i.e., sensitization). More important, APO-treated rats displayed significant sensitization on Day 9 in both the wheel and open-field tests regardless of their prior training environment. In Exp. 2, rats were treated daily with APO (5 mg/kg) or VEH and again tested in the running wheel. For one-half the rats in each drug condition the wheel was free to move and for the remainder the wheel was immobilized. On Day 10 of testing, all rats received a challenge injection of APO (5 mg/kg) and the wheel was free to move for all rats. APO-treated rats in the free wheel displayed significant sensitization over the 10 test days. In contrast, rats pretreated with APO and placed in the immobilized wheel for 9 days were not sensitized when tested on Day 10. These results suggest that the ability to express the drug-induced locomotor response may be more important than drug-associated cues in the induction of behavioral sensitization.

420.20

EFFECTS OF WITHDRAWAL FROM NICOTINE ON INTRACRANIAL SELF-STIMULATION. M. Legault* and R. A. Wise. Center For Studies in Behavioral Neurobiology, Concordia University., Montreal, QC, CANADA H3G 1M8.

Elevated intracranial self-stimulation (ICSS) thresholds following withdrawal from repeated treatments with cocaine, amphetamine or morphine are correlates of the dysphoria and anhedonia that characterize human drug dependence. Dysphoria is also a feature of nicotine dependence and we now report the effect on ICSS of nicotine withdrawal. Animals were repeatedly injected with either 0.5 or 1.5 mg/kg of nicotine or saline. Injections were given once on the first day of treatment then twice daily for the next 13 days (equaling a dose of 1.0 or 3.0 mg/kg/day) and on the morning of the final day (day 15). Two measures of rate-frequency threshold were determined every 4 hours for 32 hours following the last injection. Nicotine withdrawal caused non-parallel rightward shifts in the rate-frequency functions. Peak effects occurred between 20 and 24 hours following the final injection. During this time period threshold determined by the lowest frequency required to reinforce responding was elevated to approximately 120% of baseline in each nicotine group, whereas the peak elevation in the frequency required to maintain half-maximal responding was only 10%. These data suggest that repeated nicotine treatment results in a dependence syndrome charaterized by a phasic depression of the neural substrate underlying ICSS that is detectable at moderately reinforcing stimulation frequencies.

DEGENERATIVE DISEASE: ALZHEIMER'S—MECHANISMS OF CELLULAR INJURY I

421.1

INHIBITION OF APOLIPOPROTEIN E mRNA TRANSLATION WITH ANTISENSE OLIGONUCLEOTIDES. S. Yajima and M. M. Mouradian*. Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Alzheimer disease patients have increased frequency of apolipoprotein E (ApoE) epsilon 4, suggesting this allele is a risk factor for the disease. ApoE, which interacts strongly with amyloid beta protein in vitro, is immunochemically localized to the senile plaques, vascular amyloid and neurofibrillary tangles of Alzheimer disease. Thus, decreased expression of the ApoE gene might be of therapeutic value in minimizing amyloid deposition in this disease. The antisense oligodeoxynucleotide (ODN) strategy can be used as an effective tool to regulate the expression of specific genes. In this investigation, we used phosphorothioate antisense ODNs to down-regulate ApoE gene expression. An expression vector was constructed containing the 5' untranslated and part of the translated region of the human ApoE cDNA fused, in frame, with the reporter gene encoding luciferase and used to generate stably transformed CHO cells. A series of 21 mer antisense ODNs targeted to various portions of the ApoE message were used. Nonsense ODNs having the same nucleotide composition but in scrambled sequence were used as controls. Among the tested ODNs, two molecules spanning the ATG codon added to the culture medium at 5μM four times every 12 hours decreased luciferase activity by about 20% ($p<0.05$). Shorter time points and a lower concentration (1μM) were ineffective. No apparent toxicity of the ODNs to the cultured cells were noted. These findings suggest that the antisense approach could be useful to study the potential pathogenetic link between ApoE and Alzheimer disease.

421.2

HUMAN CEREBRAL CORTICAL NEURONS DISPLAY APOLIPOPROTEIN E IMMUNOREACTIVITY. R.E. Metzger, M.J. LaDu, M.T. Falduto, J.B. Pan, E.J. Mufson, G.S. Getz, and D.E. Frail*. Dept. of Neuroscience, Abbott Laboratories, Abbott Park, IL 60064, Dept. of Neurological Sciences, Rush Presbyterian-St. Lukes Medical Center, Chicago, IL 60612, and Dept. of Pathology, Univ. of Chicago, Chicago, IL 60637.

Apolipoprotein E (apoE) immunoreactivity has been identified in senile plaques (SP) of Alzheimer's disease (AD). Although apoE is synthesized in the brain in high abundance, the cellular localization of apoE in specific brain regions has not been clearly defined. We have used immunohistochemistry to determine cell types containing apoE in paraformaldehyde fixed frontal cortex sections from normal aged and AD brains. In normal brain, double-staining with apoE and microtubule associated protein-2 (MAP-2) antibodies revealed intense, specific apoE immunoreactivity in a subpopulation of projection neurons in cortical layers 3 and 5. In contrast, AD sections displayed apoE reactive SP, neurofibrillary tangles and fewer immunoreactive neurons, while MAP-2 stained neuropil threads but not neurons. Co-staining of sections with glial fibrillary acidic protein (GFAP) antibody demonstrated that apoE was not present in the GFAP positive cells found in layers 1 and 2 of control or layers 1-5 of AD sections. These results indicate that apoE may be produced or internalized by a select population of neurons in human cerebral cortex and that this process is altered in AD pathology.

421.3

APOLIPOPROTEIN E_4 ALLELE ASSOCIATION WITH ALZHEIMER'S DISEASE. S.E. Poduslo*, J.D. Schwankhaus. Dept. of Neurology, Texas Tech University Health Sciences Center and Dept. of Veterans Affairs, Lubbock, TX 79430.

The apolipoprotein E_4 allele has been strongly implicated in late onset familial and sporadic Alzheimer's Disease (Saunders, et al. *Neurol* 43:1467, 1993). The authors found the E_4 allele frequency to be 0.42 for late onset Alzheimer's patients, 0.19 for early onset patients, and 0.36 for sporadic cases, compared with 0.16 for CEPH control grandparents. In our studies we have obtained blood samples from early and late onset familial and sporadic Alzheimer's patients and spouses as well as Parkinson's patients. The patients were diagnosed as probable Alzheimer's after a CAT scan, EEG, extensive blood work, and examination by a neurologist. The diagnosis was made according to the NINCDS-ADRDA criteria (McKhann et al. *Neurol* 34:939, 1989). The apolipoprotein E_4 polymorphism was detected after PCR amplification of genomic DNA, restriction enzyme digestion with HhaI, and polyacrylamide gel electrophoresis. Ethidium bromide stained bands at 91 bp were designated as allele 3, at 83 bp as allele 2, and at 72 bp as allele 4. Of the 84 probable Alzheimer's Disease patients (all of whom were Caucasian), 45 had the 3/4 genotype, 13 had the 4/4 genotype, and 4 had the 2/4 genotype. There were 25 early onset patients and 59 late onset patients. The frequencies for the E_4 allele in the late onset familial or sporadic patients was approximately 0.4; while that for early onset sporadic patients was 0.29 and for familial was 0.64. We analyzed 77 spouses as controls, and of these, 51 had the 3/3 genotype and 15 had at least one E_4 allele for an E_4 frequency of 0.097. In a survey of 53 Parkinson's patients as another neurological control group, only 9 had the E_4 allele. Our findings support the association of the $ApoE_4$ allele with Alzheimer's Disease. Supported by the State of Texas DNA Bank for Genetic Studies of Alzheimer's Disease.

421.4

HIPPOCAMPAL VOLUME LOSS ON MRI SCANS IN ALZHEIMER PATIENTS AND NONDEMENTED ELDERLY SUBJECTS WITH APOLIPOPROTEIN E ε4 ALLELE. H. Soininen*, M. Lehtovirta, M. Laakso, K. Partanen, S. Helisalmi, A. Mannermaa, M. Hallikainen, T. Hänninen, A. Pitkänen, K. Koivisto, M. Ryynänen, and P.J. Riekkinen Sr. Depts. of Neurology, Radiology, Unit of Clinical Genetics, Univ. and Univ. Hospital of Kuopio, Kuopio, 70211 Finland

Several studies have indicated apolipoprotein E (apoE) ε4 as a risk factor for late-onset sporadic and familial Alzheimer's disease (AD). AD is characterized by structural damage, particularly, in the medial temporal lobe structures. Previously, apoE was implicated in regeneration of the nerve tissue and in synaptogenesis of the hippocampus in experimental animals. Here, we studied whether AD patients and nondemented elderly subjects carrying apoE ε4 allele have more severe hippocampal damage than those without ε4 allele. We measured hippocampal volumes using a 1.5 T MRI imager in 26 AD patients at the early stage of the disease and 32 healthy nondemented elderly individuals. The apoE genotype was determined using genomic DNA extraction and PCR amplification from venous EDTA-blood. We found that AD patients with apoE ε4/4 genotype had smaller hippocampal volumes (the right hippocampus, -54% of control) than AD patients with ε3/4 or ε3/3 in spite of equal clinical severity. Nondemented elderly subjects with one or two apoE ε4 alleles had a decreased volume difference between the right and left hippocampus ($p<0.01$) compared to those without ε4. Our data suggest that apoE ε4 allele is associated with severe hippocampal damage in AD and minor hippocampal changes can be detected in nondemented elderly, particularly, in those with ε4/4 genotype.

421.5

APOLIPOPROTEIN E3- AND E4-INDUCED DIFFERENCES IN NEURITE OUTGROWTH ARE ASSOCIATED WITH DIFFERENCES IN THE SUBCELLULAR LOCALIZATION OF APOLIPOPROTEIN E. B. P. Nathan*, S. Bellosta, R. W. Mahley†‡, and R. E. Pitas‡. Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, Departments of †Medicine and ‡Pathology, University of California, San Francisco, CA 94141-9100.

Apolipoprotein (apo) E4, one of the three common isoforms of apoE, has been implicated in Alzheimer's disease. Previously, we demonstrated differential effects of apoE3 and apoE4 on neurite outgrowth in cultures of dorsal root ganglion (DRG) neurons: In the presence of β-migrating very low density lipoproteins (β-VLDL), apoE3 increased neurite extension and decreased branching, whereas apoE4 decreased outgrowth (Nathan *et al., Science* 1994, 264:850–852). In the present study, we examined the effects of apoE3 and apoE4 on neurite outgrowth from a murine neuroblastoma cell line (Neuro-2a). The cells were cultured in serum-free N2 medium for 48 hours in the presence of β-VLDL (40 μg cholesterol/ml) alone or with apoE3 or apoE4 (30 μg/ml) purified from human plasma. Consistent with the results obtained with the DRG neurons, neurite extension in Neuro-2a cells increased with apoE3 but decreased with apoE4 as compared with that seen in the cells grown in medium containing β-VLDL alone. Immunocytochemical localization of apoE in the Neuro-2a cells treated with apoE3 or apoE4 with β-VLDL revealed a differential localization of apoE. In apoE3-treated cells, intense apoE immunoreactivity was observed within the cell bodies and neurites. In contrast, apoE4-treated cells showed little, if any, immunoreactivity within the neurites. The data suggest that the localization of apoE3 (but not apoE4) in the neurites may play a role in enhancing neurite outgrowth.

421.6

EFFECT OF APOLIPOPROTEIN E EXPRESSION ON SECRETION OF AMYLOID PRECURSOR PROTEIN. M.J. LaDu*, M.T. Falduto, A.M. Manelli, C.A. Reardon, G.S. Getz, D.E. Frail. Dept. of Neuroscience, Abbott Laboratories, Abbott Park, IL 60064 and Dept. of Pathology, University of Chicago, Chicago, IL 60637.

A recent series of observations has linked apolipoprotein E (apoE), particularly the e4 allele, to the pathogenesis of Alzheimer's disease. Amyloid precursor protein (APP) undergoes proteolytic processing to form ß-amyloid (Aß), a major component of Alzheimer's disease plaques. The purpose of this work was to determine whether ApoE could influence the proteolysis of APP. HEK293 cells were transiently transfected with the cDNAs encoding human apoE, human APP (751 amino acid isoform) or both cDNAs. Secreted APP relative to cellular APP was reduced by 55% (±11.3, n=6) in apoE3/APP co-transfected cells compared to cells transfected with APP alone. Secreted Aß was also reduced in co-transfected cells. The amount of cellular and secreted apoE3 was unchanged between apoE and apoE/APP transfected cells. The effect of apoE on APP secretion appears to be independent of apoE isoform, as cotransfection of apoE3 or apoE4 with APP equally reduced APP secretion in a dose-dependent manner. These data suggest a role for apoE in APP processing. ApoE could be affecting either the proteolytic cleavage of APP or the subsequent uptake and degradation of APP and Aß.

421.7

MICROVASCULAR PATHOLOGIC CHANGES IN HIPPOCAMPAL FORMATION IN ALZHEIMER DISEASE. B. Leveugle[1]*, C. Yang[1], J. Eisler[1,2], C. Bouras[3], P.R. Hof[1,2], and H.M. Fillit[1,2]. Depts of Geriatrics[1] and Neurobiology[2], Mount Sinai Med Ctr, New York, NY 10029; [3]Dept of Psychiatry, IUPG Bel-Air, Univ of Geneva Sch Med, Switzerland.

Heparan sulfate proteoglycan (HSPG), a major component of the endothelial cell surface and extracellular matrix, plays a fundamental role in the integrity of the blood-brain barrier. Using an antibody to vascular HSPG (7E12), we recently described angio-architectural changes of cerebral capillaries in various regions of the cerebral cortex in Alzheimer's disease (AD). In addition, a decrease of the vascularization was observed in all of the cortical areas in AD cases when compared with control cases. Although the entorhinal cortex is particularly affected in AD, very little is known about pathological vascular changes in this area. Using two different antibodies to vascular HSPG (7E12, EP01), we investigated the alterations of the microvasculature in the entorhinal cortex of 12 AD cases and 13 elderly control cases. Similar staining patterns were obtained with the two antibodies. Interestingly, a weaker staining of the microvasculature was observed in the control cases in comparison with AD cases suggesting that an overexpression of vascular HSPG occurs in AD. In addition to the microvessels, some senile plaques and neurofibrillary tangles were also labeled in AD cases. The laminar distribution and relative densities of the microvessels showed a correlation between angio- and cytoarchitecture. In particular, microvascular density was higher in layer II of the entorhinal cortex and in the parvocellular layer of the presubiculum and subiculum. Numerous morphological abnormalities such as string vessels and distorted vessels were observed in AD cases. The control cases exhibited some pathological changes, however to a much lesser degree than AD cases. Preliminary data suggest that a consistent decrease of the vascular density occurs in the hippocampal formation in AD. In conclusion, the morphological and biochemical changes that were observed in the microvasculature of AD brains may profoundly affect the properties of the blood-brain barrier and cause detrimental effects to specific neuronal populations.

421.8

ELEVATED CSF IMMUNOGLOBULIN LEVELS AND BLOOD CEREBROSPINAL FLUID BARRIER IMPAIRMENT IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE, MULTI- INFARCT DEMENTIA AND MAJOR DEPRESSION H. Hampel, M. Ackenheil, A. Haberl, W. Unger*, F. Müller-Spahn and C. Hock. Department of Psychiatry and Neurochemistry, Psychiatric Hospital, University of Munich, 80336 Munich, Germany.

Other groups of dementias than the presenile onset type Alzheimer described have been included in the diagnostical concept of Alzheimer's disease (DAT). Several reports indicate possible subgroups within the DSM-IIIR and NINCDS-ADRDA diagnosis. In the present study serum and CSF of 46 patients with DAT, 8 patients with Multi-infarct dementia (MID) and 17 with Major Depression (MD) were examined to investigate the intrathecal synthesis of immunoglobulins, elevated CSF levels and blood CSF barrier impairment (BCB). The DAT group was subdivided into an early onset (EO with 17 patients) and a late onset type (LO with 29 patients). Intrathecal immunoglobulin G synthesis was defined as an elevated IgG index (greater than 0.7) and/or presence of oligoclonal IgG in CSF by isoelectric focussing. The concentrations of albumin and IgG in serum and CSF have been measured to evaluate the integrity of the BCB. In total, only 1 (2.2%) of the DAT patients, subdivided in 0 EO patient and 1 (3.5%) LO patient, showed an intrathecal immunoglobulin synthesis and none among MID and MD patients. Beside these findings 8 (18%) DAT patients, 3 (18%) EO patients and 5 (17%) LO patients, 2 (25%) MID and 6 (35%) MD patients showed elevated CSF immunoglobulin levels. 5 DAT- (11%), 3 LO- (10%), 2 EO- (12%), 1 MID- (13%) and 3 MD- (18%) patients showed an impaired integrity of the BCB. Elevated CSF immunoglobulins combined with BCB impairment could be caused by a generalized immune activation. Our findings support the hypothesis that immunological mechanisms may play a role in the etiology and/or pathogenesis in at least a subgroup of patients with DAT, MID and MD.

421.9

MHC CLASS II ANTIGEN EXPRESSION BY MICROGLIA AFTER DEAFFERENTATION IN AGED RATS. M.N. Gordon*, L.A. Holcomb, W.A. Schreier and D.G. Morgan. Dept. of Pharmacology, Univ. South Florida, Tampa, FL 33612-4799.

To examine microglial reactions after brain injury in the aged rat, 6-hydroxydopamine (6-OHDA; 8 μg) was injected into the right medial forebrain bundle of male F344 rats aged 6,15 or 24 mo. Rats were killed 2,4,7 or 14d after lesioning. Unlesioned rats served as controls. Microglial cell number and staining intensity were evaluated by microscopic videodensitometry. Sections stained immunocytochemically for tyrosine hydroxylase (TH) confirmed the virtually complete loss of nigrostriatal TH in all age groups. Striatal sections stained for the microglial specific markers MHC class II antigen (MHC-II; OX6) and complement receptor 3 (CR3; OX42) indicate that MHC-II is induced by the lesion, while CR3 is not. In addition, the MHC-II induction increases in magnitude and duration progressively with age.

In 6-mo-old rats, the density of microglia expressing MHC-II was 5±2 cells/striatal section in control, uninjured rats. MHC-II+ microglia doubled in number 2d after 6-OHDA injection, reached a maximum 10-fold increase 4d after injury, then declined to control levels 14d after injection. In aged control rats, the density of MHC-II labeled microglia was greater than in young controls. In addition, aged rats displayed the highest number of microglia after deafferentation (207±21 cells) with maximal increases observed at 14d. Importantly, middle-aged rats were intermediate in responsivity and time course. Similar results were obtained measuring area occupied by reaction product or total reaction product (aka integrated optical density). In contrast, area occupied by CR3 and optical density of that reaction product were not affected by aging or by brain injury. These findings suggest that microglia do not increase in number during aging or after the modest deafferentation injury produced here, but that a larger fraction of existing microglia begin expressing the MHC class II antigen. Supported by AFAR. DGM is an Established Investigator of the American Heart Assn.

421.10

DISTRIBUTION OF MICROGLIA IN NORMAL AND ALZHEIMER'S DISEASE (AD) BRAIN SUPPORTS MICROGLIAL INVOLVEMENT IN NEURODEGENERATION. L.G. Sheffield* and N.E.J. Berman, Dept. of Anatomy & Cell Biology, Univ. of Kansas Med. Ctr., 3901 Rainbow Blvd., Kansas City, KS 66160-7400.

Microglia are the macrophages of the central nervous system and are activated in response to disease and injury. While activated, they phagocytose debris and secrete factors which may be cytotoxic. In Alzheimer's disease (AD), microglia have been found in direct association with neuritic plaques (NPs), one of the hallmarks of the disease. The region-specific localization of neurofibrillary tangles (NFTs) and NPs is used to diagnose AD in postmortem brain tissue. In early stages of AD, NFTs are found in the entorhinal region of the parahippocampal gyrus. As the disease progresses, NFTs are also found in limbic structures and eventually in selected neocortical areas, but are almost entirely excluded from the primary motor cortex.

Our studies have focused on the distribution of microglia in both normal and AD brains. Microglia were visualized by lectin histochemistry using the lectin *Ricinus communis* agglutinin-1 (RCA-1). In control brains, the density of microglia was greater in the regions which are most severely affected by AD, i.e. the parahippocampal gyrus and hippocampus. The motor cortex and area 22 exhibited a much lower density. In AD brains microglial density increased in all areas of the brain with longer periods of dementia, but the pattern of distribution seen in control brains was maintained (motor cortex < area 22 < parahippocampal gyrus < hippocampus). Morphology and size of microglia also varied according to the stage of the disease and the cortical region examined. These results provide further support for microglial involvement in the degeneration caused by AD. (Supported by MH38399 and HD02528.)

421.11

QUANTIFICATION OF THE IL-1β INDUCED MESSAGES, PLASMINOGEN ACTIVATOR INHIBITOR TYPE-1 AND PROSTAGLANDIN G/H SYNTHASE-2, IN ALZHEIMER'S DISEASE. J.W. Chang*, D.J. Selski, P.D. Coleman, and M.K. O'Banion. Departments of Neurobiology and Anatomy and Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

In response to CNS injury or neurodegenerative disorders, cytokines such as interleukin-1β (IL-1β) are released into the microenvironment. One role of IL-1β is to activate astrocytes and to increase the synthesis/secretion of astrocytic proteins. We have previously demonstrated that IL-1β promotes astrocytes to secrete plasminogen activator inhibitor type-1 (PAI-1), which may influence neuritogenesis, and synthesize prostaglandin G/H synthase-2 (PGHS-2), an inflammatory mediator. Thus levels of PAI-1 and PGHS-2 may serve as markers for astrocyte activation by IL-1β. Since IL-1β levels are elevated in Alzheimer's disease (AD) brains, we hypothesized that PAI-1 and PGHS-2 mRNA may be increased in AD brains relative to age-matched controls.

We carried out Northern hybridization of poly A^+ RNA from the putamen and subcallosal area (neocortex area ventral to genu of corpus callosum) of AD and age-matched control brains utilizing ^{32}P labeled cDNA probes for human PAI-1, PGHS-2, and G3PDH. Hybridization signals, quantified using a Molecular Dynamics Phosphoimager, were normalized for RNA loading to G3PDH signals. Our preliminary data indicate little or no alteration in PAI-1 and PGHS-2 mRNA levels with disease in the putamen (N=5 AD cases, 4 controls). However, in the subcallosal area (Brodmann's areas 12/32), PAI-1 and PGHS-2 mRNA levels are decreased 1.5-fold and 3-fold, respectively, between AD (N=3) and age-matched control (N=5) brains.

These observations are somewhat surprising given the detection of IL-1β in AD brain. Further studies in other affected brain regions are in progress. [Supported by LEAD award AG09016, R01 AG1121, and training grant T32 AG107]

421.12

HUMAN ASTROCYTES CAN EXPRESS COMPLEMENT C4 mRNA AND SECRETE COMPLEMENT C4 PROTEIN. D.G. Walker[1]*, S.U. Kim[2] and P.L. McGeer[1]. [1]Kinsmen Lab. of Neurological Research, Department of Psychiatry, [2]Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, B.C. Canada, V6T1Z3.

Studies of brains from Alzheimer Disease (AD) cases have shown evidence of inflammatory changes in affected tissue, as demonstrated by complement system activation and the presence of reactive microglia. These features indicate that a chronic inflammatory response occurs in AD brains which may be contributing to the neurodegenerative process. In addition, a pronounced astrocytic gliosis occurs in affected AD tissue. There is now increasing evidence that astrocytes may be contributing to the inflammatory changes by the production of certain cytokines and complement proteins.

In this study, we showed that astrocytes isolated from human fetal brains, and cultured in vitro, can express the mRNA for complement C4, and can also secrete the complement C4 protein. Using the reverse transcription-polymerase chain reaction (RT-PCR) technique, the expression of C4 mRNA was shown to occur in six separate sets of cultures that were highly enriched for astrocytes (>95% of cells immunoreactive for glial acidic fibrillary protein). Expression was detected in unstimulated astrocytes and this was increased by treatment of cells with Γ interferon (100 or 1000 units/ml of recombinant Γ interferon). In addition, by culturing the cells in serum-free media and by using immunoblotting and ELISA techniques, we showed that human astrocytes can secrete C4 protein into the culture media. Increased secretion of C4 occurred after treatment of astrocytes with Γ interferon, but not with interleukin 1 β (20 or 200 units/ml).

Supported by a grant from the British Columbia Health Research Foundation.

421.13

DOUBLE IMMUNOLABELING OF COMPLEMENT PROTEINS AND NEUROFIBRILLARY TANGLE MARKER PHF-1 AT LIGHT AND ELECTRON MICROSCOPIC LEVELS IN ALZHEIMER'S DISEASE BRAIN. L.-F. Lue*[1], S. Webster[1], S. Greenberg[2], J. Rogers [1]. Sun Health Research Institute, Sun City, AZ 85372[1] and Burke Medical Research Institute, White Plains, NY 10605[2]

A pathogenic role of complement activation in Alzheimer's disease (AD) has been suggested by recent evidence that the complement cascade is activated at sites of amyloid ß peptide (Aß) deposition through Aß/C1q binding. This process also appears to accelerate aggregation of soluble Aß into its pathogenic fibrillar, ß-pleated conformation. However, activated complement components C1q, C4d, C3d, and C5b-9 also co-localize with cells exhibiting neuronal morphology, particularly neurons containing neurofibrillary tangles (NFTs).

In this study we evaluated the percentage of complement immunoreactive, NFT containing neurons in entorhinal cortex samples from four pathologically confirmed AD patients. Light and electron microscopic assays suggest that the majority of complement immunoreactive cells in AD brain contain NFTs. Taken together with certain features of Aß/C1q binding and binding sites, these data suggest that complement activation may occur at the cellular surface of degenerating neurons, with lethal consequences. An interaction with NFT formation is also possible given the mechanism of C5b-9 attack on targeted cells.

(Supported by NIA AGO 7367)

421.14

ALZHEIMER'S DISEASE LIKE PHORYLATION OF TAU PROTEIN IN RESPONSE TO INTERLEUKIN-1β TREATMENT OF MIXED CELL POPULATIONS FROM RAT CORTEX. S. Wyoral and M.L. Shelanski*. Department of Pathology and Alzheimer's Disease Research Center, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Immune related processes have been associated with Alzheimer's disease (AD) as have significant reductions in the prevalence of AD among patients treated with anti-inflammatory therapies. However, a physiologically relevant mechanism for the involvement of immune responses in producing AD-like pathology has not been demonstrated. Using antibodies (Abs) to phosphorylation sensitive epitopes of tau, we have now demonstrated AD-like changes in the tau protein in response to the inflammatory cytokine interleukin-1β.

Mixed cell cultures from postnatal day one (PND-1) rat brain cortex were incubated in serum free medium augmented with the cytokine IL-1β (500 U/ml). Whole cell homogenates were then analyzed by Western blotting with tau antibodies. IL-1β produced an increase in high molecular weight immunoreactivity to the paired helical filament (PHF) antibody PHF-1. Antigenicity to the unphosphorylated tau specific Ab Tau-1 was lost upon prolonged incubation. These changes in antigenicity parallel the changes seen in human AD brain.

Decreased gel mobility of tau isoforms was seen upon IL-1β treatment. Conversely, co-incubation of tissue with IL-1β and increasing amounts of IL-1β receptor antagonist showed a dose dependent increase in tau mobility.

These results demonstrate that IL-1β may contribute to the abnormal phosphorylation of tau, which subsequently could decrease the stability of tau-microtubule interactions and contribute to PHF formation. Supported by NS-15076.

DEGENERATIVE DISEASE: ALZHEIMER'S—TAU PHOSPHORYLATION AND NEUROFIBRILLARY DEGENERATION

422.1

CONFOCAL LASER SPECTROMETRY FOR 3-D ANALYSIS OF ALZHEIMER PATHOLOGY Q. S. Deng[1]*, M. A. DeCoster[2], A.W. de Feijter[3], D. R. Brady[1], and Q. R. Smith[1], [1]Lab of Neurosciences, NIH, Bethesda, MD 20892; [2]Dept of Med. Neurosciences, Walter Reed Army Inst. of Res, Washington, DC 20307; [3]Meridian Instruments, Inc. Okemos, MI 48864

Senile plaques (SPs), neurofibrillary tangles (NFTs) and neuropil threads (NTs) are commonly studied using light or fluorescent microscopic methods that provide 2-D images of their distribution within tissue sections. Such methods are of limited value in studies of antigen or element localization where reagent penetration or surface structure identification are important. In theory, confocal laser scanning microscopy (CLSM) provides one means to identify the 3-D location of structures within specimens, from surface to surface. To evaluate this, the morphology and distribution of SPs, NFTs and NTs were examined in the brain of one 75-yr old patient with AD. Frozen tissue sections (3-30 μm) were cut from the hippocampus and entorhinal cortex, and stained with thioflavine S (Guntern et al., Experientia, 1992). Serial images (Z axis increment: 0.5-1.0 μm) of individual SPs, NFTs and NTs (n=20) were generated with CLSM, revealing complete penetration (3-30 μm) of the dye. SPs, NFTs and NTs on the surface were easily distinguished from those buried deeper within the section. The results demonstrate that CLSM provides a basis for determining the penetration of reagents in tissue staining and is useful as a guide for surface location of trace element distributions using SIMS.

422.2

ABNORMALITIES OF THE CALPAIN SYSTEM IN ALZHEIMER DISEASE. S. Katayama, K. Saito, A. Cataldo, T. Honda, F. Grynspan, W. Griffin, P.S. Mohan*, A.L. Schwagerl, R.A. Nixon. McLean Hospital, Harvard Medical School, Belmont, MA

Calcium-activated neutral proteinases (CANPs or calpains) are believed to be key enzymes in intracellular signaling cascades and potential mediators of calcium-induced neuronal degeneration. We have reported that the activated isoform of calpain I is significantly increased and the precursor isoform is significantly reduced in Alzheimer brain. To investigate calpain I alterations further, we raised antibodies against the four functional domains of calpain I. Using immunoblot analysis, we confirmed our previous finding (Saito et al., PNAS 1993;90:26-28) of a nearly 3-fold increase in the ratio of activated calpain I isoforms to the precursor isoform in prefrontal cortex from 13 Alzheimer patients and 9 normal control matched for age and postmortem interval ($p < 0.02$). In membrane fractions solubilized with Triton X-100, the ratio of activated to precursor isoforms was elevated 3.9-fold in Alzheimer brains ($p < 0.05$). The results with different calpain I antibodies, including an antibody specific for the precursor isoforms, were significantly correlated. Consistent with increased calpain I activation, levels of calpastatin activity were markedly reduced (Mohan et al., these proceedings). A significant correlation was observed between the degree of abnormal calpain I activation and reduction in levels of soluble APP. APP_s was not generated from membrane APP by purified calpain. By immunocytochemistry, calpain I antibodies preferentially stained neurons in human, monkey and mouse brain. Calpain I immunoreactivity was abnormally increased in some neurons displaying degenerative changes in prefrontal cortex from Alzheimer brains. These results provide further evidence that the calpain system may be activated in at-risk neurons in Alzheimer disease and play a critical role in neurodegeneration. Support: AG10916.

422.3

REDUCED CALPASTATIN ACTIVITY AND CONTENT IN ALZHEIMER BRAIN. P.S. Mohan, T.B. Shea* and R.A. Nixon, Mailman Research Ctr., Harvard Medical School, Belmont, MA 02178.

Calpains are believed to be important regulators of membrane skeleton dynamics and membrane trafficking, but mediate neurodegeneration in certain pathologic states. We previously reported evidence for abnormal activation of the calpain-calpastatin system in Alzheimer brain. Also, calpastatin, the specific protein inhibitor of calpain, was shown by immunocytochemistry to be markedly depleted in Alzheimer neocortex (Nixon et al., Soc. Neurosci. 19:198, 1992). To confirm this biochemically, we measured calpastatin levels in homogenates of prefrontal cortex from 13 Alzheimer patients and 9 normal controls matched by age and postmortem interval. Calpastatin immunoreactivity and inhibitory activity were measured in soluble and membrane fractions. In normal human brain, membrane fractions solubilized by Triton X-100 contained 40% of the total calpastatin (204 $\pm$ 15.6 units/g tissue). Calpastatin was identified by immunoblotting using affinity purified antibodies to be composed of 110, 70 and 41 kDa forms. Calpastatin inhibitory activity in membrane fractions of Alzheimer prefrontal cortex was reduced to 50% of control values ($p < 0.001$). By immunoblot analysis the most abundant calpastatin form isolated from human brain (M_r = 41 kDa) was reduced $\geq$ 65% in membrane fractions ($p < 0.001$). Cytosolic calpastatin activity was 25% lower ($p < 0.05$) but major immunoreactive forms were markedly reduced (75%, $p < 0.01$) reflecting proteolytic processing to smaller molecular weight inhibitory units. Calpastatin was not significantly altered in cerebellum. These results, together with the abnormal activation of μ-calpain reported earlier (Saito et al., PNAS 90:2628, 1993), implicate calpains in the disruption of membrane dynamics possibly leading to altered membrane protein processing and neurodegeneration in Alzheimer Disease. Supported by NIH (AG10916).

422.4

TAU IS A GLYCOPROTEIN. C.S. Arnold, R.N. Cole, G.V.W. Johnson* and G.W. Hart. Depts. of Biochemistry and *Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294

Tau is a family of microtubule-associate proteins which play a crucial role in the structure and function of the neuronal cytoskeleton. Tau in hyperphosphorylated states also forms the paired helical filaments (PHFs) of Alzheimer's disease. Although the phosphorylation of tau has been extensively delineated, other modifications have not been clearly defined. Recently a protein modification has been identified at Ser and Thr residues consisting of an O-linked N-acetylglucosamine (O-GlcNAc). This modification is found on a variety of proteins, including neurofilaments. The sites of O-GlcNAc glycosylation are indistinguishable from the phosphorylation sites of Ser/Thr kinases. Purified bovine tau was analyzed using galactyosyl transferase as a probe for terminal GlcNAcs, followed by peptide-N-glycosidase F treatment and β-elimination for linkage analysis. The results of these studies clearly demonstrate that tau is modified by O-GlcNAc glycosylation. This unique form of glycosylation may modulate tau function, and in specific neurodegenerative disorders could contribute to neuronal dysfunction and the formation of pathological lesions.

Supported by NIH grants CA42486 (GWH) and NS27538 (GVWJ).

422.5

IN-VITRO UBIQUITINATION AND DEGRADATION OF TAU PROTEINS. RS Black* Cornell University Medical College at Burke Medical Research Institute, White Plains, NY 10605.

The conjugation of ubiquitin to abnormally phosphorylated tau proteins, and the subsequent failure of their ubiquitin-mediated proteolysis is an important feature of the Alzheimer disease process. The DEAE-cellulose fraction of rabbit reticulocyte lysates containing enzymes of the ubiquitin conjugation system (Fraction II) was used to conjugate bovine ubiquitin to tau proteins prepared from twice-cycled bovine brain microtubules. In the presence of ATP, an ATP regenerating system, and hemin, which inhibits the breakdown of ubiquitin conjugates, high molecular weight ubiquitin-conjugated tau proteins accumulated. In the absence of hemin the ubiquitin conjugates did not accumulate and the tau proteins appeared to be degraded in an ATP-dependent fashion. In this system, ubiquitin-ubiquitin conjugates were detectable almost immediately in hemin-inhibited lysates, whereas tau-ubiquitin conjugates were detectable only after 1-2 hour incubations. Bovine MAP-2 was also a substrate for ubiquitin conjugation in this system. This system is being used to evaluate the effects of post-translational modifications of tau proteins on ubiquitin conjugation. Supported by the NIA (AG00504).

422.6

THE ABNORMAL PHOSPHORYLATION OF TAU PROTEIN AT SER- 202 IS PREFERENTIALLY LOCATED IN NEURITES AND PRECEDES ABNORMAL PHOSPHORYLATION AT SER- 396 IN ALZHEIMER'S DISEASE Joseph H. Su*, Brian J. Cummings and Carl W. Cotman. *IRU in Brain Aging, UCI, Irvine, CA 92717 USA.*

Both neurofibrillary tangles (NFTs) and dystrophic neurites (DNs) contain paired helical filament (PHFs) which are composed of abnormally phosphorylated PHF-tau. During formation of NFTs and DNs in AD, the cytoskeleton undergoes a sequence of changes, including hyper-phosphorylation of tau protein (for review, see Trojanowski *et al.*, '93). We sought to determine more specifically at what tau protein residue the earliest changes take place; and whether these alterations first occur within distal processes or within the soma. We used two monoclonal antibodies, AT8 and PHF-1, which selectively recognize phosphorylated Ser-202 and Ser-396 of PHF-tau protein respectively. Both antibodies stained NFTs and DNs, including dendrites and axons; however the quantity and distribution of immunoreactive deposits was different. AT8 immunoreactivity was usually found within intracellular NFTs as well as pretangle neurons, whereas PHF-1 fibrillar inclusions were detected within both intracellular and extracellular NFTs. Some strongly labeled AT8 DNs were continuous with "normal" neuronal soma in which AT8 immunoreactivity was absent or weak. However, PHF-1 positive DNs were only continuous with PHF-1 positive NFTs. In AD cases with mild dementia, numerous AT8 deposits were detected in the outer two-thirds of the molecular layer of the dentate gyrus, whereas no PHF-1 positive deposits could be found within the same region in adjacent sections. These results suggest that abnormal phosphorylation at Ser-202 of PHF-tau in DNs represents one of the earliest neuropathological changes within the neurites of vulnerable neurons and might play an important role in the initial pathogenesis of AD.

422.7

CONSTITUTIVE ALZHEIMER'S-TYPE TAU EPITOPES IN A NEURITOGENIC RAT CNS CELL LINE. M.P. Lambert*, S. Sabo, C.Zhang, S.A. Enam, and W. L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Paired helical filaments (PHFs) of Alzheimer's disease (AD) largely comprise hyperphosphorylated forms of the cytoskeletal protein tau. AD-type tau phosphoepitopes are absent from normal adult neurons, but recent studies have shown that their expression may contribute to neuritogenesis and axon differentiation in the developing nervous system. Therefore, we examined a brain nerve cell line that is spontaneously neuritogenic for possible expression of AD-type tau epitopes. The B103 rat CNS cell line was found to produce constitutively two AD-related epitopes of tau, detected by cellular immunofluorescence with the PHF-1 and Alz-50 monoclonal antibodies. Biochemical studies showed that the antibodies bound to proteins within the molecular weight range expected for phosphorylated tau isoforms. Further verification was established by use of tau antisense oligomers, which eliminated immunofluorescence due to the AD-related monoclonals and polyclonal anti-tau, but did not eliminate fluorescence due to anti-tubulin. Cells treated with tau antisense were not neurite-free. Neurites that remained were abnormal, generally short, and wavy in appearance. Cellular distribution of the tau epitopes was particularly interesting. Alz-50 immunoreactivity was found only in the cytoplasm, while PHF-1 immunoreactivity was found in the nucleus as well as the cytoplasm. Thus the two epitopes are morphologically segregated within the cell. Because subcellular segregation of tau is compromised in Alzheimer's disease, mechanisms that segregate AD-type phosphotau epitopes in B103 cells may have relevance to this neurodegenerative disorder.

422.8

OKADAIC ACID INDUCES MICROTUBULE DEPOLYMERIZATION AND THE DEGENERATION OF AXONS AND DENDRITES IN THE NT2N CULTURE SYSTEM: IMPLICATION IN ALZHEIMER'S DISEASE. S.E. Merrick* and V.M.-Y. Lee. Institute of Neurological Sciences, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The building blocks of Alzheimer's disease (AD) neurofibrillary tangles (NFTs), are paired helical filaments that are comprised of hyper-phosphorylated forms of tau (i.e. PHF-tau) and the accumulation of PHF-tau may be due to an inactivation or down-regulation of brain phosphatases in NFT bearing neurons. Since excessively phosphorylated PHF-tau is unable to bind to microtubules (MTs), then conversion of normal tau into PHF-tau by the inhibition of protein phosphatases could lower the level of tau that binds MT, destabilize MTs, disrupt axonal transport, and lead to the "dying back" of axons in AD. To test this hypothesis, we developed an *in vitro* neuronal culture model that allows us to examine the consequences of the inhibition of protein phosphatases on the neuronal cytoskeleton. The model is based on neurons (NT2N) derived from a human teratocarcinoma cell line (NT2) that are treated with retinoic acid to induce differentiation and then exposed to okadaic acid (OKA), a potent phosphatase inhibitor. OKA treatment increased tau phosphorylation in NT2N neurons resulting in the inability of highly phosphorylated tau to bind MTs. Concomitant with the increase in tau phosphorylation, there was an increase in the depolymerization of MTs which was due to the increased conversion of stable Glu-tubulin in MTs to monomeric Tyr-tubulin. As a consequence of the loss of Glu-MTs, there was a "dying back" of the axons of NT2N neurons prior to the complete destruction of dendritic and cellular MTs. Our results suggest that the inhibition of protein phosphatases in neurons leads to the destabilization of the MT network in axons by: (1) Increased tau phosphorylation thus reducing MT binding; and (2) Activation of the tubulin-tyrosine ligase that converts Glu-tubulin in MTs to free Tyr-tubulin.

422.9

THE IN VITRO AND IN SITU PROTEOLYSIS OF TAU BY CALPAIN. R.P. Guttmann* and G.V.W. Johnson. Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Tau is a microtubule-associated protein that is found in the nucleus and associated with ribosomes, as well as bound to microtubules. In Alzheimer's disease (AD) a hyperphosphorylated form of tau forms the characteristic paired helical filaments (PHFs). Calpain is a calcium-dependent protease that is found in high concentrations in neurons, and is postulated to be involved in the limited hydrolysis of specific structural proteins. In addition, the levels of the calcium-activated form of calpain are increased in AD brain. Previously we have demonstrated that in vitro bovine tau and human tau recombinant tau isoforms are substrates for calpain. In addition, phosphorylation of bovine tau in vitro by cAMP-dependent protein kinase (cAMP-PK) increases its resistance to calpain mediated degradation. In this study we demonstrate that phosphorylation of the longest human tau isoform (441 a.a., T4L) by cAMP-PK or cyclin-dependent protein kinase 1 ($p34^{cdc2}$/cyclin B) significantly inhibits calpain I-mediated degradation. In contrast, phosphorylation of T4L by the proline-directed protein kinase, ERK1 ($p44^{mpk}$) did not alter calpain sensitivity, although alterations in electrophoretic mobility were clearly evident. Evidence is also provided to indicate that tau is a substrate for calpain in situ. Treatment of human neuroblastoma LA-N-5 cells, which have been differentiated into a neuronal phenotype, with the calcium ionophor, ionomycin, results in the degradation of tau. This calcium-induced proteolysis of tau was apparently inhibited by the selective calpain inhibitor, cbz-leu-leucinal, suggesting that the loss of tau was due to calpain-mediated hydrolysis. These results indicate that tau is an in situ substrate for calpai,n and that site-selective phosphorylation modulates the susceptibility of tau to calpain-mediated hydrolysis. Supported by NIH grants #NS27538 and AG06569.

422.10

TAU IS DEPHOSPHORYLATED IN SITU BY THE CALCIUM-DEPENDENT PHOSPHATASE, CALCINEURIN, IN RAT BRAIN CEREBRAL CORTICAL SLICES. L. M. Fleming* and G. V. W. Johnson, Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Tau is a family of closely related microtubule-associated phosphoprotpeins which are involved in the development and maintenance of neurons. The phosphorylation state of tau modulates it's ability to promote microtubule assembly, and aberrant phosphorylation has been postulated to contribute to the formation of paired helical filaments (PHFs) in Alzheimer's disease (AD). In vitro, tau has been shown to be phosphorylated by a variety of protein kinases. Recently, the in vitro dephosphorylation of tau by specific phosphatases (PP) has been studied. PP1 and 2C dephosphorylate tau which has been phosphorylated by cAMP dependent protein kinase (cAMP-PK), while PP2A and the calcium/calmodulin phosphatase, calcineurin (PP2B), dephosphorylate tau at sites phosphorylated by either Ca^{2+}/calmodulin dependent protein kinase or cAMP-PK. Additionally, PP2A, PP1 and calcineurin have been shown to dephosphorylate sites on PHF-tau. However, the in vivo role of these phosphatases is unclear.

In this study, we examined the effect of increasing intracellular calcium concentrations on the phosphorylation state of tau in rat brain cerebral cortical slices using the glutamate analog, NMDA, and depolarizing conditions (50 mM KCl). Rat brain slices were incubated in the presence of $^{32}P_i$ prior to drug addition. After addition of appropriate agents, incubations were terminated and tau was immunoprecipitated from 250 µg of each sample. Tau was ^{32}P labeled under basal conditions. Addition of KCl and NMDA resulted in a 40 % decrease in ^{32}P incorporation into tau while addition of KCl or NMDA alone had no effect on tau phosphorylation. The noncompetitive NMDA receptor antagonist, MK801, completely inhibited the effect of KCl and NMDA. Cyclosporin A (CsA) has been shown in vitro to complex with cyclophilin and specifically bind to and inhibit calcineurin. In our study, CsA completely abolished the dephosphorylation of tau induced by KCl and NMDA treatment. Taken together, these results indicate that depolarization was required prior to activation of NMDA receptors to activate calcineurin, which in turn dephosphorylated tau. These effects were specifically blocked by MK801 and CsA. These results demonstrate that in situ, tau is dephosphorylated by calcineurin. Supported by NIH grants AG05643, AG066569 and NS27538.

422.11

PHOSPHORYLATION STATE OF TAU IN RAT BRAIN DURING DEVELOPMENT IS REGULATED BY PHOSPHOPROTEIN PHOSPHATASES. M. Mawal-Dewan*, J. Henley, J.Q. Trojanowski, and V. M.-Y. Lee. Dept. Path. Lab. Med., Univ. of Penn. Sch. of Med., Phila, PA - 19104.

The paired helical filaments (PHFs) of Alzheimer's disease (AD) are composed of highly phosphorylated tau proteins. Several of these sites were found to be phosphorylated in PHF-tau, fetal-tau, but not in adult brain tau. To determine the regulation of phosphorylation at these sites during development, we isolated tau from fresh rat brain in the presence of the phosphatase inhibitor okadaic acid (OK) to obtain tau in its native state of phosphorylation *in situ*. Fetal tau isoform was highly phosphorylated from embryonic day 18 (E18) until post-natal day 11 (P11) after which the immature isoform diminished concomitant with the decrease in phosphorylation and the appearance of the mature isoforms. In contrast to previous studies, adult and aged rats (20 mths) were found to be phosphorylated at reduced levels suggesting that Thr^{181},Ser^{202}, Thr^{231}, Ser^{396} and Ser^{404} are normal sites of phosphorylation in adult rats. We also show that the inclusion of OK in the assembly buffer has a transient effect on the abilities of tau to bind MTs at any developmental stages. However, an activation of phosphatases was detected after P12 since in the absence of OK tau was shown to be partially dephosphorylated. We further demonstrate that protein phosphatase 2A (PP2A) and 2B (PP2B) from the adult rat brain could dephosphorylate tau efficiently in a site specific manner, whereas protein phosphatases from P6 had no effect indicating that OK sensitive tau after P12 may be regulated by the *de novo* induction of adult brain phosphatases. Collectively, these findings suggest that PP2A-like phosphatases are involved in regulating the adult phosphorylation state of tau *in-vivo*, and they open the possibility that the generation of hyperphosphorylated tau leading to PHF formation in AD may be controlled by similar phosphatases.

422.12

ALZHEIMER TYPE PHOSPHORYLATIONS OF TAU PROTEIN ARE DEVELOPMENTALLY EXPRESSED *IN VITRO*. **C.K. Combs*, P.D. Coleman, and M.K. O'Banion. Departments of Neurobiology and Anatomy and Neurology, University of Rochester, Rochester, N Y 14642.**

It has been shown that certain Alzheimer type tau phosphorylations occur embryonically in humans and postnatally in rats. Determining the nature of the regulatory mechanisms involved in these events may provide a better understanding of the aberrent phosphorylation of tau occuring in Alzheimer's Disease.

We have utilized embryonic rat hippocampal cultures to investigate the expression of specific Alzheimer type tau phosphorylations during *in vitro* differentiation. The mouse monoclonal antibodies PHF-1 and Tau-1 recognize a phosphorylation on Ser 396 and the lack of phosphorylation on Ser 199/202, respectively. Using PHF-1 and Tau-1 we have observed a temporal expression of phosphorylation by immunocytochemical and Western blot analyses. Phosphorylation at the PHF-1 epitope is observed at the earliest time point *in vitro* (i.e. embryonic day 18). In contrast, phosphorylation at the Tau-1 epitope was detected around day 4 *in vitro* and appears to increase over the two weeks examined.

The temporal expression of these phosphorylation events suggests different regulatory mechanisms for each. Future investigations with this *in vitro* developmental system may not only define the kinases and/or phosphatases involved, but may also provide clues about the physiological roles of these phosphorylation events.

(PHF-1 and Tau-1 Abs were obtained from Peter Davies and Lester Binder, respectively)

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422.13

THE REGULATION OF TAU PHOSPHORYLATION IN ISOLATED BOVINE BRAIN MICROTUBULES: ACTIVATION BY EXCESS ATP AND INHIBITION BY APOLIPOPROTEIN E. **Q.LU ,R.Kanumury and J.G.Wood*,Dept.Anatomy and Cell Biology,Emory Univ. Sch. of Med.,Atlanta, GA 30322**

Supply of excess ATP to isolated brain microtubules induced Alzheimer type phosphorylation of tau proteins, providing an in vitro model to study tau phosphorylation and its regulation. Although immunoblot analysis indicated that MAP kinase and CDK are present in microtubules obtained by purification, neither purified MAP kinase or CDK could further increase the level of tau phosphorylation induced by excess ATP. Rather, the addition of CDK 1/cyclin B to microtubules partially reverses this type of tau phosphorylation. Furthermore, ATP induced tau phosphorylation is inhibited by treatment of microtubules with apolipoprotein E. The effect of apolipoprotein E is specific and dose dependent. Inhibitors of Ca^{2+}/calmodulin-dependent protein kinase and cAMP-dependent protein kinase have no profound effects on the phosphorylation of tau. Partial removal of tubulin from microtubules using taxol abolishes ATP induced tau phosphorylation, suggesting that tubulin may be involved in the regulation of tau phosphorylation by signaling proteins associated with microtubules.AG 11123, NS 27847, NS 30435.

422.14

ALZ-50 AND PHF-1 IMMUNOREACTIVITY NEAR β-AP INJECTIONS IN THE CEREBRAL CORTEX OF SHEEP P.T. Nelson* and C.B. Saper
Beth Israel Hospital and Harvard Medical School, Boston, MA 02115.

We have recently discovered that neurons in the cerebral cortex of sheep and goats develop Alzheimer-type neurofibrillary degeneration (NFD) in the absence of beta-amyloid peptide (β-AP) deposition during aging. Hence, these domesticated animals may provide a novel model for studying the mechanism of induction of Alzheimer-type NFD.

In an effort to induce experimental neurofibrillary pathology *in vivo*, we injected β-AP (1µl) into the cerebral cortices of two five-year old sheep. Following one or two weeks' survival, brains were formalin fixed and blocks were sectioned at 50µm. Sections were stained using the immunoperoxidase technique and the monoclonal antibodies Alz-50 and PHF-1. Some sections were visualized with electron microscopy. Adjacent series were stained with thioflavine, thionin, and antiserum against β-AP.

In the animal that survived one week, the β-AP injections caused necrosis. No Alz-50 or PHF-1 immunoreactive dystrophic neurites were seen in direct apposition to the injections within deep cortical layers. However, near the cortical surface, some Alz-50 and PHF-1 immunoreactive neurites appeared that were more swollen than normal immunoreactive neurites. Alz-50 immunoreactive neurites, when viewed via electron microscopy, contained large vesicles. In the animal that lived for two weeks, similar staining was seen, except that some PHF-1 immunoreactive neurites had inflitrated the injection regions.

The current study indicates that, under the conditions we employed, injected β-AP does not induce NFD per se, but may trigger a regenerative response in which modified tau proteins are present in neurites.

422.15

DIFFERENTIAL LOCALIZATION OF APOLIPOPROTEIN E AND PHF IMMUNOREACTIVITY IN THE NEO AND LIMBIC CORTEX, AND NUCLEUS BASALIS IN ALZHEIMER'S DISEASE (AD): IMPLICATIONS FOR TANGLE PATHOGENESIS. W.C. Benzing* and E.J. Mufson. Dept. of Neurol. Sci. Alzheimer's Disease Ctr., Rush Presb. St. Luke Med. Ctr., Chicago, IL 60612.

The localization of apolipoprotein E (ApoE) in relation to beta-amyloid (βA4) and paired helical filament (PHF) immunoreactivity was examined in the nucleus basalis (NBM), amygdala (AMY), and entorhinal (EC), temporal and insular cortices of patients with AD (n=8) and Down's syndrome (DS, n=3), age matched normals (AMC, n=8), and age matched nondemented cases with numerous senile plaques (HPND, n=5). ApoE immunostained plaques, neurons, and extracellular tangles in each group. ApoE's immunostaining of neurons differed dramatically from PHF depending upon brain region and diagnosis. In the AD and DS brain, numerous ApoE-ir neurons closely matched PHF in distribution and quantity in the NBM, AMY and EC. Although PHF-ir temporal and insular neurons were similar in density to the AMY and EC, ApoE-ir neurons were 4-fold fewer than PHF-ir neurons. In the HPND cases, many PHF-ir neurons were observed in the NBM, AMY and EC but were less than in AD. In contrast to these same regions in AD and DS, very few ApoE-ir neurons were observed relative to PHF. These findings suggest that PHF immunoreactivity precedes that of ApoE indicating ApoE may be binding to tangles only in their later stages of formation. Since in the HPND cases ApoE recognizes only a few of the PHF-ir neurons in the NBM, AMY, and EC, which are among the earliest brain regions affected in AD, it is possible that ApoE plays a secondary role in tangle formation. Supported by NIH grant AG10161-03.

422.16

NEUROFIBRILLARY TANGLE NEURONS IN ALZHEIMER'S DISEASE ARE ASSOCIATED WITH A LOSS OF SYNAPTOPHYSIN MESSAGE. (L.M. Callahan*, J.E. Cheetham, W. Vaules, and P.D. Coleman.) University of Rochester Medical Center, Neurobiology and Anatomy, Rochester, NY 14642). Recent evidence indicates the best correlate with the degree of dementia of AD may be the loss of synapses (e.g. DeKosky and Scheff, 1989, Terry et al., 1990). We hypothesized neurons containing tangles lose synapses due to the cytoskeletal disruption of the neuron. To investigate this possibility, we combined immunocytochemistry to identify tangle-containing neurons with *in situ* hybridization for selected messages. Message levels of synaptophysin, poly A, and cathepsin D were determined in serial hippocampal sections from 3 AD cases and 2 age-matched controls. A decrease in the level of synaptophysin, a synaptic vesicle associated protein, was detected in a majority of tangle-bearing neurons relative to levels in neighboring non-tangle neurons. Poly A (total) message levels were similar in many tangle-bearing neurons relative to non-tangle bearing neurons. An increase of message for cathepsin D, a lysosomal enzyme, was seen in many tangle neurons relative to non-tangle neurons. The demonstration of decreased message level for a protein related to synaptic function in NFT neurons would indicate that it is the NFT neurons that are correlated with synaptic loss in AD. (Supported by NIH AG00107,NIH AG01121, NIH LEAD AWARD AG09016, Alzheimer's Disease Center Grant NIH AG08665, and the American Health Assistance Foundation).

422.17

NEUROFIBRILLARY TANGLES (NFT) IN ALZHEIMER'S DISEASE: DOUBLE IMMUNOLABELING STUDIES OF ASSOCIATED *VS.* INTEGRAL PROTEINS. D.W. Dickson*, A. Ivanushkin, E. Wu, P. Davies and S.-H. Yen. Division of Neuropathology, Albert Einstein College of Medicine, Bronx, New York 10461.

Neurofibrillary tangles (NFT) composed of paired helical filaments (PHF) are the most characteristic histopathological feature of Alzheimer's disease. Recent molecular and biochemical studies have provided conclusive evidence that the major integral protein is an abnormal tau protein that is hyperphosphorylated, referred to as PHF-tau. PHF-tau antigens are present in NFT within the cytoplasm of living neurons [intracellular NFT (NFTi)] and also in NFT in the extracellular space after the neuron with NFT dies, so-called extracellular NFT (NFTe). Epitopes in the amino half of tau protein (*e.g.*, Alz-50 epitope) are susceptible to proteolysis and are lost in NFTe. Neurons in lamina II of the entorhinal cortex (EC-II) are unusually susceptible to NFT, while those in the dentate gyrus are unusually resistant to NFT. By the time AD is advanced, most of the NFT in the entorhinal cortex are NFTe, while most of the NFT in the dentate gyrs are NFTi. Using their distribution in the hippocampus as an index to their most probable type and double-labeling immunocytochemistry of cryostat or briefly-fixed vibratome sections, we further characterized NFT in AD. Monoclonal antibodies to NFT that do not cross-react with tau proteins (Ab39 and Ab69) were used as a marker for both NFTe and NFTi, while Alz-50 served as a marker for NFTi. NFTi, but not NFTe, were labeled by monoclonal and polyclonal antibodies to $p34^{cdc2}$. The anti-$p34^{cdc2}$ antibodies also labeled granular cytoplasmic and perinuclear structures consistent with so-called "pre-tangles". Monoclonal and polyclonal antibodies to apolipoprotein-E labeled primarily NFTe. NFTe were also inconsistently labeled with antibodies to C1q, Aβ and ubiquitin. These results suggest that in early formative stages NFT are composed of abnormal tau protein in close association with kinases, such as $p34^{cdc2}$, that are capable of phosphorylating tau and that only in later stages of evolution, after the neuron has died, is there substantial association with apolipoprotein-E, Aβ and C1q. (Supported by NIA grants AG06803, AG01136 & AG04145)

422.18

AUTODETECTION AND LARGE-AREA MAPPING OF NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE. *L.S. Hibbard*, D.W. McKeel, Jr., Joseph L. Price, Departments of Neurology and Neurological Surgery, Pathology, and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.*

Neurofibrillary tangles (NFT), seen in normal aged and Alzheimer's disease (AD) brains, are inhomogeneously distributed in brain. NFT anatomic distributions and densities have been shown to correlate with clinical dementia severity. To quantitate NFT deposition, we have developed computer programs which automatically locate NFT in digital micrographs of postmortem tissues stained with either the Gallyas silver stain or one of several immunostains (anti-PHF, anti-tau). Stained objects are detected by template correlation and extracted as discrete objects whose gray level and morphometric properties are used for classification and characterization. The NFT are sorted from all other detected objects using a conventional Bayesian classifier. NFT can be mapped over arbitrary, large areas of tissue, imaged as mosaics of adjacent, non-overlapping, digital microscope fields. Arbitrary regions of interest (ROI) are sketched, using computer graphics, in images of the entire mosaic at low magnification. The ROI guides subsequent processing to only those parts of fields coincident with the ROI. This method can map PHF/tau-immunoreactive cells in very mildly demented or normal aged brains. (Support: NIH 5P50-AG05681)

422.19

ALZHEIMER'S DISEASE-RELATED NEUROFIBRILLARY CHANGES IN INDIVIDUALS WITH AND WITHOUT A HISTORY OF ALCOHOL ABUSE. A PREVALENCE ANALYSIS OF STAGED AUTOPSY CASES. H.Müller,†, H.Braak,†, E.Braak,*†, J.Bohl,‡ and T.G.Ohm,†. †-ZMorph. J.W.Goethe Universität, 60590 Frankfurt; ‡-Abt. Neuropathologie, J.Gutenberg-Universität, 55131 Mainz, Germany.

The role of alcohol abuse as a risk factor for Alzheimer's disease (AD) is still disputed. So far, there is, however, no autopsy-based study analyzing the prevalence of AD-related neuropathological changes in alcoholics.

This study is based on a sample of 67 age-matched pairs of brains staged for AD-related neurofibrillary changes and 54 age-matched pairs of brains staged for ß/A4-amyloid deposits. Cases were included in the alcohol group when there was strong evidence for alcohol-related changes at autopsy and/or an anamnestic history of relevant alcohol abuse. Controls were negative for both criteria. The non-parametric Wilcoxon-Mann-Whitney-U-test revealed no significant differences in the prevalence of the stages of AD-related neurofibrillary changes ($U=2085$; $Z=0.7835$; $p\geq0.43$; Hodges-Lehmann-estimator for $X1-X2=0$; Mann-Whitney's-estimator for $P=P(X1<X2)=0.46$). The $\chi2$-test equally detected no differences between age-matched controls and the alcohol group ($\chi2=8.093$; $p=0.62$). Testing for differences in the prevalence of stages of ß/A4-amyloid deposits equally revealed no significant differences between the respective groups (U-test: $U=1425.5$; $Z=0.2706$; $p\geq0.78$; Hodges-Lehmann-estimator for $X1-X2=0$; Mann-Whitney's-estimator for $P=P(X1<X2)=0.5$; $\chi2$-test: $\chi2=2.214$; $p=0.696$).

A history of alcohol abuse thus does not seem to be a risk factor for the development and/or progression of Alzheimer's disease-related changes.
Supported by the DFG (TGO, HB)

423.1

THE EFFECTS OF IN VITRO ISCHEMIA ON PROTEINS FROM THE RAT HIPPOCAMPAL SLICE. J. Murata and K.M. Raley-Susman*. Dept. of Biology Vassar College, Poughkeepsie NY 12601.

Our previous work has demonstrated that total protein synthesis is dramatically inhibited following a 5 min episode of in vitro ischemia in neurons in the rat hippocampal slice. In the present report, we explored the responses of individual proteins to this insult. We prepared 500 µm slices from adult male Sprague-Dawley rats and incubated them in a Ringer's buffer equilibrated with 95% O_2-5% CO_2 at 37°C. Following a 2 hr preincubation period, experimental slices were exposed for 5 min to Ringer's lacking glucose and oxygen. Slices were allowed to recover for 30 min, 1 hr, 2 hr or 3 hr, then were moved into ice-cold homogenization buffer containing protease inhibitors. 15 µg of total protein, determined with the Bradford protein assay, was loaded onto a 7.5% polyacrylamide gel and was separated in one-dimension according to Laemmli. Some gels were silver-stained and analyzed by densitometry. Proteins from some gels were transferred to immobilon membranes and exposed to antibodies against hsp-72/73, GFAP, MAP-2 or calbindin-D-28$_K$. In some experiments, new protein synthesis was determined by exposing slices to ^{35}S-methionine and silver-stained gels were processed for autoradiography. Immunoblots and autoradiographs were also analyzed by densitometry.

In vitro ischemia inhibited new protein synthesis of most proteins analyzed. The total amount of some proteins was reduced at 2 hr postischemia, while that of others was unchanged. One notable exception was the response of hsp72/73 to in vitro ischemia. This protein showed a dramatic increase in new synthesis 30 min and 1 hr after ischemia, followed at 3 hr by a 50% decrease in new synthesis. At 2 hr following the insult, the total amount of hsp72/73 was significantly increased in postischemic slices, when compared with control slices. Thus, while total amounts of protein remains unchanged following ischemia and total new synthesis is reduced, the responses of individual proteins to ischemia varies considerably. These individual responses could play an important role in the response to ischemia.

423.2

BRIEF PRECONDITIONING HYPOXIA PROTECTS HIPPOCAMPAL NEURONS FROM SUBSEQUENT HYPOXIA-INDUCED DAMAGE VIA MECHANISMS REQUIRING ONGOING PROTEIN SYNTHESIS. A.T. Gage* and P.K. Stanton. Departments of Neuroscience & Neurology, Albert Einstein Coll. Med., Bronx, NY 10461

Pre-exposure of rat hippocampus to a short period of hypoxia increases the resistance of CA1 pyramidal neurons to a normally fatal hypoxic insult. One hypothesis for this protective effect is that a short hypoxic insult causes the synthesis of new proteins which protect neurons during subsequent, longer hypoxic insults. We tested this idea by using the reversible protein synthesis inhibitor cycloheximide (CYCLO) and determined if it prevented the priming neuroprotection during hypoxia. We used a bipolar stimulating electrode to activate Schaffer collateral axons in area CA1 of hippocampal slices every 60 s, and recorded evoked dendritic population epsps in stratum radiatum. Naïve slices subjected to a 15 min period of severe hypoxia (95% N_2/5% CO_2) showed only 1.5 ± 1.5% recovery of epsp slope upon reoxygenation. In contrast, in slices subjected to a 5 min preconditioning hypoxic insult, followed by 2 h of recovery in normal O_2, the 15 min hypoxic episode now led to 89 ± 8.12% recovery upon reoxygenation. When slices were bathed in 10µM CYCLO 1 h before the initial 5 min period of hypoxia, followed by a 2 h drug washout in normal O_2, the 15 min hypoxic challenge allowed only 20 ± 12.54% epsp recovery. In contrast, neither the NMDA receptor antagonist D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5, 20 µM) nor the AMPA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10µM) prevented the protective priming caused by brief hypoxia. Our data support the hypothesis that brief periods of hypoxia trigger compensatory protective events that require ongoing protein synthesis, but not activation of NMDA or AMPA glutamate receptors. Elucidation of neuroprotective protein products stimulated by hypoxia will have important therapeutic consequences to our understanding of stroke-induced brain damage.

423.3

THE RESPONSES OF ISCHEMIA-RESISTANT NEURONS AFTER TRANSIENT ISCHEMIA IS DIFFERENT FROM THOSE OF ISCHEMIA-VULNERABLE NEURONS: AN *IN VIVO* INTRACELLULAR RECORDING STUDY. Z. C. Xu* and W. A. Pulsinelli Dept. of Neurology, University of Tennessee Memphis. Memphis, TN 38163.

Spiny neurons in neostriatum and CA1 pyramidal neurons in hippocampus will degenerate after transient ischemia while giant aspiny neurons, dentate granule cells and CA3 neurons in the same region survive. To reveal the neurophysiological mechanisms associated with such selective neuronal injury, we compared the responses to transient ischemia in ischemia-resistant neurons with those of previously published data on ischemia-vulnerable neurons (Soc. Neurosci. Abst.682.18).

Intracellular recording *in vivo* was performed on halothane anesthetized Wistar rats. Severe forebrain ischemia was induced by 4-vessel occlusion in normothermic animals for 5-8 minutes. Responses of aspiny neurons, dentate granule cells and CA3 pyramidal neurons were studied following ischemia. The recorded neurons were identified by intracellular staining with neurobiotin.

The latency of ischemic depolarization (ID) induced in ischemia-resistant neurons (~3.5 min) is significantly longer than that of ischemia-vulnerable neurons (~2.5 min). The amplitude of ID in aspiny neurons (~30 mV) is smaller than that in spiny neurons (~50 mV) in neostriatum. Unlike ischemia-vulnerable neurons, the spike threshold and membrane input resistance of ischemia-resistant neurons did not increase after recirculation. No suppression of repetitive firing was observed in ischemia-resistant neurons. Such differences between ischemia-resistant and ischemia-vulnerable neurons may clarify mechanisms leading to cell death.

423.4

STRESS PROTEIN INDUCTION IS NOT REQUIRED TO EXPRESS ISCHEMIC TOLERANCE IN THE GERBIL. H. Abe and T.S. Nowak, Jr.* Dept. of Neurology, Univ. of Tennessee, Memphis, TN 38163.

Brief ischemia induces tolerance to subsequent more severe insults. Preferential induction of the heat shock protein, hsp72, in vulnerable hipppocampal neurons after brief ischemia suggests that the stress response could contribute to ischemic tolerance. Temperature during early recirculation can influence the severity of ischemic injury in the gerbil as well as the expression of hsp72. We have used the temperature dependence of hsp72 expression to assess its role in ischemic tolerance.

Gerbils were subjected to 2 min bilateral carotid artery occlusion followed by 90 min recirculation under halothane anesthesia, during which temperature was either maintained continuously at 37 °C (normothermic, NT) or elevated to 39.5 °C between 15 and 60 min recirculation (hyperthermic, HT). A 5 min ischemia was produced 2 d after the priming challenge, and injury to CA1 was assessed 7 d after this test insult. In other animals hsp72 induction was evaluated by in situ hybridization 3 h after 2 min ischemia in the NT and HT groups.

Control neuron density (295±11) was severely reduced after 5 min ischemia in naive animals (16±4), and striking protection was observed in both NT and HT pretreatment groups (191±96 and 92±101, respectively). Reduced cell density in the HT group reflected a modest loss seen with the priming insult alone. Most importantly, 90% of 2 min NT animals showed significant protection of CA1 neurons. In contrast, only 50% of animals subjected to 2 min NT expressed hsp72 mRNA. Hsp72 induction is therefore not required for ischemic tolerance, which apparently may be achieved following insults that remain below the threshold for neuronal injury as defined by hsp72 expression.

423.5

MAP2 IMMUNOREACTIVITY AS AN INDEX OF PATHOLOGY IN RAT HIPPOCAMPAL SLICES. Q. Zhou* and T. S. Nowak, Jr. Departments of Anatomy & Neurobiology and Neurology, University of Tennessee, Memphis, TN 38163

Loss of microtubule-associated protein 2 (MAP2) staining occurs in slices following anoxic incubation, and is a prominent feature of postischemic neuronal injury. Using various markers we have begun to systematically evaluate the pathology associated with slice preparation, with the goal of establishing a baseline for in vitro anoxia/aglycemia studies in hippocampal slices of adult rats. In this study we examined changes in MAP2 immunoreactivity during routine slice incubation.

Male Wistar rats were decapitated under halothane anesthesia and 400 µm vibratome slices cut and incubated in artificial cerebrospinal fluid equilibrated with 95% O_2/5% CO_2 at 34°C. At t=0, 1, 2, 3, 4 and 6 h slices were fixed in 4% paraformaldehyde, and 50 µm vibratome sections prepared for immunocytochemistry. MAP2 was visualized with a monoclonal antibody (Sigma M-4403) and peroxidase detection.

The normal in vivo pattern of dendritic MAP2 immunoreactivity was found in freshly cut slices (t=0). This morphology was sometimes well-preserved through 4-6 h incubation, but was lost during further incubation. In many cases, however, early changes in MAP2 staining could be detected within 1 h, with preferential loss in CA1 and the upper blade of dentate gyrus, accompanied by an increased signal in the cell body, comparable to the changes reported during anoxic incubation. Changes in MAP2 staining were well correlated with loss of Jun immunoreactivity. MAP2 therefore provides a sensitive index of slice quality that may be of general use in evaluating preparation conditions, and for comparison with other criteria of slice function.

423.6

AGING, ENERGY METABOLISM, AND THE ABILITY OF RAT HIPPOCAMPAL SLICES TO SURVIVE ANOXIA. C.-P. Chih[1] and E.L. Roberts, Jr.[1,2]*. [1]Department of Neurology, University of Miami School of Medicine, and [2]Geriatric Research, Education, and Clinical Center, Miami VA Medical Center, Miami, FL 33136

We examined whether (1) decreased ATP and PCr levels before anoxia, (2) inefficient ATP or PCr use during anoxia, (3) less recovery of ATP or PCr after anoxia, or (4) a diminished capacity for oxidative metabolism contribute to the increased vulnerability of aging brain tissue to anoxia. Hippocampal slices from 6-9, 16-19, and 26-29 month old Fischer-344 rats were exposed to physiological solutions containing 5-20 mM glucose or 20 mM sodium lactate. After an initial period of normoxia (95% O_2, 5% CO_2), slices were subjected to anoxia (95% N_2, 5% CO_2). Normoxia was returned one minute after onset of anoxic depolarization. ATP and phosphocreatine (PCr) levels, extracellular K^+ activity (K^+_o), and synaptic transmission were assessed in slices before, during, and one hour after anoxia. ATP and PCr levels in slices displayed no age- or glucose-related differences before or after anoxia, and were not correlated to postanoxic recovery of synaptic transmission. Thus, age-related vulnerability to anoxia was not due to pre- or postanoxic alterations in ATP or PCr. Slices exposed to 20 mM lactate exhibited no age-related differences in onset of anoxic depolarization during anoxia, and in recovering K^+_o homeostasis and synaptic transmission after anoxia. Thus, age-related differences in vulnerability were not due to inefficient conversion of PCr to ATP, or to a decreased capacity for oxidative metabolism. These results may mean that age-related decreases in glycolysis play a prominent role in the brain's vulnerability to anoxia. (Supported by a research grant from NIA (AG08710))

423.7

RELEASE OF (^{3}H)-D-ASPARTATE OR ENDOGENOUS GLUTAMATE FROM RAT HIPPOCAMPAL SLICES DURING IN VITRO ISCHEMIA IS MEDIATED BY A HIGH AFFINITY NA-DEPENDENT CARRIER. V. Roettger* and P. Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706.

In Vitro Ischemia (deprivation of oxygen and glucose=IVI) induces a several-fold increase in the release of endogenous glutamate (GLU) and accumulated ^{3}H-D-Aspartate (ASP). The release mechanism is unknown although reversal of the Na-coupled high-affinity glutamate transporter has been widely suggested. We examined this hypothesis using two competitively transported inhibitors of the Na-dependent carrier- D,L-threo-B-hydroxyaspartate (THA) and L-trans-pyrrolidine-2,4-dicarboxylate (tPDC).

Slices were loaded with THA or tPDC (0.5mM) for 60' and then with ^{3}H-D-ASP (0.25uCi/ml) for 30' after which they were exposed to IVI or veratridine (100uM) for 3 successive 5 minute periods at 37°C.

Both veratridine and IVI increased the release of ASP and GLU over basal levels. Intracellular THA or tPDC blocked these increases by 55% or greater.

This response differs from that seen with extracellular dihydrokainate (DHK, 0.5mM), a non-transported inhibitor, which blocked the increase in veratridine-induced ASP and GLU release but not the increase in release induced by IVI (Soc. for Neurosci Abst., 18:1267, 1992).

These results suggest that glutamate release during *in vitro ischemia* is largely mediated by a high affinity Na-dependent transporter sensitive to THA or tPDC but insensitive to DHK. This pharmacology matches that of the putative neuronal carrier (EAAC1 - Kanai and Hediger, Nature, 360:467, 1992) and suggests that the released glutamate originates from neuronal elements. The pharmacology of inhibition of veratridine-induced release suggests that veratridine releases glutamate from both neuronal and glial elements (Pines et al., Nature, 360:464, 1992).

423.8

DELAYED TREATMENT WITH APV AMELIORATES PHYSIOLOGICAL DAMAGE FOLLOWING MODERATE HYPOXIA IN RAT HIPPOCAMPAL SLICES. Chen, Z-F., Schottler, F., Kassell, N.F*., and Lee, K.S. Department of Neurosurgery, University of Virginia, Charlottesville, VA, 22908.

Continuous application of the glutamate receptor antagonist, DL-2-amino-5 phosphonovaleric acid (APV), improves substantially the recovery of population excitatory postsynaptic potentials (pEPSPs) after a moderate hypoxic challenge when treatment is initiated prior to hypoxia. (Chen *et al*, 1994 Stroke; 1994;25:161). Since pathophysiological damage under these "penumbra-like" conditions was related to the duration of hypoxia, the aim of present experiment was to examine the time course of effectiveness of APV treatment and determine whether delayed treatment with APV is neuroprotective. Rat hippocampal slices were submerged in artificial cerebrospinal fluid at 34° C, and pEPSPs were recorded in *stratum radiatum* of CA1 in response to activation of Shaffer-Collateral/commissural fibers. Stable responses were recorded for at least 20 min under normoxic conditions (PO_2=450-600mmHg) and hypoxia was induced for 1 hour (PO_2=40-70mmHg) by substituting 95%N_2:5%CO_2 for 95%O_2:5%CO_2 in the gas supply. Slices were treated with 100μM APV for 30 minute periods initiated at various points during hypoxia and reoxygenation. Treatment with APV during the last 15 minutes of hypoxia and first 15 minutes of reoxygenation enhanced significantly the recovery of pEPSP slope and amplitude relative to that observed in non-treated controls. Other post-treatment paradigms were less effective in providing neuroprotection. In agreement with previous studies, the absence of profound negative shifts in the DC potential and persistence of the fiber volley indicate that hypoxic depolarization did not occur in these experiments. These results demonstrate that delayed treatment with APV, restricted to late hypoxia and/or early reoxygenation, ameliorates substantially physiological damage occurring during prolonged moderate hypoxia. (Supported by NS30671 to KSL).

423.9

ANOXIA CAUSES CLEAVAGE AND REARRANGEMENT OF CYTOSKELETAL PROTEINS IN RAT CORTICAL NEURONS. J.E. Friedman,* J. Helenius and G.G. Haddad. Yale Univ. Sch. Med., Dept. Pediatrics, Sec. of Respiratory Medicine, New Haven, CT 06520

We have previously shown that oxygen deprivation causes an increase in intracellular Ca^{2+} and Na^+ in central neurons. Concurrent with these events, we have observed, in both hippocampal and cortical neurons, changes in morphology, including swelling, process retraction and bleb formation (Friedman & Haddad, J. Neurosci. (1993) 13:63, Brain Res. (1994) 641:57). We hypothesized that cytoskeletal elements might be cleaved during anoxia, thus making the neuron more susceptible to osmotic stress and undergo morphologic change. Microfilaments are not only involved in maintaining a cell's structural integrity, but have also been implicated in the regulation of Na^+ and Ca^{2+} influx through, e.g. NMDA-channels. We focused on actin, the major component of microfilaments, and the actin-cross-linking protein fodrin. We used cultured rat cortical neurons grown on poly-D-lysine coated coverslips in a chemically-defined media that does not support glial proliferation. We induced anoxia (PO_2=0) on the stage of our inverted confocal microscope, confirming its effect by observing morphological changes and increased intracellular Ca^{2+}, as demonstrated by fluo-3 fluorescence. After 10 min of anoxia, the sample was rapidly removed, fixed, and, using immunocytochemistry, stained for filamentous (F-) or monomeric (G-) actin, fodrin, or the 150kD cleaved subunit of fodrin. In the normoxic (control) neuron, phalloidin staining for filamentous actin was confined to the periphery. Following anoxia, the localization of F-actin was less restricted to the periphery, and the extent of staining appeared to have increased. Staining for G-actin, (with DNase-1) demonstrated an apparent decrease following 10 min of anoxia. In the normoxic neuron, staining was observed using antibodies against native fodrin, whereas the antibody RAF-150, which is specific to the cleaved subunit of fodrin did not stain. Following 10 min of anoxia, RAF-150 staining could be observed, indicating that fodrin had been cleaved. These results demonstrate that cytoskeletal proteins are affected by acute anoxia. We suggest that early cleavage of fodrin makes the microfilaments more susceptible to mechanical disruption during swelling, resulting in an increased number of actin polymers in the cytoplasm and less well localized filamentous actin in the plasma membrane region.

423.10

INCREASED NEURONAL SENSITIVITY TO ACUTE HYPOXIA IN RATS AFTER LONG-TERM EXPOSURE TO A REDUCED OXYGEN ENVIRONMENT J.P.O'Reilly* and G.G.Haddad., Depts. Biology and Pediatrics, Yale University and School of Med., New Haven, CT.

Ischemia and hypoxia have both been shown to be detrimental to the central nervous system (CNS). Previous studies have been concerned with the effect of acute episodes of ischemia or hypoxia on the CNS. However, the effect of long-term (chronic) hypoxia on the CNS has not been addressed. In order to determine the effect of chronic hypoxia on the CNS, we studied baseline electrophysiologic properties and the response to acute hypoxia in brain slices from animals that had been raised (P0 to >21 days) in a chronic hypoxic environment (FiO_2 = 9±1%). Age-matched rats raised in a normoxic environment served as controls. Intracellular recordings from neurons in layer 2/3 of the temporal cortex were performed. Resting membrane potential was significantly different between hypoxic rats (-77.9 ± 5.5 mV) and control rats (-83.4 mV ± 3.0 mV). Although both groups showed a biphasic response to acute hypoxia (an initial slow depolarization, followed by a large and rapid depolarization), only 40% of the control neurons showed a rapid depolarization by 7 minutes of hypoxia (latency = 13.3 ± 8.2 min), while all of the neurons from hypoxic animals showed a rapid depolarization by 7 minutes of hypoxia (latency = 5.4 ± 1.3 min). Also, during hypoxia the maximum depolarization (ΔVm = 61.0 ± 13.2 mV) and the maximum decrease in input resistance (ΔRn = 88.5% change from baseline) in hypoxic animals were greater than in controls (ΔVm = 27.4 ± 27.1 mV; ΔRn = 75.4%). These data suggest that chronic hypoxia results in an increased sensitivity to subsequent acute hypoxic stress. We speculate that long-term hypoxic exposure renders neurons in the CNS more susceptible to injury or death.

423.11

ARACHIDONIC ACID PARTICIPATES IN ANOXIA-INDUCED VESICULAR GLUTAMATE RELEASE IN CA1 NEURONS OF THE RAT HIPPOCAMPUS. N. Hershkowitz* and A.N. Katchman. Georgetown Univ. Hospital, Dept. Neurol., Wash. DC 20007.

Patch clamp in the whole cell configuration was used to examine the effects of agents that influence arachidonic acid metabolism on anoxia-induced vesicular glutamate release in CA1 neurons of *in vitro* hippocampal slices. Anoxia was induced by switching perfusion and ambient gas of an interface chamber from a 95%O_2/5%CO_2 to a 95%N_2/5%CO_2 mixture. As previously demonstrated, a significant increase in the frequency (340±50%,n=18) of spontaneous glutamate-mediated miniature excitatory postsynaptic currents (mEPSCs) was observed during the first 5 mins following anoxia. This increase in frequency was almost completely abolished if slices were preincubated in artificial cerebral spinal fluid containing the phospholipase C/A_2 inhibitor bromophenacyl-bromide (20 μM, n=8) or the cyclooxygenase inhibitors indomethacin (20 μM, n=16) and piroxicam (10 μM, n=4). There was no significant effect of these agents on the mean mEPSC amplitude. These data suggest that arachidonic acid (AA) and its cyclooxygenase products or by-products (oxygen free radicals) contribute to vesicular glutamate release during the early phase of anoxia. This observation may be important to our understanding of the neuroprotective action of these agents. (Supported by NINDS grant NS 14600-02)

423.12

HYPOXIA/HYPOGLYCEMIA-INDUCED PHOSPHOINOSITIDE HYDROLYSIS IN HIPPOCAMPAL SLICES *IN VITRO* : A MODEL SYSTEM FOR INVESTIGATING BIOCHEMICAL MECHANISMS Masuhiro Ikeda Department of Neuroscience, Tsukuba Research Laboratories of Eisai Co. Ltd. Ibaraki 300-26 Japan

Cerebral ischemia induces degradation of phosphatidylinositol-4,5-bisphosphate (PIP_2) and phosphatidylinositol-4-phosphate (PIP), and formation of diacylglycerol (J. Neurochem. 47, 123-132, 1986). To investigate the biochemical mechanisms of the ischemia-induced phosphoinositide hydrolysis, an *in vitro* method has been developed. Transverse slices of rat hippocampi labelled with (^{3}H)myo-inositol were incubated in nitrogen-saturated and glucose-free Krebs-Henseleit bicarbonate buffer in the presence of 10 mM LiCl. Inositol phosphates and inositol phospholipids were analyzed by SEP-PAKs column and TLC, respectively. The lack of glucose and oxygen induced a decrease in PIP_2 and PIP. PI did not change. The changes were very similar to those found in *in vivo* ischemia. Inositol bisphosphate(IP_2) transiently increased and then returned to control levels. Inositol monophosphate gradually increased. The increase in IP_2 was completely prevented by removing extracellular Ca^{2+} from the buffer. Addition of atropine, prazosin or ketanserin did not suppress the increase in IP_2. Antagonists of NMDA and nonNMDA receptors also had no effect. D-amino-3-phosphonopropionate (D-AP3), a putative antagonist of metabotropic glutamate receptors prevented the increase in IP_2, but the compound did not affect the decrease in PIP_2 and PIP. The effects of D-AP3 may be due to the inhibition of phosphatidylinositol synthesis as reported previously (Neurosci. Lett. 157, 87-90, 1993). These findings suggest that the present method is suitable for investigating the biochemical mechanism of ischemia-induced phosphoinositide hydrolysis, and finding compounds which inhibit the hydrolysis.

423.13

HYPOXIA-INDUCED CHANGES IN LIGHT TRANSMITTANCE IN HIPPOCAMPAL SLICES. N.R. Kreisman*, J.C. LaManna, S.-C. Liao, and J.R. Alcala. Depts. of Neurology and Biomedical Engineering, Case Western Reserve U. Sch. Med., Cleveland, OH 44106 and Dept. of Physiology, Tulane U. Sch. Med., New Orleans, LA 70112.

Intrinsic optical properties of in vitro brain slices change as cells swell (Andrew & MacVicar *Soc Neurosci Abstr* 18:1125, 1992; Henn & Turner *Soc Neurosci Abstr* 19:1322, 1993; Kreisman et al *FASEB J* 8:A655, 1994). In the present study we correlated neuronal activity and light transmittance during hypoxia. Field potentials and orthodromic population spikes were recorded in CA1 stratum pyramidale of 400μm-thick rat hippocampal slices in an interface chamber. Transmitted light was detected with a silicon photodiode coupled to a dissecting microscope (optical field, 0.5 X 1.5 mm). Hypoxia (15-20 min) blocked synaptic transmission within 3 min and produced 1-3 phases of intrinsic optical changes. The three phases of light transmittance change occurred in the following sequence: 1) a small increase ($\Delta T/T$= +5.4±0.7%; n=16/27) indicative of cell shrinkage, 2) a slow decrease ($\Delta T/T$= -17.6±3.2%; n=14/27) indicative of cell swelling, and 3) a rapid decrease (i.e., swelling) coincident with anoxic depolarization ($\Delta T/T$= -31.1±2.4%; n=11/27). Reoxygenation following 15-20 min of hypoxia only resulted in a 25% recovery of light transmittance toward baseline. Imaging showed that the largest optical changes occurred in the stratum radiatum, followed by strata pyramidale, oriens, and lacunosum-moleculare. The results suggest that cells in CA1 hippocampus swell after several minutes of hypoxia and that recovery from swelling is incomplete following 20 min of reoxygenation. (Supported by NS 22077)

423.14

ALTERATIONS IN MITOCHONDRIAL FUNCTION OF PC12 CELLS FOLLOWING CHEMICAL ANOXIA/REOXYGENATION. K.M. Myers*, A.N.Murphy, and G.Fiskum. Department of Biochemistry and Molecular Biology, George Washington University Medical Center, Washington, DC 20037.

PC12 cells were used to explore the alterations in mitochondrial activity following cyanide (KCN)-induced chemical anoxia of varying durations in the absence of glucose. Recovery of mitochondrial function was also charted over periods of reoxygenation in the presence of glucose as an ***in vitro*** simulation of neuronal ischemia and reperfusion. Exposure of suspended PC12 cells to 3 mM KCN for 7 minutes induced an 86% decrease in ATP levels, which plummeted to 1.3% of control after 90 minutes. Glucose prevented this fall, while incubations in aglycemic media without KCN had no effect This suggests combined oxygen and glucose deprivation are necessary to deplete ATP. Levels recovered to 56% following 30 minutes of anoxic aglycemia with 30 minutes of glycemic reoxygenation and climbed to 87% following 24 hours of reoxygenation. Viability was >80% in most cases. The rate of mitochondrial O_2 consumption observed with cells resuspended in a KCl-based medium containing the permeabilizing agent digitonin, oxidizable substrates, and ADP (State 3) following a 30 minute incubation of intact cells plus KCN minus glucose was 83% with subsequent decreases to 76% and 67% for 60 and 90 minutes. The acceptor control ratio (ACR), which compares state 3 respiration to that of state 4, or O_2 consumption minus ADP, was 71% after 30 minutes and fell to 61% following 90 minutes of anoxic aglycemia. Although short periods of glycemic reoxygenation allowed a virtual recovery of the ACR following 30 minutes of anoxic aglycemia (>80% after 30, 60, 120, and 240 min.), ACR declined to 52% following 24 hours of reoxygenation. This secondary impairment of function is similar to the multiphasic mitochondrial injury noted for certain animal models of cerebral ischemia/reperfusion. This system can therefore be used to test whether mechanisms of ischemic brain injury are responsible for mitochondrial injury evoked by anoxia/reoxygenation. (Supported by SigmaTau S.p.A. and Souers Stroke Fund).

423.15

SIMULTANEOUS PATCH-CLAMP RECORDINGS OF CA1 PYRAMIDAL CELLS AND INTERNEURONS IN RAT HIPPOCAMPAL SLICES: DIRECT DEMONSTRATION OF FUNCTIONAL DISCONNECTION OF INTERNEURONS BY ANOXIA. R. Khazipov, P. Congar, N. Ropert* and Y. Ben-Ari *INSERM Unite 29, 123 Bd de Port-Royal, 75014 Paris*

It was proposed in our previous work (Khazipov et al.,*J. Neurophysiol*, 70 : 2251-2260, 1993) that high sensitivity of polysynaptic IPSCs to anoxia is due to the functional disconnection of inhibitory interneurons from excitatory inputs. To verify this hypothesis, the effects of anoxia were studied in simultaneous whole-cell patch-clamp recordings from interneurons and pyramidal cells in CA1 region of adult rats hippocampal slices; these were both identified by their electrogenic properties and biocytin injection. Anoxia (95%N_2-5%CO_2; 5 min) generated currents in interneurons similar to pyramidal cells: the most prominent were the anoxic outward current and the postanoxic outward current. EPSCs and polysynaptic IPSCs were depressed by anoxia, and this depression was more pronounced in the interneurons. The currents evoked by pressure ejection of glutamate, AMPA and NMDA were not affected by anoxia, suggesting that the depression of EPSCs in both interneurons and pyramidal cells is due to a presynaptic mechanism. Depression of EPSCs but neither polysynaptic IPSCs nor $GABA_B$ receptors mediated component of monosynaptic IPSCs (see Congar et al. *Soc. Neurosci. Abs.* 1994) was prevented by adenosine A_1 receptors antagonist DPCPX (200nM) indicating the involvement of presynaptic adenosine receptors in anoxic depression of EPSCs. These observations indicate that anoxia preferentially depresses excitatory inputs to anoxia leading to a functional disconnection of interneurons.

423.16

SIMULTANEOUS PATCH-CLAMP RECORDINGS CA1 PYRAMIDAL CELLS AND INTERNEURONS IN RAT HIPPOCAMPAL SLICES: EFFECTS OF ANOXIA ON $GABA_A$ AND $GABA_B$ RECEPTORS MEDIATED SYNAPTIC CURRENTS. P. Congar, R. Khazipov, J. Hirsch* and Y. Ben-Ari *INSERM Unite 29, 123 Bd. de Port-Royal, 75014 Paris*.

The effects of anoxia on monosynaptic IPSCs, evoked by 'close' stimulation in the presense of glutamate receptor blockers, were studied in the CA1 region of hippocampal slices from adult rats using simultaneous whole-cell patch-clamp recording of pyramidal cells and interneurons with low-chloride pipete solution. In pyramidal cells and interneurons, anoxia (95%N_2-5%CO_2; 5 min) depressed or inverted the IPSCs, due to the shift of Cl^- reversal potential to more positive values, without any change of conductance. Anoxia also depressed the late postsynaptic $GABA_B$-receptor mediated component. Since adenosine A_1 receptors antagonist DPCPX (200μM) did not prevent the anoxic depression of $IPSC_B$, this depression was not due to 'occlusion' of $GABA_B$ receptors mediated currents by adenosine which is known to accumulate during anoxia and is responsible to the presynaptic depression of glutamate release (see Khazipov et al. *Soc. Neurosci. Abs.*. 1994). To study the effects of anoxia on the presynaptic $GABA_B$-receptor mediated mechanisms, we used a paired-pulse inhibition (PPI) protocol. In control, PPI of IPSC was observed both in pyramidal cells and interneurons. Anoxia did not affect presynaptic $GABA_B$-receptors mediated inhibition since there was no change in PPI during anoxia or upon reoxygenation. It is concluded that anoxia affects synaptic transmission of GABAergic inhibitory synapses on pyramidal cells and interneurons by (i) disturbance of Cl^- equilibrium and shift of reversal potential of $IPSC_A$ to positive values and (ii) selective block of post- but not presynaptic $GABA_B$-mediated responses.

423.17

IN UTERO ISCHEMIA CAUSES HYPERSENSITIVE RESPONSES IN NMDA-STIMULATED ACCUMULATION OF GLUTAMATE AND cGMP FORMATION IN CEREBELLAR GRANULE CELL CULTURES.
P.G. Rhodes*, Z. Cai and N. Zhu. Dept. of Pediatrics, Univ. of Miss. Med. Ctr., Jackson, MS 39206.

An experimental model of in utero hypoxic-ischemia was developed to study the effects of this insult on brain development. On gestation day 17, ischemia conditions were achieved by complete clamping of the uterine vasculature for designated times followed by removal of the clamps to permit reperfusion. Sham operation (surgery without vasculature clamping) was conducted in the control groups. After surgery, the uterus was returned to the dam's abdomen to allow for natural birth. Cerebellar granule cells were cultured from the pups at 8 days of age. Cell density and protein contents of the cultures were not significantly different between intact, sham operated and ischemic groups. N-methyl-D-aspartate (NMDA, 100 μM), but not kainate, induced a significant elevation of glutamate levels in cell cultures from 20 and 35 min-ischemic groups compared to intact, sham or 5 min-ischemic groups (30.9 and 27.0 vs. 19.4, 18.5 and 18.2 nmol/mg protein, respectively). NMDA-stimulated cGMP formation in cultures from 10, 20, 25 or 35 min-ischemic groups was also significantly higher than in control cells (62.4-78.2 vs. 49 pmol/mg of protein). The hypersensitive responses in NMDA-stimulated glutamate accumulation and cGMP formation in cells from in utero ischemic groups could be blocked by addition of L-N^G-monomethyl-arginine (L-NMMA, 150 μM), a nitric oxide synthase inhibitor, before NMDA stimulation. These results indicate that in utero ischemia may result in adverse effects on later NMDA receptor-mediated neurotransmission during brain development and that nitric oxide response is possibly involved in these effects.

423.18

DIFFERENTIAL RESPONSES OF CA1 PYRAMIDAL CELLS AND GRANULE CELLS IN THE DENTATE GYRUS TO ISCHEMIA-LIKE CONDITIONS IN RAT HIPPOCAMPAL SLICES. H.Shimizu, Y. Fujito *, A. Mizuguchi and M. Aoki Dept. Physiol., School of Med., Sapporo Med. Univ., Sapporo 060, Japan

We examined the regional difference of responses to oxygen-glucose deprivation (ischemia-like conditions, Hypo•G(-)) in the CA1 region and the dentate gyrus (DG) of rat hippocampal slices(350μm thick). Changes of resting membrane potentials(rest Vm) and antidromic action potentials (AAPs) were compared between CA1 pyramidal cells (PCs) and granule cells (GCs) in DG. $[Ca^{2+}]i$ measured by Fura-2/AM loaded slices were also compared between CA1 and DG. In PCs, Hypo•G(-) produced an initial hyperpolarization and a subsequent rapid depolarization. By contrast, GCs showed a slight initial hyperpolarization and a subsequent slowerl depolarization than PCs. In PCs and GCs, AAPs disappeared after 7 to 9-min periods of Hypo•G(-), when rest Vm depolarized to about -50mV. Exposure to Hypo•G(-) for ≥ 10 min produced an irreversible depolarization in both neurons. However, when reoxygenation was started at the time of disappearance of AAPs, repolarization occurred in 86% (12/14) of GCs and 36% (5/14) of PCs. $[Ca^{2+}]i$ remarkably increased at the depolarization phase both in CA1 and DG, but peak $[Ca^{2+}]i$ were significantly higher in CA1. Under Ca^{2+}-free medium, peak $[Ca^{2+}]i$ decreased and there was no significant difference between CA1 pyramidal cell layer and granule cell layer. These results demonstrate the differential responses to Hypo•G(-) between CA1 PCs and GCs in DG, probably due to different distributions of ion channels of these neurons.

423.19

BRIEF PERIODS OF OXYGEN AND GLUCOSE DEPRIVATION *IN VITRO* CAUSES SWELLING AND DELAYED CELL DEATH. W.J. Goldberg*. Stroke & Trauma Lab., Dept. Veterans Affairs Medical Center, Washington, DC 20422.

An in vitro system was used to mimic several aspects of ischemia, including oxygen and glucose deprivation and prolonged depolarization. We replaced normal culture medium from 3-week-old spinal cord cultures with oxygen-depleted, osmotically balanced (310 mOs) salt solution containing 55 mM Na^+, 65 mM K^+ and 1 μM Ca^{2+}. After incubation at 37°C for 15 minutes in an atmosphere of 7% CO_2/93% N_2, the cultures were returned to normal, oxygenated culture medium for 0, 1 or 96 hours. At the end of each recovery period, cultures were immunohistochemically stained using combined antibodies to 68kD, 150kD, and 200 kD neurofilament proteins. In non-deprived cultures, the neuronal density was 197 cells/100 mm^2. These cells were 96.67±6.8 μm^2 (mean±SEM) in size. More than 88% of the original neuronal population survived the 15 minute deprivation period. Two classes of cells were observed based on cell size; one group was not different from non-ischemic neurons (109±7.63 μm^2) and the other was significantly swollen (255.3±25 μm^2, $P<0.05$). The survival rate decreased to 64.7% after 1 hour of recovery. Swollen (240.1±14.3 μm^2, $p<0.05$) and non-swollen (112.1±9.3 μm^2) neuronal populations were observed at this time. After 4 days of recovery, the survival rate decreased further to 21.6%. All surviving cells were swollen to 337±47.7 μm^2 ($p<0.05$). No normal sized neurons were observed. Supported by the Department of Veterans Affairs.

423.20

EFFECTS OF OXYGEN- AND GLUCOSE- FREE CONDITIONS ON RAT HIPPOCAMPAL BRAIN SLICES : SIMULTANEOUS Ca^{2+} AND DC POTENTIAL MEASUREMENTS. M.L. Evans* and C.D. Benham. Biophysics Dept., SmithKline Beecham, Harlow, Essex CM19 5AD U.K.

To investigate synaptic responses, ischaemic depolarisation, and Ca^{2+} influx under oxygen and glucose free conditions, 400 micron hippocampal slices were loaded with the Ca^{2+} indicator dye INDO1-AM. Microspectrofluorimetry was used to monitor $[Ca^{2+}]_i$ within a 20 micron diameter window centred over the stratum pyramidale of either CA1 or CA3. Extracellular DC recordings were made in the stratum pyramidale adjacent to the window. Experimental temperature was 36°C. The relevant afferent pathway was stimulated every 15s to monitor synaptic responses. Synaptic responses in INDO1 loaded slices were similar to those of control slices. On switching from normal medium to N_2-saturated, glucose-free medium, synaptic responses were eliminated within 90s. After 276±45s, a large, transient, negative DC shift was observed in CA1 which was closely followed by a rapid and sustained rise in $[Ca^{2+}]_i$. Reperfusing with normal medium shortly after the peak $[Ca^{2+}]_i$ rise resulted in a return of $[Ca^{2+}]_i$ to baseline level over 3 to 5 min. but there was no recovery of the synaptic responses. Switching to normal medium before the depolarising shift and $[Ca^{2+}]_i$ rise resulted in a slow recovery of synaptic transmission to near baseline levels. Minislices containing only CA1 gave similar responses, indicating that the CA1 events are not driven by preceding events in CA3. The depolarising wave and $[Ca^{2+}]_i$ rise in CA3 had a longer latency (395±67s) and rose more slowly to a lower peak amplitude. Reperfusing after the peak $[Ca^{2+}]_i$ rise in CA3 resulted in a sustained recovery of synaptic responses. These regional differences in response to acute oxygen and glucose free conditions may underlie the vulnerability of CA1 to injury relative to CA3 observed with *in vivo* ischaemia models.

ISCHEMIA: MECHANISMS II

424.1

SUBCELLULAR LOCALIZATION OF ISCHEMIA-INDUCED INHIBITION OF CAM KINASE II. **J.T. Parsons*[1], S.B. Churn[2], and R.J. DeLorenzo[1, 2]. Departments of Biochemistry[1] and Neurology[2], Medical College of Virginia, Richmond, Va. 23298.**

Ischemia-induced inhibition of CaM Kinase II activity is well characterized, but ischemia-induced inhibition of CaM Kinase II activity in selected subcellular fractions has not been studied. Therefore, we examined CaM Kinase II activity in subcellular fractions isolated from control and ischemic rat forebrains. Subcellular fractions were isolated from control and ischemic animal forebrains by differential centrifugation as modified by {J. Neurosci. 5:2609-2619, 1985, J. Clin. Invest. 75:2014-2023, 1985}. CaM kinase II activity was determined as the amount of autophosphorylation of the alpha (50 kDa) subunit. Autophosphorylation was quantitated by computer-assisted densitometry (Mocha®, Jandel Scientific){J. Neurochem. 59:1221-1232, 1992}. CaM kinase II activity was determined in the nuclear fraction (P_1), the plasma membrane and mitochondrial fractions (P_2 subjected to centrifugation on a discontinuous Ficoll 400 gradient), the cytosolic fraction (S_3), and the endoplasmic reticulum fraction (P_3). Both control and ischemic subcellular fractions were normalized for protein, resolved on 8.5% SDS-PAGE gels, and visualized by autoradiography. As a result of ischemia, CaM Kinase II activity was inhibited by 30% ± 3% (n = 2) in the plasma membrane fraction, 26% ± 9% (n = 2) in the mitochondrial fraction, 38% (n = 1) in the nuclear fraction, 82% (n = 1) in the cytosolic fraction, and 75% ± 4% (n = 2) in the endoplasmic reticulum fraction. Thus, ischemia induces a significant inhibition of CaM kinase II activity in all subcellular fractions studied. The data support the hypothesis that ischemia-induced inhibition of CaM kinase II activity may play a role in the ischemia-induced, delayed neuronal cell death.

424.2

TRANSLOCATION OF Ca^{2+}/CALMODULIN PROTEIN KINASE II IN THE VULNERABLE NEURONS FOLLOWING ISCHEMIA AND HYPOGLYCEMIA IN THE RAT. Bing-Ren Hu*, Junichi Kurihara, Fredrik Kamme and Tadeusz Wieloch, Lab. for Exp. Brain Res., University Hospital, 221 85 Lund, Sweden.

Alterations of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) during and following transient cerebral ischemia (TCI) and insulin-induced hypoglycemia (HG) in the rat were studied. CaMKII is translocated to synaptic junctions after both TCI and HG as showed by immunoblots and calmodulin binding, concomitant with a decrease in enzymatic activity. The translocation is longlasting and persists for the whole period until neuronal death in vulnerable brain areas, whereas in resistant regions translocation is transient. The results suggest that a stimulation of CaMKII is ongoing in the vulnerable areas which causes translocation. The ischemia also reduces the expression of α-subunit of CaMKII mRNA in dentate gyrus as demonstrated by *in situ* hybrization at 6 to 12 hrs of reperfusion. This may be a protective mechanism of the neurons to shut off CaMKII signal after ischemia. Intraischemic hypothermia protects ischemic neuronal damage as well as reduces the translocation and increases mRNA expression of CaM-kinase II induced by ischemia. In conclusion the persistant translocation is highly correlated to neuronal damage in both ischemia and hypoglycemia.

424.3

ALTERATIONS IN Ca^{2+}/CaM-Kinase II AND OTHER PROTEINS IN POSTSYNAPTIC DENSITIES AFTER FOREBRAIN ISCHEMIA IN RATS. Jaroslaw Aronowski* and James C. Grotta. Department of Neurology, University of Texas Health Science Center, Houston, Texas 77025.

Postsynaptic densities (PSDs) located beneath the postsynaptic membrane may be the organelle for organizing ion channels, receptors and enzymes that participate in signal transduction. The major PSD protein, α-subunit of Ca^{2+}/Calmodulin-dependent protein kinase II (CaM-KII), account for up to 30-40% of total PSD protein. We have shown that cerebral ischemia causes inactivation and translocation of CaM-KII from cytosol to particulate fraction, and that these changes are reversible after brief but not prolonged duration of ischemia. The present study analyzes the amount and activity of CaM-KII in isolated PSDs after ischemia. Wistar rats were subjected to 20 min of ischemia using a four-vessel occlusion (4VO) model. After 0, 2 and 24 h of reperfusions, animals were decapitated and forebrains isolated in less then 15 sec and frozen in liquid nitrogen. We compared 4VO to 5 min decapitation ischemia. PSDs were isolated according to the method by Wu et al. Proteins in PSD were quantitated using immunostaining, and CaM-KII activity was measured by quantitation of its autophosphorylation and peptide substrate phosphorylation. 4VO produced a dramatic (300%) increase in amount of alpha CaM-KII in PSDs. This increase was not reversed after 2 h and only partially reversible after 24 h of reperfusion (possibly due to proteolysis of the kinase). The amount of α-tubulin, actin and neurofilaments in PSDs did not change. CaM-KII activity in PSDs was decreased to approximately 30 and 50% of control levels after 0 and 2 h of reperfusion, respectively. 5 min decapitation ischemia produced increased CaM-KII similar to the 4VO, but significantly less inactivation of the enzyme. We conclude that different severities of ischemia are associated with irreversible increases of CaM-KII in PSDs with varying degrees of enzyme inactivation. The origin of the increase of CaM-KII in PSDs will be discussed.

424.4

A reversible elevation of free calcium during simulated ischemia in neuronal tissue culture. J.J. Vornov,* M. Han, S.C. Jo and A.G. Thomas Depts. of Neurology and Neuroscience Johns Hopkins School of Medicine, Baltimore, MD 21287

Previously, we have described tissue culture models of ischemia using metabolic inhibition to cause rapid, reversible ATP depletion. Neurons can be protected by NMDA receptor blockade *after* simulated ischemia, allowing delayed treatment of injury. Since the accessibility of cultures is preserved, we have been able to measure free calcium changes during simulated ischemia with the calcium sensitive dyes fluo-3 or fura-2 in dissociated cortical cultures. Intracellular calcium rose reversibly during metabolic inhibition. The calcium rise was slower and the recovery faster than that caused by bath application of glutamate. Blockade of NMDA receptors with MK-801 caused no change in the calcium increase, but removal of extracellular calcium decreased the rise.

Calcium entry during recovery was investigated histochemically. Calcium permeable non-NMDA receptor channels are permeable to cobalt. Cultures were incubated in 5 mM CoCl2 during recovery and cobalt visualized with ammonium sulfide precipitation followed by silver intensification. In both dissociated cortical cultures and organotypic hippocampal cultures, some neurons accumulated cobalt during recovery from simulated ischemia. More cells were stained during bath application of kainate or glutamate. These experiments suggest that while free calcium may rise during simulated ischemia, toxic, persistent calcium entry during recovery may not be reflected by increased free intracellular calcium.

424.5

EFFECTS OF TRANSIENT CEREBRAL ISCHEMIA ON CALCIUM CALMODULIN KINASE II mRNA LEVELS IN THE GERBIL HIPPOCAMPUS. A.M. Babcock[1], J. Nollkamper[1], C.M. Paden*[2], P.E. Micevych[3] and P. Popper[3]. Dept. of Psychology[1] and Biology[2], Montana State Univ., Bozeman MT 59717; [3]Dept. of Anat. & Cell Biology, UCLA Sch. of Med., Los Angeles, CA 90024.

Previous studies have reported that hippocampal calcium calmodulin kinase II (CaM II) mRNA levels decrease at 24 hrs following transient ischemic insult in the gerbil (Hiestand & Kindy, 1992). The present study attempted to confirm this finding by evaluating regional CaM II mRNA levels using in situ hybridization. Gerbils (n=5/group) underwent a 5-min bilateral carotid occlusion or sham procedure. At 23 hrs post surgery, locomotor activity was assessed as a behavioral marker of ischemic cell damage. Gerbils were sacrificed at 24 hrs and sections containing the hippocampus were processed for in situ hybridization with cRNA probes for the α and β subunits of CaM II (separate sections). As expected, ischemic gerbils exhibited higher activity levels indicative of hippocampal damage. Contrary to previous findings however, no significant differences in hippocampal CaM II mRNA levels were observed between groups. Results are discussed in relationship to observed alterations in CaM immunoreactivity and enzyme activity following ischemia.

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424.6

SUSTAINED INHIBITION OF EXCITATORY SYNAPTIC TRANSMISSION BY ADENOSINE DURING PROLONGED MODERATE HYPOXIA, L. Arlinghaus* and K.S. Lee. Dept. of Neurological Surgery, Univ. of Virginia, Charlottesville, VA 22908.

Adenosine is a potent inhibitor of excitatory synaptic transmission at many sites in the CNS. During ischemia and hypoxia, extracellular adenosine levels increase substantially. This elevation of adenosine appears to contribute to the initial phase of the hypoxic inhibition of synaptic responses (Fowler; Brain Res. 490, 1989). The present study examined whether adenosine contributes to later phases of intra-hypoxic inhibition of synaptic responses during 1 hour of moderate hypoxia. Hippocampal slices prepared from adult Sprague-Dawley rats were submerged in medium with an oxygen tension of approximately 470 mm Hg. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the *stratum radiatum* of CA1 in response to stimulation at the CA1/CA2 border. Hypoxic conditions were established by changing the oxygen tension in the superfusion medium to 85 mm Hg. A complete inhibition of fEPSPs was observed within approximately 9 minutes of hypoxia. An inhibitor of adenosine A1 receptors, cyclopentyltheophylline (CPT; 10μM) was added to the medium at various intervals after the onset of hypoxia. The addition of CPT to the hypoxic superfusion medium after the loss of synaptic responses resulted in a substantial recovery of fEPSPs, while the loss of synaptic responses remained complete for the entire hour of hypoxia in the absence of CPT. When CPT was added 10 or 30 minutes after the loss of synaptic responses, fEPSPs recovered to 68 and 47% of their prehypoxic baseline values respectively. These results indicate that adenosine-mediated inhibition contributes significantly to the sustained loss of fEPSPs during hypoxia. The findings suggest that adenosine functions to maintain excitatory synaptic transmission at low levels during prolonged moderate hypoxia and support the concept that adenosine serves as an endogenous neuroprotectant.

424.7

Na^+ SUBSTITUTION TRIGGERS ADENOSINE EFFLUX FROM RAT HIPPOCAMPAL SLICE. J.C. Fowler*. Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Brain ischemia results in an increase in the extracellular levels of a number of endogenous substances. It is generally considered that an ischemic related loss of electrochemical gradient results in a reversal of Na^+-dependent uptake mechanisms and a consequent net efflux of transported substances including glutamate and adenosine. To examine the consequences of lessened Na^+-dependent transport we investigated the effect of substituting extracellular Na^+ with choline on extracellular levels of adenosine.

Hippocampal slices were initially superfused at 2 ml/min with physiological medium maintained at 33 - 34°C and equilibrated with 95% O_2/ 5% CO_2. Extracellularly recorded DC and evoked synaptic potentials were recorded. Slices were then exposed to medium in which NaCl and $NaHCO_3$ were replaced with the choline salts. Aliquots of superfusate were collected at 2 min intervals and adenosine was measured using absorbance HPLC.

Choline containing medium blocked synaptic transmission within a few minutes. A transient depolarization was observed between 11 - 12 minutes. Adenosine detected in the superfusate was subsequently increased. The increased adenosine but not the depolarization was prevented with the NMDA antagonist MK-801. The depolarization was not blocked by DNQX, a non-NMDA antagonist, but was blocked by the combination of DNQX and MK-801. It is concluded that diminished extracellular Na^+ results in an increase in extracellular adenosine via activation of NMDA receptors and a depolarization via a combined activation of NMDA and non-NMDA receptors.

424.8

ACTIVATION OF ADENOSINE RECEPTORS PROTECTS NEURONS FROM ISCHEMIA INDUCED DEATH. M.I.Sweeney*. Dept. of Physiology, Univ. of Saskatchewan & Sask. Stroke Research Center, Saskatoon, SK, Canada. S7H 4T3.

The neuromodulator adenosine is released from the brain in response to ischemia, and is neuroprotective in various animal models of stroke. The aim of this study was to determine whether this protective effect is mediated *via* a direct effect on neurons (*versus* an indirect action involving other cells). Primary cultures of cerebellar granule cells containing >95% neurons were exposed *in situ* to 'simulated ischemia' (substrate deprivation plus anoxia). Adenosine release was measured by HPLC, and uptake determined using [^{3}H]adenosine; cell death was quantitated by measuring extracellular lactate dehydrogenase (LDH) concentrations.

Exposure of cerebellar neurons to simulated ischemia induced a 10 to 25-fold increase in the release of endogenous adenosine. This was seen as early as 2.5 min after the onset of ischemia, persisted for 30 min, and then dropped from 2.2 $\pm$ 0.35μM to 1.3$\pm$0.28μM after 30 min of reperfusion with normoxic, substrate-enriched medium. Ischemia-evoked adenosine release was reduced by 25-55% by dipyridamole suggesting the involvement of a bi-directional nucleoside carrier. After 30 min of simulated ischemia, neurons showed a reduced ability to take up [^{3}H]adenosine, and this may have contributed to the enhanced levels of extracellular adenosine. Ischemia (30 min) also produced a 3.4-fold increase in the extracellular concentration of LDH, an intracellular enzyme. This effect was attenuated by 45% by 1mM exogenous adenosine and by 72% with 1μM cyclopentyladenosine, a potent agonist at adenosine A1 receptors. The effect of both agents was inhibited by an A1 antagonist, while the effect of adenosine was only slightly reduced by a blocker of adenosine uptake. These data suggest that cerebellar granule neurons release adenosine in response to an ischemic insult. Extracellular adenosine then may have a direct neuroprotective effect *via* the activation of A1 receptors located on neurons, and not *via* uptake and subsequent metabolism to ATP.

424.9

THE ROLE OF INTERLEUKIN-1 IN CEREBRAL ISCHAEMIA. S. Loddick and N.J. Rothwell. (SPON: Brain Research Association). Neuroscience Division, 1.124, School of biological sciences, University of Manchester, Manchester M13 9PT, U.K.

We have previously proposed a role for the cytokine interleukin-1 (IL-1) in the pathogenesis of cerebral ischaemia, since central administration of recombinant human interleukin-1 receptor antagonist (rhIL-1ra, Synergen, USA) inhibits ischaemic damage following permanent middle cerebral artery occlusion (MCAo). Also, it has been reported that administration of interleukin-1β (IL-1β) exacerbates damage following global ischaemia, (Minami *et al*, Soc. Neurosci., Abstr., 18 (1992) 425.5).

We determined if the neuroprotective action of IL-1ra in cerebral ischaemia is related to changes in body temperature, and investigated the effect of IL-1β on focal ischaemia and body temperature. Core temperature (peritoneal) was measured in conscious free-moving rats by remote radio telemetry. Temperature was recorded over the 24h preceding MCAo and for the following 24h, in animals treated with either saline or IL-1ra. These data were used to calculate the change in temperature that occurred over the 24h following MCAo. Injection of IL-1ra (2 x 10μg, icv) 30min before and 10min after MCAo significantly inhibited (60-70%) ischaemic damage (infarct volume) measured 24h after MCAo, but had no significant effect on body temperature at any time, indicating that the neuroprotection offered by IL-1ra is not dependent on a change in core temperature.

In a separate experiment, central administration of IL-1β (2 x 2.5ng, specific activity 200unit/ng) 30min and 2h after MCAo significantly potentiated (80-90%) ischaemic damage when compared to saline treated animals. However preliminary experiments reveal that this dose of IL-1β caused a large ($\approx$1.5 °C) increase in core temperature, which may contribute to the exacerbation of damage.

Thus IL-1ra causes a significant inhibition of ischaemic damage which is not dependent on temperature changes, and IL-1β causes a significant exacerbation of damage though the latter effect may be dependent on changes in core temperature.

424.10

EVALUATION OF lCBF, FORSKOLIN AND D_1 DOPAMINE RECEPTOR BINDING IN A REPERFUSION MODEL OF FOCAL CEREBRAL ISCHAEMIA. G. Gartshore, D. Dawson, J. Patterson and I.M. Macrae*. Wellcome Neuroscience Group, University of Glasgow, Glasgow, G61, U.K.

Reversible focal cerebral ischaemia can be induced by the abluminal application of endothelin-1 (Et-1) to the exposed middle cerebral artery (MCA). Neuroanatomical changes in local cerebral blood flow (lCBF) were examined in male Sprague Dawley rats (n=12) during ischaemia and reperfusion using i.v. administered ^{99m}Tc-HMPAO at 5 min and ^{14}C-iodoantipyrine (IAP) at 2 h post Et-1 application. Post ischaemic changes in forskolin and D1 dopamine receptor (labelled with SCH23390) binding sites were also evaluated by receptor autoradiography at the 2 h time point. ^{99m}Tc-HMPAO uptake was significantly reduced in the frontoparietal cortex (FPC), dorsolateral (DLC) and ventromedial (VMC) caudate nucleus ipsilateral to MCA occlusion 5 min post Et-1 application (to 21%, 26% and 36% of corresponding contralateral values, respectively). Reperfusion was clearly evident 2 h post-insult (ipsilateral lCBF increased by 75%, 171% and 78% respectively). In the same animals forskolin binding in the FPC, DLC and VMC was significantly reduced ipsilateral to MCA occlusion 2 h after Et-1 application whereas D_1 receptor binding was unchanged (table). This combination of double CBF measurement plus receptor binding autoradiography in the same animal provides a powerful means to study the consequences of ischaemia followed by reperfusion.

Specific Binding	Dopamine (pmoles/gm)		Forskolin (pmoles/gm)	
	IPSI	CONTRA	IPSI	CONTRA
FPC	18 $\pm$ 2	16 $\pm$ 2	102 $\pm$ 8*	136 $\pm$ 9
DLC	195 $\pm$ 10	198 $\pm$ 9	343 $\pm$ 25*	385 $\pm$ 20
VMC	173 $\pm$ 10	173 $\pm$ 9	313 $\pm$ 22*	344 $\pm$ 16

*P<0.05 ipsi vs contra (student's paired t-test)

424.11

CHANGE OF ACETYLCHOLINE IN RAT BRAIN FOLLOWING ISCHEMIA. H. Kanemitsu*[1], K. Kawai[1], T. Kirino[2], A. Tamura[1], K. Iwasaki[3] and M. Fujiwara[3]. [1]Department of Neurosurgery, Teikyo University School of Medicine, Tokyo, Japan. [2]University of Tokyo. [3]University of Fukuoka.

We have reported that the decrement of the acetylcholine level may be related to the disturbance of spatial cognition. The purpose of the present study is to determine change of the acetylcholine levels in ischemic center and periphery in rat brain tissue following focal ischemia. Male Wister rats weighing 280-300 g were anesthetized with 2 % halothane inhalation and the proximal portion of the left middle cerebral artery (MCA) was occluded by the microsurgical technique. The animals were treated with microwave 1, 2 and 4 weeks following MCA occlusion and the acetylcholine levels of the cerebral cortex, caudate putamen and thalamus were measured by using HPLC-ED system. The acetylcholine levels of the cerebral cortex and caudate putamen on the ischemic side in the MCA-occluded group were significantly decreased compared with those in sham-operated group. However, the levels of the opposite-side and thalamus unchanged. Those decrements of acetylcholine may be caused by interception of the neurons in the Meynert nucleus and be related to the impairment of learning behavior.

424.12

CHANGES IN TYROSINE PHOSPHORYLATION IN THE RAT BRAIN DURING AND FOLLOWING HYPOGLYCEMIC COMA. J. Kurihara, B.-R. Hu, Drake M* and T. Wieloch, Lab for Exp. Brain Res., University Hospital, S 221-85 Lund, Sweden

Hypoglycemic coma in the rat was induced by insulin and the brains were frozen in situ and the subcellular fractions of the brain regions were analyzed by immunoblot and immunoprecipitation. During 30 min hypoglycemic coma, tyrosine phosphorylation of a 43 kDa protein increased. The increase was transient in the cerebral cortex and striatum, whereas persistent in the hippocampus. During recovery period following hypoglycemic coma, a general increase in phosphotyrosine was observed in all structures studied, the increase was most obvious in the crude synaptosomal fraction. In the hippocampus, a general increase in tyrosine phosphorylation was noted even at 24 hrs of recovery, although the tyrosine phosphorylation of the 43 kDa protein normolized quickly. Immunoprecipitation study confirmed that the 43 kDa protein with increased phosphotyrosine during hypoglycemic coma was MAP kinase. The increased tyrosine phosphorylation of the brain MAP kinase during and following severe hypoglycemia may be due to increased glutamate receptor activation. This may be of significant for hypoglycemic neuronal damage.

ISCHEMIA: APOPTOSIS

425.1

APOPTOSIS OF PYRAMIDAL NEURONS IN THE CA1 ZONE OF GERBIL HIPPOCAMPUS AFTER BRIEF ISCHEMIA T. Nitatori1*, Y. Karasawa2, N. Sato1, H. Araki2, E. Kominami3 and Y. Uchiyama1. Dept. of Cell Biol. & Neuroanat.1, Sch. of Med. Iwate Med. Univ., Morioka, 020 Japan, Lab. of Pharmacol.2, Taishou Pharmaceutical Co. Ltd., Ohmiya, 330 Japan, and Dept. of Biochem.3, Sch. of Med., Juntendo Univ., Tokyo, 113 Japan

The CA1 pyramidal neurons in the hippocampus are selectively vulnerable to transient anoxic-ischemic damage. In experimental animals the CA1 pyramidal neurons undergo cell death several days after brief forebrain ischemia. It remains, however, unknown whether this delayed neuronal death is necrosis or apoptosis. We examined the degenerating process of the CA1 pyramidal neurons in gerbil hippocampus after brief ischemia. Nuclei of the CA1 neurons were nick end labeled by biotinylated dUTP mediated by terminal deoxynucleotidyl transferase 3 and 4 days after ischemic insult, but not at the prior stages. Simultaneously, dense chromatin masses appeared in nuclei of the neurons. By electrophoretic analysis, laddering of DNA occurred only in CA1 hippocampal tissues obtained 4 days after ischemic insult. The fragmented DNA in the CA1 pyramidal layer was phagocytosed by microglial cells. The results suggest that delayed death of the CA1 pyramidal neurons after brief ischemia is not necrotic but apoptotic.

425.2

VISUALIZATION OF DNA DOUBLE STRAND BREAKS IN THE GERBIL HIPPOCAMPAL CA1 FOLLOWING TRANSIENT ISCHEMIA K. Toda, S. Kihara, T. Shiraishi*, Y. Ju and K. Tabuchi. Dept. of Neurosurgery, Saga Medical School, Saga, JAPAN 849.

In order to clarify the relationship between delayed neuronal death and apoptosis, we made an attempt to detect DNA strand breaks in the gerbil hippocampal CA1 pyramidal neurons following transient ischemia by an in situ DNA strand breaks staining. Adult male gerbils were anesthetized with 2% halothane and transient forebrain global ischemia was produced by occluding bilateral common carotid arteries for 5 min using aneurysmal clips. Hippocampal specimens were stained using Apoptag in situ apoptosis detection kit. The condensed nuclei of neurons in CA1 were occasionally seen and positively stained 3 days after ischemia. The degenerating dark neurons with condensed nuclei in CA1 markedly increased in number and were clearly stained for DNA strand breaks 5 days after ischemia, whereas normal-looking nuclei remained unstained. No neurons were stained in other hippocampal subfields and dentate gyrus. The present study strongly indicates that the CA1 pyramidal neurons following transient ischemia die through an apoptotic process.

425.3

INDUCTION AND REPAIR OF DNA DAMAGE IN NORMAL RAT NEURONS, ASTROCYTES, AND CEREBRAL ENDOTHELIAL CELLS. G.T. Gobbel, T.Y-Y. Chan, M. Bellinzona, J.R. Fike, P.H. Gutin and P.H. Chan*. CNS Injury & Brain Edema Res. Center, Dept. Neurol., and Brain Tumor Res. Center, Dept. Neurol. Surg., Univ. of Calif., San Francisco, CA 94143

Brain ischemia can result in oxidative stress leading to the formation of free radicals. These free radicals can potentially exacerbate tissue injury by causing chemical modifications to lipids, proteins, and DNA. Damage to critical parts of the DNA molecule could lead to cellular dysfunction and further tissue injury. In the present study, we have begun to examine the sensitivity of normal brain cells to DNA damaging agents and the ability of those cells to repair damage. Neurons, astrocytes, and cerebral endothelial cells were isolated from Sprague-Dawley rats at day 15 of gestation, postnatal day 1, and postnatal day 14, respectively, plated into plastic culture dishes, and grown to confluence. The cells were exposed to radiation doses of 0, 8, 16, and 32 Gy to induce DNA double-strand breaks (DSBs) and then incubated for 0 min to 48 hrs to allow repair of the DNA damage. The number of DSBs remaining at the various time points was determined by subjecting the DNA to pulsed-field gel electrophoresis. There was a linear increase in the number of DSBs with radiation dose. There were two components to DSB repair: a fast component with half-times of repair of 10-15 min and a slower component with half-times of repair of 3-7 hrs. While there was no difference between astrocytes and endothelial cells in either the number of DSBs induced or the rates of repair, the number of DSBs induced in neurons was reduced by 7% and the rate of repair increased by 10% relative to the other cell types. Treatment of astrocytes with 0.25 mM dibutyryl cyclic AMP decreased the number of DSBs induced and signficantly increased their rate of repair. These results indicate that cyclic AMP can enhance repair of DNA damage and that neurons are better able to repair DNA damage than other brain cell types. Supported by NIH Grants AG 08938, NS 14543, NS 25372, CA 13525, and the American Brain Tumor Association.

425.4

DETECTION OF DNA STRAND BREAKS IN RAT CORTICAL NEURONS EXPOSED TO FOCAL ISCHEMIA-REPERFUSION IN VIVO. C. Du, R. Hu, S. Wong, D.W. Choi, C.Y. Hsu*. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

In our rat model of focal middle cerebral artery ischemia-reperfusion, coagulation necrosis occurs confined to the distribution of the artery. In the infarcted tissue, neuronal death evolves over several days after the ischemic insult. Some recent studies have suggested that neuronal DNA fragmentation may accompany focal ischemic injury, raising the issue of apoptosis. The present study was conducted to look for evidence of DNA strand breaks in our ischemia model.

Genomic DNA isolated from the ischemic cortex was subjected to non-denaturing polyacrylamide gel electrophoresis. Twenty-four and 48 hr after ischemia, DNA fragments of various sizes were seen. 3'-OH DNA ends were immunohistochemically identified in the formalin-fixed, paraffin-embedded brain slices by in situ end labeling (using terminal transferase with dUTP- digoxigenin) followed by anti-digoxigenin-peroxidase immunohistochemistry. DNA strand breaks detectable by such immunostaining was not observed in non-ischemic cortex, or in controls stained without dUTP-digoxigenin, antidigoxigenin antibody, or terminal transferase. Strong immunoreactivity was noted in the nuclear regions of injured and near normal-appearing neurons in ischemic cortex, 6 - 48 hr after the ischemic insult. The detection of DNA strand breaks by 2 different methods supports the idea that cortical neuronal death in focal ischemia may involve endonucleolytic degradation of DNA similar to that noted in several forms of apoptosis.

425.5

DNA FRAGMENTATION IS NOT AN EARLY EVENT IN ISCHEMIC NEURONAL DEATH. P. Wu* and J.N. Davis. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794-8121.

DNA fragmentation is thought to be an early, perhaps even, initial event in programmed cell death. We have been exploring the hypothesis that ischemic cell death may be a form of programmed cell death in focal and transient global ischemia in gerbils. After 5 minutes of bilateral carotid occlusion, hippocampal CA_1 neurons under go a delayed death. We studied DNA using 2% agarose gels at various times after transient carotid occlusion from whole hippocampus (3 experiments), dissected CA_1 (1 experiment), and punches of CA_1 pyramidal cells (3 experiments) . DNA from dexamethasone-treated thymocytes served as a positive control.

We did not see DNA "laddering" either at late times (48, 54, 66, 72 hours) or at earlier times (2, 4, 6, 24 hours). While this work was in progress, Okamoto *et al* (1993) reported DNA "laddering" from gerbil whole hippocampus between 54 and 96 hours after carotid occlusion. We tried to reproduce their results (2 experiments) even using their extraction technique, but were unable to confirm their findings.

We found DNA fragmentation in focal ischemia as reported by others. After permanent ligation of one carotid artery, half the gerbils sustained an infarct (as assessed by TTC staining); some also had seizures. "Stepladders" were present at 24 hours and more obvious at 48 hours, but were not present at 3 or 6 hours after ligation. In focal ischemia DNA fragmentation was a late phenomenon appearing after necrotic cell death in the infarcted area.

We conclude that DNA fragmentation does not trigger ischemic cell death as has been shown for some other forms of apoptosis. Further it appears that DNA "stepladders" can be seen as a late event in necrotic cell death. [Supported by the NIH (NS 30559) and the VA]

425.6

BCL-2 PROTECTS NEURONAL CELLS FROM HYPOXIA/REOXYGENATION INDUCED DEATH. A.N. Murphy, K.M. Myers, C.L. Miller, D.E. Bredesen[§], and G. Fiskum*, Dept. of Biochem.& Molecular Bio., The George Washington Univ. Med. Ctr., Washington, D.C. 20037, and [§]Dept. of Neurology, UCLA, Los Angeles, CA 90024.

The protooncogene *bcl-2* rescues cells from a wide variety of cellular insults that may induce either apoptotic or necrotic death. Recent evidence suggests that the mechanism of action of Bcl-2 involves antioxidant activity. The involvement of free-radicals in ischemia/reperfusion injury has led us to investigate the effect of Bcl-2 in this model of cytotoxicity. We have examined the survival of control and *bcl-2* transfectants of a hypothalamic cell line, GT1-7, exposed to chemical hypoxia/aglycemia (3 mM KCN, no glucose) with or without concurrent serum deprivation. After 30 min. of treatment, no loss of viability was evident in control or *bcl-2* transfectants under any conditions. However, Bcl-2 expressing cells were protected from delayed cell death measured after 24 hours of reoxygenation (KCN washout) in growth medium (DMEM+10% fetal bovine serum). Bcl-2 expressing cells incubated for 30 min. in KCN without glucose or serum were 68 ± 11% (s.e.m.) viable after 24 hours of reoxygenation, whereas control transfectants were 40 ± 9% viable compared to cells incubated for the same period in growth media. This protective effect was also evident if dialyzed serum was included in the 30 min. treatment with KCN (Bcl-2 expressors after 24 hours were 72 ± 9% and controls were 44 ± 8% viable). Serum deprivation for 30 minutes without the addition of KCN resulted in 83 ± 11% viability at 24 hours in Bcl-2 cells, and 66 ± 9% with control cells. These results indicate that Bcl-2 can protect neuronal cells from delayed death resulting from chemical hypoxia and reoxygenation. The data also indicate that cytotoxicity of hypoxia/reoxygenation is greater than death due to serum deprivation alone, suggesting either more extensive or additional mechanisms of cell injury than those elicited in response to serum deprivation. Prelimary experiments suggest that the effect of Bcl-2 may relate to alteration in mitochondrial electron transport. [Supp. by Sigma Tau S.p.A. and Souers Stroke Fund]

425.7

DNA FRAGMENTATION FOLLOWING GLOBAL ISCHEMIA IS NOT RANDOM NECROSIS I.E. Hill,[1] A. Buchan*[2] E. Preston,[1] I. Rasquinha,[1] T. Walker,[1] J.P. MacManus[1]. [1]Institute for Biological Sciences, National Research Council Canada, Ottawa, Ontario KIA OR6, Canada and [2]Ottawa Civic Hospital, Ottawa, Ontario KIY 4E9, Canada, from the *Canada/Fisons Fight Stroke* program.

Previous work provided evidence for an apoptotic component in the cell death in rat brain following global ischemia produced by two-vessel occlusion (2VO) (MacManus *et al.*, Neurosci. Letts. 164, 89-92 (1993)). Further studies undertaken to establish a time course for the development of this damage following 12 min 2VO showed an increase in damage with increasing reperfusion time. Cell death was quantitated by two methods. Firstly, DNA was radiolabeled with dideoxyATP at a 3' OH and the labeled DNA below 10kb following gel electrophoresis was measured. Secondly, 3' OH ends were labeled *in situ* on fixed sections taken from ischemic brains to identify those cells containing fragmented DNA. A comparison of these two parameters was undertaken for the striatum. This gave a positive correlation, thereby supporting the idea that the internucleosomally cleaved DNA seen by gel electrophoresis is derived from the cells identified by *in situ* labelling and that this provides a quantitative measurement of the cell death. It was also apparent that fragmented DNA could be detected at the same time that morphological changes in the nucleus were observed.

To further confirm an apoptotic component in the death of cells following transient ischemia a comparison was made with the necrotic cell death produced by decapitation. Degraded DNA was detected as a smear after electrophoresis, not as discrete bands. A time course study showed that selectivity in the hippocampus differed from that seen following ischemia with fragmented DNA first being detected in the dentate gyrus, followed by CA1, then CA3.

Further support for an apoptotic process being activated following ischemia is provided by these results. The time course for ischemia damage determined by conventional staining techniques correlated with that for DNA cleavage and major distinctions were observed between ischemic cell death and necrotic cell death.

425.8

APOPTOSIS AND NECROSIS CONTRIBUTE TO DELAYED CELL DEATH FOLLOWING HYPOXIC-ISCHEMIC INJURY TO THE IMMATURE RAT BRAIN. E.J.Beilharz, Chris E.Williams, M.Dragunow[+], E.S.Sirimanne, R.Faull[#], P.D.Gluckman. Research Centre for Developmental Medicine and Biology, [+]Department of Pharmacology, [#]Department of Anatomy, University of Auckland, Auckland, New Zealand.

The mechanisms leading to delayed cell death were examined in the 21 day old Wistar rat brain following either mild (15 min) or severe (60 min) unilateral hypoxic-ischemic injury. The timecourse of DNA degradation (using gel electrophoresis and in-situ end-labelling), microglial activation (lectin histochemistry), mitochondrial failure (TTC staining), cell death (acid fuchsin staining) and c-jun expression were examined. The mild injury led to selective neuronal loss. This was associated with the development of apoptotic morphology, DNA laddering and acidophilia from 3d post-hypoxia. Prolonged expression of c-jun occurred in these neurons before death. Cell death was accelerated by the severe injury, with DNA degradation and apoptotic morphology seen at 10hr in some regions. However, in the infarcted cortex a different pattern was seen: the DNA and mitochondria remained intact, and cells basophilic, until after 10hr post-hypoxia, then widespread necrosis developed by 24hr. In contrast to regions of selective neuronal loss, DNA degradation was initially random (at 24hr), with laddering not detected until 3d. Microglial activation coincided with the onset of DNA degradation in regions of selective neuronal loss (in both models) but not infarction. The results clearly indicate that there are two distinct pathways to cell death: cortical infarction was necrotic, and occurred independently of microglial activation and apoptosis. In contrast, selective neuronal death was apoptotic and closely coupled to the microglial reaction and c-jun expression.

NEUROTOXICITY: EAA I

426.1

EFFECTS OF NMDA ANTAGONISTS ON METHAMPHETAMINE-INDUCED STRIATAL DA OVERFLOW IN RATS.
JA Clikeman and KT Finnegan*, Depts of Psychiatry and Pharmacology, Univ. of Utah Sch. Med. and the VA Med. Ctr., Salt Lake City, UT 84148.

Methamphetamine (METH) damages nigrostriatal DA nerve terminals in rodent and monkey brain. Pretreatment with alpha-methyl-p-tyrosine or DA receptor antagonists protect against the toxic effects of METH, suggesting that at least one component of the neurotoxicity involves the ability of METH to increase extracellular concentrations of DA. NMDA antagonists are also capable of blocking the neuronal-damaging effects of METH, suggesting that alterations in glutamatergic neurotransmission may be involved as well. However, several of the NMDA antagonists employed (e.g., MK801, PCP) are also reported to affect DA systems, raising the possibility that a reduction in DA neurotransmission, rather than NMDA receptor blockade, underlies their neuroprotective actions. We therefore determined the abilities of two NMDA antagonists, CGS19755 and MK801, to reduce METH-induced DA release in the rat striatum using *in vivo* microdialysis, and also determined tissue levels of striatal DA in these same animals one week after drug injection. The administration of METH by itself (10 mg/kg/inj every 2 hr x 4, s.c.) caused large increases in striatal ECF DA (> 5500 %). Pretreatment with MK801 (2 mg/kg/inj every 2 hr x 4, given 30 min before METH, i.p.) did not significantly alter METH-induced DA overflow. In a similar fashion, pretreatment with CGS19755 (35 mg/kg/inj every 2 hr x 4, given 30 min before METH, i.p.) also failed to significantly alter the DA-releasing effects of METH. Nonetheless, both CGS19755 and MK801 partially prevented the DA-depleting effects of METH in rats killed one week later. The data suggest that a reduction in METH-induced DA overflow can not explain the neuroprotective actions of CGS19755 or MK801.

426.2

METHAMPHETAMINE-INDUCED DEPLETION OF GLUTAMATE-POSITIVE NEURONS IN THE SOMATOSENSORY CORTEX OF THE RAT. C. Pu and C. V. Vorhees*, Children's Hospital Research Foundation, University of Cincinnati, Cinti, OH 45229, USA.

The neurotoxic effects of methamphetamine on dopaminergic and serotonergic terminals have been well documented. However, the neurochemical characteristics of degenerating neurons in the somatosensory cortex seen by silver staining are unknown. By using glutamate immunohistochemistry, it was found that MA exposure in adult rats (10 mg/kg given 4 times i.p. at 2 hour intervals) causes localized depletion of glutamate-positive neurons three days following treatment. The affected region is in the somatosensory cortex, covering the middle one-third portion from the longitudinal fissure to the rhinal sulcus. The depletion of glutamate-positive neurons occurred in all layers of the cortex with layers II-III showing the most pronounced effects. This pattern of depletion is consistent with that demonstrated previously with silver staining following methamphetamine, amphetamine and methylenedioxymethamphetmaine (MDMA) exposures. The results indicate that MA exposure induces degeneration of glutamate neurons in the somatosensory cortex of adult rats.

426.3

TOXICITY FOLLOWING *IN VIVO* INFUSION OF ANTISENSE OLIGONUCLEOTIDE: ROLE OF THE INTER-INFUSION INTERVAL. B.J. Chiasson* and H.A. Robertson, Dept. of Pharmacology, Dalhousie Univ. Halifax, N.S., CANADA, B3H 4H7.

Activation of *c-fos* in the amygdala is observed following a single kindling stimulus to the amygdala. We have been examining the role of *c-fos* in the development of amygdala kindling using antisense technology. In these studies, animals received an infusion into the amygdala via a chronically implanted cannula either once (Group 1) or on a daily basis (Group 2) with phosphorothiaote antisense (AS) or control (C) oligonucleotides. Ten hours after the infusion rats in both groups were administered a kindling stimulus in the amygdala and an electroencephalographic afterdischarge (AD) was recorded. The AS treated animals in Group 1 showed considerable attenuation of Fos-immunoreactivity when compared to the C treated animals. However, during the course of these studies we observed that subsequent to 3-4 infusions Group 2 animals (both AS and C) no longer demonstrated ADs. Upon histological examination of these animals, lesions to the amygdala and surrounding areas were observed. Reactive gliosis was demonstrated surrounding the lesion with glial fibrillary acidic protein immunoreactivity (GFAP-IR). These detrimental effects are dose related. Further studies have now revealed that the degree of histological damage can be greatly minimized by extending the period between infusions. These results suggest that certain cautions must be taken when using antisense technology and furthermore that only certain types of experimental approaches are amenable to its use.

Supported by MRC Canada and the Savoy Foundation.

426.4

TAURINE RELEASE FROM SLICES OF CEREBRAL CORTEX AND BASAL GANGLIA IN TWO RAT MODELS WITH MODERATE HYPERAMMONEMIA. W. Hilgier, J. Albrecht, J.E. Olson*, Med. Res. Ctr., Pol. Acad. Sci., Warsaw, Poland, and Wright State Univ., Dayton, OH.

Hepatic encephalopathy (HE) causes brain edema and alters the brain concentration of taurine (TAU), an amino acid implicated in brain volume regulation. We used 400 μm slices of cerebral cortex and basal ganglia to investigate the mobilization of this amino acid by hypo-osmolality in animal models of HE. Moderate hyperammonemia was produced in rats (Wistar, 130-220 g) by 3 daily i.p. injections of 250 mg/kg thioacetamide, a hepatotoxin which causes cerebral edema or 600 mg/kg ammonium acetate (simple hyperammonemia - SHA). Both treatments produce brain ammonia of ~0.6 mmoles/kg (1). Control animals were injected with normal saline. Basal release rate of loaded [^{3}H]-taurine from cerebral cortical slices (CCS) of animals injected with TAA was 130±4% (mean±SEM) of the rate measured in control animals. Superfusion with hypoosmolar (HYPO) medium (NaCl reduced from 150 mM to 40 mM) increased [^{3}H]-taurine release from CCS of control and TAA rats by 12%. TAA injections did not alter basal or HYPO-induced [^{3}H]-taurine release from slices of basal ganglia, a structure which does not develop edema in this animal model (1). These results are consistent with the osmoregulatory function of taurine in TAA-induced brain edema. SHA decreased both basal and HYPO-induced [^{3}H]-taurine release in CCS. However, in this model of hyperammonemia, no edema or change in taurine content occurs in this brain region (1).

1. Hilgier and Olson, J Neurochem, 62:197, 1994.
Supported by NIH (NS-23218 and TW-00180).

426.5

MODULATION OF EXCITOTOXIC DAMAGE BY INTERLEUKIN-1 (IL-1) AND IL-1 RECEPTOR ANTAGONIST (IL-1ra). Lawrence C.B. and Rothwell N.J.* Neuroscience Division, 1.124, School of Biological Sciences, University of Manchester M13 9PT, UK.

We have previously suggested that the cytokine IL-1 is involved in ischaemic and excitotoxic brain damage, since recombinant human IL-1ra (rhIL-1ra, Synergen, USA) inhibits neurodegeneration caused by focal ischaemia or NMDA receptor activation in the brain. Neurodegeneration has been ascribed to overactivation of AMPA as well as NMDA receptors. In the present study we have therefore compared effects of rhIL-1ra and recombinant IL-1ß (rIL-1β) on damage induced by infusion of an NMDA (cis-2,4-methanoglutamate, MGlu) or AMPA (S-AMPA) receptor agonist into the striatum of the rat brain. Both agonists induced reproducible lesions assessed histologically 24-48h later. Coinfusion of rhIL-1ra (5μg) with the excitatory amino acid agonist (10nmol MGlu or 15nmol S-AMPA) significantly inhibited damage (lesion volume) by 46% or 43% when induced by NMDA or AMPA receptor activation respectively. In contrast, coinfusion of rIL-1ß (14pg, 100U) did not affect either form of damage (the dose of excitotoxins used were 5nmol MGlu and 10nmol S-AMPA). Additionally IL-1β did not induce neurodegeneration when infused alone. These data indicate that endogenous, but not exogenous IL-1, mediates NMDA and AMPA receptor induced neurodegeneration.

426.6

SELECTIVE VULNERABILITY OF HIPPOCAMPAL CELL FIELDS TO NEUROLOGICAL INSULTS. O.A. Ajilore, B.A. Stein-Behrens*, and R.M. Sapolsky. Dept. of BioSciences, Stanford Univ., Stanford, CA 94305

The hippocampus is a region of the brain that is particularly vulnerable to various neurological insults. Selective vulnerability occurs within the hippocampus as well. Three specific regions, CA1, CA3, and dentate gyrus, are preferentially damaged by hypoxia-ischemia, excitotoxicity and hypoglycemia, respectively. These neurological insults have an energetic component that plays an important role in neuron death. To further characterize and understand the issue of selective vulnerability in the rat hippocampus, we measured metabolic function in regions of the hippocampus during neurological insults using a device called the microphysiometer. The microphysiometer measures the acidification rate in cells in real time as an indicator of metabolic function. The acidification rate refers to the decrease in extracellular pH due to the accumulation of the acidic metabolites of ATP hydrolysis. By detecting this pH change, the microphysiometer provides a sensitive measure of cellular metabolism. Previous neurological studies have used the microphysiometer to study metabolic changes in primary fetal monolayers. In which, it was not possible to study selective vulnerability in different cell fields. In this project, we have adapted the device for use with hippocampal slices, which allows for the monitoring of the metabolic effects of specific insults on isolated cell fields with a high degree of temporal resolution. 400 μm-thick slices were generated in a standard manner and each cell field was dissected under a microscope and placed in a separate microphysiometer sensor chamber. With this method, we obtained stable metabolic rate measurements from different hippocampal cell fields using a variety of media. We also observed a dose-dependent increase in metabolic rate induced by kainic acid in CA3-derived tissue. Selective responsiveness to kainic acid was also demonstrated in that kainic acid failed to elevate metabolism in CA1-derived tissue. These studies suggest that the microphysiometer is sensitive enough for the study of selective vulnerability in the hippocampus

426.7

CIS-FLUPENTHIXOL PROTECTS STRIATAL CELLS FROM MALONATE-INDUCED LESIONS P. Hantraye*, M-C. Guyot, S. Palfi and E. Brouillet. CNRS URA 1285, Service Frédéric Joliot, DRIPP-DSV, 91401 Orsay Cedex and INSERM CJF 91-02, HÔpital Henri Mondor, Créteil 94010, FRANCE.

Malonate, a succinate deshydrogenase inhibitor, has been shown to produce striatal lesions resembling Huntington's disease striatal degeneration when administered directly into the striatum of adult rats (Beal et al. 1993, J. Neurochem. 61, 1147-50). This lesion is believed to be mediated via a glutamatergic (possibly NMDA) excitotoxic-like mechanism.

Dopamine is known to modulate glutamatergic transmission in the striatum through different mechanisms (modulation of glutamate release, inhibition of NMDA-induced excitatory post-synaptic potentials). The aim of the present study was to assess the potential protective effects of various dopamine antagonists on malonate-induced lesions.

Adult rats, injected intra-peritoneously (pretreatment at 30 min and post-treatment 60 min after) with either cis-flupenthixol (a mixed D1/D2 dopamine antagonist), SCH23390 (a selective D1 antagonist) or saline (control) received a single malonate injection (2μmoles into 1 μl) into the left striatum. At one week survival, the cis-flupenthixol group demonstrated a significant improvement in neuronal survival. Compared to controls, cis-flupenthixol provided more than 50% neuronal protection whereas SCH23390 had no significant protective effects.

It is concluded that manipulating the dopamine neurotransmission may confer efficient neuronal protection against excitotoxic-like striatal lesions induced by transient mitochondrial energy impairment.

426.8

DIFFERENT RESPONSES TO N-METHYL-D-ASPARTATE AND KAINIC ACID IN CEREBELLAR GRANULAR CELLS OF LEAD-EXPOSED PUPS. D.K. Lim,[1]* T. Ito[2] and I.K. Ho[2] [1]College of Pharmacy, Chonnam National University, Kwang-Ju, 500-757 Korea and [2]Dept of Pharmacology and Toxicology, Univ. Miss. Med. Ctr., Jackson, MS 39216 USA.

To determine changes in response to N-methyl-D-aspartate (NMDA) and kainic acid (KA) in cerebellar granular cell of offspring of lead-exposed mothers, pregnant rats received 0.25% lead acetate in the drinking water 2 days after gestation. The control group was given sodium acetate (0.125%) in drinking water. The cerebellar granular cells were cultured from 7 or 8 day old pups. Changes in the levels of cytosolic Ca^{++} and cGMP and the release of glutamic acid were measured using fure-2 spectrofluorometer, radioimmunoassay and HPLC, respectively. Increases in cytosolic Ca^{++} were less responsive (77%) to 50 μM of NMDA and KA in the lead exposed group as compared to that of the control group. However, the maximum responses to KA were not different. The NMDA-induced elevation of cGMP was not affected in the lead exposed pups. However, the degree of KA-induced increase in cGMP level in cerebellar granular cells of lead exposed pups was significantly reduced. The increases in cGMP level in lead exposed pups were 55.5 and 51.4% of the control groups when the incubations were carried out for 3 min and one hour, respectively. The NMDA- and KA-stimulated release of glutamate from cerebellar granular cells prepared from lead exposed pups was also significantly reduced. These results indicate that lead exposure to the mother might affect the excitatory amino acid systems during the the development of the offspring.

426.9

PERINATAL HYPOXIC INSULT DIMINISHES NEUROTOXICITY OF 3-NITROPROPIONIC ACID (3-NPA) IN ADULT RATS. Z. Binienda*, S.F. Ali, C.M. Fogle, W. Slikker, Jr., M.G. Paule and S.A. Ferguson. NCTR/FDA, Jefferson, AR 72079-9502.

The effects of a mitochondrial toxin 3-NPA on operant performance, dopamine (DA), serotonin and their metabolite concentrations in selected brain regions were studied in adult male Sprague-Dawley rats. Rats were delivered by cesarean section as either noninsulted (NI) or insulted (I) with hypoxia. Hypoxia was produced by submerging dissected uterine horns in saline for 15 min. NI rats were siblings of the I rats delivered from the adjacent nonsubmerged horns. Rats were cross-fostered and behaviorally assessed. At 1 year of age, NI and I rats were injected with 3-NPA at 5 mg/kg/day (Monday-Friday). The dose was increased by 5 mg weekly up to 30 mg/kg/day. After sacrifice at the end of 3-NPA treatment, brains were dissected, weighed and analyzed for neurochemistry. Operant measures significantly decreased with increasing 3-NPA doses but were not differentially affected by hypoxia. Ataxia at the highest dose of 3-NPA was observed only in NI rats (3/9 rats). A trend toward a DA concentration increase in frontal cortex was seen in both I and NI groups. While weights of brain regions in the control and 3-NPA treated I rats were similar, cerebellar weight in NI rats after 3-NPA treatment was reduced (ANOVA, $p<0.001$). Thus, perinatal hypoxic insult may decrease subsequent sensitivity to the toxic effect of 3-NPA.

426.10

HU-211, A NON-PSYCHOTROPIC CANNABINOID AS A NOVEL NEUROPROTECTANT AGENT. N. Eshhar*, S. Striem, V. Nadler and A. Biegon. Pharmos Corp., Kiryat Weizmann, Rehovot 76326, Israel.

Accumulation of evidence suggests that multiple proccesses lead to neuronal death following cerebral ischemia. These are associated with excessive activation of excitatory amino acid receptors and involve the generation of free radicals from different sources. Previous studies have demonstrated the ability of HU-211, a synthetic non-psychotropic cannabinoid, and a non competitive NMDA receptor antagonist to protect cortical neurons from damage mediated via the NMDA preferring glutamate receptors. The current study was designed to explore potential protective effects of HU-211 on neuronal degeneration following anoxia or exposure to the nitric oxide (NO) donor sodium nitroprusside (SNP) in culture. Primary cerebral cortical cell cultures were prepared from 18-20 day old fetal rats and consisted of neurons plated over a confluent glial-cell feeder layer. Toxicity experiments were performed with cells at 10 days in culture and cell viability was evaluated by morphological criteria following neuron specific enolase immunostaining and assessed quantitatively by measuring the extent of mitochondrial activity in the cells. Subjection of cultures to 1 hour deprivation of oxygen and glucose was associated with neuronal cell injury that was markedly reduced by 10 μM HU-211 or by 30 μM MK-801 when present during or following anoxia. By contrast, exposure of cells to SNP resulted in a massive glial and neuronal cell damage which was attenuated by the addition of 10 μM HU-211, while not affected by the presence of 30 μM MK-801. In conclusion, HU-211 can protect neurons from damage associated with multiple biochemical pathways, probably via more than one mechanism of action. The broad spectrum neuroprotective activity of HU-211 suggests the use of the compound for preventing ischemia-related neuronal damage in which excitatory amino acid receptors and NO have been shown to play major roles.

426.11

L-CHLOROPROPIONIC ACID NEUROTOXICITY INVOLVES A SUBTYPE OF N-METHYL-D-ASPARTATE RECEPTOR. E.A. Lock, I. Wyatt, M.G. Simpson, M. Lo* and P.S. Widdowson. Zeneca Central Toxicology Laboratory, Macclesfield, UK and Zeneca Inc, Wilmington, DE19897, USA.

Systemically administered L-chloropropionic acid (CPA) produces selective damage to cerebellar granule cells. We investigated whether CPA may produce the granule cell damage by acting at a subtype of the N-methyl-D-asparate (NMDA) receptor. Groups of rats were treated with CPA (750 mg/kg/po). Some animals received the irreversible NMDA antagonist, MK-801 alone (5mg/k.g./i.p.), saline or CPA plus MK-801. The neurotoxicity was assessed at days 3 and 5 of the study. Animals which received CPA alone showed marked locomotor retardation which became very severe by days 3 and 4. These animals showed marked weight loss (-22% of controls) and were terminated at days 3 and 4 of the study. In contrast, CPA and MK-801 treated animals although showing a small weight loss early on in the study did not show abnormal locomotor activity by days 2 to 5. Neuropathological examination of the brains from CPA-treated rats showed a marked (80-90%) loss in cerebellar granule cells by day 3 and 4 of the study. The co-administration of MK-801 with CPA was able to completely prevent damage to the cerebellum. MK-801 was also able to prevent the CPA-induced loss of NMDA receptors in the granular layer of the cerebellar cortex and concentrations of aspartate and glutamate in cerebellum. Finally there was a complete prevention of the CPA-induced cerebellar edema by MK-801. In conclusion we suggest that CPA is neurotoxic to rat cerebellum because this brain region contains a subtype of NMDA receptor with unique pharmacology and regulation.

426.12

DODECYLGLYCEROL PROVIDES PARTIAL PROTECTION AGAINST GLUTAMATE TOXICITY IN NEURONAL CULTURES DERIVED FROM DIFFERENT REGIONS OF EMBRYONIC RAT BRAIN. J.R. Dave*, F.C. Tortella, E. Fasnacht, B.P. Doctor and H.S. Ved. Divisions of Neuropsychiatry and Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Earlier studies have demonstrated that similar to platelet activating factor (PAF), a structurally related glycerol ether lipid void of platelet aggregating activity, dodecylglycerol (DDG), stimulates neuronal differentiation in primary cultures via a pathway involving activation of the *c-fos* gene. The objective of the present study was to determine if DDG treatment provided protection against glutamate-mediated neurotoxicity in neuronal cells. Primary cultures enriched in neurons dissociated from embryonic rat cerebral cortex, hippocampus and cerebellum were treated in a serum-free media with either vehicle, DDG (3 μM), glutamate (75 μM) or DDG and glutamate together for 24 hr and their morphological and biochemical [lactate dehydrogenase (LDH) released in the culture media] assessments were made. The neuronal differentiating effects of DDG, and neurotoxic effects of glutamate, were maximum in neurons from the cerebellum and minimum in neurons from the cortex. Co-treatment of cells with DDG and glutamate failed to provide significant neuronal protection against glutamate in all the three brain regions. However, preteatment of cells with DDG for 4 or 24 hr prior to glutamate treatment provided significant neuroprotection as judged by morphological changes and a decrease in LDH activity in the culture media. A neuroprotection of approximately 15-35% was observed following 4 hr DDG preteatment and approximately 60-85% protection was observed after 24 hr DDG preteatment. Although the mechanism of DDG's neuroprotective action remains to be elucidated these results demonstrate that both glutamate and DDG have differential specificity for anatomical regions of the brain.

426.13

ANTI-PHENCYCLIDINE MONOCLONAL FAB FRAGMENTS DRAMATICALLY DECREASE PHENCYCLIDINE (PCP) NEUROTOXICITY IN SPRAGUE-DAWLEY (SD) RATS. J.L. Valentine, W.D. Wessinger and S.M. Owens*. Dept. of Pharmacology & Toxicology, Univ. of Ark. for Med. Sci., Little Rock, AR 72205.

High affinity (K_d=1.8 nM) anti-PCP monoclonal Fab fragments were used to reverse *in vivo* PCP-induced behavioral toxicity. For these studies, male SD rats (n=4 per group) were administered four treatments in random crossover design. 1) An i.v. dose of 1.0 ml/kg saline vehicle, followed 5 min later by 1.0 ml saline (Saline–saline, negative control). All others received a behaviorally active dose of PCP (1.0 mg/kg) followed 5 min later (as toxicity began to maximize) with 2) saline (PCP–saline, positive control). 3) anti-PCP Fab (PCP–anti-PCP Fab) at 0.3, 1.0 and 3.0 times the PCP molar dose, and 4) nonspecific polyclonal human Fab (PCP–nonspecific Fab) at the same doses as the anti-PCP Fab. To assess PCP and treatment effects, locomotor activity was determined over 2-min intervals for a 60-min period on an Automex 2SD activity monitor. Baseline activity counts were monitored for 10 min prior to saline or PCP administration. Saline–saline injections resulted in relatively low background counts (0-25 counts per 2-min interval). PCP-saline injections caused extreme hyperactivity as determined by a maximum response of approximately 150 counts/2 min and a duration of 39 ± 7 min. When analyzed as a percentage of the total PCP–saline response, none of the nonspecific human Fab treatments or the 0.3 M anti-PCP Fab had a statistically significant effect on locomotor activity. However, 1.0 M and 3.0 M anti-PCP Fab significantly attenuated locomotor activity to approximately 20-30% of PCP–saline controls and the PCP–nonspecific Fab (ANOVA followed by Newman-Keuls, $p<.05$). The duration of PCP-induced effects measured from the time of Fab treatment was reduced from 34 ± 7 min to 13 ± 4 min after the 1.0 M and 3.0 M anti-PCP Fab treatments. These data show that anti-PCP Fab dramatically reverses PCP-induced toxicity. (Supported by NIDA grants DA 07610, DA 05474, and DA 00110.)

426.14

MEASUREMENT OF NITRIC OXIDE FORMATION BY DICHLOROFLUORESCEIN IN NEURONAL CELLS. P.G. Gunasekar, A.G. Kanthasamy*, J.L. Borowitz and G.E. Isom. Dept. of Pharmacology & Toxicology, Purdue Univ., W. Lafayette IN 47907-1334.

A simple and rapid spectrofluorometric assay of intracellular nitric oxide (NO) formation was developed for use in cultured neuronal cells. The technique was first studied in a cell free system using the non-fluorescent probe 2,7-dichlorofluorescin which is oxidized to a fluorescent product, dichlorofluorescein (DCF) upon addition of the strong oxidant NO. The fluorescent signal was significantly increased over 10 min period and the effect was dependent upon the concentration NO added. In DCF loaded PC12 cells, addition of NO markedly increased the fluorescence. However, pretreatment with hemoglobin (Hb) inhibited the NO mediated increase of fluorescence in both cell free and PC12 models. Similar trends were observed with the NO generator sodium nitroprusside (SNP) in the cell free system and PC12 cells. To rule out the possibility that reactive oxygen species (ROS) mediated the increased fluorescent signal, superoxide dismutase(SOD) and catalase were added. Neither enzyme blocked the fluorescent signal generated by NO in PC12 cells. To determine if NO is generated in response to glutamate, 10 μM glutamate was added to DCF loaded cerebellar granule cells, a rapid raise of fluorescent signal was noted. However, the increase of fluorescent by glutamate was blocked 50% after addition of Hb or SOD or by following pretreatment with l-NAME (300 μM). It was concluded that glutamate generated intracellularly both NO and ROS and at least 50% of the oxidation of DCF is attributed to NO. These results indicate that the oxidation of DCF by NO can be used to measure its intracellular generation on a real time basis and by using Hb and SOD pretreatments, the extent of oxidation of DCF attributed to NO and ROS can be determined. (Supported in part by NIH grant ES04140)

426.15

18α–GLYCYRRHETINIC ACID (GA) BLOCKS KAINIC ACID-INDUCED SEIZURES AND NEUROTOXICITY.

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The overall objective of this study was to determine the effects of 18α- and 18β-glycyrrhetinic acid (GA), potent inhibitors of adrenal and gonadal steroid metabolism, on kainic acid excitotoxicity. Nonanesthesized, adrenally-intact, male Wistar rats (N≥8/group) received intracerebroventricular injections of either kainate (940 pmol) or GA (300 pmol) plus kainate (GA+KA). Rats treated with 18α-GA had a lower incidence of seizures (0 vs 71%) and significantly reduced necrosis in the endopiriform nucleus, amygdalohippocampal area, basal, medial, central, and lateral nuclei of the amygdala, dentate gyrus, CA_3/CA_4 of the hippocampus, midline nuclei of the thalamus, lateral septum, and premammillary hypothalamus compared to rats treated with kainate alone. Preliminary data indicate that the 18β-isomer is not neuroprotective. In fact 100% of the rats treated with 18β–GA+KA had behavioral seizures and extensive damage in the amygdala, thalamus, and neocortex. Biochemical analyses indicated that neither 18α-GA or kainate inhibited 11β-hydroxysteroid dehydrogenase at the doses used in this study. We conclude 18α- and 18β-GA have differential effects on kainate excitotoxicity when administered directly into the brain.

This work was supported by NIH grant ES07031.

426.16

KAINATE-ENHANCED HYPOXIC NEURONAL DAMAGE IN VITRO IS BLOCKED BY MK-801 AND GYKI-52466 BUT NOT BY APV.

A. Schurr, R.S. Payne and B.M. Rigor*. Dept. of Anesthesiology, University of Louisville, School of Medicine, Louisville, KY 40292.

We have shown recently that hypoxic neuronal damage in rat hippocampal slices can be enhanced by various excitotoxins, an enhancement that can be blocked by specific antagonists of these excitotoxins. Thus, dizocilpine (MK-801), 2-amino-5-phosphonovalerate (APV) and 7-chlorokynurenate (7-Cl-KYN) antagonized the enhancement of hypoxic neuronal damage by NMDA, while 2,3-dihydroxy-quinoxaline (DHQX) and to a lesser degree, kynurenate (KYN), blocked such enhancement by kainate (KA) and (R,S)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA). APV was ineffective in blocking the enhancement of hypoxic neuronal damage by KA or AMPA, while DHQX was unable to block an NMDA-enhanced damage.

The rat hippocampal slice preparation and electrophysiological measurements were used in the present study as described elsewhere. We compared the abilities of MK-801, a noncompetitive NMDA receptor antagonist, and amino-phenyl-2,3-benzo-diazepine (GYKI 52466) a specific KA receptor antagonist, to block KA-enhanced hypoxic neuronal damage. When hippocampal slices were exposed to 12-min hypoxia, 72% recovered their neuronal function following a 30-min recovery period. Inclusion of 2.5 μM KA in the perfusion medium 30 min before and during the hypoxic period reduced the recovery rate of neuronal function to 7%. This KA-enhanced hypoxic neuronal damage was blocked in a dose dependent manner by GYKI-52466, where 200 μM of the drug completely abolished the effect of KA. As expected, APV (100 μM) was unable to antagonize the effect of KA (18% recovery rate). However, MK-801 (20 μM), completely reversed the effect of KA (87% recovery rate). Preliminary results also indicate that MK-801 antagonizes the enhancement of hypoxic neuronal damage by AMPA.

The ability of MK-801 to protect neuronal tissue against both hypoxic and excitotoxic damage, cannot be explained by its NMDA receptor antagonism alone but rather by a separate and distinct mechanism, yet to be elucidated.

426.17

THE ROLE OF EXCITOTOXIC GLUTATHIONE-DERIVED METABOLITE(S) IN ETHYL CHLORIDE-INDUCED HYPERACTIVITY IN MICE.

R.S. Bitner, G.E. Isom and G.K.W. Yim*. Dept. of Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907.

Pottenger and co-workers (1991) demonstrated that mice displayed a glutathione (GSH)-dependent, hyperactive syndrome within 20-30 min of exposure to high concentrations of ethyl chloride (Et-Cl) (15,000 ppm). The hyperactivity resembled the wild running seizures observed in mice following i.c.v. glutamate or NMDA. To test the possibilty that excitotoxic metabolites were responsible for Et-Cl-induced hyperactivity, known and potential GSH-derived metabolites of Et-Cl were examined for excitotoxic properties. S-carboxymethylcysteine (CMC) and cysteine (Cys), but not S-ethylcysteine (SEC), injected i.c.v. (3.2 umol) caused immediate wild running seizures in mice. 5-days later, brain sections taken from CMC- and Cys-treated mice showed selective CA1 and wide spread hippocampal damage, respectively. However, seizures caused by CMC or Cys were not blocked by MK-801 or CNQX. Systemic administration of a high dose of SEC (1 gm/kg i.p.) did not cause delayed seizures as might be excpected if the cysteine conjugate were metabolized to CMC or Cys. S-ethyl-GSH (SEG) injected i.c.v. (3 umol) failed to cause wild running seizures, indicating that it did not share the NMDA agonist properties of GSH (Leslie et al., 1992). Mice depleted of brain GSH using buthionine sulfoximine (3 mg i.c.v.) also failed to display wild running seizures following i.c.v. administration of SEG. Thus, the results obtained with these known and potential (CMC and Cys) metabolites do not support the possibility that the neurotoxic actions of Et-Cl are due to formation of excitotoxic GSH-derived metabolites.

426.18

GLUTAMATE-INDUCED CYTOTOXICITY OF A HUMAN NEUROBLASTOMA CELL-LINE THROUGH ACTIVATION OF NMDA RECEPTORS.

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The neutral red cytotoxicity assay was used on human LA-N-2 neuroblastoma cells to determine the effects of a 48 hour incubation with L-glutamate (0, 1, 10 mM), and with the specific NMDA agonist N-phthalyl glutamate (1 mM) . The results indicate that 1 mM and 10 mM glutamate caused a 27% and 37% decrease, while N-phthalyl-glutamate exhibited a 46% decrease, in neutral red uptake. Compared to non-treated controls, the glutamate and N-phthalyl-glutamate (NPG) treated cells exhibited classic signs of toxicity, including, clumping of chromatin, the appearance of vacuoles, and cell swelling and lysis. The possible mechanism of this toxic effect was further evaluated using the whole cell configuration of the patch clamp technique, and the specific NMDA agonists NPG and (±)-1-aminocyclobutane-cis-1,3-dicarboxylic acid (ACDA), . Addition of 1 mM NPG or ACDA for 5 min. resulted in an increased inward sodium current as compared to non-treated controls. These studies indicate that the toxicity of glutamate observed in the LA-N-2 human neuroblastoma cell line is due, at least in part, to the activation of NMDA receptors.

426.19

HISTAMINE-INDUCED NEUROTOXICITY IN THE RAT MIDBRAIN.

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Several findings suggest that endogenous histamine (HA) may enhance glutamate-NMDA mediated brain excitotoxic mechanisms (Bekkers, *Science*, 47:118-120, 1993; Vorobjev. *et al.*, Neuron, 11:837-844, 1993) and participate in thiamine deficiency-induced brain damage (Onodera *et al., Agents & Actions*, 27:120-122, 1989; Langlais *et al., J. Neurosci. Res.*,1994, in press). Nevertheless, neurotoxicity produced by HA in normal animals has not been directly demonstrated. To test this hypothesis, a detailed, light-microscopic histopathological study was performed. Rats received intracerebral HA via chronically implanted guide cannulae aimed at the ventral lateral border of the periaqueductal grey. Following predetermined intervals, animals were perfusion-fixed and brain sections were stained with cresyl violet. Three days after injection of 100 μg of HA, large areas within the midbrain showed a marked proliferation of glial cells (mean rostro-caudal extent = 1250 ± 152 μm). Injection of 30 and 3 μg of HA induced proportionately smaller areas of gliosis. A dose of 0.3 μg produced no detectable effects. Control injections of isotonic or hypertonic saline also induced little or no pathological changes. At shorter survival times (24 or 48 hr), 100 μg of HA induced a clear loss of neurons, commensurate with a neurotoxic response. These findings show that HA can cause lesions in the central nervous system and support the hypothesis that endogenous HA may function as a mediator of neuropathological processes (Supported by DA-03816 and NS-29771).

426.20

KAINATE NEUROTOXICITY IN VIVO IS GREATLY AMPLIFIED BY IMPAIRED SODIUM PUMP CAPACITY

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Many adult neurons exhibit excitotoxicity in which death is caused by excessive transmembrane ion fluxes. Since the sodium pump (Na-pump) directly or indirectly rectifies these disturbances, we hypothesized that reduced or impaired Na-pump capacity will poorly rectify ionic disturbances and amplify the toxic potential of excitatory agents. Results of our prior work indicates that direct impairment of pump capacity in vitro markedly sensitizes neurons to glutamate. However, this model is fetal in origin and findings may not apply to adult cells. We now extend this work to adult cells in vivo, using the rat kainate-induced seizure model in which widespread excitotoxic damage occurs in the limbic system. Na-pump function of the a2 and a3 isoforms was inhibited by the cardiac glycoside ouabain given ICV to attain a final concentration of ~1 μM. This dose decreased high affinity sodium pump sites by ~50% (assessed by quantitative autoradiography) and when given alone, produced a stereotyped pattern of extreme motor activity, followed by a brief period of hypotonia. In spite of marked motor activity, no hippocampal neuronal damage was observed, as assessed by silver staining. Although low-dose kainate (4-6mg/kg IP) was not associated with seizures or neuronal damage, when given with ouabain it produced marked epileptiform activity and neuronal death. However, unlike high dose kainate, virtually no animal mortality was observed. Delaying administration of ouabain predictably increased the latency of seizures, consistent with a primary role for ouabain in potentiating kainate. EEG recording revealed that although ouabain initially diminishes cortical activity, it later amplifies spike activity associated with lower doses of kainate. Neuronal damage under this protocol was more extensive than for kainate alone and included the CA1 subfield of the dorsal hippocampus. Treated animals subsequently suffered spontaneous seizures and also exhibited remodeling of the inner molecular layer, as assessed by Timm staining. These studies demonstrate the critical importance of the sodium pump for the survival of adult neurons after stimulation by potential excitotoxins.

427.1

EXCITOTOXIC LESIONS OF RAT STRIATUM EXACERBATE METHAMPHETAMINE-INDUCED DECREASES IN MAZINDOL-LABELED DOPAMINE UPTAKE SITES. A.J. Eisch* and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717-4550.

Repeated systemic injections of methamphetamine (m-AMPH) produce significant damage to dopaminergic terminals in the rat striatum as shown by decreases in dopamine content and [3H]mazindol-labeled dopamine uptake sites. Previous work from this laboratory has demonstrated that a unilateral infusion of the excitotoxin quinolinic acid (QA) prior to m-AMPH treatment protects the infused striata from m-AMPH-induced decreases in dopamine content (O'Dell et al., J. Pharm. Exp. Ther., *in press*). Here, we extend the characterization of the effect of striatal QA lesions in the m-AMPH-treated rat by utilizing quantitative autoradiography of striatal dopamine uptake sites. One week after administration of m-AMPH, and three weeks after receiving unilateral striatal QA, the density of [3H]mazindol-labeled dopamine uptake sites in the QA-infused hemisphere of m-AMPH-treated rats was less than that in the non-infused hemisphere. This finding suggests an important dissociation between measures of dopamine content and dopamine uptake sites in the QA-infused striata of m-AMPH-treated rats. Ongoing studies will address whether the uncoupling of these measures of dopamine terminal integrity may be attributed to QA-induced alterations in dopaminergic metabolism.

427.2

KAINIC ACID INDUCED DNA DAMAGE IS ASSOCIATED WITH A UNIQUE IMMEDIATE-EARLY GENE RESPONSE IN c-*fos*-*lacZ* TRANSGENIC RATS. G. M. Kasof*, A. Mandelzys, T. Curran, and J. I. Morgan. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07710.

To discriminate between events that are products of seizures elicited by kainic acid (KA) and those that are specifically associated with its excitotoxicitic properties, we have examined the expression of cellular immediate-early genes (clEGs) following KA or pentylenetetrazole (PTZ) treatment. While both chemoconvulsants elict seizures, only KA leads to neuronal damage as determined by the TUNEL (terminal transferase biotinylated-UTP nick end labeling) procedure. In order to unambiguously follow the expression of one of the prototypic clEGs, c-*fos*, transgenic rats were generated that produce a Fos-lacZ fusion protein. Fos-lacZ was induced by either KA or PTZ; however, its expression was more protracted following KA. In addition, within 6 hours following KA treatment, Fos-lacZ expression appeared in the cytoplasm of neurons that were destined to die 1-2 days later, a situation never observed following PTZ. Examination of other basic zipper genes, revealed a unique immediate early response following KA. These features included: the expression of *fra*-1 and *fra*-2 in KA treated animals; protracted and differential expression of several clEGs; and a biphasic elevation of c-*fos* and *junB* in which the first peak was correlated with the initial seizure and then the second peak occuring just prior to the onset of cell death. These changes in clEG expression were correlated with sustained increases in AP-1-like DNA binding activity in the hippocampus of KA treated rats. These features of the KA induced clEG response suggest that there are unique AP-1 complexes that could have a critical role in leading to, or counteracting, neuronal death.

427.3

CHRONIC CORTICO-STRIATAL GLUTAMATERGIC ACTIVATION INDUCES A SLOWLY PROGRESSING STRIATAL LESION T. To, B. Anton, C.J.Evans, and N.T. Maidment*. Dept. of Psychiatry & Biobehavioral Sciences, University of California, Los Angeles, CA 90024.

Current excitotoxic models of Huntington's Disease involve exogenous injection of glutamate analogues to induce selective neuronal loss in the striatum. A major limitation of this approach is the very rapid progression (1-2 days) of striatal neuronal loss which impairs temporal and spatial analysis of the specific patterns of neurodegeneration. We have attempted to develop a more subtle and slowly progressing striatal lesion that reflects the anatomical and neurochemical patterns of cell death seen in HD. Our experimental approach employs a regime of repeated (three times a day) unilateral electrical stimulation (unipolar anodic square wave pulses, 1mA amplitude, 30 s trains, 60 Hz within the train, 30 s between trains for a total stimulation period of 15 min) of the cortico-striatal excitatory pathway with the aim of chronically elevating the synaptic concentration of endogenous excitatory amino acids in the striatum. Microdialysis experiments showed significant increases (two-fold) of extracellular gluatamate in the striatum which remained elevated at least 2 hr after electrical stimulation. This stimulation paradigm was used for 2 weeks, 1 month, or 2 months to assess the morphological and neurochemical effects of repeatedly elevated striatal glutamate release on specific neuronal systems within the striatum. Astrocytes (labelled by GFAP staining) were increased both in size and number, this effect being evident at 2 weeks but more pronounced at 1 month and 2 months. Immunohistochemistry for the specific GABA neuronal marker GAD_{67} revealed significant decreases in staining at the cell body and fiber level. This model may permit future investigations into both the identification of mechanisms involved in the progression of HD and the plastic changes taking place in the striatum during this degenerative process.

427.4

QUINOLINIC ACID IMMUNOHISTOCHEMISTRY OF NEUROBLASTOMAS AND GLIOMAS OF THE RAT BRAIN. J.R.Moffett*, M.G.Espey, T.Els†, E.Dux† and M.A.Namboodiri. Georgetown Univ. Bio. Dept. Wash. DC 20057, †Max-Plank-Institute für Neurologische Forschung, 50866 Köln (Lindenthal).

Tumors of the central nervous system (CNS) were investigated with antibodies to quinolinic acid (QUIN), an endogenous neurotoxin produced by cells of the immune system. In advanced F98 glioblastomas and E367 neuroblastomas produced by inoculation in the striatum of rats, quinolinic acid immunoreactive (QUIN-IR) cells were observed almost exclusively within the tumors. The immunoreactive cells were round, rod-shaped or complex in morphology. No consistent differences were observed in the number, morphology or distribution of immunoreactive cells between gliomas and neuroblastomas, but more advanced tumors of both type had significantly higher numbers of stained cells. No QUIN-IR cells were observed in the contralateral hemisphere. Glial fibrillary acid protein (GFAP) immunoreactivity was strongly elevated in astrocytes in the compressed tissue surrounding the tumors, and in the contralateral corpus callosum. Immunohistochemistry with the monoclonal antibody ED1, directed against rat monocytes and macrophages, demonstrated that numerous mononuclear phagocytes had infiltrated the tumors. These results indicate that the number of infiltrating mononuclear phagocytes in CNS tumors greatly exceeds the number of QUIN-IR cells, suggesting that if quinolinic acid is produced by monocytes and their derivatives, it is a select subpopulation of these cells. The present findings are consistent with a functional role for quinolinic acid in certain infiltrating leukocytes during the immune response to CNS neoplasms.

427.5

PRECONDITIONING DOES NOT PROVIDE CEREBROPROTECTION FROM INTRASTRIATAL INJECTIONS OF AMPA OR NMDA. J.M. Gidday, J.C. Fitzgibbons, A.R. Shah, J.H. Thurston,* T.S. Park, and J.W. McDonald. Department of Neurology and Neurological Surgery, and Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

Recently, we were able to confer cerebroprotection from hypoxic-ischemic injury in the neonatal rat with a prior exposure to sublethal hypoxia, called preconditioning *(Neurosci. Lett. 168:221, 1994)*. Using this model, we sought to determine the extent to which preconditioning would prove neuroprotective against direct injections of NMDA or AMPA. 10 litters of 6-day-old rat pups were divided; half were preconditioned with 3 h of 8% oxygen, while the rest were placed in normothermic control chambers. 24 h later, each pup was anesthetized with diethyl ether, and received a stereotaxic microinjection (0.5ul) of either AMPA (10 nM) or NMDA (10 nM) into the right striatum 2.5 mm lateral to bregma,and 3.75 mm deep. Pups recovered in an incubator for 45-60 min, then were returned to the dam. Injury analyses on day 14 revealed that preconditioned animals injected with AMPA (n=28) exhibited a 14.9±1.5% difference in hemispheric weights, which did not differ significantly from injury in non-preconditioned animals (15.3±2.1%; n=23, p>0.8, unpaired t-test). Preconditioning also failed to be protective in NMDA-injected brains (n=27), yielding a hemispheric deficit of 12.0±1.3%, compared to 12.9±1.2% in non-preconditioned animals (n=25, p>0.6). These results suggest preconditioning cerebroprotection may involve events proximal to glutamate receptor activation rather than subsequent to its release.

427.6

TUMOR NECROSIS FACTOR (TNF)-α POTENTIATES GLUTAMATE NEUROTOXICITY IN HUMAN FETAL BRAIN CELL CULTURES. S. Hu* and C.C. Chao. Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, MN 55404

Cytokines released by activated microglia have been proposed to play a pathogenetic role within the brain. Since exposure of neuronal cells to the excitatory amino acid neurotransmitter glutamate induces injury to these cells, we tested the regulatory effect of cytokines on glutamate receptor-mediated toxicity in human fetal brain cell cultures. After 6 days of incubation, exogenous glutamate (5 mM) induced remarked neuronal loss (a 165% increase in release of lactate dehydrogenase and a 62% decrease in functional uptake of ^{3}H-γ-aminobutyric acid [GABA]). Glutamate neurotoxicity was dose-dependent (ED_{50} of 200 μM). Treatment of cell cultures with TNF-α (100 ng/ml) but not with a number of other cytokines, potentiated glutamate neurotoxicity (a 52% further decrease in ^{3}H-GABA uptake). Exposure of brain cells to TNF-α or to cytokines tested above alone did not significantly affect neuronal cell function. TNF-α-mediated enhancement of glutamate neurotoxicity was markedly blocked (up to 80%) by the glutamate receptor antagonists DL-2-animo-5-phosponovaleric acid (100 μM) and MK-801 (10 μM), indicating that the potentiating effect of TNF-α is mediated via glutamate receptors. Also, expsoure of neuronal cell cultures to TNF-α resulted in a 27% decrease in astrocyte glutamine synthetase and in a 50% inhibition of ^{3}H-glutamate uptake, suggesting that the effect of TNF-α indirectly involves glutamate metabolism. These findings support the hypothesis that TNF-α may impair neuronal injury by exacerbating excitotoxicity.

427.7

BCL-2 PROTECTS MN9D CELLS FROM STAUROSPORINE- BUT NOT TRIFLUOPERAZINE-INDUCED CELL DEATH. K.L. O'Malley* and Y.J. Oh. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110

Recently, we have shown that over-expression of the proto-oncogene Bcl-2 in a dopaminergic neuronal cell line, MN9D, can protect these cells from MPP+-mediated cell death. Because previous studies have demonstrated that protein kinase (PK) pathways may be involved in cell death regulation, we tested various PK inhibitors for their effects on stable transfectants expressing human Bcl-2 (MN9D/Bcl-2) or neomycin (MN9D/Neo). Cell death was monitored by measuring lactate dehydrogenase (LDH) release. In control cells, both staurosporine (0.01-1 uM) and trifluoperazine (TFP; 1-100 uM) led to cell death in a dose-dependent fashion. In contrast, the cyclic nucleotide-dependent PK inhibitor,KT-5720 (0.01-2 uM), the tyrosine kinase inhibitor, herbimycin A (0.001-10 uM), the PKC inhibitor, calphostin C (0.05-1 uM) and long term exposure to phorbol ester failed to do so. Cell death induced by TFP could be blocked by addition of cycloheximide whereas staurosporine-induced death was not. Over-expression of Bcl-2 completely rescued staurosporine-induced but not TFP-induced cell death as measured by LDH release and internucleosomal DNA fragmentation. These results suggest that in MN9D cells at least two distinct pathways can lead to cell death. Thus these cells may serve as a useful model system for studying the mechanisms associated with CNS neuronal cell death and suppression of this process by Bcl-2.

427.8

BCL-2 PROMOTES NEURITE FORMATION AND PROTECTS FROM MPP+- BUT NOT 6-HYDROXYDOPAMINE-INDUCED CELL DEATH IN A DOPAMINERGIC NEURONAL CELL LINE. Y.J. Oh*, B.C. Swarzenski[1], S.C. Wong, M. Moffat, D.E. Merry[2], S.J. Korsmeyer[2] and K.L. O'Malley. Dept. of Anatomy and Neurobiology, Psychiatry[1], Medicine[2], Washington Univ. Sch. of Med., St. Louis, MO 63110

The protooncogene bcl-2 inhibits programmed cell death in a variety of cell types. While the mechanism for this function remains unclear, it may involve the attenuation of oxidative stress. Because oxidative stress has been hypothesized to play a role in neurodegenerative disorders such as Parkinson's disease, we sought to evaluate the potential of Bcl-2 in sparing cells from the dopaminergic neurotoxins, N-methyl-4-phenylpyridinium (MPP+) and 6-hydroxydopamine (6-OHDA). To test this in a dopaminergic neuronal cell line (MN9D), stable transfectants expressing either human Bcl-2 (MN9D/Bcl-2) or neomycin (MN9D/Neo) were established. Surprisingly, it was found that overexpression of Bcl-2 led to robust neurite formation. Increases in primary neurite length and total neuritic extent were highly significant in MN9D/Bcl-2 versus MN9D/Neo Bcl-2 expressing cells also reduced cell death due to MPP+ treatment. In contrast, Bcl-2 overexpression did not block the cytotoxic effects of 6-OHDA. However, inclusion of antioxidant reagents or an iron chelator did substantially attenuate cell death in the 6-OHDA model. Because MPP+ is thought to inhibit the respiratory chain at Complex I and thus mitochondrial metabolism, rotenone was added to inhibit the activity of NADH dehydrogenase at this level. Rotenone-mediated cell death was significantly delayed in MN9D/Bcl-2. These data suggest Bcl-2 may protect cells from altered mitochondrial electron transfer processes. Thus, Bcl-2 may have dual roles in neurogenesis and in repression of cell death.

427.9

TOXIC NEURONAL EFFECTS OF THE HIV COAT PROTEIN GP120 MAY BE MEDIATED THROUGH MACROPHAGE ARACHIDONIC ACID. E. B. Dreyer* and S. A. Lipton. Harvard Medical School, Dept. of Ophthalmology, MEEI, Dept. of Neurology, Children's Hospital, Boston, MA, 02115.

Previous work in our laboratory has demonstrated a toxic effect of gp120 in mixed neuronal/glial cultures (Dreyer et al., Science 248:364). This effect is intimately associated with glutamate excitoxicity (Lipton et al., Neuron 7:111). It is now clear that under our conditions this effect is unlikely to be a direct action of gp120 on neurons, but is instead mediated through one or more other cell types. Both HIV-infected macrophages and gp120 stimulated macrophages produce arachidonate and its metabolites. Arachidonate inhibits re-uptake of glutamate by astrocytes and synaptosomes (Barbour et al., Nature 342:918; Volterra et al., J Neurochem 59:600), and is therefore an excellent candidate for potentiating neurotoxicity in mixed culture preparations. We have found that picomolar concentrations of gp120 inhibit excitatory amino acid uptake into cultured astrocytes. These cultures contain approximately 5% monocytoid cells; when these cells are depleted by the addition of L-leucine methyl ester, then gp120 no longer inhibits excitatory amino acid uptake. This finding suggests that the effect of gp120 in our system is mediated through the monocytic cells in our cultures. Furthermore, preliminary results indicate that aristocholic acid - which inhibits phospholipase A2, and thereby decreases the production of arachidonic acid - blocks the effect of gp120 on astrocytes. These results therefore suggest that gp120's effect on astrocytes depends upon an increase in arachidonate or its metabolites, probably released by macrophages.

427.10

A COMPARISON AND APPLICATION OF BICISTRONIC HERPES SIMPLEX VIRUS VECTORS. T.J. Meier*, D.Y. Ho, R. Dash, M.S. Lawrence, R.M. Sapolsky. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Monocistronic herpes simplex virus vectors offer an effective means of delivering genes to post-mitotic neurons. They do not, however, allow ready identification of targeted cells. We have employed two different strategies to coexpress both a gene of interest and the *lacZ* marker gene: in the first approach, both cistrons are placed under a single HSV a4 promoter, with the translation of the second cistron made possible by employingan internal ribosomal entry site; in the second approach, the two genes placed under the control of the opposite reading HSV a4 and a22 promoters, respectively. Both bicistronic systems show consistent coexpression of the two genes as assessed by double immunofluorescence, indicating that recombination deletions do not occur and that such vectors can be reliably used to identify targeted cells. We have employed such vectors to investigate the neuroprotective effects of various gene products. a) Cultured hippocampal neurons receiving a bicistronic vector bearing the bcl-2 gene show enhanced survivorship when challenged by an oxidative insult as compared to those receiving a control vector. b) Delivery of a bicistronic vector bearing the glucose transporter gene protects hippocampal neurons against glutamate excitotoxicityin vitro and kainate-induced seizure in vivo. c) Delivery of a vector expressing the gene for calbindin D28K causes a significant diminution and delay in excitotoxin-induced mobilization of free cytosolic calcium in cultured hippocampal neurons.

427.11

EXACERBATION OF GP120 EFFECTS ON RAT PRIMARY NEURONAL CULTURES BY CORTICOSTERONE. S. M. Brooke, J.D. Miller *, and R. M. Sapolsky. Dept. of Biological Sciences, Stanford University, Stanford, CA. 94305.

The section of the protein coat of the HIV virus known as gp120 may be the causal agent in AIDS dementia. It has been shown to cause neuronal death and increase in cytosolic calcium levels in neuronal cultures. The injury to neurons is thought to be mediated by release of a toxin from macrophages or glia that acts through the excitatory amino acid pathway. Corticosterone(CORT), the rat specific glucocorticoid (GC) secreted from the adrenals, is involved in the mediation of the stress response. CORT increases the effects of excitotoxins such as kainic acid, glutamate, and quinolinic acid. CORT also augments the mobilization of calcium in cytosol after an excitotoxic insult. We have investigated whether CORT exacerbates the effects of gp120 on neuronal death and cytosolic calcium levels in cultured neurons. As assessed immunocytochemically with Map2 staining, 200 pM gp120 killed about 20% of hippocampal neurons. The addition of 1μM cort resulted in a further significant decrease in survival of approximately 25%. Using standard imaging techniques and the dye fura-2, we were able to confirm reports that gp120 can cause, in some cortical neurons, a rise in cytosolic calcium. The time required to reach this peak (300% of baseline) was approximately 600 seconds. Twenty-four hour pretreatment with 1μM CORT resulted in doubling the magnitude of the peak and accelerating of the onset. Some hippocampal cells in the presence of gp120 peaked at 600% of baseline after 900 sec exposure. Pretreatment with CORT did not affect the peak or onset, but did significantly impair recovery from the peak. It would appear that the stress hormone CORT may exacerbate the effects of gp120 on neuronal tissue. This fact could be relevant to the use of megadoses of GC's to control the *Pneumocystis carinii* pneumonia in AIDS.

428.1

INFLUENCE OF TH2-DOMINATED CNS IMMUNITY ON TUMOR GROWTH IN THE BRAIN L. B. Gordon, H. F. Cserr, P. M. Knopf*. Dept. of Physiology, Brown University, Providence, RI, 02912.

We have developed a model to study CNS immune privilege in Balb/c mice to mastocytoma cell line P511. When introduced into the putamen of syngeneic DBA/2 or minor histo-incompatible Balb/c mice without blood-brain barrier disruption, 10^4 P511 cells form a progressively growing tumor, exhibiting angiogenesis, central necrosis, and brain tissue infiltration. Average time to death of mice is 25.7 ± 2.6 days postinfusion (dpi) in DBA/2 and 34.7 ± 1.1 dpi in Balb/c ($p<0.05$). We suspect that an ineffective anti-tumor response is prolonging survival of Balb/c mice. In contrast, injection of 2 x 10^6 P511 cells subcutaneously (SC) results in tumor formation in syngeneic DBA/2 mice, but cytotoxic T cell (CTL)-mediated rejection in Balb/c mice. Thus, we have established a model to study immune privilege to MHC compatible, minor histo-incompatible tumor cells in the brain, a subset of natural brain tumor situations. This allows us to examine the following hypothesis: tumor cells in the brain are detected by the immune system, but the response is directed along the Th2/humoral immunity pathway, whereas responses to tumors in a nonCNS setting favor a Th1/cellular immunity pathway.

In support, immunohistochemistry on brain sections showed scattered CD3+, CD4+, and CD8+ T cells present in and around Balb/c, but absent from DBA/2 tumors. A more dense distribution of F4/80+ and of Ig+ cells are present in brain tumors and surrounding neural tissue exclusively in the tumor-laden hemisphere of Balb/c and DBA/2 mice 14 dpi. Anti-P511 serum antibody responses have been detected in Balb/c mice with brain tumors and flank tumors 14 dpi. Precursor CTL activity, but no active CTL are detected in spleens of brain-infused or SC-injected Balb/c mice 14 dpi. Tumor-infiltrating lymphocytes (TIL) from SC tumors display active CTL activity. We are assaying TIL in brain tumors, and anticipate a lack of CTL activity due to an immunosuppressive environment towards Th1 responses.

428.2

ORIGIN OF PC12 PHEOCHROMOCYTOMA CELLS: EVIDENCE THAT LOSS OF THE MYC DIMERIZATION PARTNER MAX WAS A TRANSFORMING EVENT. R. Hopewell and E. B. Ziff*. Howard Hughes Medical Institute, Kaplan Cancer Center and Department of Biochemistry, New York University Medical Center, New York NY 10016.

Max formation of heterodimers with the nuclear oncoprotein Myc and the differentiation-associated proteins Mad and Mxi1 enables these factors to bind DNA and control genes implicated in cell proliferation and differentiation. We show that the PC12 tumor cell line, derived from a rat pheochromocytoma, fails to express Max protein, due to aberrant splicing of *max* mRNA. RNAse protection and Northern analysis show that only exons encoding N-terminal sequences of Max up to the loop region of the helix-loop-helix dimerization motif are expressed, whereas *max* exons 3' of this are not expressed. We have cloned a cDNA from PC12 by hybridization to a *max* probe. The cDNA encodes a truncated Max protein which we call Max^{pc12}, containing a novel C-terminal sequence. Max^{pc12} is incapable of homo- or heterodimerization *in vitro*, indicating that the protein cannot functionally substitute for wild-type Max.

Reintroduction of wild-type Max into PC12 cells is strongly growth inhibitory, suggesting that mutational loss of Max expression may have provided a growth advantage for these cells, contributing to the transformed phenotype, and that Max may therefore be a tumor suppressor in a restricted class of tumors. We have reintroduced Max into PC12 cells under the inducible metallothionine promoter. Experiments examining the effect of Myc and Max expression in these cells will be presented.

428.3

THE CLONING OF MULTIPLE PROTEIN TYROSINE KINASE GENES EXPRESSED IN HUMAN PEDIATRIC BRAIN TUMORS AND DEVELOPING BRAIN. Edward B. Ziff, Marc Rothman, Howard L. Weiner*, and Maria McCarthy. Howard Hughes Medical Institute, Department of Biochemistry, New York University Medical Center, New York, NY. 10016

Emerging evidence indicates that growth factor receptor tyrosine kinases (RTKs) play a central role in regulating normal cell growth and differentiation in the central nervous system (CNS). Aberrant expression of members of this family of proteins has been associated with tumorigenesis. However, little is known about the role of RTKs in early neural development or in human CNS tumors which arise from primitive neural precursors. Using degenerate oligonucleotide primers complementary to conserved sequences within the tyrosine kinase catalytic domain, we have cloned thirteen distinct tyrosine kinases from two surgically resected human malignant pediatric brain tumors, a malignant ependymoma and a glioblastoma multiforme. These two tumor types are presumed to derive from embryonic stem cells of the CNS. We hypothesized that the molecular phenotype, including receptor expression, of specific tumor cells is informative of early glial or neuronal precursors. Expression of nine of these kinase genes in human brain tumors has not been reported previously. Kinase clones analyzed thus far are identical or bear significant homology to: MET, FER, TIE, HEK2, FLK1, FLT4, PDGF-R, IGF-R, and EGF-R. Cytoplasmic PTKs, including TYK2, SLK, the human JAK2 homologue and a novel protein kinase C isoenzyme have also been identified. In order to compare the pattern of RTK expression in tumors of the developing CNS to that of normal brain, we have applied this technique to the isolated brains of E17 rats. From these studies several kinases have been identified, including rat homologues of the FAK and JAK1 kinases.

428.4

IMMUNOCYTOCHEMICAL ANALYSIS OF MEDULLOBLASTOMA

López-Robles EE., Feria-Velasco A**and +Ortiz GG* Centro de Biología Experimental. Universidad Autónoma de Zacatecas. Apartado Postal 12. 98600 Guadalupe, Zac**Centro Médico Nacional Siglo XXI IMSS, México, D.F. +Lab. de Neuropatología Experimental. CIBO CMNO. Guadalajara, Jal.

The histogenesis of the medulloblastoma has been controversial since the original description of the tumor in 1925 by Bailey and Cushing, recent debates have focused towards cellular lines glial or neuronal.

We studied retrospectively the histological and immunocytochemical features with stained routinely for H/E, Kluver-Barrera, Gomori's reticulin method, and with ABC technique, of 20 cases of classical medulloblastoma with a mean of 14.2 years and 5 cases of desmoplastic medulloblastoma with a mean of 25.8 years, using a panel of antibodies neuronal and neuroglial (GFAP, Neuron-Specific Enolase, Neurofilament 160 kD, S-100 and Synatophysin) in tissues fixed in formalin and embedded paraffin.

In the classical group high population of cells were observed with Homer-Wright rosettes, degeneration, necrosis and mitosis were observed and showed glial and neuroblastic differentiation. In the desmoplastic variety bands of connective tissue separating neoplastic cells into rows and surrounding reticulin-free islands of tumor cells, cytoplasmic processes and presence of perivascular pseudorosettes that showed ependymal differentiation were observed.

In the immunocytochemical study there was a reaction observed to all the antibodies in the classical group, and there was a reaction to all them except S-100 in the desmoplastic group.

This study shows that medulloblastoma is a neuroephithelial neoplasm that can diferentiate into ependymal, neuronal and neuroglial lines.

428.5

EFFECT OF CELL ADHESION MOLECULES (CAMs) AND LAMININ ON THE DIFFERENTIATION OF NEURO-2A CELLS. W.-W. Chunge, Laboratory of Molecular Pathology, Department of Pathology, Veterans General Hospital-Taipei, Taipei 112, Taiwan, Republic of China

Development of the polarity, extension of processes, and further formation of axon and dendrites are crucial steps in the differentiation of a neuron. The effects of laminin and CAMs on neurite differentiation have been demonstrated in various normal neurons. The effects of laminin and antibodies recognizing N-CAM (12F8, against polysialic acid) and L1 (gifts from Dr. C.F. Lagenaur) on the differentiation of a mouse neuroblastoma cell line, Neuro-2A, were tested. RT97, antibody against phosphorylated neurofilament, was used to label the differentiation of axons. Many multipolar cells were identified in the 80-hour culture plated on polylysine. Some cells extended short RT97(+) neurites, most were of less than 5 times the diameter of cell body. Only 5-10 % cells had RT97(+) neurites of total length more than 5 times the cell diameter. Many cells plated on laminin extended long and branching RT97(+) neurites. Up to 30 % had RT97(+) neurites of total length more than 5 times the cell diameter. N-CAM and L1 antibodies were tested either by adding into the medium as free form or coating (bound form) on the coverslips for culutre. Although there was no L1 expression detected by ABC-peroxidase method, the effect of free form L1 antibody was observed as numerous (up to 15%) cells extended fine and long RT97(+) neurites. No significant effect is noted for the bound form L1 antibody. In contrast, both the free and bound forms of 12F8 antibody do not produce significant effect, even though the expression of N-CAM is confirmed. Evidently, laminin and L1 antibody, similar to the effects on some neurons in primary culture, also promote the *in vitro* differentiation of Neuro-2A cells. (Supported by NSC-83-0412-B-075-055, TAIWAN, ROC)

428.6

Increases in Cyclic AMP Induce Morphologic Differentiation of MCD-1 Cells in Vitro. K. Moore, O. Dillon-Carter, M. Poltorak, M. Chedid[1], and W. Freed*. NIMH Neuroscience Center at St. Elizabeths, 2700 Martin Luther King Ave, Washington, D.C. 20032, and ([1]Medical College of Georgia, Augusta, GA)

Medulloblastomas are undifferentiated CNS tumors, cells from which may undergo morphologic differentiation following addition of exogenous agents such as retinoic acid and dibutyryl cAMP (dBcAMP). The potential of cultured medulloblastoma cells to differentiate following the addition of NGF alone or in combination with agents which influence the cAMP second messenger system was studied. MCD-1 cells were exposed to NGF, dBcAMP, IBMX, forskolin, retinoic acid, arginine-L-carnitine (ALCAR), and mixtures of NGF+IBMX, NGF+IBMX+dBcAMP, and NGF+Forskolin+dBcAMP, and process outgrowth was measured after 48 hr. There were statistically significant increases in process outgrowth in cells treated with IBMX, dBcAMP, as well as NGF+IBMX, NGF+IBMX+dBcAMP, and NGF+ forskolin+ dBcAMP. Cells treated with forskolin, NGF, ALCAR or retinoic acid did not show increased growth of processes. NGF alone had little effect on process outgrowth and did not augment the effects of IBMX or dBcAMP. Immunohistochemically the cells were positive for neuron-specific enolase (NSE), synaptophysin, and vimentin, but negative for GFAP. There were no changes in expression of NSE or synaptophysin following addition of differentiating agents. Thus morphologic differentiation of MCD-1 cells was induced by agents that increase cellular cAMP.

428.7

Biochemical and immunocytochemical characterization of the human medulloblastoma cell line MCD-1. Q. Dillon-Carter*, K. Moore, M. Poltorak, M. Chedid[1], and W.J. Freed. NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032, and ([1]Medical College of Georgia, Augusta, GA).

The MCD-1 medulloblastoma cell line was isolated from a human cerebellar tumor. MCD-1 cells express several cytoskeletal markers, including vimentin, alpha- and beta-tubulin, and microtubule-associated protein-2. The cells are also positive for Thy-1 and fibronectin, but negative for N-CAM and L1 antigen. The cells are negative for markers of glia and differentiated neurons, including GalC, MBP, GFAP, neurofilaments, synapsin, TH, GAD, DBH, and PNMT. The cells express glutamate, choline, and serotonin uptake, but are negative for uptake of GABA and dopamine. Glutamate uptake is sodium-dependent and blocked at 0°C and by DL-threo-ß-hydroxyaspartate. Serotonin uptake is inhibited by clomipramine, and choline uptake by hemicholinium-3. In addition, MCD-1 cells secrete the tumor-growth regulatory molecule TGFß2, and grow in serum-free medium. The presence of serotonin, glutamate, and choline uptake suggests that medulloblastoma cells may use these substances as biochemical mediators. The MCD-1 cell line may be useful for studies of medulloblastoma growth and differentiation.

428.8

NEURON-GLIAL REGULATION OF PROLIFERATION BY ACTIVATION OF TGFß2 IS ALTERED IN MALIGNANT ASTROCYTOMAS. D.L. Cooper,* C.N. Metz, D.B. Rifkin, C.J. Hodge, Jr., and M.E. Hatten. The Rockefeller University, New York, N.Y. New York University School of Medicine, New York, N.Y., SUNY Health Science Center, Syracuse, N.Y.

Understanding the control of cell proliferation during the development of the brain is crucial to our understanding of proliferation in malignant astrocytomas. Previous analyses, in which cerebellar astroglia and granule neurons were purified from early postnatal cerebellum and recombined *in vitro*, demonstrated that neuron-glial interactions inhibit astroglial proliferation and induce differentiation of normal astrocytes and some astrocytoma cell lines. In the present study, we have compared the effects of transforming growth factor-beta (TGFß) on the proliferation of postnatal cerebellar astroglia and astrocytomas. We also examined the role of neuron-glial and neuron-glioma interactions in TGFß activation.

TGFß1 or TGFß2 reduced DNA synthesis by postnatal cerebellar astroglia and by low grade G26-24 rodent glioma cells by 30-60%. In contrast, TGFßs increased DNA synthesis by two human malignant glioma lines (U87, U118), and had little effect on two other human glioma lines (U138, U373). All four human glioma lines produce latent TGFß alone, and in co-culture with neurons. Studies on activation of TGFß *in vitro* demonstrated that gliomas activate TGFß, even in the absence of neurons. By contrast, normal astrocytes require neuronal contact to activate TGFß.

These experiments demonstrate that control of TGFß activation is lost in malignant gliomas, where gliomas activate TGFß without neuronal contact. In the context of gliomas, where control of activation is lost, TGFß may interact with other cytokines to stimulate rather than inhibit proliferation.

(Supported by NIH-CIDA NS-1676 to DLC)

WEDNESDAY PM SYMPOSIA

429

SYMPOSIUM: TOWARDS A NEUROBIOLOGY OF VISUAL CONSCIOUSNESS. C. Koch, Caltech (Chairperson); S. Kosslyn, Harvard University; P. Stoerig, Ludwig-Maximillian's University, München, Germany; R. Desimone, Lab. Neuropsychology, NIMH; F. Crick, Salk Institute.

The aim of the symposium is to demonstrate that the neurological and neurophysiological substrate underlying conscious visual perception in humans can be approached in a rigorous experimental and reductionist manner. We have the tools in hand to explore this most central of our subjective "states" and its disruption in pathology in terms of particular types of (bio)-electrical activity in particular neurons, areas or pathways in the brain.

Kosslyn will discuss how the phenomenological qualities of visual mental imagery relate to activity in specific brain regions in human subjects, using PET and functional MRI studies. Stoerig will consider "blindsight", in which patients have a loss of conscious visual perception in the presence of demonstrable residual functions. A similar phenomenon exists in monkeys with striate cortical ablations. Desimone will discuss single-unit electrophysiological studies carried out in cortex of the awake and behaving monkey, suggesting ways in which prefrontal mechanisms prepare the visual system for expected stimuli, possibly providing the substrate for imagery. Crick will outline a general framework within which the problem of the Neural Correlate of Consciousness (NCC) can be approached using theoretical, neuroanatomical and electrophysiological studies.

430

SYMPOSIUM. DEVELOPMENTAL CONTROL OF ELECTRICAL EXCITABILITY A. B. Ribera, University of Colorado Health Sciences Center(Chairperson); M.E. Barish, Beckman Research Institute, City of Hope; W.I. Moody, University of Washington; G. Mandel, SUNY Stony Brook.

Although it is firmly established that electrical excitability is developmentally regulated, its role in the emerging nervous system is less well understood. The functions of electrical excitability in developing neurons and associated regulatory mechanisms will be discussed. **Barish** will present data that indicate that environmental influences, provided by cells such as glia, affect development of ion channels and membrane currents. **Moody** will present studies that test the role of electrical activity in development by embryonic misexpression of ion channels. **Ribera**'s work examines the specific functions of identified potassium channel genes in embryonic neurons. **Mandel** will discuss the genetic elements that are involved in activation of expression of a sodium channel gene during differentiation of peripheral neurons.

SECOND MESSENGERS II

433.1

MODULATION OF PLASMA MEMBRANE CALCIUM ATPASE (PMCA) ALTERS CALCIUM EFFLUX IN CULTURED SENSORY NEURONS. J.L. Werth* and S.A. Thayer. Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455.

Intracellular calcium concentration ($[Ca^{2+}]_i$) and whole cell Ca^{2+} current (I_{Ca}) were measured simultaneously in single rat dorsal root ganglion (DRG) neurons grown in primary culture. Following a modest rise in $[Ca^{2+}]_i$ (100-600 nM), recovery to basal $[Ca^{2+}]_i$ was fit well by a single exponential. The decay time constant (τ) was similar for action potential-evoked (no cell dialysis) and depolarization-induced (whole-cell configuration) Ca^{2+} loads (5.85 ± .61 s vs. 5.94 ± .56 s, respectively). Omitting ATP from the patch pipette led to large increases in $[Ca^{2+}]_i$ and blocked recovery to basal $[Ca^{2+}]_i$. Thapsigargin (100 nM), a selective inhibitor of sarco- and endoplasmic reticulum Ca^{2+} ATPases (SERCA), did not slow buffering kinetics. Inclusion of 1µM calmodulin in the pipette caused a slight decrease in τ compared to control (4.52 ±.51 vs. 5.94 ±.56, respectively). Inclusion in the patch pipette of a 28-amino acid peptide (C28R2, 10 µM) corresponding to the autoinhibitory domain of the PMCA slowed recovery and led to a use-dependent increase in $[Ca^{2+}]_i$. The increase in τ could be prevented by 100 nM phorbol dibutyrate, an activator of protein kinase C. These data indicate that small Ca^{2+} loads are buffered predominantly by PMCA's. Furthermore, the data suggest that Ca^{2+}/calmodulin and protein kinase C play a role in regulating PMCA's in intact neurons.

433.2

MICROPIPETTE LOADING OF FLUO-3 CAN ELEVATE BASELINE NUCLEAR CALCIUM SIGNALS. Mark N. Rand*, Trese Leinders-Zufall, Samuel Agulian, and Jeffery D. Kocsis, Yale University School of Medicine, Dept. Neurology, New Haven, CT 06510 and V.A. Medical Center, West Haven, CT 06516.

Several recent imaging studies of neurons have reported that nuclear Ca^{2+} signals can exceed those of the cytoplasm upon stimulation, and it has been speculated that elevated nuclear Ca^{2+} transients might play a role in gene expression. Most of these studies used the acetoxymethylester (AM) form of the Ca^{2+} indicator dye fluo-3, which crosses the cell membrane and becomes metabolically trapped, loading the cells in a non-disruptive manner. A recent report using micropipette loading of fluo-3 has been interpreted as suggesting that depolarization-induced Ca^{2+} signals are not higher in the nucleus (Al-Mohanna et al, Nature 367:745). However, we have found that mechanical methods of dye-loading can elevate baseline nuclear Ca^{2+} signals, confounding measurements of $[Ca^{2+}]_i$ relative to baseline. When cultured adult rat DRG neurons were impaled and filled with fluo-3 using sharp electrodes, slow stabilization of the resting potential was associated with baseline nuclear Ca^{2+} signals which were greater than those in the cytoplasm. In contrast, when resting potentials stabilized rapidly after impalement, nuclear Ca^{2+} signals were about equal to those in the cytoplasm at baseline and increased 1.5 times more than those in the cytoplasm upon depolarization. Whole-cell patching of retinal ganlion cells loaded with fluo-3AM indicated the same problem. Despite buffering the intracellular pipette solution to 10 nM Ca^{2+} and presetting the amplifier to the average resting potential, imaging the neurons at the moment of membrane disruption showed a large increase in intracellular Ca^{2+}, and resulted in elevated nuclear Ca^{2+} signals which lasted for many minutes. In contrast, when DRG neurons were non-disruptively loaded with fluo-3AM and depolarized with 60 mM KCl, Ca^{2+} signals were ~25% lower in the nucleus than in the cytoplasm at rest, and increased 2.5 times more than those in the cytoplasm. These data indicate that baseline nuclear Ca^{2+} signals can increase with micropipette disruption of the neuronal membrane, confounding the estimation of nuclear $[Ca^{2+}]_i$ after stimulation.

Supported by the NIH and Department of Veterans Affairs.

433.3

CHIMERIC RECOMBINANT AEQUORINS: NEW TOOLS FOR THE STUDY OF Ca^{2+} HOMEOSTASIS IN NEURONS AND MYOCYTES. R. Rizzuto, M. Brini, C. Bastianutto, L. Pasti, R. Marsault, M. Montero, P. Gambetti*[§] and T. Pozzan. Department of Biomedical Sciences, University of Padova, 35121 Padova Italy; [§]Division of Neuropathology, CWRU, 2085 Adelbert Rd, Cleveland, OH 44106.

In recent years, much interest has been devoted to the study of cytosolic Ca^{2+} homeostasis, not only because changes of Ca^{2+} concentration in this phenomena, but also because, until recently, the cytosol was the only cell compartment in which this cation could be measured quantitatively in intact cells. We here describe a new methodology, recently developed in our laboratory, for the measurement of Ca^{2+} concentration within specific subcellular regions, and its application to the study of Ca^{2+} homeostasis in cultured neurons and myocytes. The methodology is based on the modification of the cDNA encoding the Ca^{2+}-sensitive photoprotein aequorin, modified in order to include organelle specific targeting sequences, and on the recombinant expression of the chimeric photoprotein in cultured cells. Three chimeras are now available in the laboratory, which allow the monitoring of Ca^{2+} concentrations in the mitochondria ($[Ca^{2+}]m$), nucleus ($[Ca^{2+}]n$) and endoplasmic reticulum of living cells ($[Ca^{2+}]er$). Cell stimulation coupled to increases in cytosolic Ca^{2+} concentration invariably results in increases of $[Ca^{2+}]m$ and $[Ca^{2+}]n$, but the kinetics and amplitudes of these changes are very different. In neurons, large changes of $[Ca^{2+}]m$ are evoked by the activation of plasma membrane channels, while in skeletal muscle myotubes changes in $[Ca^{2+}]m$ appear closely linked to Ca^{2+} release from sarcoplasmic reticulum. $[Ca^{2+}]n$, on the other hand, seems to closely follow cytosolic Ca^{2+} both in kinetics and amplitude. Finally, a large drop in $[Ca^{2+}]er$ occurs upon depletion of intracellular Ca^{2+} stores, though the kinetics of this decrease are complex.

433.4

ACTIVATION OF PHOSPHOINOSITIDES HYDROLYSIS BY ENDOTHELIN-1, ENDOTHELIN-3 AND BIG ENDOTHELIN IN THE RAT SPINAL CORD. P. Poulat*, J. de Champlain and R. Couture. Department of Physiology, Université de Montréal, Montréal, Qué., Canada H3C 3J7.

The activation of endothelins receptors results in the activation of phosphoinositides hydrolysis in peripheral and central tissues of the rat. However, this has not yet been shown in the rat spinal cord where endothelins receptors have been shown. The present study was undertaken to examine the inositol phosphates formation induced by endothelin-1 (ET-1), endothelin-3 (ET-3), big endothelin-1 (big ET-1) with or without presence of a selective ET_A receptor antagonist (BQ-123) in the rat spinal cord. The entire spinal cord of male Wistar rat, was cross-chopped sliced at 350 µm. Slices (50 µl) were incubated with 0.13 µM of myo-[2-^{3}H] inositol (specific activity 23.4 Ci/mmol) and 7 mM LiCl for 60 min in a final volume of 500 µl of Krebs buffer and stimulated with the proper agonist for an additional 60 min. The radioactivity in the total inositol fraction (IP1, IP2 and IP3) was determined as described by Berridge et al. (Biochem. J., (1982), 212, 437-82) and blank value (before stimulation) was substracted from all values. ET-1 (1 nM - 10 µM), induced dose-dependent increases of labelled inositol phosphates. At equimolar doses (1 µM), the rank order of potency in stimulating production of inositol phosphates was ET-1 > ET-3 > big ET-1 in slices. BQ-123 (100 µM) reduced by about 50% the response to ET-1 and ET-3. These results suggest that activation of endothelin receptors in the spinal cord triggers inositol phospholipid hydrolysis which is partly mediated by an ET_A receptor. Furthermore, the stimulation obtained with big ET-1 suggests the presence of an endothelin-converting enzyme in the spinal cord. [Supported by the MRC of Canada].

433.5

INTERLEUKIN 1 ENHANCES RECEPTOR-DEPENDENT AND INDEPENDENT INDUCED RELEASE OF ARACHIDONIC ACID FROM MOUSE STRIATAL ASTROCYTES N. Stella, J. Siciliano, D. Piomelli, M. El-Etr*, J. Glowinski and J. Prémont. Collège de France, 75231 Paris, France.

The present study was undertaken to determine the interference of this cytokine in receptor-dependent and independent induced release of arachidonic acid from cultured striatal astrocytes. As previously shown, both 100 µM ATP or the combination of 0.1 µM PMA and 2 µM ionomycin stimulated the release of ^{3}H-AA from striatal astrocytes (136 ± 7% and 54 ± 3% above basal level, respectively) (N. Stella et al. (1994) J. Neurosci. 14:568-575). When 100 pM IL-1β was added simultaneously with ATP, the nucleotide-induced release of ^{3}H-AA was not changed (165 ± 8%). However, when astrocytes were pretreated for 24 hrs with IL-1β, both the ATP- and PMA+ionomycin-evoked release of ^{3}H-AA were potentiated (350 ± 20% and 252 ± 22%, respectively). The half maximal potentiating response was observed with 10 pM IL-1β. IL-1β–effect was mimicked by IL-1α , not by IL-6.

IL-1β did not alter the concentration-dependent ATP-evoked accumulation of ^{3}H-inositol phosphates. Moreover, the amount of $G\alpha_i$ or $G\alpha_o$ protein, as estimated by pertussis toxin induced-ADP-ribosylation, were not affected by IL-1β. Finally, although IL-1β treatment did not modify the Ca^{++}-independent membrane-bound phospholipase A2 (mPLA2) activity, it increased by two fold the amount of the Ca^{++}-dependent cytosolic PLA2 (cPLA2) as estimated by immunoblotting technique.

These results indicate that IL-1β treatment has a potentiating effect on the receptor-dependent and independent induced release of AA by enhancing specifically the amount of cPLA2. Considering the role of AA in the regulation of glutamate uptake into astrocytes, IL-1β could be involved in the glutamate-evoked neurotoxicity observed in brain inflammatory processes.

433.6

CYTOSOLIC PHOSPHOLIPASE A2 (cPLA2) mRNA DISTRIBUTION AND TRANSFECTION STUDIES QUESTION THE ROLE OF cPLA2 IN NEUROTRANSMISSION. L.L. Lautens, W.S. Young*, J.D. Sharp[1], D.L. White[1], X.G. Chiou[1] and C.C. Felder. Lab. of Cell Biology, NIMH, Bethesda, MD 20892 and [1]The Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

The identification of an 85 kD cPLA2 raised hopes that the effector enzyme mediating receptor-evoked release of arachidonic acid (AA) had been isolated. cPLA2 mRNA distribution was evaluated in adult mouse brain by *in situ* hybridization. cPLA2 mRNA was found in white matter of cerebellum, brain stem and fiber tracts; scattered cells in gray matter (adjacent to pia mater) of olfactory bulb and occipital cortex ; pia mater and choroid plexus. cPLA2 brain distribution did not correlate with previous reports of neurotransmitter-evoked AA release via PLA2 in neurons cultured from specific brain regions. cPLA2 mRNA expression was also detected in peripheral tissue of newborn mouse: pigment layer and ciliary body of the choroid and anterior lens capsule in eye; kidney; stomach; intestine; bronchi; ducts of salivary glands; oral and pharyngeal epithelium; hair follicle sheathes; mesenchyme about and pulp within molars; and ossifying cartilage primordium of nasal septum. To further evaluate the potential role for cPLA2 as a signal transducing effector, muscarinic m1 receptors were stably transfected into U373 astrocytoma cells which lack cPLA2. Carbachol, a muscarinic agonist, stimulated the release of AA in U373 cells suggesting that cPLA2 was not necessary for AA release and that other PLA2(s) may serve this function. Furthermore, carbachol-stimulated AA release was blocked in CHOm5 cells expressing the forward but not the backward orientation of cPLA2. These results suggest that cPLA2 is unlikely to be the primary effector of AA release in neurotransmission. The role of cPLA2 in transporting epithelium has yet to be determined. (LL was supported in part by Eli Lilly Corp.)

433.7

A DIFFUSIBLE MESSENGER MEDIATES SUBSTANCE P-INDUCED INHIBITION OF THE INWARD RECTIFIER K' CHANNEL IN NUCLEUS BASALIS NEURONS. K. Takano, P.R. Stanfield, S. Nakajima and Y. Nakajima*. Dept. of Anat. and Cell Biol., and Dept. of Pharmacol., Univ. of Illinois at Chicago, Chicago, IL 60612.

Our previous studies showed that substance P (SP) excites nucleus basalis neurons cultured from the rat forebrain by suppressing an inward rectifier K' current, and this effect is mediated by a pertussis toxin-insensitive G protein. We have further studied the signal transduction mechanism of this effect. Single channel activity (on-cell mode) of the inward rectifier was inhibited by SP applied outside the patch. With the whole-cell patch clamp, intracellular application of 20 µM of the PKC inhibitor PKC(9-36) almost abolished the SP-induced suppression of the inward rectifier, while 20 µM of the PKA inhibitor PKI(5-24) had no effect. Extracellular application of 10 µM 1,2-sn-dioctanoylglycerol (a PKC activator) mimicked the SP effect. The SP effect was suppressed by pretreatment with 5 µM Ro 31-8220 (a selective PKC inhibitor, a gift from Roche Products Ltd.) in both single channel and whole-cell experiments. These results indicate that the SP effect on the inward rectifier is mediated by a diffusible messenger, and the signal transduction involves PKC activation. Treatment with okadaic acid prolonged the SP-induced K' channel inhibition, suggesting that a serine/threonine protein phosphatase is involved in the recovery from the SP effect. SP also excites locus coeruleus neurons. However, it is still unknown whether the same signal transduction mechanism is involved. Supported by NIH grant AG06093 and NATO grant CRG 920143.

433.8

THE ACETYLCHOLINE RECEPTOR LINKED TO A CHLORIDE CHANNEL IN *APLYSIA* ALSO ACTIVATES A NOVEL NEURAL LIPOXYGENASE PATHWAY. S.J. Feinmark*, D.J. Steel, and J.H. Schwartz. Depts. of Pathology, Pharmacology, and the Center for Neurobiology & Behavior, Columbia University, NY 10032.

Homogenates of *Aplysia* nervous tissue contain an 8-lipoxygenase (8-LO). Although this pathway has not been previously described in nervous tissue, products of the 12-LO pathway have been shown to act as neuromodulators in *Aplysia* identified neurons (see Schwartz, *Biochem Soc Trans*, 19:387, 1991). 8-LO converts arachidonic acid to 8(R)-hydroperoxyeicosatetraenoic acid (8-HPETE) from which 8-ketoeicosatetraenoic acid (8-KETE) is enzymatically derived. In ganglia prelabeled with [^{3}H]arachidonic acid, this pathway is activated in a dose-dependent manner by the application of acetylcholine (ACh; 0.1-1000 µM). ACh is as effective in normal sea water as it is in a high Mg^{2+}/low Ca^{2+}, suggesting that the effect is direct and does not require activation of other neurons. Of 10 neurotransmitters tested, ACh is the only one to activate this pathway. Likewise, histamine specifically activates the 12-LO in this nervous system. Cholinergic agonists activate the *Aplysia* 8-LO, but several ACh antagonists including, tubocurarine, hexamethonium, triethylammonium and atropine are ineffective. Activation of the 8-LO pathway is blocked by α-bungarotoxin, which selectively blocks the ACh-gated chloride current in *Aplysia* (Kehoe *et al., Brain Res*, 107:527, 1976), raising the possibility that products of the 8-LO pathway may modulate neural function through the ACh-activated chloride channel. In support of this idea, suberyldicholine, a selective agonist, activates the 8-LO in *Aplysia* nervous tissue. Thus, 8- and 12-LO are selectively and independently activated by distinct neurotransmitters in *Aplysia*. Preliminary experiments suggest that the 8- and 12-LO pathways interact to generate lipid messengers that cannot be produced by either pathway alone.

433.9

A PAF ANTAGONIST OR DEXAMETHASONE INHIBITS THE SEIZURE-TRIGGERED SUSTAINED UPREGULATION OF THE INDUCIBLE PROSTAGLANDIN SYNTHASE IN THE HIPPOCAMPUS. V. L. Marcheselli* and N. G. Bazan, Louisiana State University Medical Center, LSU Neuroscience Center, New Orleans, LA, U.S.A.

Electroconvulsive shock (ECS) activates phospholipase A_2 that leads to accumulation of free arachidonic acid and platelet-activating factor (PAF). PAF is a messenger in the ECS induced transcriptional activation of c-fos and zif-268 in hippocampus (J. Neuroscience Res. 37:54-61, 1994). Here we report that ECS effects sustained upregulation of the inducible prostaglandin synthase (PGS-2, or TIS-10) expression in rat hippocampus. The increase of PGS-2 mRNA, peaking at two hours after ECS, still have elevated levels detected after 24 hours. The zif-268 mRNA in hippocampus peak at 1 hour after ECS, but return to normal after two hours. Pretreatment with dexamethasone (6.7 mg/kg body weight) produces a 34% inhibition of ECS induced PGS-2 mRNA expression. The PAF antagonist BN-50730 when injected intracerebroventricularly (icv) 15 min before ECS (30 μg/animal) produces a 70% inhibition of PGS-2 mRNA expression. It is concluded that in the hippocampus PAF is a mediator of inducible prostaglandin synthase expression and may in turn modulate synaptic function and seizure activity through prostaglandin synthesis. Supported by NIH NINDS NS23002.

433.10

CELLULAR COMPARTMENTALIZATION AND DEVELOPMENTAL PROFILE OF COX-2, AN ENZYME INDUCED AS AN EARLY GENE, IN RAT FOREBRAIN. W.E. Kaufmann* and P.F. Worley. Depts. of Neurology and Neuroscience, Johns Hopkins University, Baltimore, MD 21205.

COX-2 is the inducible form of prostaglandin-synthase, the enzyme that catalyzes the first step in prostaglandin synthesis. Previously, we demonstrated that this enzyme is regulated as an immediate early gene in rat brain (Yamagata et al, Neuron 11: 371-386, 1993). Here, we describe our further studies to characterize the cellular compartmentalization and developmental pattern of COX-2 expression.

A COX-2 selective mouse monoclonal antibody was generously provided by Monsanto/Searle, and used for single and double-labeling immunocytochemical studies of rat forebrain (P0-adult), with markers of glia, and GABAergic and excitatory neurons. In adults, COX-2 immunoreactivity is present exclusively in neurons, restricted primarily to cerebral cortex layers II-III and hippocampus, and co-localizes with both GABA and non-GABA markers including NADPH diaphorase. Most of the COX-2 cells are excitatory given their co-expression of CaI/Cam kinase II. In the hippocampus, COX-2 is discretely compartmentalized to granule cells and excitatory and GABAergic neurons of CA3-4. The developmental profile of COX-2 immunoreactivity parallels its mRNA expression, beginning at P10 and subsequently increasing to peak between P15 and P21. Overall, laminar distribution and cytological features are similar to adult animals although qualitative analysis demonstrates a higher density of COX-2 stained cells in the developing cortex (P21).

Our study indicates that COX-2 has a developmental profile that parallels the cerebrocortical "critical period". Since COX-2 immunoreactivity is present in all neuronal types of cortical layers II-II, COX-2 expression seems to be laminar-specific rather than cell type -specific. Supported by EY09374 and HD01046.

EXTRASTRIATE VISUAL CORTEX: INFERIOR TEMPORAL AREAS

434.1

MULTIPLE SUBDIVISIONS OF THE ANTERIOR INFEROTEMPORAL CORTEX OF MACAQUE MONKEYS. E. McClendon* and D.J. Felleman. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. School, Houston, TX 77030.

Anterior inferotemporal cortex (AIT) is a large portion of the temporal lobe that extends approximately 10-12 mm from just posterior to the anterior middle temporal sulcus (AMTS) to the temporal pole. Previous architectonic (Seltzer and Pandya, '78) and connectional studies (McClendon and Felleman, '93) have suggested that this region is heterogeneous and may contain several distinct cortical areas. To investigate further the organization of AIT, we examined the distribution and laminar patterns of labeled cells and/or terminals following 12 injections of neuroanatomical tracers into area CITv, PITv, or PITd in 7 hemispheres of 6 monkeys. The distribution of callosal projecting neurons and/or myeloarchitecture served as an independent reference for various areal borders. Injections of retrograde tracers into area PITv (n=6) produced 2 dorsoventrally-separated projection zones in AIT that were clearly anterior to the large projection zones in areas CITv and CITd. Characterized by infragranular labeling, these 2 zones were located near the posterior end of the AMTS: one on the IT gyrus and the other within the superior temporal sulcus (areas AITpv and AITpd, respectively). Injections of retrograde tracers into CITv (n=4) labeled 4 projection zones in AIT: labeled cells were found bilaminarly in areas AITpv and AITpd (layer 5 > layer 3). Labeled cells were found in layers 5/6 in the anteroventral zone (area AITav), while the anterodorsal zone (area AITad) was only weakly labeled. A complementary injection of ^{3}H-proline into CITv yielded robust feedforward projections (layer 4) to areas AITpv, AITpd, and AITav, with only scattered labeling in AITad. In contrast to PITv, a single injection of retrograde tracer in PITd labeled AITpv, and AITav. These data indicate that AIT consists of 4 distinct areas and extend current views of hierarchical processing within the inferotemporal cortex. Supported by NEI EY-08372, the Sloan Foundation, and the Whitehall Foundation.

434.2

CAT VISUAL CORTICAL CELLS ARE SENSITIVE TO THE ORIENTATION AND DIRECTION OF 'ILLUSORY' CONTOURS
Audie G. Leventhal* and Yifeng Zhou, Dept. of Neurobiology and Anatomy, Univ. of Utah School of Medicine, Salt Lake City, Utah 84132.

The visual responses of cells in the lateral geniculate nucleus (LGNd) and cortical areas 17, 18 and LS [lateral suprasylvian visual area (PMLS)] were studied. Visual stimuli consisted of spots, bars, sinusoidal gratings, and illusory contours (ICs). Stimuli producing ICs consisted either of two sinusoidal gratings differing only in spatial phase or a sinusoidal grating abutting a blank field of the same mean luminance (Grosof *et al.* Nature, 365: 550-552, 1993).

LGNd cells responded poorly to ICs. Most cells in areas 17 and 18 also responded poorly to ICs. Simple cells and complex cells with relatively small receptive fields were especially insensitive to ICs.

Some complex cells in areas 17 and 18 (Redies *et al.* Exp. Brain Res., 61: 469-481, 1986) and many cells in LS responded well to ICs. Their responses were tuned to the direction and/or orientation of the ICs. Thus, cells responding best to vertical bars and gratings responded best to vertical ICs produced by moving horizontal gratings. Some cells responded more strongly to moving ICs than to moving bars and were more sensitive to the orientation and direction of ICs than of bars.

Cats can discriminate IC orientation. This ability is reduced by lesions of LS as well as of areas 17 and 18 (De Weerd *et al.* Eur. J. Neurosci, 5: 1695-1710, 1993). Since more cells in LS than in areas 17 and 18 signal IC orientation, inputs from IC sensitve cells in areas 17 and 18 may produce LS cells that are sensitive to IC orientaion. (EY08523)

434.3

ELEMENTS OF FORM PROCESSING FROM MOTION IN MONKEY PRESTRIATE CORTEX. E. Peterhans* and R. Baumann, Department of Neurology, University Hospital, CH-8091 Zurich, Switzerland.

We have studied early stages of form processing from motion in the visual cortex of the alert rhesus monkey. During periods of active visual fixation we recorded the responses of single neurons to rows of dots (size 1 min arc, spacing 12-36 min arc) moving relative to a background of dots of similar size and density (area 4-6 deg2) which either was kept stationary or moved in antiphase. These stimuli induced percepts of moving bar-like objects, segregated from a dotted background. For control, all dots were either kept stationary or moved in phase, conditions in which no such object was perceived.

In prestriate cortex many neurons were selective for the orientation of such motion defined bars as they were for bars defined by luminance contrast. The control stimuli either failed to produce a response, or evoked weak activity at all orientations. Rows of dots moving on a uniform background also produced a response as long as the dots moved coherently; motion out of phase reduced or abolished this response. The critical stimulus parameter was coherence of motion and not collinearity. Dot misalignments were tolerated, sometimes up to ±30 min arc. In area V2 34% (38/111), and in the area V3/V3A complex 75% (24/32) of the neurons studied showed these properties, but so far none in area V1 (0/22). We conclude that motion and luminance contrast as cues of from perception are first integrated in prestriate cortex, and that areas V3/V3A play a special role in this process.

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434.4

NEURONAL MECHANISMS FOR PERCEPTUAL FILLING-IN.
P. De Weerd*, R. Gattass, R. Desimone & L.G. Ungerleider. Laboratory of Neuropsychology, NIMH, NIH, Bethesda, MD 20892.

When a patch of dynamic texture with a hole in the middle is viewed eccentrically, the hole eventually disappears as it fills in with the surrounding texture. We trained a rhesus monkey to maintain fixation for 14 secs while recording the activity from cells with their receptive field (7-8 deg of eccentricity) positioned in the hole. Neurons in area V3 showed a gradual increase in firing rate (climbing activity) which reached an asymptote at the moment humans report filling-in (De Weerd et al., *Soc. Neurosci. Abstr.* 19:27, 1993). Changes in the size of the hole, or in the extent to which the hole was surrounded by the texture, caused changes in the time course of the climbing activity corresponding to the time required by humans to experience filling-in.

We now report that after offset of the stimulus many V3-neurons show an after-discharge which may be related to the after-image experienced by humans after stimulus offset. In addition, we confirm that in contrast with V3, there was little evidence of climbing activity in the averaged population of V1 and V2 cells. Some cells in these areas, however, did show climbing activity. In V2, the proportion of cells showing climbing activity increased at greater receptive field eccentricities, in line with psychophysical observations that filling-in is more effective the farther out the hole is in the periphery (Ramachandran et al., *Vision Res* 33:717-721, 1993). The color, direction, and orientation selectivity of V2-cells showing climbing activity was not different from the rest of the V2 population. Therefore, even with the dynamic texture used, climbing activity is not exclusively a magnocellular phenomenon. This is supported by preliminary data in V4, where we found strongly color-selective cells with clear climbing activity.

434.5

SENSORY INTERACTIONS AND EFFECTS OF SELECTIVE SPATIAL ATTENTION IN MACAQUE AREA V2. J.Reynolds, L.Chelazzi, S.Luck, R.Desimone*. Lab of Neuropsych., NIMH, Bethesda, MD and Dept. of Neurosciences, UCSD, La Jolla, CA.

It was previously found that when two stimuli fall within a cell's receptive field, attention directed to one of them seems to filter out the influence of the other. To test this directly, we recorded the responses of V2 neurons in a macaque monkey to an optimal, or preferred, stimulus in the presence of a second, nonpreferred stimulus. We measured changes in response caused both by the sensory interaction between the two stimuli in a passive viewing condition as well as by the effects of attention directed to the first or second stimulus.

During passive viewing, a nonpreferred stimulus in the receptive field typically caused a partial suppression of response to the preferred stimulus. The suppressive effects of a nonpreferred stimulus diminished with distance from the receptive field but were still present in some cases beyond the field border. Attention directed to the preferred stimulus appeared, on average, to diminish the suppressive effects of the nonpreferred stimulus whereas attention directed to the nonpreferred stimulus seemed to magnify its suppressive effects. The time course of both the attentional and sensory effects on the response was similar. As in earlier studies (Neurosc. Abs., **19**:19.6), an increase in baseline activity typically occurred when attention was directed to a location within the receptive field.

We hypothesize that the baseline shift is due to a spatially selective attentional signal which increases the average firing rate of those cells whose receptive fields are at the attended location. We further suggest that the sensory interaction, which could serve preattentive visual processes such as separation of figure from ground, may also be the mechanism by which the attentional signal biases sensory processing in favor of attended stimuli.

434.6

RESPONSES OF V4 NEURONS DURING VISUAL SEARCH. L. Chelazzi* and R. Desimone. Lab. of Neuropsychology, NIMH, Bethesda MD, 20892 and Dept. of Neurol. and Vis. Sci., Univ. of Verona, Verona, Italy.

In a previous study of the mechanisms underlying visual search (Chelazzi et al., Nature 1993, 363:345-347) we reported that cells in inferotemporal (IT) cortex of the macaque may participate in storing a representation of a searched-for (target) stimulus and in selecting the target from distractors. In the present study we investigated whether area V4 also contributes to such operations. A total of 140 neurons with extrafoveal receptive fields (RFs) were recorded in one macaque monkey. Each trial began with a brief presentation of a cue stimulus at fixation. Following a delay of 1.5 s, during which fixation had to be maintained, a pair of test stimuli was presented in the periphery, at random locations, and the animal was required to saccade to the target stimulus that matched the earlier cue, ignoring the other (distractor) stimulus. Contrary to what was previously found in IT, V4 neurons did not display cue-selective sustained activation during the delay interval. However, similar to IT cortex, V4 responses to the distractor stimulus were suppressed well before the eye movement to the target. This effect was maximum when target and distractor both lay within the RF borders, diminished substantially when the target stimulus was moved outside the RF, and diminished further with increasing distance of the target from the RF borders. Thus, in addition to IT cortex, V4 contains mechanisms for selecting a relevant target based on its features and discarding distractors. The fact that these mechanisms are most evident when target and distractor are located within a cell's receptive field suggests that they are based on local competitive interactions within area V4. The competition between target and distractor in V4 may be biased by top-down, or feedback, signals directed at cells coding the expected target's features.

434.7

REPRESENTATION OF STIMULUS POSITION RELATIVE TO ATTENDED OBJECTS IN MACAQUE AREA V4. C.E. Connor*, J.L. Gallant and D.C. VanEssen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have previously shown that changes in the position of an attended object affect the spatial response profile and overall response level of cells in macaque visual area V4 (Connor et al., *Soc. Neurosci. Abstr.* **19**: 974 (1993)). In particular, many V4 cells respond differentially to an oriented bar stimulus depending on which side of the bar the attended object appears on (a laterality preference). We have explored this phenomenon further by varying the location of the attended object across multiple positions in 1 and 2 dimensions. The attended object was a ring, flashed briefly near the receptive field as part of a serial size comparison task. The probe stimulus was a behaviorally irrelevant bar of optimal orientation, color, and size flashed at various locations inside the receptive field. A single bar stimulus appeared 200 msec following each ring onset. In one paradigm, ring position was varied across 7 locations along an axis perpendicular to the optimal bar orientation. Responses to the bar were often tuned for distance relative to the attended ring, as well as showing a laterality preference. In a second paradigm, the position of the ring was varied across multiple locations in 2 dimensions, all equidistant from the receptive field. Bar response strength often depended on 2-dimensional position relative to the attended ring. These findings suggest that area V4 processes information about stimulus position relative to nearby attended objects.

434.8

NEURONAL RESPONSES IN MONKEY INFERIOR TEMPORAL CORTEX DURING LEARNING OF THE BEHAVIORAL RELEVANCE OF A VISUAL STIMULUS. B. Jagadeesh*, L. Chelazzi, R. Desimone, M. Mishkin, Lab. of Neuropsychology, NIMH, Bethesda, MD and Dept. of Neurol. and Vis. Sciences, Univ. of Verona, Italy.

With repeated stimulus-response-reward pairings, stimuli become behaviorally relevant and typically elicit orienting responses. To examine the neuronal mechanisms underlying the learning of stimulus relevance, we recorded from neurons in anterior inferior temporal cortex of a macaque monkey while the monkey acquired a stimulus-response-reward association. Pairs of stimuli were presented at random locations extrafoveally, and the monkey was rewarded for making a saccadic eye movement to one stimulus of each pair (the positive stimulus). During the recording session for a single cell, the animal learned "on-line" which stimulus was associated with the reward through trial and error.

Each pair was chosen on the basis of pre-testing to include one stimulus that activated the cell well on its own (good stimulus) and one that was ineffective (bad stimulus). Two pairs of stimuli were used for each cell, and good and bad stimuli were randomly assigned to be either positive or negative. The animal learned to find and saccade to the positive stimulus with a very short (150 ms) latency. Responses to the good-bad stimulus pair were averaged across the population of cells. During the 150 ms presaccadic period, responses were larger when the good stimulus of the pair was positive than when the bad stimulus was positive. The difference in the responses was present even though both good and bad stimuli were always present within the receptive field during that time. This result suggests that the competitiveness of representations of visual stimuli in anterior inferior temporal cortex can change with learning and influence the selection of targets from a scene.

434.9

SHAPE SELECTIVITY OF INFERIOR TEMPORAL NEURONS UNDER CONDITIONS OF BACKWARD MASKING. Gy. Kovács, R. Vogels, G.A. Orban*. Lab. Neuro- en Psychofysiologie, KUL, B-3000 Leuven, Belgium.

In humans, the visibility of a briefly presented shape is impaired when it is followed by another stimulus (backward masking). We observed the same phenomenon in 2 rhesus monkeys: discrimination performance for geometrical shapes presented at 20 msec exposure time (ET) was strongly impaired when the shape was immediately followed by a patterned mask. To determine the neuronal correlate of this phenomenon, we measured the responses of inferior temporal neurons in the same 2 monkeys, performing a fixation task, to these briefly presented shapes which were either followed by the mask (M) or not (NM). Although the response magnitude strongly declined with decreasing ET (tested range: 20-100 msec), shape selectivity remained present even at 20 msec ET (n = 54 units) in the NM condition. The surprising finding was that a majority of the units showed significant shape selectivity in the 20 msec M condition too. When using the initial 60 msec of the response, the difference between the response to the preferred and non-preferred shape was on average 0.6 and 1.0 spikes in M and NM conditions, respectively. This small but significant difference between the selectivity in M and NM conditions, increased notably when considering the first 200 msec of the response: average response differences of 0.6 and 2.6 spikes in M and NM conditions, respectively. Thus, longer temporal integration of the response results in a marked increase in discrimination capacity in the NM condition, but not when the briefly presented shape is followed by another stimulus. This suggests that the behaviorally observed backward masking is the result of temporal integration of the neuronal responses. (Supported by IUAP-22 & FGWO)

434.10

Area TE connections with inferior prefrontal regions responsive to complex objects and faces. J.F. Bates*, F.A.W. Wilson, S.P. Ó Scalaidhe and P.S. Goldman-Rakic. Yale Univ. Sch. Med., New Haven, CT 06510

Neurons in the inferior convexity of the prefrontal cortex have recently been found to respond selectively to complex stimuli such as pictures of objects and faces. These responses resemble those of feature-selective neurons in area TE of inferotemporal cortex. In order to investigate the connections between area TE and prefrontal cortex, we combined physiological mapping with WGA-HRP histochemistry to identify afferents of the prefrontal cortex involved in object vision. Injections were made in the inferior prefrontal cortex in three cases. Two cases received injections in area 45, one of which was first characterized electrophysiologically at the time of injection. The third animal received an injection in area 12. Following injections of area 45, we found retrogradely labeled cells and anterogradely labeled terminals in caudal TE, and in subdivisions TEa and TEm in the ventral bank of the STS. Labeling of area TE was also found in the case with an area 12 injection; in this case, the labeling was restricted to areas TEa and TEm in the ventral bank of the STS, and was located slightly rostral to that seen following injection of area 45. These findings indicate that area TE in the inferior temporal cortex, including the ventral bank of the STS, is a selective channel of object and feature information which links specific regions of the prefrontal cortex with the visual system. (Supported by MH-44866, 38546 and JSMF #91-47)

434.11

Learning a Visual Task as Reflected in Activity Dynamics of Macaque Inferotemporal Neurons Shaul Hochstein* and Volodya Yakovlev
Neurobiology Dept & Neural Computation Ctr, Hebrew Univ, Jerusalem, Israel

It is well-known that inferotemporal (IT) cortex of monkeys plays a crucial role in learning visual discrimination tasks. We now ask if the activity of IT neurons changes from session to session while the monkey's performance improves.

Multiple single-unit recordings were taken from IT cortex of macaque monkey performing a standard match-to-sample task. The stimuli were a bright solid circle and rectangle, of equal area and energy. We studied the responses of IT cells at all stages of learning: from the level of chance performance to 90% correct responses. For analysis the learning period was divided into 3 stages: I. chance performance; II. ~ 60% correct responses; and III. > 60% correct responses.

We found that the most dramatic changes in activity of IT cells occurred as the monkey progressed from stage I to stage II of the learning. For the SAME condition, in stage I the activity of IT cells was greater during the first, SAMPLE, stimulus than during the second, MATCH, stimulus while at later stages the response to the MATCH stimulus grew in size. The activity of cells during the inter-stimulus-interval (ISI) in stage I was suppressed compared to background activity, while in stages II and III for most cells ISI activity was stronger than background activity. Many cells were activated in the ISI as strongly as during stimulus presentation, implying that they may be involved in remembering the SAMPLE stimulus. Finally, in stage I, responses of IT cells to the second (MATCH) stimulus were suppressed for the SAME condition compared to the DIFFERENT condition. In later stages, the SAME response grew in size and presumably in importance. Thus, specifically the memory aspect of IT develops during learning.

supported by the Israel Ministry of Science and the Arts.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID VI

435.1

Aß-PROTEOGLYCAN INTERACTIONS ARE MEDIATED VIA CARBOHYDRATE MOIETIES. Rekha Gupta-Bansal, Robert C. A. Frederickson*, and Kurt R. Brunden. Gliatech, Inc., Cleveland, OH 44122

It is now well accepted that a variety of proteoglycans, including HSPG, CSPG and DSPG, are localized within amyloid plaques of Alzheimer's Disease. We have now fully characterized the binding of Aß to these classes of proteoglycans, utilizing a solid-phase binding assay. In a typical assay, proteoglycan is immobilized on a microtiter dish and the bound Aß is detected with an Aß antibody (4G8). The data suggest that Aß binding to proteoglycans (under physiological conditions) is dependent upon the physical state of the amyloid; proteoglycans bind fibrillar Aß while no specific interaction is observed with non-fibrillar peptide. At pH values below neutrality, both non-fibrillar and fibrillar Aß peptide bind proteoglycans. Complete inhibition of fibrillar Aß binding to immobilized proteoglycan was observed with free glycosaminoglycans at physiological pH. These data suggest that the binding of proteoglycans to Aß fibrils is mediated via carbohydrate moieties.

The demonstration of high affinity interactions between Aß and proteoglycan classes localized to senile plaques, and our previous demonstration that Aß-proteoglycan complexes are resistant to degradation, suggest a mechanism for amyloid accumulation and persistence. The development of specific compounds to disrupt Aß-proteoglycan binding may result in improved Aß clearance by naturally occurring proteases.

435.2

INFLUENCE OF APO E PROTEIN ON APP METABOLITES IN INTACT LIVING CELLS. Anja L. Biere, Douglas C. Anthony*, and Dennis J. Selkoe. Center for Neurologic Diseases, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

Both late onset familial and sporadic Alzheimer's disease (AD) show segregation with the ε4 allele of the ApoE gene, which is located on chromosome 19. The biological mechanism by which the ApoE4 protein confers a greater risk of developing AD remains unclear. Although a direct amyloid-β-peptide (Aβ)-ApoE interaction was suggested based on *in vitro* data by Strittmatter et al. (PNAS, 1993), no corroborating *in vivo* data have been reported to date. We sought to investigate under *in vivo* conditions the influence(s) of ApoE on APP and its metabolites, with special focus on differences between the two ApoE alleles, E3 and E4. As a first step, we have constructed expression plasmids with ApoE transcription under control of an RSV LTR promoter. An ApoE3 cDNA was converted to ApoE4 by mutagenesis, resulting in a substitution of arg for cys at amino acid 112. ApoE expression of both plasmids was tested by Western blotting and immunoprecipitation of transient and stable transfectants. These APP/ApoE double transfected lines are highly useful for detailed investigations of ApoE influence on APP processing and/or Aβ aggregation and stability. In preliminary experiments, we detected no effect of either ApoE allele on APP proteolytic processing in both CHO and 293 cells stably transfected with APP695. In addition, both cell types showed no major differences between ApoE3 and 4 in Aβ secretion. Additional experiments with the doubly transfected cells and with incubations of recombinant ApoE3 and 4 with intact cells are underway.

435.3

IDENTIFICATION OF PROTEINS THAT INTERACT WITH MEMBERS OF THE APP/APLP SUPERFAMILY. S. Guénette*, W. Wasco and R.E. Tanzi. Laboratory of Genetics and Aging and Molecular Neurogenetics Unit, Mass. General Hospital, Boston, MA 02129

The normal physiological role of members of the amyloid β-protein precursor (APP)/APP-like protein (APLP) superfamily is not clearly understood. However, the identification and characterization of proteins that specifically interact with select regions of APP and/or the APLPs may provide critical clues towards the elucidation of their normal cellular function(s). To identify proteins that interact with APP/APLP family members, we employed the transcription-based screening assay for interacting proteins known as the "interaction trap" (Gyuris *et al.* Cell 75:791, 1993). To this end, we have screened a human frontal cortex acid fusion cDNA library (a gift from Roger Brent's laboratory) for the purpose of identifying proteins that specifically associate with the following domains of the APP protein: 1) the extracellular Cys-rich region of APP751 (a.a. 38-187) and 2) the cytoplasmic domain of APP751 (a.a. 705-751). The cysteine-rich domain was chosen for the initial screen since its predicted secondary structure may allow for binding to brain-derived proteins acting as specific ligands for APP. Several cDNAs have been obtained which encode potentially interacting proteins for this region of APP. We will discuss the putative function of the proteins encoded by these cDNAs, the possible significance of their interaction with APP and/or the APLPs and their potential role in the neuropathogenesis of Alzheimer's disease.

435.4

BEYOND β–PROTEIN—THE ROLE OF AMYLOID-PROMOTING FACTORS, LYMPHOKINES, AND NON-NEURONAL CELLS AND TRISOMY 21 IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE. H. Potter*, S. Das, L. Geller, J. Ma, M. Niethammer, &L. Scinto, &K. Daffner, and D. Dressler. Department of Neurobiology, Harvard Medical School and *Beth Israel Hospital, Boston MA 02115

Two puzzling features of the filamentous amyloid of Alzheimer's disease and Down syndrome are the regional specificity of the deposition and the presence of other structural components, such as the protease inhibitor ACT and the lipid carrier apoE. We believe, on the basis of recent data from our own and other labs, that the solution to these puzzles lies in the brain-region-specific expression by astrocytes of amyloid-promoting agents (pathological chaperones) that catalyze the polymerization of Aβ into amyloid filaments. Specifically, we have found that the two amyloid-associated proteins, ACT and apoE, stimulate amyloid filament formation 10 to 20-fold (see Ma *et al.*). Furthermore, preliminary evidence indicates that ACT and apoE increase Aβ neurotoxicity. The expression of the amyloid catalyst ACT is induced in cultured human astrocytes by IL-1, which, in turn, is expressed by cortical microglial cells both *in vivo* and *in vitro*. Trisomy 21causes AD in Down syndrome individuals. We have found that non-Down syndrome AD patients also show an increase in trisomy 21. Furthermore, we have found that AD patients, like Down syndrome persons, show a marked hypersensitivity to the pupil dilating effect of cholinergic receptor antagonists (see Scinto *et al.*). These two results are consistent with the hypothesis that AD is a mosaic form of trisomy 21 Down syndrome.

435.5

SEQUESTRATION OF AMYLOID ß-PROTEIN BY TRANSTHYRETIN: STRUCTURAL STUDIES OF TTR-Aß INTERACTION.
A.L. Schwarzman, M. Tsiper, M.P. Vitek, M. Eisenberg, and D. Goldgaber*. Dept. of Psychiatry and Pharmacology, SUNY, Stony Brook, NY 11794 and The Picower Institute, Manhasset, NY, 11030.

Recently, we proposed that sequestration of Aß in biological fluids and extracellular space is the key step in the homeostatic mechanism which prevents Aß amyloidosis, and that failure to sequester Aß may lead to amyloid formation. We then found that in cerebral spinal fluid, TTR sequesters Aß into stable complexes. We also showed that TTR prevented amyloid formation *in vitro*. In order to investigate the interaction of the two proteins, a computer graphic model of the TTR-Aß complex was built and the Aß binding domain with several negatively charged amino acid was predicted. To verify this model, forty different mutations were introduced into the putative binding domain of recombinant TTR and the mutants were tested for binding of radioiodinated Aß. The mutants of TTR that failed to bind Aß and to prevent amyloid formation were identified and the key amino acids of TTR that interact with Aß were determined. These results enabled us to refine the model and predict possible variants of TTR that may be associated with Aß amyloidosis. The correct model of TTR-Aß complex will identify structural requirements for compounds that prevent amyloid formation.

435.6

ISOFORM-SPECIFIC BINDING OF APOLIPOPROTEIN E TO ß-AMYLOID PEPTIDES. M.T. Falduto* , M.J. LaDu, A.M. Manelli, C.A. Reardon, G.S. Getz, D.E. Frail. Dept. of Neuroscience, Abbott Laboratories, Abbott Park, IL 60064 and Dept. of Pathology, University of Chicago, Chicago, IL 60637.

Apolipoprotein E (apoE), particularly the e4 allele, is genetically linked to Alzheimer's disease (AD). ApoE has previously been shown to bind to ß-amyloid (Aß), an amyloidogenic, proteolytic product of amyloid precursor protein. To analyze the interaction of Aß and apoE, we used Western immunoblotting of Aß peptides incubated with concentrated, conditioned media from HEK293 cells stably transfected with either apoE3 or apoE4 human cDNA. Non-reducing SDS-PAGE revealed the presence of a ~45kd multi-protein complex with both Aß and apoE immunoreactivity. Using either Aß 1-40 or Aß 1-28 peptides, the level of apoE3/Aß complex was ~20-fold greater than apoE4/Aß complex. This apoE isoform-specific binding pattern was maintained at concentrations of Aß from 10uM to 1mM, from pH 5 to pH 9, and from 2 minutes to 24 hours of peptide incubation. The higher level of apoE3 binding to Aß are in contrast to previously published data using purified apoE that had been delipidated and denatured (Proc. Natl. Acad. Sci. 90:8098, 1993). These data suggest that apoE3 may play a role in protecting against AD by binding Aß and preventing aggregation or facilitating uptake/removal of this amyloidogenic fragment.

435.7

DISTRIBUTION OF APOLIPOPROTEIN E AND β-AMYLOID PROTEIN IN CULTURES OF NEURONS, MICROGLIA AND ASTROCYTES. M.D. Ard*, G.M. Cole, and A.P. Mehrle. Dept. of Anatomy, U. of Miss. Med. Ctr., Jackson, MS 39216, & Dept. of Medicine, UCLA and GRECC, VAMC, Sepulveda, CA 91343.

Understanding the mechanistic basis for apolipoprotein E (ApoE) genotype as a major risk factor for β-amyloidosis and Alzheimer's disease requires an exploration of ApoE and β-amyloid protein (Aβ) interactions with CNS cells.

Cells were cultured from embryonic (neurons) or neonatal (astrocytes) rat cerebral cortex or from adult rat spinal cord (microglia), in serum-free medium. After incubation with ApoE (Organon Teknika or Calbiochem), only microglia showed substantial cellular immunostaining with anti-ApoE, indicating a high density of receptors consistent with existing data on macrophages. Neuronal cell bodies and astrocytes were rarely immunopositive. Aβ (42 amino acids) added to culture medium was cleared from the medium and accumulated intracellularly by microglia (immunoblots and immuno-EM) and accumulated by neurons and astrocytes. Incubation with both ApoE and Aβ together resulted in increased anti-ApoE immunostaining in all three cell types, colocalized with Aβ immunoreactivity; however, by Western analysis, ApoE dramatically reduced the amount of Aβ monomer and aggregates accumulated by microglia. These results, suggesting that ApoE may be an important determinant of microglial Aβ clearance rates, provide both a mechanism for Aβ clearance and a rationale for ApoE as a risk factor for Alzheimer's. Supported by the Alzheimer's Association (MDA) and NIH AG9009-04 and the Cal. State Dept. of Health (GMC).

435.8

APOLIPOPROTEIN E (APOE) GENOTYPE AND IMMUNOHISTOCHEMICAL FINDINGS IN ALZHEIMER'S DISEASE (AD) WITH AND WITHOUT PARKINSON'S DISEASE (PD) CHANGES. M. Gearing*, J.A. Schneider, and S.S. Mirra. Department of Pathology and Laboratory Medicine, VA Medical Center and Emory University School of Medicine, Atlanta, GA 30322.

The ε4 allele of ApoE has recently been identified as a risk factor for the development of AD, and colocalization of ApoE and ßA4 has been observed in senile plaques. Genetic dysequilibrium of the ε4 allele and the ApoE-amyloid association in senile plaques were investigated in 100 dementia patients with neuropathologically confirmed AD with and without coexistent PD pathology (nigral degeneration and Lewy bodies at any site). AD+PD (n=50) and "pure AD" (n=50) cases were matched for age, sex, and duration of dementia. DNA was extracted from frozen or formalin fixed paraffin-embedded tissues, and ApoE genotype was determined using a PCR/restriction enzyme method. We found that 72% of the patients in each group had at least one ApoE ε4 allele compared with reported estimates of 20-25% in the general population. The AD vs. AD+PD groups also showed similar immunohistochemical findings. Although cortical plaques generally demonstrated ßA4-ApoE colocalization, ßA4-positive plaques in focal neocortical regions were ApoE-immunonegative. In all cases, ßA4-positive diffuse plaques in the striatum failed to label with antibody to ApoE, whereas cerebellar diffuse plaques showed consistent colocalization of ßA4 and ApoE. These discrepancies may be explained in several ways: (1) amyloid processing differs regionally, and the association between ßA4 and ApoE is not universal; (2) ApoE-immunonegative plaques may represent an earlier stage in plaque evolution than ApoE-positive plaques, and the ApoE-ßA4 colocalization is a later phenomenon; (3) ApoE is present in all plaques, but in some plaques the levels are too low to be detected with our methods, or (4) ApoE is more sensitive to technical vagaries than ßA4. Supported by VA Merit Award and NIH grant AG10130.

435.9

CEREBROSPINAL FLUID LEVELS OF AMYLOID ß-PROTEIN ARE INDEPENDENT OF APOLIPOPROTEIN E GENOTYPE AND ARE INVERSELY CORRELATED WITH SEVERITY OF DEMENTIA IN ALZHEIMER'S DISEASE. John H. Growdon*[1], G. William Rebeck[1], Meihua Deng[3], U. Ingrid Richardson[3], Marsha Tennis[1], Dale B. Schenk[2], Carmen Vigo-Pelfrey[2], Ivan Lieberburg[2], Richard J. Wurtman[3], Bradley T. Hyman[1] and Roger M. Nitsch[1,3].

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Alzheimer's disease (AD) is characterized by formation in brain of neurofibrillary tangles and of amyloid deposits. Brain amyloid burden is highest in AD patients homozygous for the apolipoprotein E *ε4* (*apoE ε4*) allele, inheritance of which is a major risk factor for the development of AD. To investigate the relationship of *apoE* genotype with brain metabolism of the amyloid ß-protein precursor (APP), we measured cerebrospinal fluid (CSF) levels of amyloid ß-protein (Aß) and of secretory N-terminal APP derivatives in 19 AD patients with varying degrees of dementia and with the most common combinations of *apoE* alleles, that is, *ε3/3*, *ε3/4*, and *ε4/4*. Mean CSF levels of Aß, of the 106 and the 25 kDa N-terminal APP derivatives were similar among the *apoE* genotype groups. In contrast, CSF levels of Aß were inversely and significantly correlated both to cognitive and to functional measures of dementia severity. We conclude that measurements of CSF Aß provide a useful biochemical marker which parallels dementia severity in AD but is independent of *apoE* genotype.

435.10

INCREASED APOLIPOPROTEIN E ε4 ALLELE FREQUENCY IN ALZHEIMER'S DISEASE (AD) AND IN VASCULAR DEMENTIAS (VD) S. Govoni, G.Frisoni#, A Bianchetti#, G.Franceschini°, L.Calabresi° V.Olgiati*^ and M. Trabucchi§. Inst. Pharm. Sci.and °E.Grossi Paoletti Ctr, Univ. of Milano, #Alzheimer's Disease Unit, FBF-S.Heart Hospital, Brescia, ^R&D Poli, Milano; §Dept Exptl. Med. Biochem. Sci., Univ. of Roma T.Vergata, ITALY

ApoE is the only apolipoprotein expressed in the nervous system where it may participate in the transport of cholesterol and lipids via binding to LDL and LRP receptors. In AD apolipoprotein E has been found in senile plaques and ApoE4 (Apo E is encoded by a single gene, a common polymorphism is determined by alleles ε2, ε3 and ε4) binds in vitro to β-amyloid. These observations stimulated the search of ApoE genes in AD leading to the discovery that ApoE ε4 frequency is raised in AD (Corder et al., Science 261, 921, 1993). The increased prevalence of ε4 allele is confirmed in our series of AD patients. The ApoE ε4 frequency determined in 120 clinically diagnosed AD patients was 0.43 (P<0.0001) compared to 0.09 in 64 age and sex matched controls. The presence of two copies of ApoE ε4 alleles was associated with an earlier onset of the illness . The age adjusted risk for AD increased with the allele dose. Notably a gender effect was observed, since the presence of one ε4 allele was sufficient to significantly increse the odds ratio for AD in females (5.6, P<0.009), but not in males (2.8, n.s.) suggesting an interaction between Apo E ε4 and sex hormones. This view is supported by the observation that estrogen replacement therapy may decrease the risk for AD in women (Paganini et al. Soc.Neurosci. Abs., 1993). Moreover an increased ε4 frequency (0.41, 32 patients; P<0.001)was observed in VD . This last observation indicates that the ApoE ε4 allele specificity for AD is doubtful and that the modified allele frequency may have some more fundamental role in altering the sensitivity of neurons to damage, perhaps through an impairment of repair processes in the brain.

435.11

SECRETION OF β-APP INTO THE RAT CSF: AGED RATS SECRETE SIGNIFICANTLY HIGHER LEVELS OF β-APP FOLLOWING FOREBRAIN CHOLINERGIC LESIONS THAN YOUNG RATS. V. Haroutunain*, K.L. Davis, R. Gluck, E. Fiber & W.C. Wallace. Department. of Psychiatry, Mount Sinai School of Medicine. NY, NY, and Laboratory of Biochem. Genetics, NIMH, Washington, D.C.

Experimental lesions of various subcortical neurotransmitter systems (cholinergic, noradrenergic, serotonergic) induce β-APP expression in the cortex of the rat. This induction results in the persistent secretion of β-APP into the cerebrospinal fluid (CSF). In the current series of experiments young (2 month old) and old (24 month old) Fischer 344 rats received unilateral N-methyl-d-aspartic acid lesions of the cholinergic basal forebrain. One week after lesioning, cisternal CSF was collected and the rats were sacrificed. Analysis of cortical choline acetyltransferase activity (ChAT) showed that cortical cholinergic marker activity was significantly ($ps<0.001$) reduced by approximately 50-56% in both groups. Immunoblots of the CSF using an antibody (α5) to the secreted β-APP showed that significantly higher levels of β-APP were present in the CSF collected from lesioned rats relative to sham operated controls ($p<0.0001$). In addition, a significantly ($p<0.0002$) greater amount of β-APP was present in the CSF of lesioned aged rats relative to lesioned young rats (118% increase vs 66% increase). Basal levels of β-APP in the CSF of young and old sham lesioned rats did not differ ($p>0.2$). The levels of β-APP in the CSF were highly, and inversely, correlated with the degree of cortical ChAT depletion ($r=-0.55$, $p<0.001$). Since secreted β-APP contains the potentially amyloidogenic $A\beta_{1-28}$ amino acid sequence, these results suggest that the consequences of cholinergic system dysfunction may be more deleterious in the old than in the young.

435.12

CHOLINE ACETYLTRANSFERASE ACTIVITY IS DECREASED IN FRONTAL CORTEX BIOPSY SAMPLES OF DEMENTED PATIENTS WITH ELEVATED EXTRACELLULAR β-AMYLOID.

O.S. Jørgensen, G. Waldemar#, M. Juhler# (SPON: European Neuroscience Association). Laboratory of Neuropsychiatry, Department of Pharmacology, University of Copenhagen, Rigshospitalet-6102, DK-2100 Copenhagen, Denmark, and #Departsments of Neurology and Neurosurgery, University Hospital, Rigshospitalet, Copenhagen, Denmark.

Demented patients suspected to suffer from normal-pressure hydrocephalus were evaluated for shunt surgery and a small biopsy was taken from the right frontal cortex of fourteen patients. One patient with tumour cerebri was excluded. Slight-to-moderate Alzheimer disease (AD) was demonstrated in three of the patients (β-amyloid peptide containing plaques, a few tangles) and diffuse β-amyloid peptide staining was shown in one patient. The specific activity of choline acetyltransferase (ChAT) in the frontal cortex of these four patients (mean age 68 years, range 63-70) was decreased 33 percent in comparison with the other nine patients ($p<0.05$; mean age 64 years, range 53-75) which apparently had a normal level of ChAT (68 ± 5 pmol/min/mg protein, 37°C). The decrease in ChAT was only half the decrease previously observed in autopsy frontal cortex samples of AD patients. Glial fibrillary acidic protein and glutamine synthetase were not significantly changed. Blood flow in the right frontal cortex was decreased in eight of the patients, including the patients with positive β-amyloid peptide staining. In the low-flow patients ChAT was decreased 26 percent ($p=0.08$, two-tailed t-test). The results cannot exclude that precipitation of β-amyloid peptide and down-regulation of the cholinergic function are associated early in the pathogenesis of AD and might be a consequence of a decreased regional blood flow (Supported by The Danish Medical Research Council).

435.13

IMAGING STUDIES OF A TC-99M ANTI-Aß FAB MONOCLONAL ANTIBODY (MAB) IN ALZHEIMER'S DISEASE (AD) USING SPECT. R.P.Friedland, J.M.Reno, R.E.Majocha, M.S.Berridge, F.Miraldi, P.Hedera*, L.R.Lyle, C.A.Marotta. Alzheimer Center, University Hospitals, CWRU, Cleveland, OH 44106; NeoRx Corp., Seattle, WA; Mallinckrodt Medical, St.Louis, MO; Brown University, Providence RI.

A Mab (10H3) targeting the AD Aß protein has been developed as a tool for the in vivo assessment of amyloid angiopathy using SPECT. Results from immunocytochemical studies using the 10H3 antibody suggest that microvascular Aß is accessible to an imaging agent delivered via the IV route. The 10H3 antibody is stably radiolabeled by conjugation of a preformed Tc 99m diamine dithiolate chelate active ester with the protein amino groups, and fractionated to Fab fragments. In vitro and animal studies in vivo have demonstrated the safety, sensitivity, specificity and satisfactory biodistribution of the labeled 10H3 Fab (**J Nuc Med** 33:2184,1992; **Mol Neurosci**, in press). SPECT and gamma camera imaging in 4 patients with AD demonstrate rapid appearance of activity in the blood pool in the thorax and brain, with rapid uptake in liver and kidney. Clearance of the label from serum is slower than expected, with an initial half life of 3 to 4 hours. No binding to rbc's could be shown, and free Tc-99m was minimal. Tomographic brain images obtained up to 15 hours after injection show retained activity in the venous sinuses with patchy activity in the brain parenchyma. Studies are currently underway to compare the brain distribution of the label to known blood volume markers, and compare patients in the early and later stages of the disease. A label with a longer physical half life may be needed to allow for imaging at later times following clearance of the radiopharmaceutical from the blood.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION

436.1

DO THYROID HORMONES REGULATE THE ONSET OF THE BREEDING SEASON IN THE EWE? L. T. Thrun, G. E. Dahl*, F. J. Karsch. Repro. Sci. Prog., Dept. of Biology, and Dept. of Physiol., Univ. of Michigan, Ann Arbor, MI 48109.

Thyroid hormones are required for the transition from the breeding to non-breeding state in a variety of species ranging from starlings to sheep. The onset of the breeding season appears to be thyroid hormone-dependent as well in the starling because premature testicular recrudescence occurs in birds thyroidecomized (THX) as they enter the non-breeding state (J. Exp. Zool., 179: 331-338). In the present study, we tested the hypothesis that thyroid hormones are involved in the transition from the non-breeding season (anestrus) to the breeding season in the ewe, a species in which both onset and end of the breeding season are generated by an endogenous rhythm. We determined the influence of thyroidectomy on the onset of the breeding season in ewes maintained outdoors and ewes held in a fixed short-day photoperiod in which the endogenous reproductive rhythm would be unmasked. All animals were ovariectomized (OVX) and received s.c. Silastic implants of estradiol. As an index of reproductive neuroendocrine activity, luteinizing hormone (LH) was measured in twice weekly blood samples. A sustained increase in circulating LH above 1 ng/ml denoted the onset of the breeding season. Ewes maintained outdoors were either THX in late anestrus (Aug., n=8), THX in early anestrus (Mar., n=4), or remained thyroid intact (n=7). There was no group difference in the mean date of increase in LH (20 Sept ± 10, 16 Sept ± 6, and 14 Sept ± 3, respectively). Ewes held in constant photoperiod were either THX early in anestrus (a month after LH decreased; n=3) or remained thyroid intact (n=3). Again, there was no difference in the onset of the subsequent breeding season, with LH rising 6 mo. after the previous decline. We conclude that thyroid hormones do not influence the transition into the breeding season in the ewe. Rather, thyroid hormone action on the reproductive neuroendocrine axis appears to be limited to the processes involved in the development of anestrus. (Supported by NIH HD18337, 18258 and MH10506; NSF IBN 9206510).

436.2

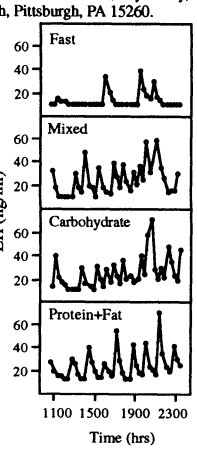
A PHYSIOLOGICAL ROLE FOR EXCITATORY AMINO ACIDS IN THE CONTROL OF PULSATILE LH SECRETION. V.B. Mahesh*, L. Ping and D.W. Brann. Department of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA 30912.

The purpose of the present study was to determine the role of excitatory amino acids (EAAs) such as glutamate and aspartate in the regulation of pulsatile luteinizing hormone (LH) secretion in adult male and female rats. To achieve this aim, specific EAA receptor antagonists were injected into the third ventricle of castrated adult rats and the effect on pulsatile LH secretion was determined. The specific N-methyl-D-aspartate (NMDA) receptor antagonist AP5 (2-amino-5-phosphono-pentanoic acid, 10μg/rat) and the specific non-NMDA receptor antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione, 30nM) were utilized in these studies. In female rats, administration of either AP5 or DNQX resulted in a significant suppression of mean LH levels which was due to a suppression of both LH pulse frequency and amplitude. In male rats, AP5 suppressed mean LH levels and amplitude with no effect on frequency, while DNQX had no effect on any parameter of LH secretion. Increasing the duration of exposure to the antagonists by administering three injections (instead of one) at 20 minute intervals yielded similar results to a single injection for AP5, while three injections of DNQX (20 nM each injection) now caused a significant suppression of mean LH levels and pulse amplitude with no effect on frequency. These studies provide evidence that EAAs acting through both NMDA and non-NMDA receptors play a significant role in regulating pulsatile LH secretion and that female animals have a higher sensitivity than male animals to this regulation.

436.3

EFFECT OF MACRONUTRIENT COMPOSITION ON FEEDING-INDUCED LUTEINIZING HORMONE SECRETION IN RHESUS MONKEYS. D. A. Schreihofer*, F. Renda, and J. L. Cameron. Departments of Neuroscience, Psychiatry, and Cell Biology and Physiology. University of Pittsburgh, Pittsburgh, PA 15260.

One day of fasting significantly suppresses pulsatile LH secretion in adult male rhesus monkeys. Feeding on the day following a fast (refeeding) leads to a rapid resumption of pulsatile LH release that is dependent on the amount eaten. The current study examined the role of macronutrient content of the refeed meal on LH secretion. Seven adult male rhesus monkeys were fitted with indwelling venous and intragastric catheters and maintained on standard jacket-tether-swivel systems. Monkeys were fasted on the day before the study. On the following day monkeys were either fasted for a second day or fed a liquid diet through the indwelling gastric catheter. Three diets of equal volume (600 ml) and caloric content (600 Cal) were used. One was equal in macronutrient content to their normal monkey chow (mixed meal); one consisted of a pure carbohydrate source (dextrose); and one consisted of a mixture of protein (casein) and fat (Liposyn) in the same ratio as the mixed meal. Blood samples were collected every 20 min from 1100-2400 h, and LH was measured by RIA. On a day of fasting LH secretion was low, however, administration of all three liquid diets significantly stimulated pulsatile LH secretion in a similar manner (see example). These results suggest that the feeding-induced stimulation of LH secretion is more dependent on caloric content than macronutrient composition.



436.4

INFLUENCE OF ETHANOL (ETOH) ON INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) AND IGF-1 RECEPTOR (IGF-1R) SYNTHESIS DURING FEMALE PUBERTY. V. Srivastava, J.K. Hiney, C.L. Nyberg, N.H. McArthur*, and W.L. Dees. Dept. of Vet. Anatomy, Texas A&M University, College Station, TX 77843-4458.

Recently, we showed that most IGF-1 available to the hypothalamus at puberty is derived from peripheral sources and that IGF-1 activates the LHRH/LH releasing system of prepubertal female rats; thus, indicating IGF-1 may participate in the control of LH secretion at the time of puberty. Since ETOH delays puberty and is associated with depressions in GH and LH, we investigated in this study whether ETOH can affect a) IGF-1 gene expression in liver, b) IGF-1R gene expression in the median eminence (ME) and c) serum levels of IGF-1 and LH. Prepubertal female rats were implanted with gastric cannulae on day 24 and began receiving control or ETOH liquid diets on day 29. On day 34, rats were killed, determined to be anestrus, and tissues and blood collected. Results indicate that the ETOH-fed rats showed a decrease ($p < .001$) in liver IGF-1 mRNA levels compared with the controls, and this paralleled depressions in both serum IGF-1 ($p < .01$) and LH ($p < .05$). No changes were detected in IGF-1R mRNA within the ME. These results suggest that ETOH may alter the pubertal process, at least in part, by blocking the peripubertal increase in the synthesis of IGF-1, a peptide suggested as a metabolic signal involved in the onset of puberty. (Supported by NIH-AA07216)

436.5

EFFECTS OF ETHANOL (ETOH) ON NMA-INDUCED SECRETION OF LH AT THE TIME OF FEMALE PUBERTY. C.L. Nyberg, J.K. Hiney, V. Srivastava, W.L. Dees*, Department of Vet. Anatomy, Texas A&M Univ., College Station, TX. 77843

We have shown that EtOH can delay puberty and block LHRH secretion *in vitro*. Additionally, we have shown that EtOH blocks LHRH secretion induced by NMA *in vitro* and delays NMA-induced precocious puberty. The present study determined if EtOH could block NMA's ability to elicit LH release *in vivo*. Prepubertal female rats were implanted with jugular cannulae and blood samples were drawn at 10 min intervals on the following day. Four samples were taken to establish basal release before a 3g/kg dose of EtOH (or an equal volume of saline) was administered by gastric gavage. After 90 min to allow for EtOH absorption, the animals recieved NMA (2.5 mg/kg IV) then four more blood samples were taken. Rats were killed and confirmed to be in first proestrus. In the EtOH-treated animals, the peak LH pulse after NMA was blunted to a 24.0 $\pm$ 12.1% increase over basal. In the saline control animals, the peak LH pulse was increased by 167.8 $\pm$ 58.1%, significantly ($p < 0.01$) greater than the EtOH-treated animals.These results suggest that ETOH is capable of altering the excitatory amino acid-induced LH secretion at the time of first proestrus. (Supported by NIH-AA07216)

436.6

PULSATILE FSH SECRETION IN THE OVARIECTOMIZED EWE IS CONTROLLED BY BOTH GnRH-DEPENDENT AND GnRH-INDEPENDENT MECHANISMS. V. Padmanabhan*, K.L. McFadden, N.P. Evans, G.E. Dahl, D.T. Mauger, F.J. Karsch. Department of Pediatrics and Physiology, Reproductive Sciences Program and the National Center for Infertility Research at Michigan. University of Michigan, Ann Arbor, MI.

The objectives of this study were to determine: i) if FSH secretion is episodic and ii) if a GnRH-independent control of episodic FSH secretion exists. Hypophyseal portal blood and peripheral blood were collected from five short-term ovariectomized ewes at 5-min intervals for 6 h before and 6 h after a single i.v. injection of Nal-Glu, a GnRH antagonist (10 μg/kg body weight). GnRH in portal blood, and LH and FSH in both jugular and portal blood were determined by radioimmunoassays. Pulsatile GnRH secretion was evident both before and after Nal-Glu administration. Pulses of LH in the portal circulation coincided with those seen in the peripheral circulation but were larger and more discrete in nature. The pattern of FSH in peripheral blood was not episodic. In marked contrast, FSH in portal blood was unambiguously pulsatile. Prior to Nal-Glu treatment, each pulse of GnRH correlated with a pulse not only of LH but also of FSH in portal blood. However, 49% of the total FSH pulses occurred in the absence of corresponding GnRH pulses. Following Nal-Glu administration, LH pulsatility was completely eliminated, demonstrating its dependence on GnRH. In contrast, Nal-Glu administration blocked only GnRH associated pulses of FSH; it did not block other episodes of FSH release. We conclude that in the ovariectomized ewe: i) FSH secretion is episodic; ii) all GnRH pulses induce an FSH pulse; iii) there exists a GnRH-independent episodic component of FSH release. Our results are consistent with the hypothesis of a dual regulation of FSH release. Supported by NIH grants U54 HD 29184, P30 HD 18258.

436.7

APPROXIMATE ENTROPY STATISTICS REVEAL NON-GNRH-DEPENDENT SUBORDINATE ACTIVITY IN FSH CONCENTRATIONS IN THE PERIPHERAL AND HYPOPHYSEAL PORTAL CIRCULATIONS OF OVARIECTOMIZED SHEEP. A.R. Midgley*, S.M. Pincus and V. Padmanabhan. Reproductive Sciences Program, National Center for Infertility Research, Univ. of Michigan, Ann Arbor, MI 48109 and 990 Moose Hill Rd., Guilford, CT 06437 (SMP)

Continuous sampling of hypophyseal portal blood (HPB) from unrestrained sheep is not only providing an unprecedented means for defining the secretory profiles of GnRH, this method can also be used to reveal the dynamics of pituitary hormone secretion without deconvolution (Midgley et al., Program, Endocrine Soc., 1992). These studies have shown that, while GnRH pulses are followed by secretion of LH and FSH, non-GnRH-driven pulses of FSH can also be observed in HPB. In contrast, discrete pulses of FSH cannot be discerned reliably in peripheral blood. Recently, a novel algorithmic method was developed that provides an estimation of the regularity in a data set by means of calculating the approximate entropy or ApEn statistic (Pincus, PNAS 88:2297, 1991). The objective of this study was to determine if the ApEn statistic would be able to discern in peripheral blood the dissociations in LH and FSH secretory bursts revealed in the HPB. We calculated the ApEn statistic for concentrations of LH and FSH in 7 sets of 72 samples of peripheral and portal blood obtained every 5 min from ovariectomized ewes. Group comparisons of ApEN for secreted HPB FSH (1.415±0.097 SD) and HPB LH (0.822±0.213 SD) differed with P = 0.000077 by paired t-test. Of greater interest was the persistence of these differences in the periphery, FSH = 1.431±0.101 and LH = 1.252±0.086 with P = 0.024. These highly significant differences in ApEn suggest that FSH has considerably more irregularity than LH, a result that confirms our measurements at the site of secretion. This approach may help delineate differences in LH and FSH secretory patterns in the peripheral blood of humans, in whom HPB cannot be obtained readily. (Supported by NIH U54 HD29184)

436.8

INHIBITION OF ENDOGENOUS NEUROPEPTIDE Y (NPY) SYNTHESIS BY ANTISENSE OLIGODEOXYNUCLEOTIDE ADMINISTRATION SUPPRESSES THE PROGESTERONE-INDUCED LH SURGE. *Kalra, P.S.*, Bonavera, J.J., Dube, M.G., Crowley, W.R. and Kalra, S.P.*, Departments of Physiology and Neuroscience, Univ. Fla., Gainesville, FL 32610 and Department of Pharmacology, Univ. Tenn, Memphis, TN 38163

Evidence shows that NPY is involved in stimulation of the LHRH and LH surges induced by progesterone (P) in estradiol benzoate (EB)-primed ovariectomized (ovx) rats. We have also reported that P stimulates NPY peptide content and preproNPY mRNA levels in the hypothalami of these rats, thereby suggesting P-induced activation of NPY synthesis. To evaluate whether newly synthesized NPY is critical in induction of the P-induced LH surge, ovx rats fitted with permanent cannulae in the lateral cerebroventricle (icv) were primed with EB (30 μg/rat s.c.). Two days later, these rats received P at 1000 h (2 mg/rat s.c.). Additionally, they were injected icv at 1000, 1200 and 1400 h with either NPY antisense or scrambled oligodeoxy-nucleotides (OLIGO); controls were injected with saline. Blood samples were withdrawn via pre-implanted intra-atrial cannulae at 1000 h and at hourly intervals between 1400-1800 h for LH analysis. Results show that robust LH surges occurred in controls and in rats injected with scrambled OLIGO. However, in rats injected with NPY antisense OLIGO to block NPY synthesis, the P-induced LH surge was completely suppressed. These results support the hypothesis that P activates *de novo* synthesis of NPY, which is necessary to evoke LHRH and LH surges. (Supported by NIH HD 08634).

436.9

FOOD RESTRICTION INCREASES NPY GENE EXPRESSION IN A SUBPOPULATION OF NPY NEURONS IN THE ARCUATE NUCLEUS OF PREPUBERTAL RATS. J.L. Cameron*, D.J. Hollingshead and M.S. Smith. Departments of Psychiatry, Neuroscience and Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261.

Food restriction has been shown to delay the onset of puberty in female rats. Others have reported that food restriction increases hypothalamic NPY activity. These studies examined whether changes in NPY gene expression were associated with the delayed onset of puberty induced by food restriction. Three groups of prepubertal rats (n=6 each) began treatment on day 28 of age and were sacrificed on day 42: 1) controls (C), ad lib feeding; 2) food restriction (FR); 3) food restriction + 24 hrs of ad lib feeding before sacrifice (FR + 24 hr refeed). Average body weights on day 42 were C, 136.6 ± 3.3 gm; FR, 74.2 ± 0.7 gm; and FR + 24 hr refeed, 99.6 ± 1.3 gm. At the time of sacrifice, C animals were in various stages of the estrous cycle, whereas none of the FR groups had exhibited first estrus. *In situ* hybridization was performed on 20 mM brain sections using a ^{35}S riboprobe for rat NPY mRNA. The data were analyzed by measuring the area occupied by silver grains over the arcuate nucleus. NPY mRNA levels did not differ among the three groups in the rostral portions of the arcuate nucleus. However, in the caudal portions of the arcuate nucleus, NPY mRNA levels were increased by 76% in response to food restriction: C = 59,108 ± 5,774 (area occupied by silver grains); FR = 104,042 ± 6715. FR + 24 hr refeed resulted in NPY mRNA levels that were in-between C and FR values (FR + 24 hr refeed = 84,302 ± 8769). These data demonstrate that the delay in the onset of puberty induced by food restriction is associated with an increase in NPY mRNA levels in a specific population of NPY neurons in the caudal portion of the arcuate nucleus. Further studies will be necessary to determine whether these NPY neurons play a role in causing the suppression of GnRH secretion that underlies the delay of puberty induced by food restriction.

436.10

IDENTIFICATION, ANALYSIS AND CLONING OF NEUROPEPTIDE Y1 RECEPTOR (NPY1r) TRANSCRIPTS IN THE PITUITARY. J.H. Urban*, J.E. Levine. Dept. Neurobiol & Physiol, Northwestern Univ., Evanston, IL 60208.

Neuropeptide Y (NPY) regulates preovulatory luteinizing hormone secretion in female rats. The receptor which mediates NPY's pituitary actions remain uncharacterized. The present studies were designed to determine whether the pituitary expresses NPY1r. RT-PCR, with primers derived from extracellular loops 3 & 4, identified the presence of NPY1r by producing an appropriately sized PCR product within the hippocampus as well as in whole and anterior pituitary. To determine whether full length NPY1r transcripts are present within the pituitary, Northern analysis was performed on mRNA obtained from pituitary with hippocampal tissues as a control. Using a cDNA probe complementary to the 3' end of the coding region (TM7 and intracellular tail) of NPY1r, Northern analysis revealed the presence of a full length transcript (approx. 3.7 kb) for NPY1r in both pituitary and hippocampus. In addition, a smaller size transcript (approx. 1.6 kb) was also seen in pituitary but not hippocampus. RNA mapping was used to identify sequence homology of the coding region of the smaller pituitary transcript with the known NPY1r sequence. Restriction fragments along the coding region of NPY1r (5' and 3' of the 80 bp intron splice site) hybridized with the full length transcript in both pitutiary and hippocampus; but only the probe encoding the 3' end of NPY1r hybridized to the small transcript. Screening and partial sequence analysis of clones from a rat pituitary cDNA library to identify NPY1r transcripts have verified the presence of a full length NPY1r. These studies suggest that mRNA for NPY1r is expressed within the pituitary and that an additional transcript within the pituitary may also encode a region, or splice variant, of the NPY receptor. The functional significance and complete identity of this transcript is unknown, and studies are in progress to identify this gene product. (NIH HD20677, HD219121, HD28048)

436.11

AXONAL TRANSCRIPTS IN THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL TRACT: G.F. Jirikowski* Dept. of Neuroendocrinology, Max Planck Inst. for Psychiatry, Klin. Inst., Munich, Germany.

With light- and electron microscopical in situ hybridization and non radioactive detection methods, we could obtain evidence that oxytocin- and vasopressin- encoding mRNA is present in the axonal pathway of magnocellular hypothalamic perikarya, probably associated with a fraction of the large secretory vesicles. mRNA is likely to be subject to rapid axonal transport, since osmotic challenge changes axonal concentrations of transcripts, while colchizine treatment blocks such effects. In recent studies we could demonstrate that several other transripts occur in magnocellular hypothalamic axons in the median eminence and the posterior lobe: mRNA coding for cfos and cjun as well as tyrosine hydroxylase mRNA could be found in the median eminence and the posterior lobe. Salt loading resulted in a shift of hybridization signal to the magnocellular perikarya within 15min, which could be prevented by pretreatment with colchizine. Pretreatment with a polymerase II inhibitor did not affect increase of hybridization signals in the paraventricular and supraoptic nuclei upon osmotic stimulation. It is likely that neuroendocrine systems with high secretory capabilities, like the hypothalamo neurohypophysial system, utilize their large axonal volume for storage of transcripts, which are perhaps compartimentalized in vesicles, to circumvent degradation. Retrograde transport upon specific stimulation may allow for immediate de novo translation, to replenish depleted peptide pools in terminal sites, prior to the onset de novo gene expression.

436.12

SEX DIFFERENCE IN COEXPRESSION BY GALANIN NEURONS ACCOUNTS FOR SEXUAL DIMORPHISM OF VASOPRESSIN IN THE BED NUCLEUS OF THE STRIA TERMINALIS. B. Planas*, P.E. Kolb, M.A. Raskind and M.A. Miller. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

Vasopressin (VP) neurons in the bed nucleus of the stria terminalis (BNST) are steroid sensitive and the number of VP mRNA expressing neurons is larger in males compared to female rats. This difference in VP cell number has been attributed to proliferation and/or survival of VP neurons following exposure to gonadal steroids during the critical period. We have shown that galanin (GAL) and VP mRNAs are coexpressed in the BNST of the adult male rat and recently, that GAL mRNA gene expression in the BNST is not sexually dimorphic. These results suggest that the index of coexpression of these neuropeptides differs in male and female rats.

Here, we hypothesized that VP neurons represent a subset of GAL cells in the BNST and that the incidence of coexpression of VP mRNA by GAL mRNA expressing neurons in the BNST would be reduced in female rats compared to male rats. We performed double in situ hybridization histochemistry on sections through the BNST from male and female Wistar rats (90 d). Brain sections were hybridized with ^{35}S-labeled and digoxigenin-labeled cRNA probes complementary to GAL and VP mRNAs, respectively. Radioactive grains were detected over VP cells indicating the presence of GAL gene expression. We divided the BNST into two anatomical regions which are part of separate functional pathways: medial (BSTM) and lateral (BSTL) divisions. We found a significant sex difference in the number of GAL cells which coexpressed VP in the BSTM (Mean±SEM; male: 124±8, female: 56±6; $p \leq 0.0001$). However, no difference was found in the BSTL (male: 80±9, female: 83±15). Likewise, the number of cells expressing GAL mRNA only was significantly higher ($p \leq 0.002$) in the BSTM of female (85±9) compared to male rats (43±5). We provide evidence that VP is coexpressed by a subset of GAL neurons and that the reduced incidence of coexpression of VP by GAL neurons in the medial BNST accounts for the sex differences in VP cell number in this limbic region.

436.13

GAP JUNCTION PROTEINS ARE DIFFERENTIALLY EXPRESSED IN NEURONS OF THE MATURING MONKEY HYPOTHALAMUS. P.C. Goldsmith* and K.K. Thind. Reproductive Endocrinology Center, Univ. California Sch. of Med., San Francisco, CA 94143-0556.

Gap junctions (GJ) establish cytoplasmic continuity and synchronize activity between adjacent cells. In the brain, the GJ proteins, connexins (Cx), exhibit developmentally regulated expression and restricted distribution. Although Cx26 and Cx43 were thought to occur predominantly in astrocytes, a recent report demonstrated functional GJs and Cx26 expression in GnRH GT1-7 neurons *in vitro* [*Neuroendocrinology (1993) 58:485-492*]. To reevaluate which cell types express Cx isoforms in the intact brain, we analyzed the distribution and expression of Cx26 and Cx43 in the primate hypothalamus during development. Vibratome sections were immunostained with optimal dilutions of affinity-purified polyclonal antibodies against Cx26, residues 108-117 (from J.A. Germak) or 112-125 (from N.B. Guilula), and a mouse monoclonal IgG_1 against 19 residues of Cx43 (Zymed). Two fetal males (142-150 dga),had few Cx immunoreactive (-ir) fibers. However, in 4 infant females (4.5-7.5 mo), faint or speckled Cx26-ir and Cx43-ir perikarya also were seen, especially in animals pre-treated with colchicine. In 3 juvenile females (14.7-19.2 mo), Cx26-ir and Cx43-ir somata had light to intense, uniform or speckled staining. In 3 adults, Cx43-ir perikarya were absent, but speckled Cx26-ir somata remained. In addition, Cx-ir periventricular and Cx26-ir fibers in the ventral hypothalamic tract continued into the infundibulum (INF) and median eminence. Considering that Cx-ir was found in perikarya retrogradely labeled from the INF, and staining mimicked that for neuronal but not astrocytic markers, we conclude that Cx26 and Cx43 are present in neurons. Since GJs create electrotonic connections and allow small molecular diffusion between coupled cells, developmental changes in Cx expression may help coordinate maturation and synchronize specialized functions of hypothalamic neurons. Supported by HD10907 and HD11979.

437.1

REGIONAL DISTRIBUTION OF AGMATINE AND ARGININE DECARBOXYLASE IN RAT BRAIN. H. Wang*, K. Otake, D.A. Ruggiero, T.A. Milner, G. Li, W. Raasch, C. Youngson, S. Regunathan and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. and Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Agmatine, an amine, is an endogenous ligand for imidazoline and α_2-adrenergic receptors recently detected in bovine brain (Li et al., *Science* 263:966, 1994). We investigated if agmatine is present in rat brain and if it, and its biosynthetic enzyme, arginine decarboxylase (ADC), are regionally distributed. Agmatine, measured by HPLC, is present in rat brain (2.4±0.6 ng/gm wet wt). A polyclonal antibody (Ab) was produced in rabbits by injection of an agmatine-KLH complex. Specificity of the agmatine Ab was established by: (a) dot blots; (b) immunoprecipitation; (c) demonstration that cultured bovine chromaffin cells (which contain agmatine) exhibited positive immunostaining with immune but not pre-immune serum; (d) loss of immunostaining after preabsorption with agmatine. Immunoreactive agmatine was localized to neurons preponderantly in cerebral cortex in laminae VI>V>III and the subiculum. The greatest staining was in retrosplenial, cingulate and primary somatosensory cortices. A few neurons were detectable in posterior hypothalamus. ADC activity, measured in regions by conversion of ^{14}C-arginine to $^{14}CO_2$ (nmol CO_2/h/mg protein) (see Li et al., *Soc. Neurosci. Abstr.*, 1994), ranked: cortex (16) > hippocampal formation (subiculum, hippocampus and entorhinal cortex) (8.5) > hypothalamus (6.3) > brainstem = cerebellum (3.5) in agreement with the immunocytochemical distribution of agmatine. We conclude: agmatine is synthesized in rat brain, is neuronal, unevenly distributed and most concentrated in cerebral cortex. The findings are consistent with the hypothesis that agmatine may be a novel neurotransmitter.

437.2

ENZYMATIC SYNTHESIS OF ANANDAMIDE, AN ENDOGENOUS LIGAND FOR THE CANNABINOID RECEPTOR; PROPERTIES AND DISTRIBUTION IN THE BRAIN. W.A. Devane* and J. Axelrod, Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

Anandamide (arachidonylethanolamide) was the first endogenous cannabimimetic compound to be identified in brain (Devane *et al.*, *Science* 1992). The purpose of this study was to characterize the enzymatic synthesis of anandamide using brain membranes.

Bovine membranes (P_2 fraction) were incubated with [^{14}C]ethanolamine and varying concentrations of ethanolamine and arachidonic acid for 20-40 min at 37°C. This resulted in the formation of a radioactive product which had the same R_f value as authentic anandamide using both normal and reverse phase thin layer chromatography. Incubation of [^{3}H]arachidonic acid and ethanolamine also resulted in the synthesis of a radioactive product having the same R_f value as anandamide in both chromatographic systems. The synthesis of anandamide had a pH optimum of 9 to 10, and the amount of product formed was linear up to 50 min, using 70 µg of membrane protein/200 µl.

When varying the concentration of arachidonic acid, the enzymatic activity exhibited a biphasic curve, suggesting substrate inhibition. When compared to a number of related fatty acids, arachidonic acid proved to be the best substrate with the lowest EC_{50} and the highest V_{max}. A distribution study in bovine brain indicated that anandamide synthase levels are high in the hippocampus, followed by cortex, striatum, and thalamus. Lower levels of enzymatic activity were observed in the pons, cerebellum, and medulla. These findings suggest the presence of anandaergic neurons.

437.3

MOLECULAR CLONING OF A NEW MEMBER OF G PROTEIN-COUPLED RECEPTOR FAMILY EXPRESSED IN MOUSE CENTRAL NERVOUS SYSTEM. Y. Saeki, S. Ueno, R. Mizuno, H. Fujimura and T. Yanagihara*, Dept. of Neurology, Osaka Univ. Med. Sch., Osaka 565, Japan.

A novel cDNA clone encoding a putative G protein-coupled receptor (named GPCR21) was isolated from mouse brain cDNA library along with its homologue, GPCR01 (the mouse counterpart of previously reported rat receptor R334 [(1991) FEBS Lett. 292, 243-248.]) by the polymerase chain reaction using degenerate oligonucleotide primers. Both polypeptides encoded by these clones had seven hydrophobic transmembrane domains which are characteristic for G protein-coupled receptor superfamily. Northern blotting and reverse transcription-polymerase chain reaction analyses showed predominant expression of these two receptors in the central nervous system. *In situ* hybridization analysis revealed their prominent expression in the limbic system and further demonstrated the differential distribution of their mRNAs in mouse brain. Although the ligands for these receptors are yet to be identified, the significant sequence homology between these receptors suggests that they constitute a new receptor subfamily and they possibly represent different receptor subtypes for an unknown neurotransmitter. The prominent expression of GPCR21 in medial habenular nucleus, shown by *in situ* hybridization analysis, suggested that further characterization of GPCR21 may facilitate our understanding of the function of the habenular nuclei.

437.4

INVESTIGATION OF VASOPRESSIN RECEPTORS IN CELLS FROM CIRCUMVENTRICULAR ORGANS USING CALCIUM MEASUREMENTS. M. Jurzak, A. R. Müller and R. Gerstberger . MPI for Physiol. & Clin. Res., W.G. Kerckhoff-Inst., Parkstr. 1, 61231 Bad Nauheim, Germany.

Circumventricular organs (CVO) of the rat brain lamina terminalis lacking a blood-brain-barrier, the subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT), are involved in body fluid and cardiovascular control by sensing blood-borne substances. *In vitro* autoradiography on brain tissue sections showed binding sites for vasopressin (AVP) and its AVP(4-9)-fragment within the SFO and OVLT. For characterization of AVP and AVP(4-9) receptors in cells cultured from the SFO and OVLT, rises in [Ca^{++}]i were measured in fura-2 loaded single neurons and glial cells. The investigated cell type was verified by immunofluorescence using NSE- and GFAP-specific antibodies. SFO- (n=19) and OVLT- (n=25) neurons were found to increase their [Ca^{++}]i after exposure to AVP in a dose-dependent manner (0.1-100 nM). Glial cells from SFO (n=21) and OVLT (n=10) were found to respond to AVP with similar sensitivity. The pharmacological characterization revealed the presence of a V1 receptor subtype, since specific antagonists inhibited the AVP evoked responses in neurons (SFO n=4, OVLT n=9) and in glial cells (SFO n=9, OVLT n=3). Ca^{++}-transients measured in the absence of extracellular Ca^{++} suggest intracellular stores to be the Ca^{++}-source. An additional AVP receptor subtype in OVLT-neurons is implicated by the finding of Ca^{++}-signals induced by the V2 receptor agonist dDAVP (n=7). Furthermore, Ca^{++}-transients were evoked with AVP(4-9) (1-100 nM) in SFO- (n=6) and OVLT- (n=7) neurons as well as in SFO- (n=2) and OVLT- (n=6) astrocytes. A significant number of cells (n=9) responded exclusively to the fragment peptide, indicating the existence of yet another member of the AVP receptor family.

437.5

RECEPTOR AUTORADIOGRAPHIC ANALYSIS OF α_2-ADRENOCEPTOR DISTRIBUTION IN RAT HYPOTHALAMUS. M. Savola*, M. Kulatunga, P. Panula and M. Scheinin, Dept. of Pharmacology, Univ. of Turku, and Dept. of Biology, Åbo Akademi University, FIN-20520 Turku, Finland.

Hypothalamic α_2-adrenoceptors (AR) participate in endocrine and cardiovascular regulation, and have also been implicated in complex integrative behaviors and adaptive functions. Receptor autoradiography with [^{3}H]*para*-aminoclonidine has revealed relatively dense binding in most hypothalamic nuclei, some of which may have been to non-adrenergic imidazole sites, e.g. in the arcuate nucleus(1). Intense immunohistochemical α_{2A}-AR labeling has been seen in some hypothalamic nuclei(2). *In situ* hybridization also indicates that the main α_2-AR subtype in the rat hypothalamus would be α_{2A}(3). We have performed receptor autoradiography with brain sections of adult male Sprague-Dawley rats using the subtype-nonselective α_2-AR radioligand [^{3}H]RX821002(4) (in K^+-phosphate buffer). Specificity of binding was determined with 100 µM adrenaline. Regional receptor binding was quantitated with the MCID image analysis system. B_{max} and K_d values were derived from saturation assays with 7 concentrations of [^{3}H]RX821002 (0.1-15 nM). Specific α_2-AR binding (B_{max} 50-185 fmol/mg tissue) was detected in all hypothalamic regions analyzed (35 regions). The paraventricular and dorsomedial hypothalamic nuclei had the highest receptor densities. Similar K_d values (0.5-0.6 nM) were found in all regions. Co-incubation with 100 nM oxymetazoline indicated that α_{2A} is the predominant α_2-AR subtype in rat hypothalamus. References: (1)Unnerstall, Brain Res Rev 7:69, 1984; (2)Go, Soc Neurosci Abstr 196.4, 1992; (3)Scheinin, Mol Brain Res 21:133, 1994; (4)Hudson, Mol Neuropharmacol 1:219, 1992.

437.6

ALTERED α_{2C}-ADRENOCEPTOR GENE EXPRESSION IN BRAINS OF TRANSGENIC MICE. M. Scheinin*, R. Link, M. Kulatunga, J. Marttila, M. Savola, G. Barsh and B.K. Kobilka, Dept. of Pharmacology, Univ. of Turku, FIN-20520 Turku, Finland, and Howard Hughes Medical Institute and Stanford University, Stanford, CA 94305.

Three α_2-adrenoceptor (AR) genes are expressed in brains of rodents and are evolutionarily conserved in humans. One of the subtypes, α_{2A}, mediates both presynaptic regulatory functions and postsynaptic effects. To probe the neurophysiologic roles of α_{2C}, mice were genetically altered to express increased or decreased levels of α_{2C}. Mice that overexpress α_{2C} were generated by pronuclear injection of the α_{2C} gene flanked by 10 kb of genomic DNA, and α_{2C} knockout mice were generated by homologous recombination in embryonic stem cells using a gene targeting vector that introduced stop codons into all three frames of the gene. Total α_2-AR density was determined with the subtype-nonselective ligand [^{3}H]RX821002. The proportion of α_{2A}-binding was determined by co-incubation with 100 nM oxymetazoline, an α_{2A}-selective ligand. α_{2C}-ARs were selectively labeled by low concentrations of the α_{2C} -preferring ligand [^{3}H]rauwolscine + 100 nM oxymetazoline. Homozygotes for the α_{2C} knockout had no detectable α_{2C} binding in brain, and overexpressing transgenic mice had several-fold increased α_{2C} binding in the brain regions normally expressing α_{2C}, i.e. the caudate-putamen, olfactory tubercle, cerebral cortex, and parts of the hippocampal formation. Levels of α_{2A}-binding were not altered in either strain. Thus, 10 kb of flanking genomic DNA is sufficient to confer regional tissue specificity for expression of the α_{2C} gene. The α_{2A} gene does not compensate for altered levels of α_{2C} expression. These mouse strains may provide useful models for studies on the physiological and pharmacological functions of the α_{2C}-AR.

437.7

LOCALIZATION OF CALCIUM RECEPTOR mRNA IN RAT BRAIN USING *IN SITU* HYBRIDIZATION HISTOCHEMISTRY.

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The capacity to sense changes in the level of extracellular Ca^{2+} is an important function in several cell types. For example, hormone secretion by parathyroid cells and thyroid C cells is primarily regulated by extracellular Ca^{2+}. The protein that enables these cells to detect and respond to extracellular calcium has recently been cloned and shown to be a 7-transmembrane, G-protein-coupled receptor linked to the mobilization of intracellular Ca^{2+} in response to increases in extracellular Ca^{2+}. Northern blot hybridization and PCR analysis have indicated that transcripts for this Ca^{2+} receptor (CaR) are present in several tissues including thyroid, kidney and brain. We have used *in situ* hybridization histochemistry to localize CaR mRNA in the adult rat CNS. *In situ* hybridization was performed on 20 um brain slices using ^{33}P-labeled riboprobes complimentary either to the entire rat CaR coding region or to the 5' most 1.2 kb portion of the cDNA . The results with both probes were identical. Cells expressing CaR mRNA were scattered throughout the CNS including the cortex, midbrain, hindbrain, and spinal cord; however, the density of CaR expressing cells within these regions varied considerably. Particularly high numbers of cells were present in the hippocampus, corpus callosum, piriform cortex, caudate-putamen, amygdala, and several hypothalamic nuclei. These data indicate that a subset of cells within the CNS express parathyroid CaR mRNA and that this expression may have important functional consequences in the brain.

437.8

IMMUNOHISTOCHEMICAL STUDIES OF SITES IN THE VENTROLATERAL MEDULLA THAT EXERT CONTROL OVER CARDIOVASCULAR FUNCTION

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Physiological studies using microinjections of L-glutamate into specific sites in the rostral venrolateral medulla and monitoring of cardiac function and vascular blood flow (Doppler flow probes) of pentobarbital-vagotomized dogs revealed two sites where consistent responses were obtained. The first is an area located 3.5 to 5.8 mm rostral to obex, 4.2 to 5.1 mm lateral to midline, 0.5 to 1.5 mm below the ventral surface [referred to as the subretrofacial area (SRFN)] and the second is an area located 6.2 to 7.1 mm rostral to obex, 2.9 to 5.4 mm lateral to midline, 0.5 to 2.0 mm below the ventral surface (referred to as rostroventromedial area). L-glutamate microinjected in the subretrofacial area produces increases in arterial blood pressure associated with decreases in femoral and renal vascular bed conductances. L-glutamate microinjected into the rostroventromedial area produces only a slight presser response associated with a decrease in renal arterial bed conductances but an increase in femoral arterial bed conductance. Using immunohistochemical methods and specifically tyrosine hydroxylase (TH) and phenolethanolamine-N-methyltransferase (PNMT) colormetric immunohistochemistry, we determined whether TH and/or PNMT immunoreactive neurons were present at the two microinjection sites. Results indicate that neurons in the canine subretrofacial nucleus are immunoreactive to antibodies against PNMT and therefore contain epinephrine. Further analysis show that neurons in the rostroventromedial area are mainly composed of neurons exclusively immunoreactive to antibodies against tyrosine hydroxylase (TH) although some neurons also contain PNMT. Previous investigators have included this region anatomically as part of the SRFN (Dampney et al, 1992). However, physiologic responses to L-glutamate microinjection in this area are characterized primarily by a significant increase in femoral conductance consistent with vasodilation.

437.9

STIMULATION OF C-FOS EXPRESSION IN SELECTIVE BRAIN NUCLEI BY EXOGENOUS ARGININE-8-VASOPRESSIN: QUANTITATIVE IMMUNOCYTOCHEMICAL STUDY.

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Peripheral administration of arginine-8-vasopressin (AVP) has been shown to retard the decay of learned behaviors and to maintain tolerance to ethanol by the action on AVP-V1 receptors. Others found that i.c.v. injection of AVP increased c-fos mRNA in the septum and hippocampus. However, the precise site(s) of action of peripherally injected AVP remain unknown. Adult male Sprague Dawley rats were injected with saline (S, 0.3 ml, s.c., n=6), AVP (10 μg/0.3 ml, s.c., n=6), saline (2 ml, i.p., n=1) or pentylenetetrazole (PTZ, 50 mg/kg, 2.0 ml, i.p., n=2) and were sacrificed 2 h later. Serial coronal brain sections were processed with antibodies against Fos and Fos-like proteins and AVP. Brain areas containing nuclei of similar size and shape were quantified by counting the number of neuronal cell bodies showing Fos immunoreactivity (Fos-IR) in each nucleus in left or right side under a Nikon Optiphot microscope with 20 X objective and CFPL ocular (2.5 X). The average Fos-IR cell counts/side/section (Fos-IRCSS) for the suprachiasmatic nucleus (SCh) were 9.2 ± 5.9 (S, s.c.) and 96.8 ± 16.9 (AVP, s.c.) [$p<0.001$, t-test]. In SCh, the i.p. injection of S and PTZ yielded Fos-IRCSS of 43.5 and 33.3 respectively. The supraoptic nucleus (SON) had 0.72 ± 0.3 (S) and 60.6 ± 11 (AVP) [$p<0.019$, t-test], but PTZ did not stimulate Fos expression. AVP significantly increased Fos-IRCSS in the paraventricular nucleus (PVN)[19.4 ± 5.9 (S) vs 164.3 ± 44.7 (AVP), $p<0.023$, t-test] and so did PTZ. The lateral septum showed no Fos-IR either in the S- or AVP-treated rats. Fluorescent labelling of AVP cell bodies revealed that Fos-IR cells were also AVP-IR cells. Results indicate that AVP cells in the SCh, SON and PVN are potential sites of action for the exogenous AVP. Supported by NIAAA grant #1 R01-AA08212-05.

437.10

ESTROGEN-SPECIFIC TARGET SITE IDENTIFIED BY PROGESTERONE-11α-HEMISUCCINATE-(2-[^{125}I]-IODOHISTAMINE IN MOUSE BRAIN MEMBRANES.

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Using photoaffinity labeling with the progesterone analogue, progesterone-11α-hemisuccinate-(2-[^{125}I]-iodohistamine ([^{125}I]-his-PG), we have recently identified a protein band of MW 29 kDa (band 4) in mouse cerebellar membranes. Inhibition of the labeling was specific with respect to steroid structure and was most effectively inhibited by preincubation with estradiol. Among estrogens, the labeling was strongly inhibited by estradiol 17β, estradiol 17α. The antiestrogen tamoxifen and the synthetic estrogen diethylstilbestrol were also effective. Other estrogens (estriol, estrone) were less effective and the steroids, dihydroandrosterone, androsterone and aldosterone were inactive. Preincubation with estradiol 17α or estradiol 17β inhibited the labeling in a dose-dependent manner with IC_{50} values of 0.3 and 2.0 μM; respectively. The extent of labeling was three times as high in male as in female cerebellar membranes. In males labeling of cerebellar membranes was greater than that of cortex or limbic region. In contrast to the well described steroid receptors which are in the cytosolic fraction; the newly identified estrogen target site (band 4) has a membrane localization.

ISCHEMIA: MECHANISMS III

438.1

EFFECT OF MK-801 IN FOCAL CEREBRAL ISCHEMIA IN THE MONKEY.

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To determine the effectiveness of MK-801 in primate focal ischemia, transorbital occlusion of the middle cerebral artery (MCA) was done in female squirrel monkeys (*Saimiri sciureus*). Clip occlusion was verified under the operating microscope, while blood pressure was controlled to 60 mm Hg using halothane. No other drugs were administered. Ambient temperature was controlled under the operating drapes to roughly 33°C, and temporalis temperature was controlled to 37.5°C. MK-801 (1 mg/kg) was administered 20 minutes after occlusion. Following 110 minutes of occlusion, the clip was released and restoration of flow was verified visually. Quantitative histopathology at 52 coronal planes was performed after 2 weeks. There was no statistically significant difference in the infarct volumes between treated and untreated animals. Neuropsychologic testing also revealed no difference between the groups.

The cingulate cortex was carefully examined in the oldest animals, and no evidence of acidophilic neuronal necrosis, microglial nodules, or other evidence of MK-801 induced cell death was seen.

We conclude firstly that MK-801 is ineffective in reducing cerebral infarct volume in this model of transient focal ischemia. Secondly, although 1 mg/kg is a relatively high dose of MK-801 in the primate, we conclude that the neuronal necrotizing effect of this NMDA antagonist, which has been well-described in the cingulate cortex of the rodent, does not occur in this primate.

438.2

IMMUNOCYTOCHEMICAL DETECTION OF GLUT1 IN BRAIN MICROVESSELS AND PARENCHYMA: EFFECTS OF FOREBRAIN ISCHEMIA.

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Transport across the blood-brain barrier and into some brain parenchymal cells is believed to be mediated by two differently glycosylated forms of GLUT1. The effects of cerebral ischemia for GLUT1 mRNA have been described but not for the protein. Using a high affinity, isoform-specific, polyclonal antiserum (ALM1-K) generated by a special intravenous secondary immunization protocol, we have examined immunocytochemical distribution of GLUT1 in rat brain 1 and 4 days after global forebrain ischemia using a 2-vessel occlusion/hypotension model. In non-ischemic rats, GLUT1 immunoreactivity was intensely concentrated in cerebral microvessels as previously noted. In addition, diffuse parenchymal staining was also detectable and was completely inhibited by preincubation of antisera with 10 μg/mL of peptide. Preliminary electron micrographic localization (ALM1-K; 1:5000) confirmed the asymmetric distribution of GLUT1 with ablumenal > cytoplasmic > lumenal concentrations. After ischemia, both microvascular and non-microvascular GLUT1 concentrations increased markedly by as soon as 1 day and persisted through 4 days after ischemia. **Conclusions:** GLUT1 may be detected in both cerebral microvessels and parenchyma using a high affinity, isoform-specific antiserum. Global forebrain ischemia rapidly and persistently increases GLUT1 expression of both forms. Supported by NS22213 and NS17493.

438.3

INDUCTION OF BASIC FIBROBLAST GROWTH FACTOR EXPRESSION FOLLOWING FOCAL CEREBRAL ISCHEMIA-REPERFUSION INJURY. T.N. Lin*, J. Te, M. Lee, C.Y. Hsu. Neuroscience Research Division, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC.

Basic fibroblast growth factor (bFGF) is a biologically active polypeptide with mitogenic, angiogenic, and neurotrophic properties. Significant increase in the level of bFGF immuno-reactivity were found after neuronal injury. In the present study, we examined the temporal and spatial expression of bFGF mRNA during ischemia-reperfusion, using a three-vessel occlusion focal cerebral ischemia model. Northern blot analysis showed that severe ischemia induced a two- to three-fold increase in a single species of 6.0 kb bFGF mRNA transcript in the ischemic cortex, which peaked at 12 h during reperfusion. There was no obvious increase in the contralateral cortex. Similar two- to three-fold enhanced expression of bFGF mRNA also found in the ipsilateral hippocampus, however, unlike the ipsilateral cortex, this higher expression were maintained up to 2 weeks of reperfusion. In situ hybridization studies further revealed a constitutive expression of bFGF mRNA in discrete areas within the central nervous system (CNS). Ischemia-reperfusion increase the expression of bFGF mRNA in three distinct areas: (1) rostral area of the infarct, close to the outer layer of cerebral cortex, (2) area surrounding the infarction, penumbral area, and (3) dentate gyrus of hippocampus. The mechanisms leading to this discrete enhancement is not clear, however, it is likely that the effect of bFGF is regulated in the transcriptional level not the post-translational modification level. These results suggest that during ischemia-reperfusion, bFGF may be released from different pools of cells and synergistically performed their beneficial effect through different mechanisms.

438.4

High $+G_z$ exposure followed by expression of heat shock protein 70 (HSP70) in rat brain: A RT-PCR study. A. R. Shahed*, S. Galindo Jr., R. Echon, J. A. Barber and P. M. Werchan. Operational Tech. Corp., Krug Life Sci. and Armstrong Laboratory, Brooks AFB, TX. 78235.

We have previously reported that a 30 s, high $+G_z$ (head-to-foot inertial load) exposure of > +20 G_z causes global cerebral ischemia in rats (Werchan and Shahed, The Physiologist, 35: S143-146, 1992). Also six +25 G_z exposures have been shown to cause transient brain edema (Shahed et al. Aviat. Space Environ. Med. 63:5, 1992). In the present study *HSP70* expression was investigated as an early marker of cellular stress. ***Methods: Protocol 1:*** Rats were exposed to +22.5 G_z for either 15, 30 or 60 s in a small animal centrifuge, and brains were collected immediately and 0.5 to 24 hr after the run. Control rats were exposed to +0.5 G_z. **Protocol 2**: rats were subjected to six 30 s exposures of +22.5G_z and brains were collected as in Protocol 1. Total RNA was extracted by RNAzol B method. RT-PCR was performed using 1 µg of RNA for cDNA synthesis and specific *HSP 70* primers were used for subsequent amplification. The PCR products (235 bp) were visualized on agarose gels. ***Results:*** Expression of *HSP 70* did not increase above the control level after 15 or 30 s exposure. Following the 60 s exposure, *HSP 70* expression increased at 60 and 180 min after the centrifuge run. In protocol 2, expression of HSP 70 increased at 60 and 180 s min after the run. ***Discussion:*** High $+G_z$ exposure of at least 60 s duration or six exposures of +22.5G_z were required to increase expression of *HSP70.* The time points of increased *HSP70* expression were similar to those previously reported for brain edema. This suggests that *HSP70* expression could be a suitable marker for monitoring post $+G_z$ exposure effects.

438.5

INTERCELLULAR ADHESION MOLECULE-1 IS UPREGULATED ON BRAIN MICROVASCULAR ENDOTHELIAL CELLS IN FOCAL ISCHEMIA. F.C. Barone*, A-L. Siren, T. Liu, T-L. Yue, G.Z. Feuerstein and X.K. Wang. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406 and Uniformed Services University of the Health Sciences, Bethesda, MD 20814

The adhesion of leukocytes to endothelium is the first step for cellular infiltration in the inflammatory response to ischemic tissue injury. Therefore, the expression of intercellular adhesion molecule-1 (ICAM-1) was studied in rat focal ischemic cortex and compared to the known inflammatory response in this model. A significant increase in ICAM-1 mRNA expression in the ischemic cortex over levels in contralateral (control) cortex was observed by means of Northern blot analysis following either permanent or temporary (160 min) occlusion with reperfusion of the middle cerebral artery (PMCAO or MCAO with reperfusion) in spontaneously hypertensive rats. In the ischemic cortex, levels of ICAM-1 mRNA increased significantly at 3 hours (2.6-fold, N=3, $p<0.05$), was peaked at 6 and 12 hrs (6.0-fold, $p<0.01$) and remained elevated up to 5 days (2.5-fold, $p<0.05$) after PMCAO. A similar profile of ICAM-1 mRNA expression in the ischemic cortex following MCAO with reperfusion was observed, except that ICAM-1 mRNA increased significantly as early as 1 hr (6.3-fold, N=3, $p<0.05$) and then more gradually reached a peak at 12 hrs (12-fold, $p<0.01$) after reperfusion. ICAM-1 mRNA expression in ischemic cortex following PMCAO was significantly greater in hypertensive rats, that are more sensitive to focal ischemia, than in two normotensive rat strains. Immunohistochemical staining using anti-ICAM-1 antibodies indicated that ICAM-1 was localized to endothelial cells of intraparenchymal blood vessels in the ischemic but not control cortex. The increased expression of ICAM-1 message and protein significantly preceeds the influx of leukocytes into ischemic tissue which occurs at 12 hr post-PMCAO and 6 hr post-MCAO with reperfusion. These data suggest that an upregulation of ICAM-1 mRNA and protein in brain capillary endothelium plays an important role in leukocyte migration into the ischemic brain.

438.6

Time Course of Basic Fibroblast Growth Factor (bFGF) Expression Following Focal Cerebral Ischemia in the Rat. E.K Speliotes*, C.G. Caday, J. Weise, T. Do, P. Ge, N.W. Kowall, and S.P. Finklestein. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129 ; Veteran's Administration Medical Center, Bedford, MA 01730 and Boston University Medical School, Boston, MA 02118.

Basic fibroblast growth factor (bFGF) is a polypeptide found in brain that has potent trophic effects on neurons. In this study, we examined changes in bFGF expression in tissue surrounding focal infarcts of the right cerebral hemisphere of rats sacrificed at 4hr,1,3,7, and 14 days following permanent occlusion of the middle cerebral artery. Northern blot analysis of the right cerebral cortex revealed a three fold increase in bFGF mRNA levels at 24 hours after ischemia that declined somewhat by 3 days and returned to control levels at 7 and 14 days after ischemia. *In situ* hybridization corroborated the Northern blot findings and revealed that the upregulation of bFGF mRNA occurred throughout the entire right cerebral cortex, and in the contralateral cingulate cortex. At 1 and 3 days following ischemia increased numbers of bFGF immunoreactive astrocytes were detected in the right cerebral cortex concomitant with a shift from nuclear to nuclear plus cytoplasmic localization of bFGF immunoreactivity. The time course and distribution of bFGF expression suggests that this factor may play a role in cytoprotective and /or reparative processes following ischemia.

438.7

Suppression of Hippocampus Fos Expression and Activator Protein-1 (AP-1) Activity during Focal Cerebral Ischemia using Antisense Strategy. P.K. Liu, A. Salminen, Y.Y. He, I.M. Tarkka* and C.Y. Hsu. Div. of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

An activation of *c-fos* and the subsequent expression of the Fos protein were noted following focal cerebral ischemia. Fos and Jun form a heterodimer as AP–1 which modulates the expressions of several genes. To study the post-ischemic events related to *c-fos* expression, we sought to suppress the post-ischemic expression of *c-fos* by intraventricular infusion of an antisense phosphothioated DNA of *c-fos* mRNA. The antisense DNA blocked in vitro *c-fos* mRNA translation into Fos. In the uptake study, we noted that the ^{32}P-labelled antisense DNA was internalized within 6 h and detectable in the RNA fraction up to 41 h after intraventricular delivery. The antisense DNA formed DNA:RNA hybrid after uptake. When focal cerebral ischemia was induced 6-16 h after the infusion of the antisense DNA, the post-ischemic increase in Fos expression and AP–1 binding activity were suppressed. The binding activity of a cyclic AMP response element was not blocked by the antisense DNA. The results support that Fos expression is at least partially responsible for an increase in AP–1 binding activity after focal cerebral ischemia.

438.8

DIFFERENTIAL EXPRESSION OF mRNA ENCODING PROSTAGLANDIN ENDOPEROXIDE SYNTHASE AND c-fos IN REVERSIBLE CEREBRAL ISCHEMIA. M.H.Ruppert(1,3), M.S.Kindy(1), H.H.Tai(2), and L.C.Pettigrew(1,3)*. (1) Stroke Program, Sanders-Brown Center on Aging and (2) College of Pharmacy, University of Kentucky, (3) Department of Veterans Affairs Medical Center, Lexington, Kentucky.

Postischemic reperfusion of brain activates PGH Synthase (PGHS), known previously as cyclooxygenase, to generate vasoactive eicosanoids from arachidonic acid. Recent studies have identified two isoforms of PGHS, denoted PGHS-1 and PGHS-2. Knowing that inducible PGHS-2 is transcriptionally regulated at its AP-1 gene sequence by c-fos translational products, we hypothesized that the constitutive isoform, PGHS-1, is under separate transcriptional control in ischemic brain. We examined levels of PGHS-1 and c-fos mRNA immediately after reversible cerebral ischemia by the 4-vessel occlusion (4VO) technique or after 60 or 180 min of postischemic reperfusion. Levels of PGHS-1 mRNA peaked immediately after ischemia (263±85; mean±SD, expressed as %sham; $p\leq0.01$), which was significantly different from levels at 60 (80±22) and 180 (77±40) min. In contrast, c-fos mRNA levels peaked after 60 min of reperfusion (743±317; $p\leq0.01$) compared to levels immediately after ischemia and after 180 min of reperfusion. We conclude that prior induction of the c-fos gene (with probable translation into Fos protein) does not precede transcription of PGHS-1 mRNA in ischemic brain.

438.9

DNA FRAGMENTATION INDICATIVE OF APOPTOSIS OCCURS IN A NEONATAL MODEL OF CEREBRAL HYPOXIA-ISCHEMIA. U.I. Tuor*, I.E. Hill, I. Rasquinha and J.P. MacManus. Institutes for Biological Sciences and Biodiagnostics, NRC, Ottawa and 435 Ellice Ave., Winnipeg, Manitoba, R3B 1Y6

A well established model of neonatal cerebral hypoxia-ischemia involves subjecting 7 day old rats to unilateral carotid artery occlusion and exposure to 3 hr. of hypoxia which results in damage ipsilateral to the carotid occulsion. In several adult animal models of cerebral ischemia, DNA fragmentation indicative of apoptosis has been observed. Whether programmed cell death contributes to the damage which occurs following hypoxia-ischemia in neonates is not known.

The pattern and incidence of DNA fragmentation was investigated by in situ end-labelling with terminal transferase. Brains of 7 d rats which had been subjected to either 0, 2 or 3 hrs of hypoxia-ischemia (unilateral carotid occlusion + hypoxia) were perfusion fixed either 0 or 18 hr post hypoxia. Paraffin embedded sections were stained with hematoxylin&eosin or processed for in situ labelling. In additional animals, the brains were dissected and frozen 0 or 18 hr after the hypoxia.The tissue was homogenized and DNA extracted and processed for detection of DNA laddering by gel electrophoresis.

Breaks in DNA were observed in brain sections ipsilateral to the carotid artery occlusion in rats exposed to 3 hrs of hypoxia-ischemia corresponding to extensive damage observed in the H&E sections. In some animals subjected to 2 hr of hypoxia-ischemia, the damage was less severe and selective areas of positive DNA fragmentation were observed in the hippocampus, thalamus, caudate nucleus and cerebral cortex. Tissue analysis of these regions confirmed the presence of laddered DNA fragmentation. Thus, in this model of neonatal hypoxia-ischemia there is evidence that DNA fragmentation indicative of apoptosis can occur suggesting that programmed cell death contributes to the damage observed.

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438.10

PHOTOCHEMICAL UNILATERAL CORTICAL STROKE IN RATS CAUSES DNA FRAGMENTATION AND INCREASES p53 IMMUNOREACTIVITY IN THE AREA PENUMBRA. A. Kharlamov*, D.M. Armstrong, D.R. Grayson, C.M. Cagnoli and H. Maney. Allegheny-Singer Res. Inst., Med. Coll. of Pennsylvania, Pittsburgh, PA 15212

Apoptosis, or programmed cell death (PCD), is the process by which certain stimuli can force a cell to commit suicide. PCD is usually associated with intranucleosomal DNA fragmentation, which may lead to an upregulation of certain proteins such as p53, a member of the heat-shock protein family. We have shown that following unilateral focal stroke, induced in rats by photochemical thrombosis (rose bengal is injected intravenously and the skull is irradiated with a beam of focussed light; *Neuroscience* 1993, 55: 473; *J Neurosci* 1993, 13: 2483) there is in the cortex a primary thrombotic/necrotic core and an area penumbra where the cells are injured and may eventually die, but which also can be pharmacologically protected. In the present study we investigated whether PCD may contribute to cell death in the area penumbra. We collected brain samples at different times post-stroke and determined immunohistochemically: a) in-situ DNA fragmentation by staining the brain slices with anti-digoxigenin antibody to detect the digoxigenin-dUTP-extended DNA complexes (ApopTag, Oncor); b) the presence of p53 (antibody PAb240, Oncogene Science Inc.); and c) the presence of 72 kD heat-shock protein (72 kD antimouse monoclonal antibody, Amersham). Following stroke, we observed increases in DNA fragmentation, p53, and 72 kD protein in the cortex ipsilateral to the thrombotic core. These increases occurred in different brain regions: DNA fragmentation and p53 increased only in the area penumbra, while 72 kD heat-shock protein increased throughout the ipsilateral cortex, except in the area penumbra. DNA fragmentation was evident as early as 6 hrs post-stroke, peaking at 8-12 hrs, and leveling off at 24 hrs. In contrast, p53 and 72 kD immunoreactivity was not increased until 8 hrs and peaked at 12-24 hrs post-stroke. These results suggest that stroke-triggered p53 upregulation might be responsible for the PCD in the area penumbra.

438.11

THE EFFECT OF TRANSIENT GLOBAL ISCHEMIA ON ACTION POTENTIAL AND SYNAPTIC INDUCED CALCIUM TRANSIENTS IN CA1 PYRAMIDAL NEURONS. Y. Schiller*[1], C. Sommer[2] and M. Kiessling[2] Abt. Zellphysiologie, MPI Med. Forschung[1] and Dept. Neuropath., Univ. of Heidelberg[2], Heidelberg, Germany.

Elevation of the $[Ca]_i$ were implicated to play an important role in the ischemic induced delayed cell death. We were interested in characterising the effect ischemia on calcium transients evoked by electrical and synaptic activity in CA1 pyramidal neurons. Brain slices were prepared from normal and ischemic gerbils (5 minute transient global forebrain ischemia). Simultaneous recordings of $[Ca]_i$ and the electrical activity were performed in CA1 neurons using patch clamp and flourometric fura-2 techniques. Calcium transients were measured at the soma and at different location along the apical dendrite (10-300μm)

The major findings of this study are: 1) The resting $[Ca]_i$ was not significantly different in control and ischemic gerbils up to 24 hours after the ischemic event. 48-72 hours after ischemia a small increase of the resting $[Ca]_i$ was observed. 2). The $[Ca]_i$ transients evoked by a train of 5 action potentials (synaptic or electrically evoked) were not significantly altered up to 24 hours after the ischemic event. 48-72 hours after the ischemic event a 30% prolongation of these $[Ca]_i$ transients was observed. 3). Following a 0.5 second 100Hz synaptic stimulation the peak and decay of the $[Ca]_i$ transient did not differ significantly in normal and ischemic gerbils 24 hours after the ischemic event. In both groups about 85% of this $[Ca]_i$ transient was blocked by APV. From these findings we concluded that during the first and potentially reversible period after the ischemic event (24h) no significant abnormalities were detected in the amplitude of synaptic and action potential induced $[Ca]_i$ transients and in the ability of CA1 pyramidal neurons to eliminate this calcium load.

438.12

ROLE OF THE Na^+-Ca^{2+} EXCHANGER DURING ANOXIC AND GLUCOPENIC CONDITIONS IN C6 GLIOMA CELLS. S. Amoroso, E. Iannotti, N. Caso, G. Russo, A. Bassi, G.F. Di Renzo and L. Annunziato*. Unit of Pharmacology, Dept. of Neuroscience, School of Medicine, University "Federico II" of Naples, Via S. Pansini 5, 80131 Naples, Italy.

Under anoxic and glucopenic conditions the homeostasis of intracellular Ca^{2+} and Na^+ ions is altered. The Na^+-Ca^{2+} exchanger is a bidirectional pathway which couples the extrusion of Ca^{2+} to the entrance of Na^+ ions or viceversa Na^+ ion efflux to Ca^{2+} ion influx. The aim of the present study was to characterize the mode of operation of the Na^+-Ca^{2+} exchanger, under anoxic and glucopenic conditions, and the role played by this exchanger in anoxia-glucopenia induced neurotoxicity.

In C6 glioma cells, a cell line that has been extensively used as a model for cerebral glia, anoxic-glucopenic conditions, mimicked by adding a mixture of oligomycin plus 2-deoxyglucose to the incubation medium, induced a massive release of intracellular LDH, used as an index of neurotoxicity. The activation of the Na^+-Ca^{2+} exchanger as a Na^+ extrusion pathway coupled to Ca^{2+} ion entry, obtained by the removal of Na^+ ions from the extracellular medium, reduced the release of LDH, induced by anoxic-glucopenic treatment. CB-DMB, a rather specific inhibitor of the Na^+-Ca^{2+} exchanger, in a dose-dependent way, completely reverted the reduction of LDH release, induced by the removal of Na^+ ions from the extracellular medium, under anoxic-glucopenic conditions. These results suggest that the Na^+-Ca^{2+} exchanger during anoxic-glucopenic conditions operates as a Na^+ efflux mechanism coupled to Ca^{2+} ion entry and that this mode of operation, although intracellular Ca^{2+} ion concentrations increase, may be protective against anoxia-glucopenia-induced brain cell death. (Supported by C.N.R. and 40-60% MURST Grant to L. Annunziato and G.F. Di Renzo)

AXON GUIDANCE I

439.1

GENETIC MANIPULATION OF ORGANOTYPIC COCULTURES WITH RECOMBINANT ADENOVIRUS DOES NOT ALTER THALAMOCORTICAL PROJECTIONS. M. Wilkemeyer* and K. Angelides. Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The structure and organization of thalamocortical (TC) axons in the developing mammalian neocortex is the focus of continued investigation. Organotypic cocultures of fetal thalamic and neonatal cortical explants permit the *in vitro* dissection of the signals and mechanisms underlying the development of TC projections. Genetic manipulation of various receptor and signal transduction molecules is a potentially powerful tool for examining the development of this circuitry. Recently, several labs have demonstrated the utility of using viral vectors (herpes simplex virus I and adenovirus) to transfect neurons *in vivo* and *in vitro* . Replication-defective, recombinant (RDR)-adenoviruses have several advantages over herpes virus vectors, including: i) high titre, ii) safety and iii) relative long-term expression of the recombinant gene. To explore the possibility for genetic manipulation of organotypic cocultures, rat cortical explants, cocultured with thalamic explants, were infected with Ad.RSVβgal. Infection of cortical explants, via both bulk and focal application, resulted in relatively long-term expression (2 weeks) of the reporter gene (β-galactosidase) with little gross, structural alterations in the explant. DiI-labeled thalamic ingrowth was also assayed 1-2 weeks post infection. In 22 cocultures where the thalamic explants were sandwiched between infected and control cortical explants, no gross differences in the extent of thalamic ingrowth or axonal arborization were detected. These data suggest that adenoviruses are suitable gene transfer vectors for genetic manipulation of target tissue. Future experiments will concentrate on designing recombinant adenovirus for studying target selection and circuitry formation in thalamocortical cocultures. Supported by NIH grant NS 26733. M. W. is an NIH postdoctoral fellow (NS 09306).

439.2

DISRUPTION OF ONE CELL ADHESION SIGNAL IS SUFFICIENT TO HINDER AXONAL NAVIGATION IN DEVELOPING VISUAL CORTEX. Fidelma A. Nazif* and Kimon J. Angelides. Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Synaptic patterning in the developing visual cortex requires precise spatial and temporal pathfinding by afferent axons. The initial stages of axonal targeting are directed by activity-independent signals, such as cell adhesion molecules (CAM). In cultured neurons the presence or absence of a single CAM can drastically affect axonal growth and targeting. In the more complex environment of the visual cortex, it is not known whether a single molecule can have such a strong effect.

In organotypic co-cultures of fetal mouse thalamic lateral geniculate nucleus (LGN) and neonatal visual cortex (VC) thalamic afferents grow into the VC and terminate in layer IV, as *in vivo*. This preparation allows direct manipulation of the cortical environment. NCAM is expressed throughout the cortex during development. The anionic polysialic acid (PSA) extracellular moiety of NCAM is thought to attenuate cell interactions, allowing neuritic elongation during development. In this study, co-cultures were treated with the enzyme neuraminidase, which cleaves the PSA moiety off NCAM, and axonal growth and targeting were examined.

Co-cultures were treated daily, beginning either 20 hr or 48 hr after plating. Controls were treated with vehicle alone. After one week thalamocortical afferents were labelled with DiI. In those co-cultures treated after 20 hr, bridges of tissue formed between the LGN and VC. In 66% of co-cultures very short axons reached the proximal cortex but did not grow into the cortex. In 50% of co-cultures outgrowth from LGN over the collagen coated membrane was extensive. In 66% of control experiments ingrowth into the cortex was observed. In co-cultures treated with neuraminidase after 2 days in culture 83% showed growth into the proximal cortex and 17% showed axons extending deeper into the cortex, but not as deep as layer IV. 100% of control co-cultures showed thalamocortical afferents growing deep into the cortex, mostly terminating in layer IV, some extending almost to the pial surface. We conclude that disruption of one signal which regulates axonal navigation may be sufficient to disrupt axonal targeting in the visual cortex. Supported by NS 26733.

439.3

LAMINAR SPECIFIC ATTACHMENT AND NEURITE OUTGROWTH OF THALAMIC NEURONS ON CULTURED SLICES OF DEVELOPING CEREBRAL NEOCORTEX. D. E. Emerling and A. D. Lander*, Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

In nervous system development, the growth cones of advancing axons are thought to navigate to their targets by recognizing cell-surface and extracellular matrix molecules that act as specific guidance cues. To identify and map cues that guide the growth of a particular axonal system, the thalamocortical afferents, an assay was devised to examine short-term interactions of dissociated embryonic thalamic cells with living, ~150 μm slices of developing mouse forebrain. Thalamic cells rapidly (<3 hours) and efficiently attached to and extended neurites on pre- and postnatal slices, but a broad zone throughout the neocortex was generally non-permissive for both thalamic cell attachment and the ingrowth of neurites. This zone coincided with the cortical plate at early stages (embryonic day 15), but later became restricted, in rostral-to-caudal fashion, to cortical laminae 2/3. Thus, at each stage, thalamic cells *in vitro* avoided just that area that thalamic axons confront, but generally do not enter, *in vivo*. In addition, neurites that extended on some layers were found to be significantly oriented in directions that coincide with the pathways that thalamic axons follow *in vivo*. Furthermore, these laminar specific behaviors of thalamic cells on cortical tissue were not observed when fresh frozen cryostat sections were substituted for living brain slices. The results imply that local adhesive cues and signals that affect process outgrowth are distributed among developing cortical laminae in a manner that could underlie much of the temporal and spatial patterning of thalamocortical innervation. By investigating what cell-surface and extracellular matrix molecules account for the behavior of thalamic cells in this rapid *in vitro* assay, we hope to elucidate some of the molecules that control the *in vivo* development of the thalamocortical pathway.

439.4

CNS-ASSOCIATED CUES ARE REQUIRED FOR CENTRALLY-DIRECTED NAVIGATION BY PERIPHERAL SENSORY NEURONS IN THE EMBRYONIC MEDICINAL LEECH. J. Jellies*, K. Johansen† & J. Johansen†, Neurobiol. Res. Center and Dept. of Physiol. and Biophysics, Univ. of Alabama, Birmingham, AL 35294 & †Dept. of Zool. and Genetics, Iowa State Univ., Ames, IA 50011.

The axons of peripheral sensory neurons in *Hirudo medicinalis* navigate through several potential choice points toward the CNS where they then segregate into discrete axon tracts which can be distinguished by differential expression of surface antigens. The earliest population of sensory cells arise in discrete clusters called sensilla and pathway selection by individually dye-filled sensillar growth cones is highly specific. We have shown earlier that one potential source of guidance information proximal to the CNS was the outgrowing axons of central pioneer neurons. To examine the potential influence of CNS-associated cues, we performed CNS ablations early on embryonic day 10. Ganglionic primordia were removed from anesthetized embryos which were allowed to survive for 6-8 days before labeling them with the monoclonal antibody Lan3-2 to stain all sensillar axons. Control preparations involved surgically opening the body wall but not removing the ganglionic primordia. Pathfinding and selective fasciculation in control preparations were indistinguishable from normal. In contrast, in each of 110 hemisegments deprived of CNS (15 animals), the sensillar axons appeared undirected. In all cases the majority of sensillar axons fasciculated extensively upon one another-following a tight circular pathway around the nephridiopore. Normally these axons project some distance from this pore. There were also a few instances where normally separate peripheral fascicles became tightly fasciculated along abnormal pathways. These results demonstrate that the CNS normally provides cues necessary for correct navigation, and in their absence the peripheral neurons default to fasciculate with each other. Further evidence in support of this is that later-developing extrasensillar neurons do differentiate and project axons in the absence of the CNS, but appear unable to identify an appropriate direction for growth. Instead they form short bundles of axons that do not join their normal pathways. Thus, the peripheral sensory neurons of leeches require CNS-derived guidance information. We are currently further examining the nature of the cues provided by CNS neurons necessary to direct afferent ingrowth. Supported by NSF 9209237 and a Sloan Foundation Fellowship (JJe) and NIH 28857 (JJo).

439.5

NETRINS: A FAMILY OF AXON OUTGROWTH-PROMOTING PROTEINS HOMOLOGOUS TO *C. ELEGANS* UNC-6. T. Serafini*, T.E. Kennedy, M.J. Galko, C.M. Mirzayan, T. Jessell† and M. Tessier-Lavigne. Dept. of Anatomy, University of California, San Francisco, CA 94143-0452; †Howard Hughes Medical Inst., Columbia University, New York, NY 10032.

In the embryonic spinal cord, commissural axons pioneer a circumferential pathway from dorsal regions to the floor plate at the ventral midline. Floor plate cells secrete a factor that attracts these axons *in vitro*, suggesting that the ventral growth of these axons may be directed partly by chemotropism. Floor plate cells have also been shown to secrete a (possibly distinct) diffusible factor that promotes the outgrowth of commissural axons into collagen gels *in vitro*. Using outgrowth of commissural axons into collagen as an assay, we have purified from embryonic brain two proteins, netrin-1 and netrin-2 (78 kD and 75 kD, respectively, by SDS-PAGE), that each mimic the outgrowth-promoting activity of the floor plate. We have also identified a distinct activity in embryonic brain that potentiates their effects. Cloning of cDNAs encoding the two netrins show that they are homologous proteins (72% identical), which are structurally related (50% identical) to UNC-6, a laminin-related protein required for the circumferential migration of cells and axons in the nematode *C. elegans*. This homology suggests that the guidance of growth cones in the vertebrate spinal cord and the nematode are directed by similar molecular cues.

439.6

NETRINS ARE CHEMOTROPIC FACTORS THAT MAY GUIDE COMMISSURAL AXONS IN THE DEVELOPING SPINAL CORD. T. E. Kennedy*, T. Serafini, J. R. de la Torre, C. Mirzayan, M. J. Galko, and M. Tessier-Lavigne. Dept. of Anatomy, University of California, San Francisco, CA 94143-0452

The guidance of axons to their targets in the developing nervous system is believed to involve diffusible chemotropic factors secreted by target cells. Floor plate cells at the ventral midline of the spinal cord secrete a diffusible factor (or factors) that promotes the outgrowth of spinal commissural axons and attracts these axons *in vitro*. Two proteins from embryonic brain, netrin-1 and netrin-2, possess the outgrowth-promoting activity of floor plate cells. Here we report that during the period of commissural axon growth to the ventral midline, *netrin-1* is expressed at high levels in the floor plate region, whereas *netrin-2* is expressed more widely and at lower levels in the ventral spinal cord. Moreover, heterologous cells secreting either netrin can mimic both the outgrowth promoting and the long-range chemotropic activities of floor plate cells. Thus, netrin-1 and netrin-2 are chemotropic factors that may guide commissural axons in the developing spinal cord.

439.7

GENETIC ANALYSIS OF GROWTH CONE GUIDANCE AT THE MIDLINE IN DROSOPHILA: EXPRESSION AND FUNCTION OF D-NETRIN. K.J. Mitchell+, J. Doyle+, G. Tear*+, T. Serafini#, T. E. Kennedy#, C. Mirzyan#, M. Tessier-Lavigne#, and C.S. Goodman+, +HHMI, Neuro. Div., Dept. of Mol. and Cell Biol., U. C. Berkeley, CA 94720; # Dept. of Anatomy, U. C.S.F., CA 94143

We have been characterizing the expression and function of several genes involved in growth cone guidance at the midline of the Drosophila central nervous system (CNS) . In mutations in the *commissureless* (*comm*) gene, growth cones that would normally cross the midline instead stay on their own side (Seeger et al., 1993). In mutations in the *roundabout* (*robo*) gene, growth cones that would normally stay on their own side instead now cross the midline. The *comm* gene encodes a novel transmembrane protein expressed by a subset of midline cells (Tear, Seeger, and Goodman, unpublished results). Cloning of the *robo* gene is underway.

Here we describe the characterization of a third gene -- *D-netrin* -- that was cloned on the basis of homology to the two vertebrate netrin genes and the nematode *unc-6* gene. In the nematode, UNC-6 appears to control circumferential guidance. In vertebrates, netrin-1 and netrin -2 are expressed by the floor plate and ventral neural tube, respectively, and can function in vitro to attract commissural growth cones. The *D-netrin* gene is located on the X chromosome. It is strongly expressed by a subset of midline cells during the period of axon outgrowth, and by visceral mesoderm. Based on its homology to the netrins/UNC-6 and its expression along the Drosophila CNS midline, we propose that D-Netrin plays an important role in midline guidance. To test this model, we are using genetic approaches, including the analysis of specific growth cones in loss-of-function mutations and in embryos carrying transgenic constructs which produce specific patterns of ectopic D-netrin expression.

439.8

GENETIC ANALYSIS OF SEMAPHORIN II FUNCTION DURING GROWTH CONE GUIDANCE AND TARGET RECOGNITION IN DROSOPHILA. D.J. Matthes, A. L. Kolodkin*, and C.S. Goodman, HHMI, Neuro. Div., Dept. of Mol. and Cell Biol., U. C. Berkeley, CA 94720

The Semaphorin genes encode a family of highly related secreted and transmembrane proteins which appear to function during growth cone guidance. Antibody blocking experiments have shown that grasshopper Semaphorin I (formerly Fasciclin IV) is required for the proper guidance and fasciculation of the Ti1 growth cones in the limb bud of the grasshopper embryo (Kolokdin et al., 1992). Chick collapsin, a secreted member of the Semaphorin family, is capable of causing growth cone collapse in an in vitro assay (Luo, et al., 1993).

We previously identified two members of the Semaphorin gene family in Drosophila (Kolodkin et al., 1993). Semaphorin II (Sema II) is a secreted protein that is structurally similar to chick collapsin; both are comprised of a signal sequence, a 500 amino acid Semaphorin domain, a single immunoglobulin domain, and a short C-terminal region. In the embryo, Sema II is expressed by a small subset of cells in the CNS and by muscle 31 in the body wall of the T3 segment, suggesting a role in the generation of neuromuscular connectivity.

We have taken a genetic approach to characterize the function of Sema II. Adult flies homozygous for loss-of-function mutations in *semaII* have a variety of behavioral phenotypes including partially penetrant flightlessness, visual orienting defects, and abnormal drinking behavior. To gain insight into the origins of these behavioral defects, we have examined the effect of the *semaII* loss-of-function mutations on the innervation of muscle 31 in the embyro and larva. To complement these studies, we have used transgenic constructs to ectopically express Sema II by novel sets of muscles. In this way, we hope to determine the function of this secreted Semaphorin during growth cone guidance and target recognition.

439.9

EXPRESSION OF THE SEMAPHORIN III GENE DURING DEVELOPMENT OF THE MOUSE NERVOUS SYSTEM. E. Messersmith*, A. L. Kolodkin, C.S. Goodman, and C.J. Shatz. Division of Neurobiology, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720

The Semaphorin genes encode a family of highly related secreted and transmembrane proteins that appear to function during growth cone guidance. Semaphorin I (Sema I; formerly Fas IV) in the grasshopper is a transmembrane protein that functions in vivo in growth cone guidance (Kolodkin et al., Neuron, 1992). Sema II in Drosophila is a secreted protein that is essential for adult behavior and survival (Kolodkin et al., Cell, 1993). Chick collapsin, a secreted protein that is closely related to D-Sema II, can function in vitro to cause DRG growth cone collapse (Luo et al., Cell, 1993).

Based on the sequence of human Sema III (Kolodkin et al., Cell, 1993), we used PCR to clone mouse Sema III which is likely to be the homologue of chick collapsin. Here we conduct in situ hybridization using a mouse Sema III cDNA to analyze Sema III expression during mammalian development. Whole-mount embryos (E9.5) and tissue sections (E13.5, E15.5, P0, P7, and P21) were hybridized with antisense and sense riboprobes. Some of the structures showing the most prominent labeling with the M-Sema III probe include posterior dermomyotome and branchial arches at E9.5, ventral neural tube motoneurons, cells surrounding dorsal root ganglia, pons, and cortical plate at E13.5, retina and olfactory tract at E15.5, and olfactory tract, cerebellar Purkinje cells, and cortical layers 6b and 5 at P0. The spatial and temporal patterns of M-Sema III expression correlate with periods of axon outgrowth and are thus consistent with its proposed role as a growth cone guidance molecule. Supported by Fight for Sight (EM), NIH NS 18366 (ALK), Howard Hughes Medical Institute (CSG), and March of Dimes and NIH EY02858 (CJS).

439.10

RETINAL AXONS SUDDENLY STOP ELONGATING AFTER SIGNIFICANT EXTENSION ONTO GRADIENTS OF POSTERIOR TECTAL MATERIAL. R.W. Davenport*, J. Löschinger, J. Huf, J. Jüng, F. Bonhoeffer. Max-Planck-Institut für Entwicklungsbiologie, Spemannstr. 35/I, D72076 Tübingen, Germany.

Fundamental questions remain concerning the cellular phenomena responsible for development of topographic retinal projections. By a matching of coordinate molecular gradients across retinal and tectal surfaces, growth cones could uniquely identify their target location (Sperry, 1963)[1]. Recently, Baier and Bonhoeffer (1992)[2] developed an assay to present exponentially increasing gradients of tectal material to retinal ganglion axons extending *in vitro*. Cultures fixed at a given interval after retinal explantation clearly demonstrated that retinal axons can respond to graded molecular cues; temporal axons were shorter than their nasal counterparts when faced with increasing amounts of posterior tectal material. To begin to understand cellular mechanisms that underpin guidance in this system, technical modifications of the methods of Baier and Bonhoeffer (1992) were made in the present investigation. Retinal axons were forced to extend in lanes with relatively linearly increasing amounts of repulsive guidance activities which were bounded by non-substrate lanes. Temporal axons extended considerable distances onto the repulsive material, yet not to lengths as great as their nasal counterparts. To investigate what characteristics of continuous, long-range guidance cues were responsible for such changes in axon behavior, systematic modifications were made of gradient amplitude, slope and the growth supportive material encountered prior to the gradient. The modifications led to the following results: temporal growth cones stop at approximately the same point in a given gradient, the stop position is slope-dependent and independent of the repulsive materials' absolute amount. High concentrations of repulsive material at the site of axon initiation seem to shift the stop position higher into the gradient. Time-lapse movies reveal that upon entering the gradient axonal extension rates do not gradually diminish. These results suggest that growth cones may sense a particular difference between amounts of guidance material measured at positions near the site of axonal initiation and termination.

[1]*PNAS* (1963) 50:703-710; [2]*Science* (1992) 255:472-475.

439.11

AXONIN-1- AND Ng-CAM-LIKE IMMUNOREACTIVITY DURING THE DEVELOPMENT OF THE RETINOTECTAL SYSTEM IN THE CHICK. G. Rager*, P. Morino and P. Sonderegger[1]. Institute of Anatomy, University of Fribourg, CH-1700 Fribourg, and [1]Institute of Biochemistry, University of Zürich, CH-8057 Zürich, Switzerland.

The development of the retinotectal system seems to follow the rule of chronotopy (Rager et al., Anat. Embryol. *179*, 135-148, 1988; Rager et al., J. Comp. Neurol. *334*, 529-544, 1993). To produce this type of order, the expression of cell adhesion molecules could play an important role. Recently, two axonal cell adhesion molecules, axonin-1 and Ng-CAM, have been found to interact during the process of outgrowth (for review see Sonderegger and Rathjen, J. Cell Biol. *119*, 1387-1394, 1992). We have analyzed the expression of axonin-1 and compared it with the distribution of Ng-CAM in the retina and in the retinotectal system of the chick throughout the period of development. At stage 18 both axonin-like (A-LI) and Ng-CAM-like immunoreactivity (Ng-CAM-LI) are clearly present in the area where first retinal ganglion cells (RGC) are generated. The immunoreactivity spreads synchronously with the formation of RGCs over the developing retina. From stage 32 on the inner plexiform layer was also stained according to its temporospatial gradient of maturation. In later stages the outer plexiform layer and the inner segments of photoreceptors show immunoreactivity, too. The development of A-LI and Ng-CAM-LI along the optic nerve, chiasm, optic tract, and in the superficial layers of the optic tectum seem to follow the chronotopic distribution of axons as it was found by earlier morphological investigations. Older axons seem to loose their A-LI which allows us to localize the position of newly formed axons. The fact that A-LI and Ng-CAM-LI go in parallel with the formation and maturation of axons allows us to suggest that axonin-1 and Ng-CAM may play an important role in the organization of the retinotectal system. Supported by Swiss N.S.F. grant 31-36468.92.

439.12

A GENETIC MODEL FOR RETINOTOPIC MAP DEVELOPMENT: THE DROSOPHILA R7/R8 VISUAL PROJECTIONS. F.N. Katz* and J.A. Ashley. Dept. of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235-9038.

The *Drosophila* compound eye is composed of 800 simple eyes which each contain eight photoreceptor neurons. Two of these neurons, R7 and R8, project axons to the medulla of the optic lobe, where they establish a retinotopic map. In a search for mutations that disturb map formation, the *gap1*^{mip} mutation was recovered (Buckles et al., 1992: Neuron 8, 1015-1029). Loss of function for this gene results in stable hyperinnervation of the optic lobe, caused by the differentiation of extra R7 neurons. Using somatic mosaic constructions, wild-type or hyperinnervated terminal sites were juxtaposed with areas lacking innervation. Collateral sprouts were observed to invade unoccupied sites only in the hyperinnervated configuration, suggesting a balance between intrasite and intersite competition may be involved. During the third instar, the growth cones of wild-type R7/R8 axons send out promiscuous filopodia that overlap surrounding terminal sites and provide a means of assessing neighboring site occupancy. This is followed by a period of terminal refinement, which does not require visually evoked electrical activity for maintenance. R7/R8 axon behavior in the absence of neighbors suggests that photoreceptor neurons have positional labels that influence their connectivity in the brain, and a set of genes that have regional expression in the eye consistent with such positional subdivisions have been recovered. We propose that the position and morphology of each axon terminal is determined by the positional specification of its soma, the synaptic sufficiency of its termination site, and the availability of adjacent sites.

439.13

ISOLATION OF DROSOPHILA VISUAL SYSTEM CONNECTIVITY MUTATIONS. K. A. Martin, H. Roth, A. Ebens S. L. Zipursky*. Dept. of Biological Chemistry, UCLA Sch. of Med., Los Angeles, CA 90024.

Orderly neuronal maps of visual space are created in the fly brain by precise retinal cell (R cell) contacts to the optic lobe. To identify genes that regulate R cell connectivity we screened Drosophila larvae for mutations affecting the R cell projection pattern. We analyzed over 5000 EMS mutagenized lines for aberrant R cell projections and isolated 44 mutations. Each mutation was characterized to determine if the gene underlying the defect 1) is required in the R cells, 2) affects R cell fate determination, 3) affects cell fate determination in the optic lobe. A genetic mosaic analysis was used to determine if the gene is required in the eye. In this procedure a patch of mutant eye tissue is generated in an otherwise normal fly. Those mutations that produce a defect within the optic lobe region innervated by a mutant patch are required in the eye. This class of mutations is particularly interesting because it is likely to include genes that function in the R cell growth cones. Neuronal fate determination was assessed in both the eye and the optic lobe. In particular we wanted to eliminate from further analysis those mutations that disrupt R cell fate determination because connectivity defects may be a secondary consequence of changes in neuronal identity. Three mutants were isolated that are required in the eye but do not affect the determination of R cell fate. These mutants represent candidate genes which function in the R cells to generate precise connections with their targets.

439.14

DROSOPHILA RECEPTOR TYROSINE PHOSPHATASES ARE EXPRESSED IN THE DEVELOPING VISUAL SYSTEM C. Desai, E. Popova, B. Hamilton and K. Zinn*. Dept. of Biol., Caltech, Pasadena, CA 91125.

Several receptor-linked protein tyrosine phosphatases (R-PTPs) appear to be expressed on most or all CNS axons in *Drosophila*. We purified one such R-PTP, DPTP69D, using antiserum against horseradish peroxidase (HRP) as immunaffinity reagent. Anti-HRP antibodies recognize a carbohydrate epitope selectively expressed in insect nervous systems. Many neuronal glycoproteins (denoted 'HRP proteins') apparently bear the HRP carbohydrate epitope. Protein sequence data also defines neurotactin and fasciclin I as HRP proteins. Western blotting data suggest that fasciclin II, neuroglian, DPTP10D, and DPTP99A are also HRP proteins.

Like previously characterized R-PTPs, DPTP69D is localized to CNS axons in the embryo. In third instar larvae, DPTP69D expression is restricted to subsets of neuronal processes in the brain, ventral nerve cord, and eye disc. In particular, DPTP69D is expressed on photoreceptor axons and on the neuropils of the developing lamina, medulla and lobula complex. Interestingly, DPTP99A is also expressed in the optic lobes in a similar pattern, but at lower levels. We have made DPTP99A mutants; these are viable with normal embryonic CNSs. We are examining the visual system in larvae and adults lacking DPTP99A for possible defects. We are also trying to disrupt *DPTP69D* by mobilizing a *P* element located 8 kb downstream from DPTP69D.

440.1

UP-REGULATION OF [^{125}I]-NCQ 298 BINDING SITES IN RAT BRAIN FOLLOWING CHRONIC TREATMENT WITH REMOXIPRIDE OR HALOPERIDOL. H. Eriksson*, G. Ericsson and A. Westlind-Danielsson, Department of Neuropharmacology, CNS Preclinical R & D, ASTRA ARCUS AB, S-151 85 Södertälje, Sweden.

As the antipsychotic effect of neuroleptic drugs is believed to be a result of dopamine D2 receptor blockade it can be assumed that chronic treatment with such drugs leads to an up-regulation of D2 receptors. We tested this hypothesis for remoxipride, with haloperidol as a positive control.

Rats were administered remoxipride (10 mg/24 hrs/rat) or haloperidol (0.1 mg/24 hrs/rat) for 14 days with osmotic pumps implanted s.c. After this period dopamine D2 receptor concentrations were determined in six different brain regions using the specific high affinity D2 antagonist [^{125}I]-NCQ 298.

[^{125}I]-NCQ 298 binding sites increased in striatum by 40 % after remoxipride treatment while a 50 % increase was apparent after the haloperidol treatment. In addition, a clear increase in [^{125}I]-NCQ 298 binding sites were also measurable in the olfactory tubercle and nucleus accumbens with remoxipride treatment by 35-41 % over control values. A slight increase in [^{125}I]-NCQ 298 binding sites was also notable in both the medial prefrontal cortex and hippocampus. However, the entorhinal cortex displayed about a 40 % decline in [^{125}I]-NCQ 298 binding sites with either treatment. The control levels of [^{125}I]-NCQ 298 binding was found in the range 6.4-254 fmol/mg protein. The lowest level in the hippocampus and the highest in the striatum. This report is the first account of dopamine D2 receptor up-regulation in several different brain regions of the rat after chronic remoxipride administration. The results strengthen the notion that postsynaptic D2 receptor blockade is involved in antipsychotic action.

440.2

REGULATION OF DOPAMINE D3 RECEPTORS EXPRESSED IN HEK-293 CELLS. M. A. Pacheco*, K. D. Burris and P. B. Molinoff, Department of Pharmacology, Univiversity of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.

We have previously reported a drug-induced increase in the density of D2 receptors expressed in transfected HEK-293 cells (Mol. Pharmacol. 44:371-379). D2 and D3 dopamine receptors have similar structural and pharmacological properties. It was of interest to determine whether the D3 receptor regulates in a fashion similar to the D2 receptor. A stable cell line was created by transfecting cDNA encoding the D3 receptor, under the control of a cytomegalovirus promoter, into human embryonic kidney cells (HEK-293). Changes in the density of receptors after treatment with compounds known to be agonists or antagonists at D2 receptors were measured in homogenates using radioligand binding assays with the D2 agonist [^{125}I](R)*trans*-7-hydroxy-PIPAT or the D2 antagonist [^{125}I]NCQ-298. HEK-293-D3 cells express a density of receptors of 551 ± 45 fmole receptor per mg of protein. Exposure of HEK-293-D3 cells to quinpirole (5.0 μM) for 18 hr resulted in a 77% increase in the density of binding sites for both [^{125}I](R)*trans*-7-hydroxy-PIPAT and [^{125}I]NCQ-298. This increase in density was both time- and concentration-dependent. Exposure of cells to 7-hydroxy-DPAT (5.0 μM) for 18 hr, resulted in a 57% increase in the density of D3 binding sites. Treatment with the dopamine antagonist haloperidol (5.0 μM) for 18 hr resulted in a 145% increase in the density of D3 receptors. The increase in the number of D3 binding sites induced by haloperidol was also concentration-dependent. The data suggest that both D2 agonists and antagonists induce an increase in the density of D3 receptors expressed in transfected HEK-293 cells. (Supported by MH14654, and NS18591.)

440.3

PRODUCTION OF MICE WITH A CONSTITUTIVE DEFICIENCY OF THE β ADRENERGIC RECEPTOR KINASE BY HOMOLOGOUS RECOMBINATION. B. Giros, R. J. Lefkowitz and M.G. Caron*. HHMI, Duke University Medical Center, Box 3287, Durham, NC 27710, USA.

Protein phosphorylation represents one of the most prominent molecular mechanisms responsible for cellular plasticity and adaptability. Within the large family of G-protein-coupled receptors, phosphorylation of the agonist-activated state of the receptor underlies rapid desensitization, a condition which leads to a blunted efficiency of coupling and signal transduction. G protein-coupled Receptor Kinases (GRKs), specifically recognize the activated form of the receptor as the prefered substrate for phosphorylation, and are therefore responsible for homologous desensitization. In order to study the physiological role of this homologous desensitization, we decided to produce a mouse line in which a targeted disruption of the β-adrenergic receptor kinase (βARK-1, GRK-2) would be engineered. First, the entire genomic organization of the gene encoding the murine βARK-1 was established. It is composed of 21 exons in the coding part, interspread with introns ranging from 68 bp to more than 12 kb. A plasmid was constructed in which four exons covering part of the catalytic domain of βARK-1 were substituted with the neomycin resistance gene. This plasmid was used to transfect embryonnic stem cells from 129/Sv mice, and clones with one event of homologous recombination were selected to inject into blastocystes. Chimeric males obtained from these manipulations were mated with wild-type females, and successfully produced animals with a genotype βARK-1 (+/-). Heterozygote animals are being bred to produce homozygotes βARK-1 (-/-). The detailed study of these animals should provide a much better understanding of the biological functions which are dependent on the βARK-1 phosphorylation processes.

440.4

ENDOCYTOSIS, DESENSITIZATION AND RESENSITIZATION OF NK_1 AND NK_2 RECEPTORS. A.M. Garland, E.F. Grady, M. Lovett, J.E. Krause, and N.W. Bunnett*. Depts. of Surg. and Physiol., UCSF, San Francisco, CA 94143-0660 and Depts. of Anat. and Neurobiol., Washington University, Saint Louis, MO, 63110.

Endocytosis of NK_1 and NK_2 receptors, stably expressed in CHO cells, was studied by confocal microscopy using substance P (SP) or neurokinin A (NKA) labeled with the fluorophore cyanine 3 (cy3). When cells expressing the NK_1 receptor or the NK_2 receptor were incubated with cy3-SP or cy3-NKA, respectively, for 2 h at 4°C, fluorescence was confined to the plasma membrane. By 5 min of warming to 37°C, a marked reduction in cell surface labeling occurred and fluorescence was observed in endosomes beneath the plasma membrane. At 30 min, the signal was located in larger, more central vesicles. Endocytosis was quantified by binding with ^{125}I-SP or ^{125}I-NKA, using an acid wash to separate cell surface from internalized label. At 4°C, label was mostly found in the cell-surface fraction. After 10 min at 37°C, 90±1% of specifically bound ^{125}I-SP and 85±2% of specifically bound ^{125}I-NKA were internalized by cells expressing the NK_1 and NK_2 receptors, respectively. Truncated mutant receptors, lacking portions of the C-terminal tails (NK_1-Δ325, NK_2-Δ367, NK_2-Δ347), internalized normally, as assessed by microscopy and ligand binding. Desensitization and resensitization of wild type NK_1 and NK_2 receptors to SP and NKA, respectively, were examined by measuring IP3 generation. When cells were exposed to a test dose of agonist 10 min after a desensitizing dose, the IP3 response diminished to 28.8±7.9% for the NK_1 receptor and 48.6±17.8% for the NK_2 receptor of the control response (no desensitizing dose). The response recovered to control levels after 30 min. Treatment of cells with phenylarsine oxide, to block endocytosis, had no effect on desensitization but prevented resensitization. In summary, fluorescently labeled peptides can be used to localize receptors. Wild type NK_1 and NK_2 receptors internalize within minutes of binding ligand, and mutant receptors lacking portions of the C-terminal tail internalize normally. Endocytosis of NK_1 and NK_2 receptors is not required for desensitization of the IP3 response but is necessary for resensitization.

440.5

MODULATION OF THE SUBCELLULAR DISTRIBUTION OF THE BETA-ADRENERGIC RECEPTOR KINASE IN CELLS OVEREXPRESSING THE BETA2-ADRENERGIC RECEPTOR. A. Ruiz-Gómez[2], C.Murga[2], J.A. De Carlos*and F.Mayor, jr[2]. [1]Instituto Cajal, 28002 Madrid and [2]Centro de Biología Molecular, "Severo Ochoa" (CSIC-UAM) Universidad Autónoma de Madrid, 28049 Madrid, Spain.

ß-adrenergic receptor kinase (ßARK) is a regulatory enzyme involved in the modulation of the ß-adrenergic and other G protein-coupled receptors in the brain and many other tissues. ßARK has been described as a soluble, cytosolic protein that transiently translocates to the plasma membrane in order to specifically phosphorylate agonist-occupied receptors. In addition, we have recently shown that a significant amount of ßARK is associated to microsomal membranes (García-Higuera et al (1994) J.Biol.Chem.269, 1348-1355). The existence of different ßARK pools (i.e., soluble, microsome-bound and plasma membrane-bound) has been investigated further in HEK-293 cells overexpressing ßARK either alone or in combination with ß2-adrenergic receptors (ß2AR),by using both immunofluorescence assays with specific ßARK antibodies and subcellular fractionation followed by Western blot and ßARK activity determination. These studies confirm the complex subcellular distribution of ßARK both in wild type and stably ßARK-transfected cells.To examine the role of the ß-adrenergic receptor in modulating the subcellular localization of ßARK, cells were cotransfected with plasmids encoding for ßARK and ß2AR. All stable clones expressing both ßARK and ß2AR display a different distribution of the kinase. In these cells, the ratio between the pools of ßARK protein changed from microsome-bound >plasma membrane-bound >soluble, in cells expressing ßARK alone, to plasma membrane-bound>microsome-bound>soluble in cells expressing both ßARK and ß2AR. This experimental system may prove useful in identifying the role of the ß2-adrenergic receptor presence and activation in regulating ßARK subcellular distribution. Supported by DGICYT PB92-0135, EC Biotech CT930083, Boehringer Ingelheim and Fundación Ramón Areces.

440.6

CATECHOLAMINE DENERVATION BY 6-OHDA INCREASES THE mRNA EXPRESSION OF ßARK AND ß-ARRESTIN IN DIFFERENT RAT BRAIN AREAS. A. De Blasi, M. Sallese, P. Liguori, R. Marinacci and E. Esposito*, Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Sud, S. Maria Imbaro (Chieti), Italy.

Two types of proteins play a major role in determining homologous desensitization of G-coupled receptors: ß-adrenergic receptor kinase (ßARK), which phosphorylates the agonist-occupied receptor and its functional cofactor, ß-arrestin. ßARK is a member of a multigene family, consisting of six known subtypes, which have also been named G-protein coupled receptor kinase (GRK1 to 6) due to the apparently unique functional association of such kinases with this receptor family. The four members of the arrestin/ß-arrestin gene family identified so far are: arrestin, X-arrestin, ß-arrestin1 and ß-arrestin2. Both ßARK and ß-arrestin are highly expressed in the central nervous system (CNS), suggesting a relevant role of these proteins in synaptic receptor-mediated responses. However, informations regarding the role played by ßARK/ß-arrestin in the modulation of CNS receptors are still lacking. To investigate this point we treated intracerebroventricularly male Sprague-Dawley rats with 6-hydroxydopamine (6-OHDA), a neurotoxin which destroys catecholaminergic neurons. Ten days after 6-OHDA (200 μg/20 μl of a saline solution containing 0.05% ascorbate) or vehicle administration, animals were killed and several brain areas were rapidly removed. In lesioned rats, the mRNA expression of ß-arrestin was significantly increased in corpus striatum, nucleus accumbens, hippocampus and brainstem. The mRNA expression of ßARK was increased in the hippocampus, while it was only slightly, if at all, affected in different other areas. These preliminary data suggest that ßARK and ß-arrestin are selectively modulated in response to reduced catecholaminergic tone.

440.7

NUCLEAR PROTEINS FROM YOUNG AND OLD RAT TISSUES INTERACT DIFFERENTLY WITH THE D2 DOPAMINE RECEPTOR GENE PROMOTER. J. M. Chernak,* G. S. Roth, T. Minowa and M. M. Mouradian. Molecular Physiology and Genetics Section, Laboratory of Cellular and Molecular Biology, NIA, NIH, Baltimore, MD 21224; Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Loss of D2 dopamine receptors and/or changes in their activity may lead to compromised neuronal functioning in aging and certain neurodegenerative diseases. To study the mechanisms of regulation of the D2 dopamine receptor gene in different tissues with aging, we carried out gel mobility shift experiments using nuclear extracts from young and old rats and DNA fragments or oligonucleotides containing different parts of the rat D2 promoter. A 415 bp DNA fragment containing sequences from -490 to -76 relative to the major transcriptional start point and a 378 bp fragment containing sequences from -75 to +302 bound to protein(s) present in nuclear extracts prepared from rat cortex, cerebellum, hippocampus, striatum, kidney and liver. In addition, the 378 bp probe bound to purified AP2 protein as well as protein(s) present in AP2-enriched extracts. We have also shown that an oligonucleotide containing sequences from -75 to -30 binds protein(s) present in extracts from rat cortex, hippocampus, striatum and liver. In several cases, nuclear factor(s) present in extracts from cortex, cerebellum, hippocampus, or striatum formed DNA-protein complexes with different gel mobilities than those formed with liver extracts, suggesting that the identity of some of the nuclear factors binding to the D2 promoter may differ between brain and peripheral tissues. Furthermore, with equal amounts of total nuclear protein, greater binding was observed using cerebellar and liver extracts from young rats than from old rats. These results suggest that the concentration and/or binding activity of nuclear factors interacting with the D2 dopamine promoter may change with age. Further characterization of putative DNA regulatory sites and regulatory proteins in different tissues of young and old animals is in progress.

440.8

PINEAL α–1B ADRENERGIC RECEPTOR mRNA: HIGH ABUNDANCE, DEVELOPMENT AND ADRENERGIC REGULATION. S.K. McCune*, S. L. Coon, D. E. Brenneman and D. C. Klein. Section on Developmental and Molecular Pharmacology and Section on Neuroendocrinology, Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Adrenergic receptors mediate tissue responses to catecholamines. Binding studies have documented the presence of α-1 adrenergic receptors in the pineal gland and pharmacological studies have demonstrated a role of these receptors in the adrenergic regulation of many aspects of pineal function, including melatonin production. In an effort to better define α-adrenergic receptors in the pineal gland, α-1B adrenergic receptor expression was examined using *in situ* hybridization and Northern blot analysis.

Developmental expression of the α-1B adrenergic receptor subtype was determined by Northern blot and densitometric analysis of *in situ* hybridization of brain sections through the pineal as well as sections of individual pineal glands. Alpha-1B receptor mRNA was highest in the early perinatal time periods and decreased with maturation. The abundance of mRNA encoding this receptor subtype in the pineal gland was extremely high as compared to brain and other tissues; a strong hybridization signal was detected on Northern blot with 2 μg of total RNA.

Northern blots of pineal mRNA from day and night animals showed a 2.5-3 fold increase in the α-1B adrenergic receptor mRNA at night without a similar increase in the mRNA encoding G3-PDH. This change is probably due to nocturnal adrenergic stimulation of the pineal gland, because *in vitro* stimulation of the pineal gland for 4 hours with norepinephrine (1 μM) induced a selective 2-fold increase in the abundance of α-1B adrenergic receptor mRNA.

The presence of high levels of α-1B adrenergic receptor mRNA in the pineal gland and evidence of neural regulation of expression make this tissue an excellent model for further analysis of this receptor subtype.

440.9

DOWNREGULATION OF THE HISTAMINE H_2 RECEPTOR EXPRESSED IN CHINESE HAMSTER OVARY CELLS M.J. Smit*, R. Leurs, Y. van de Vrede and H. Timmerman Leiden/Amsterdam Center for Drug Research, Department of Pharmacochemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

Under several pathophysiological conditions, where histamine is released in large quantities, or during treatment with histaminergic drugs in various disorders regulatory processes such as desensitization and downregulation may become apparent.

Long-term exposure (24 hrs) of CHO cells, overexpressing the rat histamine H_2 receptor ($CHORH_2$), to histamine resulted in a dose dependent decrease in the number of H_2 receptors (max. reduction 50 %). The receptor affinity remained unaltered. This process appeared to be a H_2 receptor mediated process as dimaprit induced downregulation and its structural analogues, homo- and nordimaprit, which are devoid of H_2 activity, did not. All compounds generating cAMP upon stimulation, such as H_2 agonists, forskolin and cholera toxin, induced histamine H_2 receptor downregulation, suggesting the possible involvement of protein kinase A in the process of receptor downregulation. In contrast, treatment of $CHORH_2$ cells with various H_2 antagonists resulted in an upregulation of H_2 receptors (200 %). This effect could probably explain therapeutic observations of reduced efficacy after some period of time.

Currently, we examine the possible role of different kinases in this process and determine which structural elements of the receptor are essential for H_2 receptor downregulation.

440.10

GSH RECEPTOR REGULATION IN RAT CORTICAL ASTROCYTES. C.A. Shaw*, H. Bining, R.A. Lanius, and B.A. Pasqualotto. Depts. of Ophthalmology and Physiology and Neuroscience Program, c/o Dept. of Anatomy, Univ. British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

Recent studies have suggested that glutathione (GSH) may act as a neurotransmitter at specific receptors in the mammalian CNS. One of the highest concentrations of GSH receptors (GSHR) are on cortical astrocytes (Guo & Shaw, 1992, *Mol. Brain Res.*, 15, 207) and activation by GSH stimulates an increase in IP3 activity. In other studies, the regulation of GSHR in spinal cord appears to depend on protein kinase C (PKC) (Lanius et al., 1994, *J. Neurochem.*, in press). We have now examined the actions of GSH, a PKC activator (phorbol ester (PB)), and cellular depolarization (veratridine) in the presence or absence of kinase or phosphatase blockers on GSHR in cultured rat cortical astrocytes. Exposure of astrocytes to GSH (10-5M) produced a significant decrease in [35S]-GSH binding (-17.9%+-2.4%, $n=21$, $p<0.05$) compared to untreated cells. This effect was time and concentration dependent and could be blocked by co-incubation with the ser/thr phosphatase inhibitor N-B-D-glycerophosphate but not the tyr-selective inhibitor sodium orthovanadate. The effects of PKC activation by PB produced a significant increase in [35S]-GSH of 21.4%+-3.7% ($n=17$, $p<0.05$) which could be blocked by co-incubation with PKC inhibitors. Veratridine was without significant effect. These data suggest that GSHR may be regulated by opposing actions of kinase/phosphatase activity in a manner similar to that reported for several neurotransmitter receptors in brain.

440.11

STEROID RECEPTOR MEDIATED EFFECTS OF NEUROSTEROIDS. R. Rupprecht, J. M. H. M. Reul, T. Trapp, B. van Steensel, C. Wetzel, W. Zieglgänsberger and F. Holsboer*. Max-Planck-Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Kraepelinstr. 2-10, 80804 Munich, FRG.

Several 3α-hydroxy steroids accumulate in the brain after local synthesis or after metabolization of steroids that are provided from the adrenals. As this may occur independently from peripheral sources these steroids are called neurosteroids. The 3α-hydroxy ring A-reduced pregnane steroids allopregnanolone and tetrahydrodeoxycorticosterone are believed not to interact with intracellular receptors but enhance γ-aminobutyric acid (GABA)-mediated chloride currents.

The present study shows that these neurosteroids can regulate gene expression via the progesterone receptor (PR). Although they do not bind to the PR of either species, they confer an exclusively nuclear localization of the PR. However, the induction of DNA-binding and transcriptional activation of the PR requires intracellular oxidation of the neurosteroids into PR-active 5α-pregnane steroids. Thus, in physiological concentrations, these neurosteroids regulate neuronal function through their concurrent influence on transmitter-gated ion channels and gene expression. These findings extend the concept of a "cross-talk" between membrane and nuclear hormone effects and provide a new lead for the therapeutic application of these steroids in neurology and psychiatry.

441.1

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CULTURED, COLCHICINE-TREATED CRF-CONTAINING NEURONS OF THE RAT AMYGDALA. R. W. Gabr*, A. K. Salm[1], and D. L. Birkle. Departments of Pharmacology and Toxicology and [1]Anatomy, West Virginia University School of Medicine, Morgantown, WV, 26506.

CRF is a 41 amino acid peptide first isolated and characterized in 1981 (Vale et al). CRF is part of a dynamic system which regulates central and peripheral components of the stress response (Owens and Nemeroff, 1991). Depolarization-induced, calcium-dependent release of CRF from fetal neuronal cultures of the amygdala has been previously demonstrated (Cratty and Birkle, 1994). Fetal cell culture (E17-E18) of the rat amygdala on glass coverslips (2 x 10^6 cells/slip) yielded a 5:1 mixture of neurons and glia after 15-18 DIC. Immunostaining with anti-neurofilament marker, anti-glial acidic protein and DAPI allowed quantification of total cell types in the culture system. The proportion of CRF-containing neurons was determined using indirect immunofluorescence with rabbit anti-rat/human CRF (rC70) followed by anti-rabbit IgG conjugated to FITC. Total cells per 330 μm^2 field were 32 $\pm$ 4 (n=122), with 6 $\pm$ 1 (n=53) cells staining positive for GFAP. NFM immunostaining revealed long bundles of fibers coursing from neuron cluster to cluster, as well as the distinct bead-like processes of peptidergic neurons in culture. 0.65 $\pm$ 0.25 cells (n=40) stained positive for CRF (2%). 24 hr incubation with colchicine (0.01mM) dramatically increased staining intensity, resulting in positive anti-CRF staining in 19% of cultured cells. Preliminary studies further indicate that a majority of fetal neuronal cells cultured from the amygdala contain glutamate. These results indicate that a high proportion of amygdala neurons (24%) may contain CRF, as revealed by colchicine treatment. Furthermore, immunohistochemical characterization of these cells in culture demonstrates compartmentalization of neurons into clusters connected by networks of fiber bundles, providing evidence that the heterogeneity of the amygdala is maintained in the in vitro culture system. Supported in part by NSF (IBN-9222263)

441.2

SUPPRESSION OF CORTICOTROPIN-RELEASING FACTOR IN THE AMYGDALA ATTENUATES AVERSIVE CONSEQUENCES OF MORPHINE WITHDRAWAL. F. Menzaghi*, G. Schulteis, G.F. Koob, L. Stinus[1] and S.C. Heinrichs[2]. The Scripps Research Institute, Dept. of Neuropharmacology, CVN7, 10666 N. Torrey Pines Rd., La Jolla CA, 92037, [1]Psychobiologie des Comportements Adaptatifs, INSERM Unité 259, Bordeaux, France and [2]Neurocrine Biosciences, Inc., 3050 Science Park Rd., San Diego CA, 92121.

The central nucleus of the amygdala is a CRF-containing limbic brain site which mediates both fear-like and avoidance behaviors, and intra-amygdala administration of a CRF antagonist blocks the increase in anxiogenic-like behavior characteristic of ethanol withdrawal. In order to evaluate the role of brain CRF in negative motivational states associated with other classes of abused drugs, the present studies examined the effects of suppression of amygdala CRF systems on the characteristic aversive state of precipitated withdrawal in morphine-dependent subjects. In a place conditioning paradigm, administration of a CRF antagonist, α-helical CRF (9-41) [250 ng], bilaterally into the central nucleus of amygdala reversed the withdrawal-induced conditioned place aversion produced by injection of the opiate antagonist, methylnaloxonium [500 ng], into the same site. Impairment of CRF neurons by immunotargeted toxins administered into the central nucleus of amygdala one month prior to testing attenuated conditioned morphine withdrawal as measured by a decrease in response rate produced by exposure to distinctive sensory cues associated previously with systemic administration of naloxone [25 μg/kg SC] in morphine-dependent subjects. These results indicate that suppression of intra-amygdala CRF systems weakens the aversive stimulus properties of conditioned opiate withdrawal and suggest a general role for CRF in coordinating behavioral responses to negative motivational effects of drug withdrawal.

This research was supported in part by grants DK 26741 and DA 04043 to GFK.

441.3

LEARNING DEFICITS IN TRANSGENIC MICE WITH CENTRAL OVEREXPRESSION OF CORTICOTROPIN-RELEASING FACTOR. S.C. Heinrichs[1*], M.P. Stenzel-Poore[2], L.H. Gold, E. Battenberg, F.E. Bloom, G.F. Koob, W.W. Vale[3] and E. Merlo Pich. The Scripps Research Institute, Dept. of Neuropsychopharmacology, 10666 N. Torrey Pines Rd., La Jolla CA 92037, [1]Neurocrine Biosciences, Inc., 3050 Science Park Rd., San Diego CA 92121, [2]Oregon Health Sciences University, Dept. of Microbiology and Immunology, P.O. Box L220, 3181 Sam Jackson Park Rd., Portland OR 97201 and [3]The Salk Institute, Clayton Foundation Laboratories for Peptide Biology, 10100 North Torrey Pines Rd., La Jolla CA, 92037 .

Transgenic (Tg) mice with central overexpression of corticotropin-releasing factor (CRF) exhibit a Cushing's Disease-like picture, elevated plasma levels of ACTH and corticosterone, increased behavioral reactivity to stressors and an anxiogenic-like state which is reversed by central administration of a CRF antagonist. Moreover, centrally administered CRF alters learning/memory processes in animal behavioral models and CRF substrates are altered in post-mortem tissue taken from Alzheimer's patients exhibiting learning/memory pathology. The present studies were designed to test the cognitive capacity of CRF Tg mice in a forced alternation water T-maze task and in the Morris water maze. In T-maze testing, littermate control mice reached criterion after five days of trials while the performance of CRF Tg subjects was random after the same training. In Morris maze testing, control subjects reached the submerged platform significantly faster after three days of trials while the performance of CRF Tg mice was unimproved over the same period. The deficit in Morris maze performance in CRF Tg mice was reversed when the platform was visible above the surface of the water and by pre-test administration of chlordiazepoxide (10 mg/kg IP) prior to acquisition training. Finally, the was no evidence of hippocampal cell loss in CRF Tg animals. Taken together, these results suggest that disregulation of brain CRF may produce hyperemotionality that interferes with cognitive performance.

This research was supported in part by grants DK 26741 to WWV and GFK and by the Kleberg Foundation.

441.4

CENTRAL AMYGDALOID CORTICOTROPIN-RELEASING HORMONE MODULATION OF THE STRESS RESPONSE IN ROMAN HIGH- AND ROMAN LOW AVOIDANCE RATS.

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The central nucleus of the amygdala (CeA) is known to be involved in the regulation of the parasympathetic and passive coping response to conditioned and acute stressors. Neuroanatomical studies revealed that the majority of the corticotropin-releasing hormone (CRH) containing neurons in the CeA have direct connections with autonomic regulatory nuclei in the brainstem.

In this study we used the Roman High Avoidance (RHA/verh) and the Roman Low Avoidance (RLA/verh) rats (kindly provided by P.Driscoll, Zürich) to examine the effects of locally applied CRH into the CeA in both a stress-free and a conditioned stress situation. The RHA/verh and RLA/verh rats are considered to use an active and a passive coping style respectively.

A 7-min infusion of 30 ng CRH (in 0.5μl aCSF) into the CeA of freely moving male RHA/RLA rats under stress-free conditions, led to an increase in heart rate and behavioural activation only in the RHA treated animals, leaving the RLA rats unaffected. This is in contrast with the conditioned situation in which only the RLA/verh males responded. These results suggest a differential CRH central amygdaloid control of the behavioural and physiological stress response in the two selected strains of rats.

Subsequently experiments using CRH mRNA and Fos immunocytochemistry are in progress to obtain futher experimental evidence for a differential central amygdala CRH modulation in the RHA/RLA rats.

441.5

PRENATAL STRESS CAUSES HYPERSECRETION OF CORTICOTROPIN-RELEASING FACTOR (CRF) FROM AMYGDALA. MS Cratty*, HE Ward, EA Johnson, AJ Azzaro, DL Birkle. Depts. of Pharmacology/Toxicology, Behavioral Medicine/Psychiatry, and Neurology, West Virginia University School of Medicine, Morgantown, WV 26506.

The neuropeptide, corticotropin-releasing factor (CRF), has been found distributed throughout the central nervous system. CRF has a proposed role in emotional and behavioral states including stress and anxiety. The amygdala, a limbic structure important in the delicate control of emotions and autonomic responses to stress, contains CRF nerve terminals and CRF receptors. We examined CRF release from the amygdala of adult, male offspring of dams exposed to daily saline injection (0.1 ml, s.c.) from G14 to G21. This prenatal stress model produces offspring that are hyper-responsive to stress (Ward et al, this volume). In light of the role of amygdala CRF release in anxiogenic responses, we hypothesized that exposure to prenatal stress would increase CRF release from the amygdala. CRF release from amygdala slices (1 mm^3) was time-dependent, depolarization-induced and calcium-dependent. There was a 32% increase in depolarization-induced CRF release from the amygdala of prenatally stressed rats. Prenatally stressed offspring also had elevated CRF concentrations in the amygdala. The data suggest that CRFergic neurotransmission in amygdala is upregulated after prenatal stress, an effect that may contribute to the hyperemotional state observed in these animals. Supported in part by NIH (2S07RR05433-31 and GM07039) and UHA.

441.6

CORTICOTROPIN-RELEASING FACTOR INFUSED INTO THE LOCUS COERULEUS INCREASES NOREPINEPHRINE RELEASE IN MEDIAL PREFRONTAL CORTEX. Gennady N. Smagin, Artur H. Swiergiel, Glenn Guerin* and Adrian J. Dunn. Dept Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Previous studies have indicated that intracerebroventricular administration of corticotropin-releasing factor (CRF) increases the firing rate of noradrenergic neurons in the brain stem locus coeruleus (LC) and metabolites of norepinephrine (NE) metabolism in terminal regions in the forebrain, as well as extracellular concentrations of NE. To assess the possibility of direct effects of CRF in the LC, 100 ng of CRF was infused into the LC and concentrations of NE and metabolites were measured in microdialysates collected from ipsilateral or contralateral medial prefrontal cortex (PFM) with a 22 min sampling interval. Infusion of CRF significantly increased concentrations of NE in dialysates from the ipsilateral PFM in the first three postinfusion samples (26, 82 and 54% of baseline, respectively). No changes were observed with infusions of aCSF, or when CRF infusions occurred outside the LC - in the parabrachial nucleus, the tegmental area, the fourth ventricle or the cerebellum. Although dialysate concentrations of NE were slightly increased in the contralateral PFM, this effect was not statistically significant. The effect of CRF on NE release was prevented by a simultaneous infusion into the LC of 1 μg of the CRF antagonist, alpha-helical CRF_{9-41}. These results are consistent with anatomical and electrophysiological evidence suggesting that CRF may directly activate noradrenergic neurons in or close to the LC, affecting cortical NE secretion and behavior.

Supported by grants from NINDS and the Air Force AFOSR.

441.7

SENSITIZATION OF THE EXCITATORY EFFECTS OF VASOPRESSIN OR CORTICOTROPIN RELEASING FACTOR ON THE ACOUSTIC STARTLE REFLEX BY VASOPRESSIN. G.H. Pelton, Y. Lee, M. Bowers*, M. Davis. Dept of Psychiatry, Yale Univ. School of Med. 34 Park St., New Haven, CT 06508

In the rat, evidence now suggests a neurotransmitter function for arginine vasopressin (AVP), implicating it in various autonomic, behavioral, and neuroendocrine responses. One characteristic of AVP's central effect is a sensitization phenomenon, in which repeated intracerebroventricular (ICV) injections of AVP lead to an increase of its initial effect on behavior. This has begun to be characterized by other investigators via a profoundly abnormal motor response called "barrel rolling", which occurs with repeated ICV AVP administration.

We used a simpler, neuroanatomically-defined behavior, the acoustic startle reflex in the rat, to evaluate whether repeated ICV AVP would cause sensitization. Different groups of rats were infused with various doses of AVP (30, 300, 3000 pg) or vehicle on Day 1. On Day 2, 48 hours later, all animals were infused with a single test dose of AVP (300 pg) or vehicle. The 300 pg dose of AVP was subthreshold for increasing baseline startle on Day 1. The results showed that 30 pg of AVP on Day 1 did not sensitize the efffect of AVP on Day 2, but 3000 pg did ($p<0.05$).

Because stress is known to activate release of both AVP and corticotropin releasing factor (CRF) and ICV CRF is known to increase baseline startle, we wondered if AVP would also sensitize the excitatory effect of CRF on the acoustic startle reflex. To test this, various doses of AVP (3, 30, 300, 3000 pg) were infused ICV on Day 1. On Day 2, 48 hours later, all rats were infused with a dose of CRF (0.25 µg) that usually has no effect on baseline startle. The results showed a non-monotonic excitatory effect of CRF on startle, with the combination of 30 pg AVP/0.25 ug CRF showing a significant increase ($p<0.05$) over vehicle/vehicle or vehicle/0.25 µg CRF. The time course of the effect was similar to that usually seen when 1 µg of CRF is given ICV.

Taken together, these results show that a single infusion of AVP sensitized the excitatory effect of either AVP or CRF on the acoustic startle reflex, when given 48 hours later. This may provide a model system for analyzing how prior stress leads to enhanced reactions to subsequent stressors and dysregulation of the stress response.

441.8

THE DISTRIBUTION OF OXYTOCIN RECEPTOR BINDING IN THE BRAIN OF COMMON MARMOSETS. K.K.Moody* J.D Newman and T.R.Insel. Lab of Comparative Ethology, NICHD and Lab of Clinical Science, NIMH, NIH, Poolesville, MD 20837.

The neurohypophyseal peptide oxytocin has been implicated in the mediation of several forms of affiliative behavior. Marked species and gender differences have been found in monogamous and polygamous rodents, suggesting that oxytocin may play a role in species-typical patterns of social behavior. Using *in vitro* receptor autoradiography with a selective oxytocin receptor ligand [^{125}I]d(CH$_2$)5[Tyr(Me)2 Tyr-NH^9]OVT ([^{125}I]OTA) we examined the brains of a monogamous nonhuman primate, the common marmoset. [^{125}I]OTA binding sites were apparent in several brain areas including the diagonal band, lateral septum, nucleus accumbens and brain stem. In addition, neonates showed a marked pattern of [^{125}I]OTA binding in cortical regions which was not apparent in adults. The distribution of [^{125}I]OTA binding is consistent with those found in several highly affiliative species of rodents and represents the first evidence of oxytocin receptor binding in a monogamous nonhuman primate.

441.9

PROXIMAL SEPARATION FROM PUPS REESTABLISHES OXYTOCIN CONTROL OF MATERNAL BEHAVIOR IN EXPERIENCED RAT MOTHERS. C.A. Pedersen*, J.M. Johns, B.M. Faggin, G. Ayers and J.D. Caldwell. BDRC, Dept. of Psychiatry and The Neurobiology Curriculum, School of Medicine, the University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27599.

We previously reported that subjecting primiparous rat dams to proximal separation (PS), a condition in which pups were isolated in a small metal mesh cage within each dam's cage so that the dam could smell, hear and see pups but could not touch them, for 6 days beginning on Day 5 of lactation markedly depleted immunostaining of oxytocin (OT) neural cell bodies in many forebrain sites compared to rat dams subjected to total separation (TS) or no separation (NS) from pups. In recent studies, we found that all rats subjected to 4-6 days of PS (10/10) or NS (10/10), but only 4/11 rats subjected to TS, retrieved in the first 3 hrs after being given free access to pups (Fisher's exact probability, $p<.01$). Infusion of OTA, an OT antagonist, but not normal saline, into the VTA (a site that receives projections from OT neurons located in the forebrain) significantly inhibited the rapid reemergence of retrieval and crouching behavior in dams subjected to PS for 4-6 days. In contrast, infusion of OTA into the VTA had no inhibitory effect on the reemergence of maternal behavior (MB) in rats that were separated from pups for only 1 hr. Additional experiments have established that 4 or more days of PS are necessary to reinstate OT control of MB. Our behavioral pharmacological findings indicate that the depletion of OT immunostaining by PS probably results from an increased rate of OT release. These observations also suggest that, shortly after parturition and the OT dependent onset of MB, either OT control of MB is inhibited and a nonOT control mechanism is activated by some aspect(s) of physical contact with pups (suckling?), or a nonOT mechanism that depends upon physical contact with pups emerges and maintains MB in parallel with the OT mechanism. Supported by HD25255 and MH33127.

441.10

COLOCALIZATION OF OXYTOCIN (OXT) AND C-FOS IMMUNOREACTIVITY IN FEMALE HAMSTER BRAIN. D.C. Whitman* and H.E. Albers. Lab. of Neuroendocrinol. & Behav., Dept. of Biol., Georgia State Univ., Atlanta, Ga. 30303.

Central administration of OXT facilitates the expression of lordosis in rodents. In addition, central administration of an OXT antagonist reduces or eliminates gonadal hormone-stimulated lordosis. Since OXT appears to mimic the effects of progesterone (P) on lordosis, the present study tested the hypothesis that OXT influences lordosis by mediating the effects of P on lordosis. Double immunocytochemical techniques were used to examine whether OXT neurons also exhibit c-fos immunoreactivity (a marker of cellular activity) in the brains of OVX hamsters treated with estradiol benzoate (EB) (unmated), EB+P (unmated) and EB+P (mated). Preliminary analysis indicates that OXT- and c-fos-immunoreactivity were colocalized in the paraventricular and supraoptic nuclei of mated hamsters. C-fos immunoreactivity was not observed in either of the unmated groups. This study was supported by NSF IBN-9222099.

441.11

POLYMORPHISM AND GENETIC MAPPING OF THE HUMAN OXYTOCIN RECEPTOR GENE ON CHROMOSOME 3. S. Michelini*, M. Urbanek, M. Dean and D. Goldman. Laboratory of Neurogenetics, National Institute of Health-National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland.

Centrally administered oxytocin has been reported to facilitate affiliative and social behaviours, in functional harmony with its well known peripheral effects on uterine contraction and milk ejection. The biological effects of oxytocin could be perturbed by mutations occurring in the sequence of the oxytocin receptor gene, and it would be of interest to establish the position of this gene on the human linkage map. Therefore we identified a polymorphism at the human oxytocin receptor gene. A portion of the 3' untranslated region containing a 30 bp CA repeat was amplified by polymerase chain reaction (PCR) and evaluated by electrophoresis, revealing a polymorphism with two alleles occurring with frequencies of 0.77 and 0.23 in a sample of Caucasian CEPH parents (n=70). The CA repeat polymorphism we detected was used to map the human oxytocin receptor to chromosome 3p25-3p26, in a region which contains several important genes, including loci for Von Hippel-Lindau disease (VHL) and renal cell carcinoma.

411.12

AN ANXIOLYTIC ACTION OF OXYTOCIN IS ENHANCED BY ESTROGEN IN THE MOUSE. M.M. McCarthy* & D. Goldman, Department of Physiology, University of Maryland, Baltimore, MD and Laboratory of Neurogenetics, NIAAA, Rockville, MD.

The neuropeptide oxytocin exerts broad effects on social and affiliative behaviors and has been speculated to exert an anxiolytic action but this hypothesis has not been rigorously tested. In Experiment 1, NIH-Swiss ovariectomized mice were pretreated with 10µg estradiol (E2) in oil or oil alone 48 & 24 hr prior to i.p. injection of either 3mg/kg oxytocin (Sigma) or saline. Forty min later animals were tested for 5 min each on a hole-board apparatus followed by an elevated plus maze using a computerized activity monitor. Data were analysed by 2-way ANOVA and post-hoc Tukey's. A combination of E2+oxytocin significantly increased percent and total time spent on the open arms and the number of entries onto the open arms. The amount of time spent on closed arms was decreased by oxytocin in both E2 and oil-treated females. There was no effect on motor activity or head dips in the hole board apparatus in this experiment. I.P. injection of oxytocin in NIH swiss males did not significantly alter behavior but baseline levels of activity were comparable to E2-treated females. In Experiment 2, NIH-swiss ovx'd females bearing in-dwelling cannula aimed at the lateral ventricle were pretreated with E2 or oil as before and infused ICV with either oxytocin or vasopressin (8µg in 4µl saline) and behaviorally tested 20 min post-inj. The percent and total time spent on open arms was significantly increased by oxytocin regardless of steroid treatment and the percent and number of entries onto the open arms was increased by the combination of E2+oxytocin over all other groups. The combination of E2+vasopressin significantly decreased the percent time spent on the open arms compared to other groups. This combination in general exerted opposite effects to that of E2+oxytocin. There was an increase in time spent head-dipping in the hole board apparatus in E2-treated females but this was not affected by oxytocin or vasopressin. These findings indicate that oxytocin can act as an anxiolytic and that E2 enhances that effect. Furthermore, E2 may be exerting opposite effects on vasopressin, causing it to act as an anxiogenic. Brains are prepared for receptor autoradiography and results will be reported.

442.1

ASTROCYTES IN THE DEVELOPING MACAQUE MONKEY RETINA C. Distler* and M.A. Kirby, Zoology & Neurobiology, Ruhr-University Bochum, 44780 Bochum, FRG and Dept. of Pediatrics & Div. Perinatal Biology, Loma Linda University, Loma Linda CA 92350

In mammals, two types of macroglia are known to exist in the retina, Müller cells and astrocytes. While Müller cells are ubiquitous throughout the retina, astrocytes are only found in vascularized retinae or vascularized retinal regions. It has recently been shown that there is an exception to this rule: in the parafoveal region of the adult macaque monkey retina no astrocytes are found even though this retinal region is heavily vascularized. To test if the parafoveal region is astrocyte-free at all stages of development or if it is secondarily deserted by astrocytes, we investigated the spatial distribution of astrocytes in fetal macaque monkey retinae using GFAP immunocytochemistry. Fetuses were obtained by Caesarian section under aseptic conditions, deeply anesthetized with pentobarbital and perfused through the heart with 3-4% paraformaldehyde. The retinae were dissected free and processed for GFAP immunocytochemistry as wholemounts.

At embryonic day 120 (term gestation is 165 days), i.e. shortly after formation of the foveal pit, a distributional pattern of astrocytes similar to that found in adults is evident. Astrocytes are found at all retinal eccentricities with the exception of the parafoveal region. Fetal astrocytes are small with delicate processes, but already adultlike in their association with axon bundles and blood vessels and their segregation into two subpopulations, one to be found in the ganglion cell layer, the other in the nerve fiber layer. These data together with preliminary observations in even younger fetuses (E90) suggest that the parafoveal region of the monkey retina is avoided by astrocytes prior to the time of foveal pit formation.

Supported by grant Di 391/2-1 of the Deutsche Forschungsgemeinschaft to C. Distler.

442.2

CHANGES IN RPTP-β EXPRESSION DURING GLIAL CELL DIFFERENTIATION. P.D. Canoll, S. Petanceska, G. Barnea, J. Schlessinger J.M. Musacchio* Dept. of Pharmocology, N.Y.U Medical Center, New York, NY 10016.

RPTP-β is a receptor type tyrosine phosphatase that is expressed in glia and glial progenitors (Canoll et al., Dev Brain Res 75:293, 1993). Nucleotide probes to RPTP-β hybridize to three major transcripts on northern blot analysis. Two of the transcripts (9.5 kb and 6.4 kb) encode transmembrane glycoproteins which contain a carbonic anhydrase related domain in the extracellular portion and tandem phosphatase domains in the intracellular portion. The third transcript (8.4 kb) encodes a secreted chondroitin sulfate proteoglycan which contains the extracellular portion of RPTP-β, but does not contain the intracellular phosphatase domains (Maurel et al. PNAS 91:2512, 1994). In situ hybridization and northern analysis show that the relative abundance of the 3 transcripts changes during brain development. During postnatal development, the 9.5 and 6.4 kb transcripts (transmembrane forms) are predominantly expressed in glial progenitors located in the proliferative subventricular zone, whereas high levels of the 8.4 transcript (secreted form) are seen in differentiated glia that have migrated out of the subventricular zone. A similar transition in expression can be induced by treating cultured glial cells with the differentiating agent dibutyryl cAMP. The different forms of RPTP-β have very different structure, and most likely perform different functions. The shift in the relative abundance seen during glial cell differentiation may relate to important changes in the role glial cells play during brain development and regeneration.

442.3

SCHWANN CELL EXPRESSION OF CD9 IS REGULATED BY NERVE CRUSH AND SUBSEQUENT REGENERATION. S. A. Banerjee* and P. H. Patterson. Division of Biology, Caltech, Pasadena, CA 91125.

CD9 is a 26 kD cell surface glycoprotein with four transmembrane domains that has been implicated in signaling in platelet activation, and is present in both the peripheral and central nervous systems (PNS and CNS). In the PNS, CD9 is expressed in several neuronal populations and in Schwann cells of the sciatic nerve. CD9 expression follows a developmental time-course in the nerve that closely parallels that of the myelin genes P_0 and myelin basic protein. Here we report the response of CD9 following injury to the nerve.

CD9 and P_0 mRNA levels were analyzed following nerve crush in 30-day old rats. CD9 mRNA drops approximately 10-fold in the nerve distal to the crush, similar to the change in P_0 mRNA. By two weeks following injury, when axons are known to be rapidly regenerating, CD9 and P_0 mRNAs recover to levels slightly higher than those found in the normal adult. By 30 days, levels reach those found in the normal adult. A smaller, but significant response is also observed in the part of the nerve proximal to the crush.

These results suggest that Schwann cell expression of CD9, like P_0 expression, requires the presence of axons. This would also be consistent with our observation that when primary Schwann cells are placed in culture, CD9 immunoreactivity is lost. We are currently investigating the role of axons on CD9 expression in cultured Schwann cells.

442.4

Human Fetal Spinal Cord Slice Cultures: A Model for the Study of Neurodevelopment and Neuropathology.

W.E. Grever*, K.M. Weidenheim and W.D. Lyman. Department of Pathology, *Albert Einstein College of Medicine, Bronx, New York, 10461.*

Human fetal spinal cords are obtained from abortuses that range in age from 20 to 24 weeks of gestation. Spinal cords are stripped of meningies and cut into 800μm transverse sections. The slices are cultured on collagen-coated tissue culture inserts in chemically-defined media. Slice cultures have been grown for up to 28 days under these conditions. Histological and immunocytochemical examination of the cultures reveal the presence of neurons, oligodendrocytes, microglia, astrocytes, ependyma and endothelial cells. Indirect immunofluorescent confocal microscopy shows intact myelin basic protein-positive sheaths around neurofilament-positive axons. These myelinated axons can be observed by summation of optical sections and span over 60μm. Additional evidence of active myelination during the culture period is observed by light and electron microscopy. Some axons contain mitochondria with distinct cristae and are surrounded by large inner loops of a myelinating oligodendrocyte process. Inner loops are found inside layers of compacted myelin. After one week in culture, the slices are surrounded by an outgrowth of cells. The outgrowth area contains microglia, astrocytes, and oligodendrocytes as determined by morphology and immunocytochemical labeling. This culture system provides a unique model to study the development of the human central nervous system and neuropathologic changes that result from exposure to inflammatory cytokines, infectious agents such as HIV, drugs of abuse, or physical damage.

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442.5

REGULATION OF TRANSFORMING GROWTH FACTOR ALPHA (TGFα) GENE EXPRESSION IN HYPOTHALAMIC ASTROCYTES BY METABOTROPIC GLUTAMATE RECEPTORS. Y.J. Ma*, F. Rage and S.R. Ojeda. Division of Neuroscience, Oregon Regional Primate Res. Ctr., Beaverton, OR 97006.

Excitatory amino acid (EAA) neurotransmitters influence the initiation of mammalian puberty by stimulating the secretory activity of neurons producing luteinizing hormone releasing hormone (LHRH). Evidence also exists that TGFα, a trophic factor of glial origin, contributes to this activation via a paracrine mechanism involving glial production of eicosanoids. The functional relationships that may exist between these two regulatory systems are not known. Since glial cells are endowed with both ionotropic and metabotropic glutamate receptors, we have initiated experiments to determine if glial expression of the TGFα gene is subjected to EAA-mediated regulatory inputs. Purified astrocytes from neonatal rat hypothalamus were exposed to glutamate receptor agonists including glutamate itself, N-methyl-D-aspartate (NMDA) and the metabotropic receptor agonists quisqualate and ACPD (aminocyclopentane-1S.3R-dicarboxylic acid) (100 μM each). Changes in TGFα mRNA levels were measured by quantitative reverse transcription-polymerase chain reaction at different intervals after initiation of the treatment (15 min-24h). The results showed that both glutamate and metabotropic receptor agonists, but not NMDA, rapidly upregulated TGFα gene expression (within 15 min exposure), with maximal mRNA values being reached between 8-16h. These initial findings suggest that hypothalamic astrocytes are targets for EAA acting via metabotropic receptors, and that EAA may represent one of the neuronal signals involved in the control of glial expression of the TGFα gene during development. Supported by NIH Grants HD25123, HD18185 and RR00163).

442.6

IMMUNOGENICITY OF SCHWANN CELL POPULATED ACELLULAR BASAL LAMINA NERVE GRAFTS. D.R. Rai, D. W. Sickles * and A.K. Gulati . Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

Acellular basal lamina (BL) allografts are known to exhibit reduced immunogenicity. It is also known, that the extent of host axonal regeneration through acellular BL grafts is reduced in comparison to cellular grafts. In the present study, isogeneic (genetically identical) and allogeneic (genetically different) Schwann cell populated acellular BL grafts were transplanted into host rats to define their immunogenicity. Inbred strains of Fischer (FR) and Buffalo (BF) rats were used. Acellular BL grafts were prepared, by freeze-thawing (5x), 6-week predegenerated FR rat nerve. Acellular BL nerves were placed in dishes with cultured Schwann cells established from FR or BF rat nerves. After 7 days, 2 cm long Schwann cell cocultured nerves were transplanted in a surgically created gap in the FR host peroneal nerve. Transplanted nerves were analyzed at 1, 2, 4 and 8 weeks later to determine their immunological fate and axonal regeneration through them with light microscopy Acellular BL grafts populated with isogeneic FR Schwann cells survived and were supportive of host axonal regeneration through them. On the other hand, acellular BL grafts with allogeneic BF Schwann cells underwent rejection and were unsuccessful. The results indicate that cultured Schwann cells continue to exhibit immunogenicity. Thus, it is important to employ host matched (isogeneic) Schwann cells to populate acellular BL grafts in attempts to enhance their axonal growth supporting function. (Supported by NIH Grant NS-24834).

442.7

DEVELOPMENTAL AND GROWTH FACTOR-INDUCED REGULATION OF THE NEURAL STEM CELL MARKER NESTIN IN OLIGODENDROCYTE LINEAGE CELLS. V. Gallo and R. C. Armstrong* Lab. of Cell. Mol. Neurophysiol., NICHD, NIH, Bethesda, MD 20892 and Dept. Anat. Cell Biol., USUHS, Bethesda, MD 20814.

The expression and regulation of the neural stem cell marker nestin was studied in cells of the oligodendrocyte lineage. Glial cells from late embryonic rat cerebral cortex, tissue prints from neonatal brains and the oligodendrocyte cell line CG4 were used. In both culture systems, nestin mRNA was highly expressed in proliferating O-2A progenitors, but down-regulated in differentiated oligodendrocytes. Immunocytochemistry of cultured cells and tissue prints, demonstrated that O-2A progenitors express high levels of the nestin protein. In addition, rapidly dividing pre-O-2A cells, which were not labeled with anti-GD3 antibody, expressed nestin. In contrast, the majority of $O4^+$-pro-oligodendroblasts and $O1^+$-oligodendrocytes were not labeled with anti-nestin antibodies. In cortical astrocyte cultures, type-1 astrocytes, but not type-2, expressed nestin mRNA and protein. Culture conditions which prevented O-2A progenitors from differentiating (PDGF+bFGF or B104-conditioned medium), upregulated nestin at the mRNA and protein levels. These results demonstrate PDGF, bFGF and B104-secreted mitogens up-regulate expression of nestin in O-2A progenitors, which is indicative of a proliferative state.

442.8

I_{HA} IN CULTURED RAT ASTROCYTES. D. Janigro[‡‡], D.L. Tinklepaugh[‡], L.G. Costa[‡] and H.R. Winn[†*]. Depts. of Neurosurgery[†] and Environmental Health[‡], University of Washington, Seattle, WA 98104

Hyperpolarization-activated, cesium-sensitive Na^+/K^+ currents play an important role in the regulation of neuronal excitability. However, these ionic currents (named I_h or I_q) are not expressed only in excitable cells. We have recently described a similar ionic current (I_{HA}) in non-excitable brain microvascular endothelial cells (*Neurosci. Abstr. 19, 692, 1993*). We here describe a hyperpolarization-activated current recorded under voltage clamp from cultured rat astrocytes after wash out of inward potassium currents with 0.2 mM GTP-γ-S. Astrocytes were obtained from purified glial cultures obtained from E21 rats and were visually identified as non-OX 42 positive cells. In astrocytes, I_{HA} was activated at around -55 mV and had a reversal potential of -25 mV under physiological ionic conditions. I_{HA} conductance was greatly increased by increases of $[K^+]_o$ from 5 to 15-30 mM. In addition, the current was blocked by extracellular (5 mM) but not intracellularly applied (140 mM) Cs^+. The whole cell current was decreased by acetyl-β-methyl choline or carbachol (0.1-1 mM) and these effects were prevented by atropine. Our results suggest that non-excitable CNS cells express a hyperpolarization-activated current previously associated with the regulation of neuronal firing and resting potential. Due to its ionic nature, sensitivity to $[K^+]_o$ and strategically important expression in both endothelial and glial cells, I_{HA} may play a role in the regulation of Na/K homeostasis in the brain. Supported by NS 51614, NS30305, NS21076, NS07144 and AA08154.

442.9

MICROGLIAL REACTIONS TO FOCAL RETINAL INJURY IN THE RABBIT. M.F. Humphrey* and S. Moore. WA Retinitis Pigmentosa Research Centre, Lions Eye Institute, Perth, Western Australia 6009.

As preliminary experiments to examine the role of microglia in retinal injury reactions, the microglia were labelled using nucleoside diphosphatase (NDP-ase) histochemistry following argon laser photocoagulation lesions in the peripheral rabbit retina. At 2, 6, 24 and 48hrs, and 7, 14 and 21 days after moderate photocoagulation lesions (argon green laser at 300-400mW for 0.2sec, 200μm diameter) in pigmented rabbits, animals were anaesthetised, perfused and the retinas immersion fixed in 4% paraformaldehyde containing 8% sucrose and 5% DMSO. The next day the retinas were processed for NDP-ase activity, using cytidine diphosphate as a substrate, wholemounted and analysed. Normal retinas had microglia in the nerve fiber layer (NFL) the inner plexiform layer (IPL) and outer plexiform layer (OPL). The distributions were similar to those previously reported (Schnitzer, J. Comp. Neurol., 282, 1990, p779). Following lesioning the IPL microglia had processes pointing into the lesion by 2hrs and had migrated to leave a vacant zone around the lesion by 6hrs, this vacant zone was gradually filled by 7 days. The NFL microglia did not react until 7 days when they became aligned with degenerating axons between the lesion and optic disc. By 14 days these cells were no longer reactive but reactive cells were now found extending from the lesion to the periphery and by 21 days the reaction was largely over. The OPL contained increased numbers of microglia from 6hrs after lesion until 14 days but by 21 days the levels were reduced again. Thus, the microglia in each layer of the retina reacted in different ways reflecting the injury response of the other cells in each layer.

442.10

INTRACELLULAR CALCIUM REGULATON IN ASTROCYTES IN ADULT RAT SPINAL CORD PREPARATIONS. E.Theriault*[1,2], M.G. Fehlings[2], S. Agrawal[2] and L.R. Mills[2]. The Toronto Hospital Arthritis Centre[1] and Playfair Neuroscience Unit[2], University of Toronto, Toronto, Ontario CANADA, M5T 2S8.

We have previously described the presence of a metabotropic glutamate receptor subtype (mGluR1α) on a subpopulation of astrocytes in the rat spinal cord (Theriault et al., '93a,b). The subpial location of these astrocytes is correlated with the characteristic region of tissue survival following traumatic spinal cord injuries (Theriault, '93), and our electrophysiological studies demonstrate a neuroprotective effect of mGluR activation following *in vivo* and *in vitro* clip compression injuries (Theriault et al., in prep.). To determine the mechanism(s) underlying the neuroprotection, we have developed an *in vitro* preparation of the spinal cord. Adult female Wistar rats (n=5, 280-300 gms) were anaesthetized, a thoracic laminectomy performed and a 20mm by 400 μm strip of the dorsal columns was removed. The isolated strip was secured in a chamber and incubated in 95-5% oxygenated Ringers with Fluo3/AM. Using confocal microscopy we have examined changes in intracellular calcium $[Ca^{2+}]_i$ levels of spinal cord astrocytes in response to K^+, bromo-A23187, and *trans*-ACPD. Rest levels of fluorescence in spinal cord astrocytes were increased two-fold following application of 100 mM K^+, and five-fold after ionophore. Interestingly, preliminary experiments with 100 μM *trans*-ACPD in *uninjured cords* revealed no detectable changes in $[Ca^{2+}]_i$ levels. Ongoing studies are examining $[Ca^{2+}]_i$ regulation in clip compression-injured spinal cord preparations. **Theriault et al. ('93a)** 2nd Int. Neurotrauma Symp. July 4-9, Glasgow, Scotland; **Theriault and Hampson, ('93b)** Soc. Neurosci. 19:748; **Theriault ('93)** 5th Int. Symp. Neural Regen., Dec.8-12, Asilomar Conf. Centre, P#59.

INVERTEBRATE LEARNING AND BEHAVIOR IV

443.1

EFFECTS OF apNPY ON BEHAVIOR IN *APLYSIA*. Valerie L. Begnoche, Samuel K. Moore and Earl Mayeri* Dept. of Physiology, University of California, San Francisco, CA 94143-0444 USA.

Aplysia neuropeptide Y (apNPY) is a 40 amino acid peptide that is homologous to NPY in vertebrates. Immunoreactivity for apNPY is widespread in the neuropile of all central ganglia, suggesting that the peptide may have many roles. In the eye, stained neurons may correspond to circadian pacemaker neurons.

To explore a possible role for the peptide in control of circadian rhythms, we did a behavioral study in which animals were kept in constant darkness for 21 days, and then injected with the peptide 3 times a day for 11 days. We found that apNPY was capable of phase-shifting the endogenous rhythm of locomotor activity. In another experiment, eyes were removed from animals that had been kept in a 24 hr light-dark cycle for 4 days. In the days following eye removal, the animals were kept in constant darkness and apNPY was injected each day at projected dawn. We found that injection of the peptide each day was followed by increased locomotion for several hours, but that this effect wore off after several days. These initial behavioral results are consistent with a role for apNPY in imposing a circadian rhythm of behavior, but more work will be needed before a firm relationship is established. Lastly, injection of apNPY causes an increase in the rate of spontaneous respiratory pumping that lasts for one hour. This suggests that apNPY may have a role in other behavioral processes as well.

443.2

HOMOSYNAPTIC LTP AND PTP OF SENSORIMOTOR SYNAPSES MEDIATING THE TAIL WITHDRAWAL REFLEX IN *APLYSIA* ARE REDUCED BY POSTSYNAPTIC HYPERPOLARIZATION. M. Cui* and E. T. Walters. Dept. of Physiology & Cell Biology, University of Texas Medical School at Houston, TX. 77225.

Tetanization of nociceptive tail sensory neurons (TSNs) causes LTP of monosynaptic connections to tail motor neurons (TMNs) lasting > 90 min in isolated ganglia (Walters & Byrne, *JNeurosci* 5:662, 1985). It was not known if this potentiation was produced homo- or heterosynaptically, but Lin & Glanzman (*ProcRSocLondB*, 255:113, 1994) recently showed clear homosynaptic LTP of sensorimotor synapses in dissociated cell culture. The LTP in culture was blocked by postsynaptic hyperpolarization during tetanization. As a first step in determining mechanisms and functions of homosynaptic LTP in defensive circuits, we have examined the dependence of TSN-TMN potentiation upon TMN membrane potential in whole ganglia. After 2 baseline tests, half the TSNs were tetanized (10 x 0.4 ms trains, 25 Hz each) with the TMN at normal resting potential. The other TSNs received identical tetanization, except that the TMN was hyperpolarized to -120 mV during the tetanus. EPSPs were then tested at 5 min intervals for at least 25 min afterwards. As predicted, LTP in the ganglia was significantly reduced by TMN hyperpolarization during the tetanus (120% of baseline vs 70% of baseline at 25 min; t_8=3.77, $p<0.05$). Interestingly, PTP observed 1 min after tetanization was also reduced by TMN hyperpolarization (387% vs 242%; t_8=3.04 $p<0.05$). Observations of homosynaptic LTP in nociceptor synapses of *Aplysia* and rat (Randic et al., *JNeurosci* 13:5228, 1993) suggest that LTP (perhaps NMDA receptor-mediated) might be a primitive mechanism for storing memory of injury.

443.3

TEMPORAL SPECIFICITY OF HEBBIAN LONG-TERM POTENTIATION OF *APLYSIA* SENSORIMOTOR SYNAPSES IN CELL CULTURE. X. Y. Lin and D. L. Glanzman*. Dept. of Physiological Sci., UCLA, Los Angeles, CA 90024.

Long-term potentiation (LTP) of in vitro *Aplysia* sensorimotor synapses can be induced by pairing brief stimulation of the presynaptic sensory neuron with strong depolarization of the postsynaptic motor neuron (Lin & Glanzman, *Proc. R. Soc. Lond.* B 255:215-221, 1994). This pattern of neuronal activity resembles that which occurs during classical conditioning of the *Aplysia* withdrawal reflex (Carew et al., 1981, 1983). Specifically, presentation of the CS (weak stimulation of the siphon) and US (tail shock) yields brief sensory neuron activation together with strong motor neuron depolarization (Frost et al., 1988). Classical conditioning of the withdrawal reflex might therefore be mediated in part by Hebbian potentiation of sensorimotor synapses. However, Lin and Glanzman (1994) used simultaneous pre- and postsynaptic stimulation to induce Hebbian LTP, whereas during behavioral conditioning the onset of the CS occurred 0.5 sec before the onset of the US.

We have systematically varied the interval between sensory neuron stimulation and motor neuron depolarization to investigate whether the induction of Hebbian LTP of in vitro sensorimotor synapses exhibits temporal specificity. The interval between pre- and postsynaptic stimulation was varied from –5.0 sec (the onset of motor neuron depolarization preceded the onset of sensory neuron stimulation by 5.0 sec) to + 5.0 sec (the onset of sensory neuron stimulation preceded the onset of motor neuron depolarization by 5.0 sec). We find that an interstimulus interval of +0.5 sec produces significant LTP of sensorimotor synapses. But the temporal specificity of Hebbian LTP for these synapses does not match that reported for the CS–US interval in conditioning of the reflex (Hawkins et al., 1986). Therefore, although Hebbian modulation of sensorimotor synapses may contribute to classical conditioning (see Murphy & Glanzman, 1994), other cellular mechanisms must also be involved.

443.4

INFUSION OF BAPTA INTO POSTSYNAPTIC SIPHON MOTOR NEURONS BLOCKS THE CELLULAR ANALOGUE OF CLASSICAL CONDITIONING IN *APLYSIA*. G. G. Murphy* and D. L. Glanzman. Interdepartmental Program for Neurosci. and Dept. of Physiological Sci., UCLA, Los Angeles, CA 90024.

Classical conditioning of the *Aplysia* siphon withdrawal reflex (Carew et al., 1981, 1983) is thought to be due to a presynaptic mechanism—activity-dependent presynaptic facilitation of sensorimotor connections (Hawkins et al., 1983; Walters & Byrne, 1983). Recent work on sensorimotor synapses in cell culture (Lin & Glanzman, *Proc. R. Soc. Lond.* B 255:215-221, 1994), however, provides another cellular mechanism for classical conditioning—Hebbian potentiation of sensorimotor connections. Lin and Glanzman's data indicate that Hebbian LTP of these connections is mediated by activation of NMDA-related receptors and requires the postsynaptic elevation of intracellular Ca^{2+} because it can be blocked by APV or by infusing the motor neuron with BAPTA, a specific chelator of intracellular Ca^{2+}.

To determine whether the cellular mechanism of classical conditioning in *Aplysia* also involves elevation of intracellular Ca^{2+} in the motor neurons, we carried out experiments on preparations consisting of the CNS of *Aplysia* together with the posterior pedal nerves, which innervate the animal's tail. We examined changes in the strength of siphon sensorimotor connections following an analogue of classical conditioning training like that used by Hawkins et al. (1983). Preparations which received paired sensory neuron activation (CS) and pedal nerve shock (US) exhibited significant enhancement of monosynaptic sensorimotor EPSPs 60 min after the last US compared to preparations which received only the test stimuli alone (Test Alone) ($p < 0.03$, Mann-Whitney U test). By contrast, the size of the monosynaptic EPSPs in preparations which also received CS+ training, but which had BAPTA (50 mM) infused into the motor neuron, were not significantly different from those of EPSPs in Test Alone preparations at the 60-min posttest ($p > 0.9$). These results implicate a postsynaptic, possibly Hebbian, mechanism in classical conditioning in *Aplysia*.

443.5

REGULATION OF UBIQUITIN-MEDIATED PROTEIN DEGRADATION DURING DEVELOPMENT OF LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS. A.N. Hegde*, K. Inokuchi, N. Yamamoto, D.G. Chain, E.R. Kandel and J.H. Schwartz. Center for Neurobiology & Behavior and Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, New York, NY, 10032.

Persistent activation of the cAMP-dependent protein kinase (PKA), which occurs during the development of long-term presynaptic facilitation of the sensory-to-motor neuron synapses underlying the defensive reflexes, results from proteolysis of dissociated regulatory (R) subunits. Persistence of kinase activity, which requires new protein synthesis, lasts for at least 24 h. Hegde et al (*PNAS* 90:7436) presented evidence that this proteolysis is mediated by the ATP-ubiquitin-proteasome pathway. Using a variety of subcellular fractionation and immunochemical techniques, we find that both ubiquitin and the proteasome complex are abundant in all parts of the neuron: cell body, axons and terminals. We presume that the degradation of R subunits is regulated by induction of some new proteins. Consistent with this idea, we find that one of the immediate early genes induced by 5-HT encodes a 29,000-molecular-weight protein with similarity to L1-type vertebrate ubiquitin C-terminal hydrolases (UCH). Peptide antibodies against the predicted sequence encoded by the *Aplysia* mRNA recognize an M_r 29,000 protein, which, like vertebrate L1, is expressed only in nervous tissue. Low molecular-weight UCHs are believed to process polyubiquitin precursors to monoubiquitin. Nonetheless, we find this *Aplysia* UCH-immunoreactivity to be associated with proteasomes. Eytan et al (*JBC* 268:4668) showed that high molecular-weight UCHs are associated with proteasome and postulated that the multiubiquitin chains cleaved from degraded proteins clog the complex, thereby slowing degradation. It is attractive to think that the low molecular-weight UCH (which we also find to be associated with proteasome in rat brain) also can enhance degradation. We are also looking for the induction of other proteins that might regulate R subunit degradation during the development of long-term memory.

443.6

POSTSYNAPTIC MODIFICATIONS IN LONG-TERM FACILITATION IN *APLYSIA*: UP-REGULATION OF EXCITATORY AMINO ACID RESPONSES. L.-E. Trudeau* and V.F. Castellucci. Lab. of Neurobiology and Behavior, IRCM, Univ. de Montréal, Montréal, Québec, H2W 1R7.

Long-term sensitization of the gill and siphon withdrawal in *Aplysia* is accompanied by facilitation of sensory-motor synaptic connections in the abdominal ganglion which depends on new protein synthesis. This phenomenon has been previously shown to involve presynaptic growth and an increase in transmitter release without any change in the size of miniature EPSPs. At the postsynaptic level, a reorganization should occur to parallel the formation of new synaptic contacts. We show here that 24h following an application of 5-HT which produces long-term synaptic facilitation (LTF), the response of the motoneuron to an excitatory amino acid agonist of the synaptic receptors is increased (day2/day1= +60.4 +/- 11.9%; n=9). Inhibition of protein synthesis in the whole ganglion using anisomycin or limited to the postsynaptic neuron by injection of gelonin, a ribosome-inactivating toxin, blocks this enhancement (-12.5 +/- 19.2; n=5, and -21.5 +/- 4.4%; n=6). The postsynaptic inhibition of protein synthesis however fails to block 24h LTF. Although these results show that 24h LTF is independent of postsynaptic protein synthesis, the data are still compatible with a model of LTF that involves coordinate pre- and postsynaptic changes. The latter alterations may be initially independent of protein synthesis, but may gradually become dependent on transcription for stages of LTF lasting more than 24h. An increase in the number of functional postsynaptic receptors in a reserve pool may also prime the postsynaptic neuron for subsequent learning-associated plasticity. Funded by MRC of Canada (MT-12099).

443.7

EARLY STEPS IN LEARNING-RELATED SYNAPTIC GROWTH: 5-HT INDUCES INTERNALIZATION OF THE TRANSMEMBRANE BUT NOT THE GPI-LINKED ISOFORM OF apCAM IN *APLYSIA* SENSORY NEURONS. C.H. Bailey*, B.-K. Kaang, M. Chen, and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032, & Inst. Mol. Biol. & Genetics, Seoul Natl. Univ., Korea.

The synaptic growth that accompanies 5-HT-induced long-term facilitation of the sensory-to-motor connection in dissociated cell culture is associated with a down-regulation of cell adhesion molecules (apCAMs) on the surface membrane of the sensory neuron (Mayford at al., 1992). Down-regulation is achieved by activation of the endosomal pathway leading to internalization and apparent degradation of apCAM (Bailey et al., 1992). Which of the two types of isoforms is internalized, the transmembrane (TM) or the phosphoinositol-linked? To address this question, we selectively expressed epitope-tagged constructs of the two isoforms in cultured sensory neurons. By combining thin section EM with gold-conjugated Ab we have found 5-HT leads to a 68% decrease in the density of gold-labeled complexes bound to the TM form at the surface membrane (8.7/μm ± 0.5, n = 8, vs. Control 27.7/μm ± 2.9, n = 7, $p < 0.001$) and to a 24-fold increase in their internalization (53 ± 4.4 vs. 2.2 ± 0.7, $p < 0.001$) By contrast, 5-HT has no effect on either the surface distribution (26.8/μm ± 1.8, n = 7 vs. 25.6 ± 2.4, n = 6) or internalization (1.7 ± 0.5 vs. 2.2 ± 0.9) of the GPI-linked isoform. The selective internalization of the TM form highlights the potential regulatory significance of its intracellular domain, which contains a PEST sequence (thought to target degradation) and has two consensus sites for MAP kinase phosphorylation (Michael and Kandel, 1994). The availability of TM constructs with deletions of, or mutations in, the cytoplasmic tail should now allow us to determine which part of this molecule triggers internalization and which part targets degradatio.

443.8

TAIL SHOCK DIFFERENTIALLY MODULATES TWO FORMS OF SYNAPTIC PLASTICITY IN INHIBITORY INTERNEURON L30 OF *APLYSIA* T.M. Fischer *[1] and T.J. Carew[1,2], Yale University, Departments. of Psychology[1] and Biology[2], New Haven, CT 06520.

In the siphon withdrawal network of *Aplysia*, activation of the L30 inhibitory interneurons, either directly or by mild tail stimulation, produces significant inhibition which is mediated through post-tetanic potentiation (PTP) of the L30 IPSP (Fischer and Carew 1993a;b). In the present study we describe the suppression of PTP in L30 by a modulatory stimulus, tail shock.

Synaptic transmission from L30 was measured as inhibitory post-synaptic currents (IPSCs) in L29 excitatory interneurons (voltage clamped at -85 mV). Following a baseline measurement, L30 was directly activated for 5 sec (8 to 12 Hz) to potentiate the synapse. Two forms of L30 activity-induced plasticity were examined: (1) frequency facilitation (FF), the enhancement of the L30 IPSC during intracellular activation (measured as the mean of the last 5 evoked IPSCs in the train); and (2) post-tetanic potentiation (PTP), the additional enhancement of the IPSC following activation (measured 20 sec after the train). Prior to tail shock, the largest increase in the L30 IPSC occurred 20 sec following activation: PTP was three times greater than FF ($p < 0.05$, N = 6). 90 sec following tail shock, the baseline IPSC was significantly reduced ($p < 0.05$, N = 5; also see Frost et al., 1988), yet the IPSC still exhibited significant FF ($p < 0.05$; compared to baseline) that was not significantly different from pre-shock FF values ($p = 0.22$). However, no significant enhancement of the IPSC was observed following activation: there was no difference between FF and PTP ($p = 0.41$). Tail shock thus appears to selectively suppress one form of plasticity, potentiation of the L30 IPSC following activation (PTP), while sparing another form (FF). Additional data indicate that tail-shock suppression of PTP may last at least 40 min. We are currently examining the cellular mechanisms of this suppression, as well as its functional consequences (see Blazis et al., this volume).

443.9

HOMOSYNAPTIC DEPRESSION IN TAIL SENSORY NEURONS IS NOT THE MECHANISM OF HABITUATION OF TAIL-INDUCED TAIL OR SIPHON WITHDRAWAL IN *APLYSIA*. M. Stopfer* and T.J. Carew. Departments of Psychology and Biology, Yale University, New Haven, CT. 06520

Homosynaptic depression of sensory neuron (SN) output is considered a primary cellular mechanism of habituation in several systems. We have examined the contribution of homosynaptic depression in tail SNs to habituation of two tail-induced reflexes in *Aplysia*: (1) tail withdrawal, which has both direct and indirect SN input to tail motor neurons (MNs); and (2) siphon withdrawal, which has only indirect SN input to siphon MNs. In behavioral experiments, repetitive, mild tail stimulation (30s ISI) significantly habituated both tail and siphon reflexes ($p<0.001$ in each case). In parallel cellular experiments, the same repetitive stimuli induced progressively fewer action potentials in both tail and siphon MNs ($p<0.001$ in each case).

When tail SNs were repeatedly activated (30s ISI) with intracellular pulses, significant homosynaptic depression of the monosynaptic EPSP onto tail MNs was exhibited ($p<0.001$); depression did not generalize to other, non-activated SNs connected to the same MN. However, when the same SN EPSP was examined during behavioral training that caused significant reflex habituation ($p<0.001$), SNs activated by tail stimulation showed significant *facilitation* of their output to tail MNs ($\bar{x}$ EPSP increase = 463%, $p<0.02$). SNs not activated by tail stimulation also showed significantly facilitated EPSPs ($\bar{x}$ EPSP increase = 385%, $p<0.05$), indicating that SN facilitation was heterosynaptic.

In summary, by using behaviorally relevant stimuli and simultaneously measuring reflex behavior, MN output, and SN output we found that, while the behavioral responses and MN output significantly decreased during habituation, SN output significantly increased. We are currently exploring whether: (1) increased SN output onto inhibitory interneurons; and (2) increased inhibition and/or homosynaptic depression at interneuronal sites contribute to habituation in these reflex systems.

443.10

DISTINCT COMPONENT OF PRESYNAPTIC CALCIUM CURRENT IS INCREASED BY CYCLIC AMP AT APLYSIA SENSORIMOTOR SYNAPSES IN CULTURE. M. Klein*, Lab. of Neurobiology & Behavior, Clinical Research Inst. of Montreal & Univ. of Montreal, Montreal, Quebec H2W 1R7, Canada.

Synaptic augmentation of transmitter release by serotonin (5HT) at Aplysia sensorimotor synapses can be mimicked by activation of adenylyl cyclase and is blocked by inhibiting cyclic AMP-dependent protein kinase. Two components of calcium current have previously been identified in Aplysia sensory neurons, a slowly-inactivating current that is enhanced by 5HT and blocked by dihydropyridines and a rapidly-inactivating current that is reduced by FMRFamide; of the two, only the rapidly-inactivating component appears to participate in transmitter release caused by single action potentials under most conditions. Chlorophenylthio-cyclic AMP augments synaptic currents at sensorimotor synapses in culture and also causes an increase in a slowly-inactivating calcium current in the sensory neurons. However, this latter current differs from the major current modulated by 5HT in that it is not affected by dihydropyridine antagonists, and, unlike the current described earlier, is not blocked by H7, an inhibitor of protein kinase C in these neurons. The current modulated by the cyclic AMP analog is also not affected by FMRFamide. These findings thus raise the question of whether an increase in a cyclic-AMP-sensitive calcium current may contribute to the enhancement of transmitter release that occurs during synaptic augmentation by 5HT.

Supported by NIMH, NSERC, and a Sloan fellowship.

443.11

ARE LEARNED BIASES IN APLYSIA HEAD-WAVING BEHAVIOR DUE TO ASSOCIATIVE OR NONASSOCIATIVE MECHANISMS? J. Chey, P. Cisek, P. Gaudiano*, R. Wood. Boston University, Dept. of Cognitive and Neural Systems, Boston, MA 02215.

A long term bias in the exploratory head-waving behavior of Aplysia can be induced using flashed lights as an aversive stimulus (Cook and Carew, 1986, 1989). Coupling onset of the lights with a particular direction of head movement results in a bias away from that direction. This bias has been interpreted as a form of operant conditioning, and has been simulated with a neural network model based on associative synaptic facilitation (Raymond *et al.*, 1992). We simulate the head-waving behavior using a *recurrent gated dipole*, a nonlinear dynamical neural model previously used to explain various data including oscillatory behavior in biological pacemakers. In our model two recurrent neural circuits inhibit each other to generate oscillations, which drive the side-to-side head-waving. Within each channel the frequency and amplitude of oscillations depend on transmitter mobilization dynamics, which exhibit both short- and long-term adaptation. We assume that light onset results in an increase in nonspecific arousal to both sides of the dipole. Repeated pairing of arousal increments with activation of one side (the "paired" side) of the dipole leads to a bias in transmitter dynamics, which causes the oscillation to last a shorter time on the paired side than on the unpaired side. This model provides a parsimonious explanation of the observed behavior, and it avoids some of the unexpected results obtained with the Raymond *et al.* model. In addition, our model makes predictions concerning the rate of onset and extinction of the biases, and suggests new lines of experimentation to test the nature of the head-waving behavior.

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443.12

PLASTICITY OF LE AND NON-LE SIPHON SENSORY NEURONS DURING HABITUATION AND DISHABITUATION OF THE *APLYSIA* GILL-WITHDRAWAL REFLEX. S.W. Kaplan*, E.R. Kandel, and R.D. Hawkins. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032

We have developed a simplified preparation, consisting of the isolated mantle organs of *Aplysia*, that undergoes habituation, dishabituation, sensitization, and classical conditioning of the gill-withdrawal reflex. We previously found that the LE siphon sensory neurons contribute to the reflex elicited by controlled force stimulation of the siphon. However, a weak water movement stimulus that can produce PSPs in motor neurons and withdrawal of the gill does not cause the LE cells to fire, suggesting that other, unidentified sensory neurons can also contribute to the reflex (Cohen et al., 1991). To compare plasticity of PSPs from the LE and non-LE sensory neurons we stimulated the siphon with either a controlled force tap or weak water movement and measured the complex PSP in a gill motor neuron. The ganglion was perfused with hi Ca^{2+}, hi Mg^{2+} ASW, which blocks most of the polysynaptic component of the PSP. With the controlled force tap there was a decrease in the remaining monosynaptic component during habituation, and an increase 2.5 min after shock during dishabituation. With the weak water movement stimulus, the results were very similar. Furthermore, like the PSP from LE sensory cells, the PSP produced by the weak water movement stimulus was facilitated when the ganglion was perfused with serotonin, which mediates some of the effects of the shock during dishabituation. These results suggest that PSPs from the LE sensory neurons and the unidentified sensory neurons have basically similar plasticity during habituation and dishabituation of the reflex.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS VIII

444.1

NEURONS IN RHESUS MONKEY VENTRAL STRIATUM SHOW RESPONSE CHANGES RELATED TO ASSOCIATIVE LEARNING. M. Shidara*, T. G. Aigner & B. J. Richmond. Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

The ventral striatum is considered to have a role in motivation. Neurons in the ventral striatum respond during a task in which monkeys earn water reward by releasing their grip on a bar when a visual target changes color. The number of correct trials required to get a reward was randomly varied from 1 to 3. A cue located above the target signaled progress toward obtaining a reward, with the brightest cue indicating that a reward was forthcoming if the current trial were completed successfully (brightening paradigm). The mean reaction time to bar release decreased as the cue brightness approached the level that signaled reward, suggesting that the monkey was more motivated. When we randomized the sequence so that cue brightness lost its meaning (random paradigm), the mean reaction time was always near the shortest value.

Some ventral striatal neurons responded when the cue brightness changed, some when target changed color, and others when the reward was delivered in the brightening paradigm. About one-third of the neurons showed significantly different firing frequencies after the cue brightness lost its meaning. Some neurons responded most vigorously to the cue brightness for the rewarded trial in the brightening paradigm and decreased their firing in the random paradigm. Others responded most vigorously for the non-rewarded trial in the brightening paradigm and also decreased their firing in the random paradigm. These changes in firing frequency paralleled the changes in reaction times, suggesting that the firing frequencies of these neurons are closely related to the current meaning of the cue, i.e., they show effects related to associative learning.

444.2

EVIDENCE FOR DIFFERENT FORMS OF MEMORY RETENTION IN MACAQUES FOR TASKS NEEDING JUDGEMENT FOR RECENCY COMPARED WITH FAMILIARITY. J.L. Ringo* and Mark D. Diltz, Department of Physiology, University of Rochester School of Medicine.

When a single pair of images is repeatedly re-used in a delayed matching to sample task, the subject must choose on the basis of recency. When trial-unique images are used, the subject may use simple familiarity. The mechanisms mediating these kinds of memory were investigated by disruption confined to the retention period.

Disruption was provided by 1-sec of electrical stimulation to the medial temporal lobe in the middle of the delay period. Sessions with repeated images and sessions with trial-unique images were interleaved. Delays of 4, 8, 16 and 32 sec were used. The duration of the sample image presentation was varied in order to approximately equalize performances (shorter sample durations for trial-unique than for repeated images, and shorter sample durations for shorter delays). Control performances, without stimulation, were roughly 80-85% correct.

The same levels of electrical stimulation delivered to the same electrodes in the same monkeys produced significantly ($P<0.01$) more disruption when delivered during testing with repeated images than testing with trial-unique images. Compared to interlaced trial without stimulation, electrical stimulation caused a 15% reduction of performance with repeated images. A smaller but still significant 7% reduction of performance was found with trial-unique images. These effects were seen at all delays. Built in controls indicate that the effects of the stimulation occurred at the time of stimulation, i.e., were not due to lingering aftereffects.

These data suggest that active memory, (e.g., the persistent neuronal firing often seen in memory tasks) may be an important mediator of memory in these monkeys when repeated images were used.

444.3

CRUCIAL ROLE OF GLUTAMATE-MEDIATED TRANSMISSION IN MONKEY PERIRHINAL CORTEX FOR VISUAL RECOGNITION MEMORY. K. Gale[1], M. Frank-Molnia[1], D. Olson, D. Dybdal[1], F. Fornai[1], M. Dubach* and V. Gunderson. Regional Primate Ctr., Univ. of Washington, Seattle, WA 98195 and [1]Dept. of Pharmacology, Georgetown Univ. Med. Ctr., Washington, DC 20007.

In an attempt to identify brain regions in which glutamate receptors may mediate memory processes, we examined the effect of focal intracerebral application of a glutamate antagonist on the performance of pigtailed macaques in a delayed nonmatch-to-sample (DNMS) task. Kynurenate, an antagonist at NMDA and non-NMDA glutamate receptors, was microinjected into various sites within the inferotemporal cortex and hippocampus of awake, behaving juvenile Macaca nemestrina (n = 5). All subjects were trained to a stable performance (>90%) at 10, 30 and 60 sec delays prior to the experiments. Kynurenate infusions into the perirhinal cortex, but not into the neighboring hippocampus or parahippocampal cortex, produced reversible, delay-dependent impairment of performance (90% correct at 10 sec delays and 65% correct at 60 sec delays). This impairment required bilateral infusions of kynurenate and was not obtained with control infusions of saline. Spontaneous behavior and motor function were not altered by the focal infusions of kynurenate. These findings indicate that glutamate transmission in a relatively selective region of perirhinal cortex is a crucial component of the neural substrate for visual recognition memory. Moreover, these results demonstrate the utility of focal drug application for exploring neural networks subserving cognitive function in the primate brain.

Supported by an NSF Visiting Professorship award to K. G.

444.4

COLOUR BLINDNESS IN MACAQUES AFTER ANTERIOR TEMPORAL CORTEX ABLATIONS: FEATURE PERCEPTION AND OBJECT PERCEPTION. D. Gaffan*, C. A. Heywood, M. Buckley, A. Cowey and S. A. Gutnikov. Exp Psychology, Oxford University, Oxford OX1 3UD, U.K.

Colour blndness resulting from cortical lesions occurs clinically in man but has not until now been demonstrated in the monkey. Previous experiments have shown, for example, that monkeys with ablations of area V4 have only mild impairments in colour vision. In three preoperatively trained macaques (M. fascicularis) we made ablations of temporal neocortex anterior to V4. These ablations extended from the upper bank of the superior temporal sulcus to the parahippocampal gyrus, including the entire cortical area TE and also some cortex adjacent to area TE, but sparing V4. All three monkeys were unable postoperatively to discriminate hue (red from green) or saturation (red from grey). They were only mildly impaired in brightness discrimination. One further monkey (M. mulatta) with total ablation of area TE, and with the remaining temporal cortex intact, also showed colour blindness. Three monkeys with cortical ablations restricted to the inferior temporal gyrus (perirhinal cortex) had no impairment in colour vision, but demonstrated impairments in object discrimination in a concurrent learning task with 24-h intertrial intervals. These results show that the role of perirhinal cortex in object perception is not limited to short-term recognition memory (matching or nonmatching to sample), and is not based on the detection of simple features such as colour, while the cortex between V4 and perirhinal cortex is essential for colour vision. Thus, the temporal cortex contains both a mechanism for perception of object features such as colour and also a mechanism for the individual identification of complex objects. These mechanisms may not necessarily be hierarchically arranged, but may represent parallel specializations for aspects of visual perception and memory.

444.5

MONKEYS WITH RHINAL CORTEX LESIONS ARE IMPAIRED ON MATCHING-TO-SAMPLE WITH LARGE STIMULUS SETS, EVEN AT 0-SEC DELAYS, BUT ARE NOT IMPAIRED ON MATCHING-TO-SAMPLE WITH SMALL STIMULUS SETS. E.A. Murray*, M.J. Eacott, and D. Gaffan, Lab. of Neuropsychology, NIMH, NIH, Bethesda, MD 20892, U.S.A. and Dept. of Experimental Psychology, Oxford OX1 3UD, U.K.

Seven cynomolgus monkeys were trained to perform simultaneous and delayed matching-to-sample (DMS) both with a large stimulus set and with a small one. Preoperatively, the monkeys were given performance tests in which 4 delays (0, 5, 15, and 30 sec) and the simultaneous condition were mixed within sessions. After training, monkeys either received bilateral ablations of the rhinal cortex (Group Rh, N=3) or were retained as unoperated controls (Group Con, N=4), and were then retrained on DMS with 0-sec delay. After relearning, the monkeys were again given the performance tests, which were presented in the same way that they had been preoperatively. Group Rh scored significantly more poorly than Group Con on the performance test with the large stimulus set (Group Con, mean = 93% correct; Group Rh, mean = 77% correct). Further, there was a significant interaction of Group and delay, which was still the case even if the data from the delay used for relearning (0-sec delay) were omitted from the analysis. However, Group Rh was also impaired relative to Group Con in the 0-sec delay and simultaneous matching conditions. When data from two versions of DMS with trial-unique stimuli (regular task plus "difficult" version in which physical discriminability of stimuli was reduced) were considered together, Group Con scored 93% correct on simultaneous and 0-sec delay conditions combined whereas Group Rh scored 86% correct. Surprisingly, when the stimulus set was restricted to a single pair, the DMS performance test scores of Group Rh and Group Con did not differ. The results indicate that the rhinal cortex is not specialized for performance on DMS or DNMS, but instead plays a broader role in the processing of visual stimuli. The rhinal cortex appears to be critical both for storing information about novel visual stimuli, and for identification of those stimuli.

444.6

ABNORMAL PERFORMANCE DURING THE FIRST 10 TRIALS OF LEARNING THE TRIAL UNIQUE DELAYED NONMATCHING TO SAMPLE TASK BY MONKEYS WITH LESIONS LIMITED TO THE HIPPOCAMPAL REGION. S. Zola-Morgan*, L.R. Squire and P. Alvarez. V.A. Medical Center, San Diego, and Depts. of Psychiatry and Neurosciences, U.C.S.D. School of Medicine, La Jolla, CA 92093.

Work with humans, monkeys, and rodents has shown that damage limited to the hippocampal region (i.e., the hippocampus proper, dentate gyrus and the subicular complex; referred to as the H lesion), is sufficient to impair memory. On the delayed nonmatching to sample task (DNMS), monkeys with H lesions were impaired at each of the two longest delay intervals (10 min and 40 min). By contrast, the monkeys with H lesions learned the task at a normal rate with a delay interval of 8 sec. In addition, the H monkeys performed as well as normal monkeys at the shorter delays of 15 sec and 60 sec. The DNMS task takes advantage of the normal tendency of monkeys to prefer the novel object in a 2-choice test. Tasks that depend on spontaneous tendencies have proven especially sensitive to hippocampal lesions. Accordingly, we examined the 20 trials that comprise the first day of training on DNMS. During this very early stage of training, performance primarily reflects the spontaneous tendency to select the novel object rather than acquisition of the nonmatching rule. Monkeys with H lesions were impaired during the first 10 trials of training on the first day. Normal monkeys scored 81% correct. Monkeys with H lesions scored 60% correct ($P<.05$) These findings show that damage to the hippocampal region can impair memory when the delay interval between sample and choice is only 8 sec.

444.7

EFFECTS OF LESIONS OF PERIRHINAL CORTEX OR PARAHIPPOCAMPAL CORTEX ON MEMORY IN MONKEYS. S.J. Ramus*, S. Zola-Morgan, and L.R. Squire. UCSD Depts. of Neurosciences and Psychiatry, La Jolla, CA 92093, and V.A. Medical Center, San Diego, CA 92161.

The perirhinal cortex (PR) and the parahippocampal cortex (PH) are part of the medial temporal lobe memory system and are themselves important for memory. For example, previous studies have shown that conjoint lesions of PR and PH impaired memory performance in both the visual and the tactual modalities. Recent anatomical studies raise the possibility that PR and PH may contribute to memory in different ways. Specifically, PR receives strong inputs from unimodal visual areas in the inferotemporal cortex and the ventral bank of the superior temporal sulcus. By contrast, PH receives relatively little input from these areas and instead receives major inputs from polymodal association areas. Accordingly, one possiblity is that damage to PR could produce more impaired performance than damage to PH on tasks of visual memory. In order to assess the separate contributions to memory of PR and PH, we prepared two group of monkeys, one with lesions limited to the PR (n = 5), and one with with lesions limited to PH (n = 5). Findings with the delayed nonmatching to sample task, a test of visual object recognition memory, showed that monkeys with lesions limited to PR were impaired relative to 7 normal control monkeys ($p<.001$) and relative to monkeys with PH lesions ($p<.05$). Monkeys with lesions of PH were unimpaired ($p>.10$). These findings, together with preliminary data from other visual memory tasks, suggest that lesions of PR produce a memory impairment in the visual modality while lesions of PH do not.

444.8

INFEROTEMPORAL CORTEX AREA TE AS REDEFINED BY RECENT ANATOMICAL STUDIES. E.A. Buffalo, S. Zola-Morgan and L.R. Squire*. Departments of Philosophy, Psychiatry, and Neurosciences, University of California at San Diego, La Jolla, California, 92093 and Veterans Affairs Medical Center, San Diego, California, 92161.

Work with monkeys has helped identify the structures in the medial temporal lobe that are important for memory: the hippocampal region, and anatomically related areas, i.e., the entorhinal, perirhinal, and parahippocampal cortices. Inferotemporal cortex area TE lies adjacent to these cortical areas and has significant projections to one component of this system, the perirhinal cortex (PR). Previous studies of the effects of lesions of area TE have reported impaired pattern discrimination learning and impaired visual recognition memory. Typically, these TE lesions involved the temporal polar region anteriorly, extended posteriorly to 10 mm in front of the ascending inferior occipital sulcus, were bound dorsally by the superior temporal sulcus, and extended ventrally to the rhinal and occipito-temporal sulci. However, recent neuroanatomical studies show that PR occupies some of the temporal polar region and the ventral region previously considered part of area TE. Moreover, our laboratory and others have shown that PR plays an important role in memory function. Thus, it is possible that in previous studies of the effects of TE lesions, the inclusion of PR contributed to the reported memory impairment. We have prepared a group of monkeys (n = 5) with bilateral lesions limited to area TE that did not include PR. Preliminary behavioral findings indicate that the monkeys with TE lesions are impaired on the visual nonmatching to sample task. With additional tests, it should be possible to dissociate impairments in visual perception from impairments in memory.

444.9

DAMAGE TO THE HIPPOCAMPAL REGION IN HUMAN AMNESIA: NEUROPSYCHOLOGICAL AND NEUROANATOMICAL FINDINGS FROM TWO NEW CASES N.L. Rempel-Clower*, S. Zola-Morgan, and L.R. Squire Depts. of Neurosciences and Psychiatry, UCSD, La Jolla, CA 92093, and V.A. Medical Center, San Diego, CA 92161

Patient RB had a moderately severe and long-lasting anterograde memory deficit following an ischemic episode that resulted in a circumscribed, bilateral lesion of the entire CA1 field of the hippocampus (Zola-Morgan, et al., 1986). Two new cases of human amnesia resulting from an anoxic or ischemic event have now undergone extensive postmortem neuropathological analyses. These amnesic patients, LM and GD, were studied for 6 and 8.5 years, respectively, together with other amnesic patients using tests of anterograde memory, retrograde memory, and other cognitive functions. Both patients had exhibited significant anterograde and retrograde amnesia, and normal intellectual and cognitive abilities. Both LM and GD had complete bilateral lesions of the CA1 field of the hippocampus. In addition, LM had damage to other areas of the hippocampal region including the anterior part of the CA3 field and slight damage to the subiculum and dentate gyrus. GD did not sustain damage to any area of the hippocampal region other than the CA1 field. In both patients, damage in the medial temporal lobe was limited to the hippocampal region, with no degeneration apparent in the amygdala or entorhinal, perirhinal, and parahippocampal cortices. In neither case was damage evident in the mammillary nuclei or the medial thalamic nuclei, other areas associated with memory function. Although minor pathology was present outside the hippocampal region in both cases (e.g., in LM there was cell loss in the right medial occipitotemporal gyrus; in GD there was a small infarct in the right globus pallidus), the primary damage in LM and GD was limited to the hippocampal region. Complete bilateral loss of CA1 pyramidal cells is a consistent finding in all three amnesic cases (RB, LM, and GD) and the pathology in the hippocampal region is the only damage that can be reasonably associated with their memory deficits.

444.10

INTACT ARTIFICIAL GRAMMAR LEARNING IN PATIENTS WITH HUNTINGTON'S DISEASE. **B.J. Knowlton, L.R. Squire, & N. Butters. VA Med. Ctr., San Diego, and Depts. of Psychiatry and Neuroscience, UCSD, La Jolla, CA 92093.**

Artificial grammar learning appears to be an example of implicit learning. It is normal in amnesic patients and occurs independently of the medial temporal lobe memory system. Patients with Huntington's disease (HD), who have striatal damage, are impaired in learning certain motor and perceptuomotor skills which are also examples of implicit learning. We tested whether artificial grammar learning depends on the same kind of skill learning by testing 11 patients with HD and 12 control subjects. During the study phase, subjects were shown 23 letter strings generated by a finite-state rule system. Each letter string was presented for 9 sec, and the subjects attempted to reproduce the item. The set of training items was repeated once. Five min later, subjects were told that the training items were generated by a set of rules, and that their task would be to decide for a new set of items whether or not each adhered to these rules. This classification test consisted of 23 new grammatical items and 23 nongrammatical items. Performance by the patients on the classification test was not significantly different from the performance of the control subjects (60.3±3.4% vs. 64.3±3.6% correct). The patients were marginally impaired when asked to recognize letter strings that had been presented during the study phase (65.4±3.1% vs. 73.3±2.9% correct $p<.1$). Artificial grammar learning appears to be unaffected by the striatal pathology and dementia associated with HD.

444.11

MEMORY CONSOLIDATION AND THE MEDIAL TEMPORAL LOBE: A SIMPLE NETWORK MODEL. P. Alvarez* and L. R. Squire. V.A. Medical Center, San Diego, CA 92161, Dpts. of Psychiatry and Neuroscience, UCSD, La Jolla, CA 92093, and Computational Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Declarative memory has been shown to depend on a system of medial temporal lobe structures that includes the hippocampus and the adjacent cortical areas (entorhinal, perirhinal and parahippocampal cortex). The role of this system is only temporary, however, as shown by the fact that after damage to the medial temporal lobe, recent memories are impaired but remote memories are intact. This suggests that the medial temporal lobe memory system is involved in a process of consolidation: memories that are initially dependent on this system gradually become established elsewhere, presumably in neocortex. Several proposals for how interactions between the medial temporal lobe and neocortex may mediate consolidation have been presented, but they are often not explicit on many key points. We have attempted to synthesize a number of the existing ideas into a concrete proposal that can be implemented as a neural network model. We propose that a key role of the medial temporal lobe in memory is to bind together the different portions of a memory representation that are distributed over separate areas of neocortex. This binding is mediated by fast changes in the connections between medial temporal lobe and neocortex, and within the medial temporal lobe The medial temporal lobe then directs the slower changes in the connections between neocortical areas that underlie consolidation. We show that a simple neural network model based on this proposal behaves in a way consistent with experimental observation. This model addresses several current questions about consolidation, and provides a reference point for further experimental investigation and a starting point for improved models.

444.12

SHORT TERM MEMORY FOR DURATION IN HYPOXIC SUBJECTS. **R.O. Hopkins* and R.P. Kesner, Department of Psychology, University of Utah, Salt Lake City, Utah 84112.**

Previous research has demonstrated that an hypoxic episode can cause damage to the hippocampus as determined by volumetric MRI analysis. There are several ways in which temporal information can be represented in the brain, including temporal order and duration. Previous research has shown that hypoxic subjects are impaired for memory for novel linguistic and spatial temporal order information. Subjects who experienced an hypoxic episode and matched control subjects (N=9) were tested for short term memory for duration (STMD). The STMD task assessed memory for duration across a variety of delays. Subjects were presented with a single object (square, circle, etc.) on a computer screen, for a duration of 1 or 3 seconds. Subjects were instructed to remember the duration of presentation of the object. After a delay of 1, 4, 8, 12, 16 or 20 seconds, the same object reappeared for 1 or 3 seconds and the subject determined if the object appeared for the same or a different duration. There were 48 trials which allowed for 8 observations for each interval. Hypoxic subjects were impaired relative to control subjects in short term memory for duration. In order to determine if the deficits were due to impaired memory for the items, instead of duration, a control task was developed. The same subjects were presented with a single object for a duration of 1 to 3 seconds and were asked to remember the object. After a delay of 1, 4, 8, 12, 16 or 20 seconds, either the identical object or a different object appeared on the screen. The subjects were asked to determine if it was the same object or a different object. No significant differences between hypoxic and control subjects were found on this task. These results suggest that hypoxic subjects are impaired for memory for duration information, but not for memory for single objects. Hypoxic subjects , with damage to the hippocampus, appear to be impaired in the temporal coding of information.

DEGENERATIVE DISEASE: OTHER I

445.1

TRINUCLEOTIDE REPEAT EXPANSION AND DRPLA (SMITH'S DISEASE): MOLECULAR CHARACTERIZATION OF ATROPHIN-1. R.L. Margolis*, S-H. Li, C.A. Ross. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave., Ross 615, Baltimore, MD 21205.

Smith's disease (also known as dentatorubral pallidoluysian atrophy or DRPLA) is a rare, progressive, fatal neuropsychiatric disorder similar to Huntington's disease. Smith's disease is characterized by ataxia, choreoathetosis, myoclonic epilepsy, dementia, and the genetic phenomenon of anticipation. A few cases have been misidentified as schizophrenia. Neuropathological findings include prominent cell loss in the dentate nucleus of the cerebellum, the globus pallidus, the red nucleus, and the subthalamic nucleus. An expansion of a CAG trinucleotide repeat encoding polyglutamine in a gene originally identified in our laboratory (CTG-B37, Li et al, Genomics, 1993) has now been identified as the cause of this disorder (Nagafuchi et al and Koide et al, Nature Genetics, 1994). Northern analysis indicates that the gene, which we have termed atrophin-1, is widely expressed as a 5 kb mRNA in human tissue. Cloning and sequencing of the open reading frame from inserts contained in brain cDNA libraries is in progress. In addition to the CAG repeat, the ORF contains an unusual region of alternating acidic and basic amino acids and a region just 5' to the CAG repeat remarkably prone to chimerism during cDNA library construction. Further characterization of atrophin-1 cDNA should lead to a better understanding of the normal and mutant forms of this gene.

445.2

THE PRION PROTEIN 178^{Asn} MUTATION ALTERS PROCESSING IN A TRANSFECTED HUMAN CELL LINE. R.B. Petersen*, S.L. Richardson, S. G. Chen, P. Parchi, C.B. Urig, and P. Gambetti. Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106

The prion protein, PrP, has been implicated in a number of neurodegenerative diseases which can occur sporadically, by transmission, or through inheritance of a mutated allele. A unifying feature of all these diseases is the presence of protease resistant PrP in the brain of affected individuals. The protease resistance is a result of the altered conformation of the PrP; as is the case in Alzheimer disease the change seems to involve a transition of alpha helix to beta pleated sheet. The mechanism by which this transition occurs and is subsequently propagated is unknown. We previously described two subtypes of prion diseases that share a single mutation, Asp to Asn, at codon 178 of the PrP, but are differentiated by a common Met/Val polymorphism at codon 129. We created transfected cell lines expressing either normal PrP or PrP with the 178^{Asn} mutation to determine if the processing of the resulting protein was altered. We used a homologous system, the human PrP coding sequence and the human neuroblastoma cell line M17, to simplify analysis. Our findings show that the 178^{Asn} mutation results in an alteration of the metabolism of the prion protein. This work was supported by NIH grant AG-08992 and AG-08155.

445.3

SCRAPIE PRION PROPAGATION ALTERS THE HEAT SHOCK RESPONSE IN NEUROBLASTOMA CELLS. J. Tatzelt#, W. J. Welch#, J. Zuo^, R. Voellmy^, S. B. Prusiner*#. #University of California, San Francisco, ^University of Miami.

The fundamental event underlying scrapie infection seems to be a conformational change in the prion protein (PrP). Because changes in the conformation of PrP might require the participation of molecular chaperones, we examined the expression of heat shock proteins (Hsp) that are known to function as molecular chaperones in scrapie infected cells. Neither heat shock nor exposure to sodium arsenite induced Hsp synthesis in scrapie infected neuroblastoma (ScN2a) cells in contrast to uninfected, control neuroblastoma (N2a) cells. Constitutively expressed Hsp 73 was observed primarily within distinct, localized areas in the cytoplasm of ScN2a cells; upon heat shock it was dispersed throughout the cytoplasm and failed to be redistributed into the nucleus/nucleolus. Hsp 73 was widely dispersed throughout the cytoplasm and was translocated into the nucleus/nucleolus after heat shock in the N2a cells as observed for other cell types. While most of Hsp 73 could be removed following nonionic detergent extraction of the N2a cells, significant amounts of Hsp 73 remained within a detergent insoluble fraction of the ScN2a cells. These alterations in the stress response observed for the ScN2a cells may feature not only in the pathogenesis of the prion diseases but also in the formation of PrP^{Sc}.

445.4

TRUNCATED FORMS OF PRION PROTEIN IN NORMAL AND PATHOLOGICAL HUMAN BRAINS. S.G. Chen*, P. Parchi, R. Petersen, L. Monari, W. Wang, P. Gambetti, L. Gambetti. Case Western Reserve University, Cleveland, Ohio 44106.

The prion protein (PrP) is a glycosyl-phosphatidylinositol (GPI) anchored membrane protein of unknown function. An abnormal PrP isoform, partially resistant to proteases, is a hallmark of prion diseases in humans and animals. Immunoblot analysis of normal human brains using antibodies specific to various regions of PrP, has revealed the presence of truncated PrP forms. These fragments are not the product of autolysis, since they were also present in neuroblastoma cells in culture. The most abundant of these fragments is N-terminal truncated and is found in amounts comparable to that of full length PrP. This truncated form is also attached to the cell membrane through a GPI anchor, since it can be released from neuroblastoma cells in culture as well as from brain microsomal fractions by treatment with PIPLC. Our ongoing studies indicate that the relative amount and type of these truncated forms vary according to severity and type of pathology in brains from subjects with human prion diseases, such as Creutzfeld-Jakob disease and Fatal Familial Insomnia. Studies of these fragments may help understand the role of PrP processing in normal and pathological conditions. Supported by NIH grants AG-08155 and AG-08992.

445.5

IS THE APOLIPOPROTEIN E (APOE) ε4 ALLELE OVERREPRESENTED IN DIVERSE NEURODEGENERATIVE DISORDERS (ND)? J.A. Schneider, M. Gearing, and S. S. Mirra*. Dept. of Pathology and Laboratory Medicine, VA Med. Center & Emory University School of Medicine, Atlanta, GA 30322.

The apoE ε4 allele is overrepresented in late onset familial and sporadic Alzheimer's disease (AD). While other amyloid-associated disorders, e.g. Creutzfeldt-Jakob disease and amyloid polyneuropathy, have failed to demonstrate this dysequilibrium (Saunders et al. Lancet 1993;342:710), we sought evidence of increased representation of the ε4 allele in ND that, like AD, are associated with increased age, neuronal loss, and cytoskeletal pathology. DNA was extracted from paraffin blocks or frozen tissue, and ApoE genotype was determined using a polymerase chain reaction/restriction enzyme method in 48 cases of ND: progressive supranuclear palsy (PSP; n=17), Pick's disease (n=6), corticobasal ganglionic degeneration (CBGD; n=7), Parkinson's disease (PD; n=7), diffuse Lewy body disease (DLBD; n=1), multisystem degenerations (MSD; n=7), lobar atrophy without Pick bodies (n=2), and entorhinal sclerosis (n=1). Coexistent AD pathology of varying severity was present in 16 of the 48 ND cases. We found at least one ApoE ε4 allele in 27 of 48 cases of ND (56%) as compared with estimates of 20-25% in the general population and 60-80% in AD (72% in our own series). When the 16 cases with coexisting AD were excluded, 15 of the remaining 32 ND cases (47%) had at least one ApoE ε4 allele. The proportion of cases with the ε4 allele increased to 56%, when only those ND with neuronal cytoskeletal abnormalities or inclusions (PSP, Pick's disease, PD, DLBD, and CBGD; n=25) but without AD were analyzed. In the seven ND cases without concomitant AD or neuronal inclusions, however, an ε4 allele was found in only one case (14%). These findings suggest that the ApoE ε4 allele is overrepresented in ND other than AD, particularly those with neuronal cytoskeletal abnormalities. Further genetic analysis of additional cases is needed to clarify the role of ApoE in these diverse disorders. Supported by AG10130 and VA Merit Award.

445.6

QUANTITATIVE ANALYSIS OF SENILE PLAQUES: EFFECT OF APOLIPOPROTEIN E GENOTYPE. B.T. Hyman*, H.L. West, G. W. Rebeck, S. Buldyrev, R.N. Mantegna, S. Havlin and H.E. Stanley. Mass General Hospital, Boston, MA 02214 & Center for Polymer Studies, Boston Univ, Boston, MA 02215

Quantitative analysis of senile plaque (SP) number, size, and amyloid burden (percent of cortex covered by Aß) in Alzheimer disease (AD) show that the amount of Aß deposited correlates with apoE genotype, with apoE ε4/4 > apoE ε3/4 > apoE ε3/3. To test the hypotheses that this genotype effect is due either to differences in nucleation events or to alterations in the kinetics of SP growth and dissolution, we used computerized image analysis techniques to analyze the size distribution of about 400 SP/case in the superior temporal sulcus of 42 AD cases. We discovered a remarkable homogeneity of size distribution that fits a log-normal curve. This size distribution profile suggests that SP growth is not dependent on time or simple surface area, but instead that the interior of SP may be available for Aß deposition and resolution. Comparing the Aß measures among AD patients with different genotypes, we find increased total Aß deposition with apoE ε4 but no difference in the size distribution curves among genotypes. This suggests that the major difference accounting for the larger amount of Aß in apoE ε4 is formation and stabilization of SP nucleation rather than alterations in ongoing deposition of Aß. Supported by NIH AG08487.

445.7

APOLIPOPROTEIN E, LRP, AND ALZHEIMER DISEASE: NEW OBSERVATIONS OF SHED RECEPTORS AND ISOFORM SPECIFIC COMPLEX FORMATION IN CSF. G. W. Rebeck*, S. Harr, H.L. West, A. J. Mendez, J. H. Growdon, M. Kounnas, W. S. Argraves, D. Strickland and B.T. Hyman. Mass. General Hospital, Boston, MA 02214 and the Holland Laboratories, American Red Cross, Bethesda, MD.20855

Increased risk of Alzheimer disease (AD)is associated with inheritance of different alleles of the apolipoprotein E (apoE) gene (apoε4 > apoε3 > apoε2). The apoE isoforms differ by single amino acid changes in the receptor binding domain: apoE2 has two Cys, apoE3 one Cys, and apoE4 none. ApoE binds Aß in vitro and immunostains senile plaques (SP). We previously found that an apoE receptor, the low density lipoprotein receptor-related protein (LRP) also stains SP, and proposed that apoE acts as a transport mechanism for Aß. We have now studied apoE and LRP in CSF. Two new results are evident: Surprisingly, LRP is present in CSF, as a novel shed form of the receptor. This form appears to contain the extracellular ligand binding domain but not the intracellular internalization domain of LRP. Second, in addition to being present as monomers, apoE2 > apoE3 exist as large complexes due to disulfide bonds forming homodimers and apoA-II heterodimers; ApoE4 does not form these complexes. Because dimers may bind receptors less well than monomers, this difference may be of biological importance.

445.8

SUPEROXIDE DISMUTASE, AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND ASTROCYTES. S.A. O'Reilly, J. Roedica, D. Nagy, R. Hallewell, K. Alderson, S. Marklund, J. Kuby and P.D. Kushner*. Dept of Biol, San Francisco State Univ, San Francisco, CA, Dept of Mol Biol, Imperial Col, London, England, Dept of Neurol, Univ of Utah Sch of Med, Salt Lake City UT, and Dept of Clin Chem, Umea Univ, Umea, Sweden.

Copper, zinc superoxide dismutase (SOD1) is involved in neutralizing free radicals within cells and has recently been shown to be defective in some familial ALS cases. In this study, we have analyzed SOD1 in sporadic ALS with activity assays and immunocytochemisty. We found no difference in erythrocyte SOD1 activity between 13 ALS cases and 4 controls. Spinal cord sections from 6 ALS cases, 1 primary lateral sclerosis (PLS) case and 1 control case were stained using 3 different antibodies to SOD1; since astrocytes are involved in neutralizing free radical damage, antibodies to glial fibrillary acidic protein (GFAP) and vimentin were used as independent monitors of astrocytes. Our three principal findings from localizations are 1) ALS spinal cord displayed a reduction or absence of SOD1-reactive astrocytes, compared to the control and PLS cases, and 2) examination of GFAP-stained sections showed that, besides the reactive gliosis in the lateral tracts and ventral white and gray matters in ALS and PLS cases, there was a close relationship between astrocytic processes and motor neuron somata in the control case, which was decreased in the ALS and PLS cases and confirmed with morphometry. 3) Vimentin-positive astrocytes were found either in the lateral tracts or in the ventral white matter or in both, in all ALS cases; the case of PLS had none. We conclude that an absence of detectable SOD1 in subpopulations of spinal cord astrocytes and alterations of the motor neuron-glia relationship may play an important role in ALS.

445.9

ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY IN SPORADIC AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS. S. Przedborski*, D. Donaldson, O. Hirsch, D.J. Lange, N. Latov, P.L. Murphy and L.P. Rowland. Department of Neurology, Columbia University, New York, NY 10032

Free radicals have been implicated in several neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, and more recently amyotrophic lateral sclerosis (ALS). Reduced activity of superoxide dismutase (SOD), an enzyme that protects the cell against free radical oxidative damage, has been detected in some patients with the familial form of ALS (FALS) as a result of gene mutations. Thus far, whether SOD activity is changed in patients with the sporadic form of ALS (SALS) is unknown. We have therefore measured the levels of erythrocyte SOD activity in 29 SALS patients, 4 FALS patients and 19 normal controls by a spectrophotometric assay. We found that erythrocyte SOD activity was significantly reduced ($F=7.55$, $p=0.014$) in both SALS (mean ± SEM, 934.88 ± 61.54 U/mg protein; -19%) and FALS (529.88 ± 60.90 U/mg protein; -54%) patients compared to controls (1161.57 ± 71.02 U/mg protein). Values of SOD activity measured in both controls and FALS are closely clustered around their respective means. In contrast, in SALS the distribution of SOD activity seemed to be in two clusters: one in the range of normal activities (n=18, 1139.66 ± 55.33 U/mg protein) and a second (n=11, 599.80 ± 55.33 U/mg protein) close to FALS values. Although, additional investigation is required to clarify the underlying mechanism responsible for the bimodal distribution of SOD activity in SALS as well as for the reduction in SOD activity in some of these patients, our data support the hypothesis that free radicals may play a role in the sporadic form of the disease. Supported by Grants from the Muscular Dystrophy Association and NINDS (1-K08-NS01724-01)

445.10

IN VIVO NEUROCHEMICAL ABNORMALITIES IN HUNTINGTON'S DISEASE: EVIDENCE FOR A METABOLIC DEFICIT? WJ Koroshetz, BG Jenkins, BR Rosen, MF Beal. Neurology and radiology, Massachusetts General Hospital, Harvard Medical School, Boston Ma. 02114

Huntingtons disease (HD) is a genetic neurodegeneration that preferentially affects the striatum. Patients with Huntington's disease appear hypermetabolic with large caloric requirements, atrophy of body fat and muscle. We have searched for a neurochemical evidence consistent with a generalized metabolic defect.

^1H magnetic resonance spectroscopy demonstrated that persons with clinical Huntington's disease have elevated lactate levels in striatum and occipital cortex. Persons who are assymptomatic but gene positive have normal lactate levels in the occipit al cortex; but lactate may be elevated in striatum. Photic stimulation caused a 3 fold increase in occipital lactate in controls but a decrease of occipital lactate (50% decrease) in HD. 12/14 HD pts treated with ubiquinone demonstrated a lowering of occipital lactate (29% decrease). In some, treatment with riboflavin/nicotinamide also lowered brain lactate levels. Blood lactate or pyruvate levels were normal. CSF lactate/pyruvate ratios were mildly elevated ($12.67\pm$ 0.38 HD vs 15.82 ± 1.03 controls). Ratios of resting organic/inorganic phosphate measured by MRS in calf muscle are decreased in HD affected (6.5 ± 1.4 HD vs. $9.8\pm$ 1.4 control).

Many of these abnormalities occur in patients with known mitochondrial disorders. We hypothesize that elevated lactate may be a measure of a pathologic metabolic strain leading to neuronal death and pharmacologic stratagies which lower lactate might slow the neurodegeneration.

CELL LINEAGE AND DETERMINATION II

446.1

MOLECULAR CLONING OF PROTEIN TYROSINE KINASES FROM AN IMMORTALIZED, DIENCEPHALIC CELL LINE AND DIFFERENTIATION BY TROPHIC FACTORS. K. Shimoda*[1], T. Kitagawa[1], H. Takahashi[2] and JW. Commissiong[3]. [1]Div. Neurol., Natl., NishiTottori Hospital, Tottori 68902, Japan, [2]Dept. Path. and Microbiol., Tokyo Metropol. Inst. Geront., Tokyo 163, Japan. [3]NTU, LMCN, NINDS, NIH; Bldg 10/5N214, Bethesda, MD 20892.

Diencephalic cells from the brain of the E13 rat fetus were immortalized by transfection with the SV40 LT antigen temperature sensitive mutant, carried by a retroviral vector. Degenerate, oligoprimers were proliferated by the PCR method, and used to clone protein tyrosine kinases (PTK). We sequenced the clones from one cell line that we designated S1, that was cultured at the permissive temperature (39-40°C) and identified the following PTKs:Jak1, Jak2, bFGFR, hck/bmk and Tyro10. At the non-permissive temperature (33-35°C), when the cells were cultured with bFGF or IL-3(both are coupled to Jak2), the cell extended processes and differentiated to a neuron-like morphology. These results strongly suggest that PTKs may be involved in controlling cell growth and differentiation in this S1 cell line. They may also have a similar function during normal development of the CNS. This S1 cell line may become a useful model for studies involving the molecular aspects of neuronal differentiation and signal transduction.

446.2

REGULATION OF NEURONAL DETERMINATION GENES IN PC12 AND P19 CELLS. P.S. Issack*, and E.B. Ziff. Dept. of Biochemistry, New York Univ. Sch. of Med., NY, NY 10016.

The role of neuronal determination genes in maintaining the neuronal phenotype was assayed by northern analysis. cDNAs of several genes important in neurogenesis were used to probe RNA from PC12 rat pheochromocytoma cells, P19 murine embryonal carcinoma cells, and E1a- or Wnt-1 transformed PC12 cells. The above cell lines are considered model systems of neuronal determination, differentiation and tumorigenesis respectively. We have observed a complete down regulation of the mRNA of the neuronal determination basic-helix-loop-helix factor MASH-1 in E1a-transformed PC12 and Wnt-1- transformed PC12 cells; expression of MASH-2, another bHLH factor, is unaffected. This is consistent with the hypothesis that oncogenic factors may disrupt the neuronal phenotype by repressing neuronal determination genes which may function to maintain the neuronal phenotype. We are currently analyzing (1) the possible cell-state specific ability of the MASH proteins to transactivate from a reporter construct containing a multimerized E box, a sequence which specifically binds bHLH proteins (2) the effects of abolishing MASH-1 expression in PC12 cells by antisense and (3) the effects of overexpressing MASH-1 in E1a- and Wnt-1 PC12.

446.3

CHARACTERIZATION OF THE RT4 NEURONAL CELL LINES. M.A. Turnbow* and L.M. Donahue. Dept. of Cell Biology and Anatomy, Texas Tech Univ. H.S.C. Lubbock, TX 79430.

We are interested in the molecular mechanisms whereby multipotential stem cells in the mammalian nervous system give rise to differentiated daughter cell types. To study differentiation we are using an *in vitro* system called RT4: a family of four cell lines isolated from an ethylnitrosourea-induced rat peripheral neurotumor. The stem cell spontaneously converts in culture to three derivative cell types: two are neuronal and one is glial. Although the RT4 glial cell line has been well characterized, much less is known about the neuronal lines which were originally classified as neuronal based on (1) the ability to fire action potentials and (2) the morphology of the long neurite-like processes they extend upon treatment with cAMP and steroids. Recently we discovered that the neuronal derivatives express an unusual voltage-sensitive sodium channel, the cardiac or SkM2 channel. The SkM2 channel gives rise to a sodium current that is relatively resistant to tetrodotoxin (TTX). Based on the fact that the RT4 neuronal derivatives express SkM2 but do not express neurofilament proteins, we hypothesized that they might be related to small cell neurons from dorsal root ganglia (DRG). Small cell DRG neurons do not express neurofilament proteins and have TTX-insensitive sodium currents. Therefore, we analyzed the following sensory neuron markers in the RT4 cell lines: peripherin, CGRP, KH10, LA4 and Brn-3. All were negative. We have since extended our analysis to include markers for adrenergic, cholinergic and serotonergic neurons. In addition, we are examining the cells for the expression of markers of very immature neural cells such as the intermediate filament protein nestin.

446.4

MULTIPLE TROPHIC SIGNALS ARE REQUIRED FOR DIFFERENTIATION OF OLIGODENDROCYTE PRECURSORS DERIVED FROM AN IMMORTALIZED NEURAL STEM CELL LINE. R. Marmur, M. F. Mehler, S. M. Crain*, and J. A. Kessler. Depts. of Neurology & Neuroscience, Albert Einstein Coll. of Med., Bronx, NY 10461

The trophic signals and intracellular transduction pathways that mediate oligodendroglial development are still largely unknown. To establish an *in vitro* model system to investigate these issues, we utilized a clonal, conditionally-immortalized cell line (MK-31), derived from embryonic day 17 mouse hippocampus, that exhibits neural stem cell characteristics. MK-31 has previously been shown to acquire a neuronal phenotype following application of specific subsets of cytokines (Mehler et al., *Nature*, 1993). In the current study, we test the hypothesis that the differentiation and survival of oligodendrocyte (OL) precursors derived from MK-31 stem cells is dependent on signaling from a specific subset of growth factors (GF), as suggested by Barres et al. (1993). Cells grown in the absence of GF, at non-permissive conditions (39°C), gave rise to small subpopulations of rounded cells that extend radial processes and exhibit 04 immunoreactivity, typical of pro-OLs (Pfeiffer et al, 1993). These pro-OLs normally die after several days in culture. Application of a limited subset of GFs from several subclasses: bFGF, PDGF-AA, IGF-1, NT-3 and the IL6/LIF family significantly increased survival and also promoted the differentiation from stem cells of a distinct OL precursor population with bipolar morphology, that initially exhibits GD3 and A2B5 immunoreactivity, characteristic of oligodendroblasts (OBs). These OB cells gradually extend radial processes typical of pro-OL cells and subsequently acquire immunoreactivity for the Ranscht monoclonal antibody, indicative of pre-OLs. Some of these pre-OLs progress to the immature OL stage and exhibit galactocerebroside immunoreactivity. These observations establish an *in vitro* model system for molecular analysis of graded stages of early oligodendroglial differentiation.

446.5

PREFERENTIAL EXPRESSION OF MEF2C IN INHIBITORY INTERNEURONS IN HUMAN CEREBRAL CORTEX. D. Leifer* and K. Wehr. Dept. of Neurology, Yale University School of Medicine, New Haven, CT.

MEF2C (myocyte-specific enhancer binding factor 2C) belongs to the MADS family of transcription factors, is expressed in muscle and brain, and activates transcription by binding to the MEF2 element, a DNA regulatory element found in certain genes expressed in muscle and brain (Leifer et al., PNAS 1993; 90:1546-50). MEF2C is present at high levels in the cerebral cortex where it is found in post-mitotic neurons that have migrated to the cortical plate. We have now studied MEF2C in human brain samples resected during surgery for intractable epilepsy. Strongly MEF2C-immunoreactive (IR) nuclei occur predominantly in layers II, IV, and VI, and are also scattered in layers III and V, where more numerous weakly reactive nuclei are found. Some MEF2C-IR nuclei are present in layer I. Many of the strongly MEF2C-IR cells that predominate in layers II, IV and VI and the rarer strongly MEF2C-IR cells in layers III and V are also labeled with antibodies against parvalbumin or calbindin, calcium-binding proteins found in GABAergic inhibitory interneurons. These cells have a variety of non-pyramidal morphologies. Many of the weakly MEF2C-IR nuclei in layers III and V are in pyramidal cells, as determined by double labeling with SMI-32, a monoclonal antibody against nonphosphorylated neurofilaments found in pyramidal cells.

These results indicate that the level of MEF2C immunoreactivity correlates with neuronal phenotype and that strong immunoreactivity is present in inhibitory interneurons. The level of MEF2C expression may therefore have a role in regulating genes that control neuronal phenotype.

446.6

HIGHLY RESTRICTED EXPRESSION PATTERN OF A NOVEL ZINC FINGER PROTEIN, *ZIC*, IN CEREBELLAR GRANULE CELL LINEAGE. J. Aruga[1], N. Yokota[1], and K. Mikoshiba[1,2*] 1) Lab. of Mol. Neurobiol., Tsukuba Life Science Center, RIKEN, Tsukuba Ibaraki Japan 2) Institute of Medical Science, Univ. of Tokyo

Cerebellar granule cells are generated from the unique neuroblast-proliferative zone, the external germinal layer (EGL). To clarify the mechanism underlying the generation of the EGL and the subsequent differentiation process to cerebellar granule cells, we have cloned a gene, named *zic* as a mlecular marker for EGL-granule cell lineage. *zic* encodes 48kDa protein with a tandem repeat of five zinc finger motives. It showed a signficant homology to *Caenorhabditis elegans* sex determining gene *tra-1*, *Drosophila* segment polarity gene *cubitus interruptus Dominant* and human *GLI* oncogene.

In situ hybridization and immunohistochemical study revealed that *zic* was expressed abundantly in the EGL-granule cell lineage throughout the development of cerebellum. Before the formation of EGL, *zic* was expressed in the neuroblast proliferative zone which faces the fourth ventricle and the area of cerebellar anlage adjacent to the primordium of choroid plexus. It is expressed at an early embryonic stage in the dorsal half of the neural tube. Furthermore, the bacterially expressed fusion protein of *zic* containing the zinc finger domain showed a sequence specific DNA binding activity. The co-transfection assay using the *zic* expression vector and *zic* protmoter-driven luciferase gene indicated that *zic* acts on its own promoter.

These findings suggest that *zic* is a member of transcription factors involoved in both differentiation and maintenance of properties of the cerebellar granule cells.

446.7

CLONING AND CHARACTERIZATION OF RAT PBX1, A HOMEOBOX GENE DEVELOPMENTALLY REGULATED IN THE CNS. M.A. Morabito*, S. Hockfield and L. Redmond. Section of Neurobiology and Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510

In CNS development a homogeneous neuroepithelium gives rise to a multitude of cell types as the result of cell proliferation, differentiation and migration. The regulation of these processes is controlled by many genes whose expression is both temporally and spatially restricted during development. This specificity of expression is presumably the result of expression and activation of transcription factors. Recently a novel group of human homeobox genes, PBX1, 2 and 3, have been identified. They show a high level of divergence from previously reported homeodomains, and only 36% identity to yeast MATa1. Expression of PBX genes was observed in a variety of adult and fetal tissues and PBX1 is expressed abundantly in human fetal (18-22wk) brain (Monica, et al., 1991, Mol. Cell. Biol. 11, 6149-6157.).

To characterize the expression of PBX1 in the mammalian CNS and to investigate its putative role in nervous system development, the rat homologue of PBX1 was cloned by PCR amplification from an adult rat brain library using synthetic oligonucleotides. The clone obtained was used in Northern and in situ hybridization analysis of embryonic and postnatal rat brains. Both studies revealed expression of rat PBX1 in the CNS over the course of embryonic and postnatal development. Rat PBX1 expression is observed in the CNS at embryonic day 15 (E15), peaks in expression postnatally, and is subsequently down regulated in the adult. Rat PBX1 is detected in the cortex at all ages and in the olfactory bulb postnatally. Several structures in the midbrain, diencephalon and cortex show transient expression during development.

The temporal and spatial pattern of expression of rat PBX1 mRNA suggests a role for this homeodomain protein in mammalian brain development. (Supported by NS22807 to SH)

446.8

MOLECULAR CLONING OF CHICK BRAIN-3.0 AND THE DISTRIBUTION OF ITS mRNA DURING DEVELOPMENT OF THE CHICK NERVOUS SYSTEM. Jonas Lindeberg, Reg Williams, Anders Bäckström*, Finn Hallböök and Ted Ebendal. Department of Developmental Biology, Box 587, Uppsala University, S-751 23 Uppsala, Sweden.

Brn-3.0, a member of the POU transcription factor family, is mainly expressed at high levels in sensory neurons and retina. We wanted to isolate the chicken homologue to rat Brn-3, investigate its mRNA expression pattern development and to examine possible correlation between Brn-3 mRNA and the expression of the neurotrophin high-affinity Trk receptors. Using degenerate oligonucleotide primers and PCR we isolated the chicken Brn-3.0 from spinal ganglia cDNA. This fragment was then used to screen cDNA libraries and a chicken genomic library in order to get the full length sequence promoter and gene structure. *In situ* hybridization histochemistry was performed on consecutive sections using probes specific for the detection of mRNA for Brn-3.0 and for the neurotrophin high-affinity receptors. Brn-3.0 was expressed within neurons of many of the cranial ganglia, specifically localized to neurons derived from the epibranchial placodes, and generally absent from neurons derived from the neural crest. TrkC mRNA and Brn-3.0 mRNA were expressed in the same populations of neurons at all stages of chicken development. Whereas the expression of trkC mRNA appeared to decrease with age, no decrease in the expression of Brn-3.0 was observed. At the earliest stage examined (stage 13) Brn-3.0 mRNA expression was quite strong in the presumptive neuroblasts migrating from the trigeminal placode, while the expression of trkC mRNA was just beginning. This indicates a possible influence of Brn-3.0 on trkC expression in trigeminal placode derived neuroblasts.

446.9

THE REGULATION OF NESTIN EXPRESSION R. McKay*, T. Hayes, R. Lin #, K Johe, O. Brustle, T. Hazel, C. Vicario, R. Josephson, LMB/NINDS, Bldg36, Rm3D02, NIH, Bethesda, MD 20892; # Physiology & Biophys., Hahnemann U., Philadelphia.

Expression of the intermediate filament protein nestin is characteristic of stem cells in the mammalian CNS. This conclusion is based on extensive in vivo analysis of the expression of both the endogenous protein and the identification of a tissue specific enhancer in the nestin gene. Nestin is down-regulated in primary cultures of hippocampal cells that differentiate to express neuronal markers. Nestin is also expressed in glial precursor cells. In the adult brain, re-expression of nestin has been observed in reactive astrocytes and in neuroepithelial tumors. We are currently focussing on the identification of regulatory sequences controlling nestin expression. Both gel shift analysis and the generation of transgenic mice are being used to determine whether the same DNA elements are regulating transitions in nestin expression in different cell types..The identification of these regulatory elements in the nestin gene will establish whether the expression of nestin in different cell types reflects the action of common mechansisms.

446.10

RESTRICTED CLONAL ALLOCATION IN THE CHIMERIC MOUSE BRAIN C. Kuan*, E. Elliott#, R. Levenson+, R. Flavell#, and P. Rakic Sect. Neurobiol., #Sect. Immunobiol., +Dept. Cell Biol., Yale School of Medicine, New Haven, CT 06511

Lineage analysis in *Drosophila* wing development revealed restricted clonal allocation and led to the concept of compartmentalization. Whether similar principles apply for embryogenesis of the vertebrate nervous system remains an issue under debate. Here we address this issue by examining distribution of clonally related cells in chimeric mouse brains. Stable transfected embryonic stem(ES) cell clones were generated to express *lacZ* gene driven by chick beta-actin promoter as lineage marker. Mouse blastocyst embryos were injected with such *lacZ*(+) ES cells, transferred to foster mother mice, bred to term and sacrificed at postnatal day 10 for examination by X-gal histochemical staining. Although X-gal positive and therefore clonally related cells were intermingled with X-gal negative cells in the chimeric brain, the overall distribution was not random but highly regionalized. Each animal exhibited a distinct subset of brain regions colonized with X-gal(+) cells, and within each colonized structure the density of marked cells could be highly enriched compared to surrounding tissues. Furthermore, a bilateral symmetry in distribution and density of X-gal(+) cells was a prevailing feature in all examined animals. Thus, for example, when a group of X-gal(+) cells were localized in CA2 region of hippocampus or in a subcortical nucleus, a mirror image of distribution was also observed in the opposite side of brain. Taken together, the regionalized and bilaterally symmetric distribution of clonally related cells in chimeric mice suggests a restricted and precise clonal allocation exists in the vertebrate nervous system.

446.11

IN VIVO CHARACTERIZATION OF PROLIFERATING CELLS IN ADULT RAT WHITE MATTER. J. M. Gensert and J. E. Goldman*. Dept. of Pathology, Columbia University, New York, NY 10032.

Dividing cells persist in the adult mammalian CNS. Although these cells have been presumed to be immature glia, their nature has not been fully characterized. Furthermore, their developmental fates and migratory potential are not understood.

Proliferating cells in adult rat brain subcortical white matter were examined *in vivo* using stereotactic injections of a replication-deficient retrovirus that expresses ß-galactosidase. Cells were visualized by X-gal histochemistry or by immunocytochemistry with anti-ßgal antibodies. These cells exhibit an immature morphology and are unipolar or contain very few or no detectable processes. There are no clear astrocyte forms in the population of cells labeled, and these cells do not express the astrocyte markers glial fibrillary acidic protein (GFA), glutathione-S-transferase Y_b, or S100ß as determined with immunohistochemical techniques. A very small subset of these cells, however, does express vimentin, and a significant subpopulation expresses an isoform of glutathione-S-transferase that is oligodendrocyte-specific, Yp. However, neither of two other antibodies used to identify oligodendrocytes (carbonic anhydrase and RIP) gave a positive signal, nor had the cells produced any apparent myelin sheaths. Since the initial population of labeled cells resides only in a small area, migration of these cells can be followed. The cells, however, do not appear to migrate from the initial area of labeling, and furthermore, they continue to exhibit a simple morphology when followed *in vivo* for 30 days. Our results suggest that we are labeling a heterogeneous population of immature cells, some of which have begun to develop through an oligodendrocyte lineage pathway. It remains to be determined if these cells have the potential to differentiate into myelinating oligodendrocytes *in vivo* when provided the appropriate signals. Supported by NS17125.

446.12

HAIR CELLS AND SUPPORTING CELLS SHARE A COMMON PROGENITOR IN THE DEVELOPING CHICKEN INNER EAR S. Muthukumar and D.M. Fekete*. Dept. of Biology, Boston College, Chestnut Hill, MA 02167.

The basilar papilla, the avian counterpart to the mammalian organ of Corti, consists of a contiguous mosaic of hair cells and supporting cells. The present study sought to establish the lineage relationship between these two cell types. Individual progenitor cells were labeled by targeting replication-incompetent retroviruses encoding a marker gene (alkaline phosphatase) into the otocyst on embryonic day 3 (E3) or E4. In most cases, a library of avian retroviruses with $>10^5$ complexity was used so that clonal assignments could be verified later by PCR amplification and DNA sequencing (Cepko, Fields-Berry and Golden, unpublished). Tentative clonal assignments were made on the basis of close grouping of labeled cells in E18-20 whole mounts of the basilar papilla. These were sectioned to determine cell types. The average clone size was 4.8 $\pm$ 2.6 cells (range 1-10; n=24). The ratio of hair cells to supporting cells was 1:3.5, which is close to the overall ratio of these two cell types. 20% of the clones consisted of supporting cells only. The remainder had a mixture of hair cells and supporting cells. Since there was only a single 2-cell clone (both supporting cells) in the data set, it was not possible to determine whether a progenitor can give rise to both a hair cell and a supporting cell at the final mitotic division. In no case did a clone contain more hair cells than supporting cells. Thus it is not yet possible to distinguish between a stochastic mode of cell type determination versus an obligatory mechanism in which a hair cell is always generated in concert with a supporting cell. Supported by the March of Dimes and a Clare Boothe Luce Professorship (to DMF).

446.13

EMX2 IS SELECTIVELY EXPRESSED IN GERMINAL NEUROEPITHELIUM OF DEVELOPING DORSAL TELENCEPHALON OF THE MOUSE. M. Gulisano*[1,2], V. Broccoli[1] and E. Boncinelli[1] [1]Dibit, Scientific Institute H San Raffaele, Via Olgettina 60, 20132 Milano and [2]Istituto di Biologia Generale, Università di Catania, 95124 Catania, ITALY

We recently cloned two vertebrate homeobox genes, Emx1 and Emx2, related to *empty spiracles*, a gene controlling very anterior body regions during early *Drosophila* embryogenesis.

Both vertebrate genes are expressed in the brain of mouse embryos. They are expressed in the embryonic cerebral cortex in a developmental period, between day 10 and day 16 post coitum, corresponding to major events in cortical neurogenesis. In its full extension, days E12.5 to E13.5, their expression domain comprises cortical regions including promordia of neopallium, hippocampal and parahippocampal archipallium. In particular, Emx1 expression seems restricted to cortical regions, mainly if not exclusively hexalaminar in nature. At day E17, cortical expression of the two genes is almost exclusively confined to hippocampal germinal layers. It seems reasonable to speculate about a role of Emx1 and Emx2 in establishing the limits and identity of the embryonic cerebral cortex. In addition to presumptive cortex, Emx2 is also expressed in some embryonic neuroectodermal areas including olfactory placodes and olfactory epithelia.

We have now studied in detail their expression pattern in relation to cortical neurogenesis in E12-16 mouse embryos. Preliminary data show that Emx2 is expressed in germinal neuroepithelium but neither in the cortical plate nor in the so-called transition field. Conversely, Emx1 is expressed in both the neuroepithelium and the transition field.

PROCESS OUTGROWTH IN RETINA AND RETINAL PROSECTION SITES

447.1

DETECTION OF HIGH MOLECULAR WEIGHT MATERIAL IMMUNO-REACTIVE WITH ANTIBODIES TO THE 72 kd GELATINASE IN EMBRYONIC CHICK RETINA. H. Peng, P. Alexander[1], and J.B. Sheffield*. Department of Biology, Temple University, Philadelphia, PA 19122 and [1]Department of Ophthalmology, University of Oregon, Portland, OR 97201.

In previous studies, we have detected extracellular proteases in embryonic chick retinal cell culture supernatants and pellets by zymograms and substrate digestion. We have now used western blotting techniques to further characterize the enzymes in retinal tissue extracts. Embryonic chick retinal tissue ranging from E8 to E18 days were dissected and transferred to a Tris buffer pH 7.6 containing 0.5 M NaCl, 0.05% Brij-35, 20 mM PMSF, and 10 mM $CaCl_2$. The tissue was homogenized in 0.15 M NaCl Tris-buffer and centrifuged at 1,000 rpm for 10 minutes, and then at 50,000 rpm for 10 minutes. Western blotting was performed using an avidin-biotin-peroxidase-luminol technique. Specific antibodies made against peptides of human stromelysin and 72 Kd gelatinase and purified by affinity chromatography, as well as commercial antibodies against α-1-antitrypsin and α-2-macroglobulin were used. The extract from all ages stained intensely with antibodies to 72 Kd gelatinase. The primary band of reactivity was at an apparent molecular weight of about 230 KD. Analysis of a possible 72kd band was obscured by reaction of the extracts with avidin. Densitometry indicated that the staining intensity for the 230 Kd band increased from E8 to E10, by a factor of two, and then decreased gradually from E12 to E18. No reaction was found with antibodies to human stromelysin, α-1-antitrypsin, α-2-macroglobulin. The high molecular weight of the band that we detected may be due to a complex between the enzyme and an endogenous inhibitor.

447.2

EFFECTS OF ADDED GLYCOSAMINOGLYCANS ON NEURITE OUTGROWTH FROM REGENERATING GOLDFISH RETINAL EXPLANTS. J. S. Elam* and J. F. Challacombe. Program in Neuroscience and Department of Biological Science, Florida State University, Tallahassee, FL 32306.

Proteoglycans (PGs) and their glycosaminoglycan (GAG) chains have been shown to modulate neurite outgrowth during development of the nervous system. That PGs are multifunctional growth-regulatory molecules is becoming increasingly apparent, but few studies have focused on the functions of PGs during regeneration. Our laboratory has demonstrated that inhibition of PG synthesis by addition of 4-methylumbelliferyl-β-D-xyloside to the culture medium of goldfish retinal explants reduces regenerative axonal outgrowth on poly-L-lysine (PLYS), but not on poly-L-lysine+laminin (PLYS+LN) (Challacombe and Elam, Soc. Neurosci. Abs. 19, 877, 1993).

To extend this study, explants growing on both PLYS and PLYS+LN substrates were exposed to soluble chondroitin 4-sulfate (CS), while heparan sulfate (HS) was added to explants on PLYS. The data show that CS dose-dependently stimulates regenerative axon growth on both substrates, while the effects of HS depend on the concentration added to the culture medium. Low doses of soluble HS stimulated outgrowth, while at a high concentration, inhibition was observed. These findings suggest that PGs containing CS and HS GAG chains participate in events underlying the regeneration of goldfish optic axons following injury.

Supported by NIH Grant NS20502

447.3

DYNAMICS OF RETINAL GROWTH CONES ENCOUNTERING THEIR TARGET IN CO-CULTURE: A TIME-LAPSE STUDY. A. El-Bohy*, D. F. Chen, G. E. Schneider & S. Jhaveri, Dept. Brain & Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

Our previous studies showed that in explant co-cultures, retinal axons from embryonic hamster grow readily into tectal slices prepared from animals of all ages, including adult; however, retinal axons reveal different patterns of growth when challenged with tectal slices from animals of different ages. In embryonic tectum, embryonic retinal fibers elongate with very little branching; whereas in postnatal and adult tectum, significantly more ramifications are observed. In order to further characterize the growth of embryonic retinal axons in targets of different ages, we have used time-lapse video microscopy to examine the morphology of retinal growth cones as they encounter and begin to invade target tissue. Explants of retina and tectum were maintained in serum free medium for 15 - 72 hr. Crystals of DiI were placed in the retina 10 hr prior to the beginning of observations. Images of labeled retinal axons were recorded on a low-light SIT camera every 2-4 min for a period of 5-8 hr. In general, fibers from E14 retina growing into E14 tectum have simple growth cones which form swellings at the tip of the axons and have few filopodia. Significantly more growth cones were seen extending along other labeled axons than in the older tissue. When retinal explants were co-cultured with postnatal or adult tectum, the growth cones were typically larger (2-5 times those in embryonic tectum), more lamellate, and had longer, more numerous filopodia. Over the 8-hr observation period, some filopodia were observed to stabilize and become collaterals on trunk axons once the growth cone moved ahead. Our results suggest that the shape of growth cones on afferent fibers and the number of filopodia decorating them reflect the patterns of axonal growth, which are dependent on the maturational state of the target substrate. (Support: NIH grants T32-MH15761, EY05504, EY00126, EY02621).

447.4

CELL LINES GENERATED FROM AVIAN EMBRYONIC RETINA BY ONCOGENE-TRANSFECTION EXHIBIT MATURE GANGLION CELL CHARACTERISTICS AND RESPOND TO POSITIONAL CUES OF TECTAL MEMBRANES IN VITRO. G. E. Pollerberg*, C. Kuschel, B. Eickholt, M. Zenke, H. Beug & U. Schwarz, Dept. of Biochemistry, Max-Planck-Institute for Developmental Biology, D-72076 Tuebingen, Germany

We generated cell lines by transfecting quail embryo retina with a retroviral construct containing v-myc fused to the hormone-binding domain of the estrogen receptor, allowing for the activation of the chimeric protein by estradiol. The oncogene myc was chosen because of its proliferation-inducing capacity and differentiation compatibility. The infection was performed by cocultivation of virus-producing, proliferation-incompetent fibroblasts with embryonic day 3.5 quail eyes. In these organ-cultured retinae, cells proliferate and differentiate within a time span of less than 20 hours, a prerequisite for both the generation of stable as well as neuronally differentiated cell lines. The transfected neuroretinae were separated into nasal and temporal parts, dissociated, and kept as single-cell cultures. Individual cell colonies were isolated and expanded; these cell lines have been maintained in culture over 6 months with a doubling time of approximately 20 hours and can therefore be considered immortal.

Spontaneously differentiating cells were observed within the cell line cultures, exhibiting morphological features of mature retinal neurons in vitro. Some of these differentiated cells displayed the morphological characteristics and marker expression of mature retina ganglion cells. The differentiation rate could be substantially increased by the administration of retinoic acid or basic fibroblast growth factor. The differentiated cells maintained functional properties of ganglion cells as revealed by a substrate choice assay. When confronted with substrate stripes of membrane vesicles prepared from target and non-target parts of the tectum, the axonal processes of temporal lines preferred to grow on target stripes, indicating that the cell lines will be useful for the investigation of mechanisms underlying axon-target interactions.

447.5

GROWTH PATTERNS OF CHICK RETINAL AXONS IN RETINA-TECTUM COCULTURES. Stefan Meyer* & Friedrich Bonhoeffer, Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35/I, 72076 Tübingen, Germany.

During retinotectal map formation in the chick, growth of temporal axons is restricted to the anterior half of the tectum, whereas nasal axons invade the posterior tectum (Nakamura and O'Leary, 1989). Innervation of a terminal zone is achieved by sidebranch formation and subsequent elimination of overshooting parts of the axon. It is assumed that a repulsive guidance molecule (RGM) expressed predominantly in the posterior tectum prevents growth of temporal axons into this region (Walter et al., 1987).

In order to investigate axonal growth and branching in vitro, we developed a co-culture assay of retina with tectum. DiI-labeled pieces of retina were cultured together with a piece of anterior and posterior tectum on opposite sides in a collagen matrix. Growth patterns of labeled axons were investigated after 48 hours or continually by using time lapse video microscopy. As in vivo, temporal axons did not invade posterior tissue, but grew and branched in anterior tissue. Nasal axons grew into and branched in both tissues. These results indicate that failure of temporal axons to invade the posterior tectum is linked to repulsive molecules and not due to attraction by the anterior tectum. When cocultures were treated with the enzyme PI-PLC, temporal axons could invade posterior tissue. This is consistent with the earlier findings that RGM is GPI-linked.

In a different paradigm, both tectal pieces were placed on one side of the explant. Thus, axons first grew through anterior tissue before they reached posterior tissue. We found that temporal axons were restricted to the anterior tectal piece, whereas nasal axons continued to grow into posterior tissue. Time lapse analysis revealed that temporal growth cones collapsed after they reached the A-P border. We are presently investigating whether such encounters with repulsive tissue can also lead to the formation of new sidebranches at a distance from the growth cone.

447.6

MOLECULAR CHARACTERIZATION OF REGGIE-ANTIGEN: A GROWTH ASSOCIATED CELL SURFACE PROTEIN IN THE RETINOTECTAL SYSTEM OF GOLDFISH T. Schulte, F. Lottspeich+ and C.A.O. Stuermer* Faculty of Biology, University of Konstanz, and +MPI for Biochemistry, Munich, Germany

Reggie-Antigen, which is recognised by the monoclonal antibody M802, was identified as a cell surface protein appearing on goldfish retinal ganglion cells (RGCs) and regenerating RGC axons after optic nerve transection (ONS) (Paschke and Stuermer, Soc. Neurosc. Abstr. 1991). In addition to its expression on RGCs and regenerating RGC axons, Reggie antigen is also found on cells lining blood vessels in the CNS and on microglial cells in the injured optic nerve.

Reggie-Antigen was purified by immunoaffinity chromatography with M802 from total membrane fractions of adult goldfish brains and from total larval goldfish (age: 30-60 d).
In SDS PAGE the immunopurified protein has an apparent molecular weight of 48 kD under reducing conditions. We here report cloning of a 300 bp cDNA fragment coding for part of the Reggie-Antigen. It was obtained by PCR using degenerate oligonucleotide primers derived from internal peptide sequences of the immunopurified protein. So far the cDNA clone and the peptide sequences show no homology to known proteins.

Anchored PCR strategy as well as cDNA library screening is now being performed to obtain a full length clone.

447.7

RETINAL GANGLION CELL GROWTH CONE COLLAPSE CAUSED BY NITRIC OXIDE DONORS. R. C. Renteria, C. J. Barnstable, and M. Constantine-Paton.* Yale University, Interdepartmental Neuroscience Program, New Haven, CT 06511.

The amphibian retinotectal projection is a useful system in which to study mechanisms of activity-dependent synaptic development. Chronic application of NMDA-receptor agonists and antagonists to tectal cells *in vivo* is known to cause modification of retinal afferent arbors (Cline and Constantine-Paton, *J. Neurosci.* 10(4):1197, 1990). Nitric oxide (NO), a multifunctional molecule, is a proposed retrograde messenger in the CNS for the activity-dependent modification of synaptic efficacy. A subpopulation of rat retinal ganglion cells contains a putative cGMP-gated cation conductance which is blocked by Cd^{2+} and activated by the NO donors sodium nitroprusside (SNP) and S-nitrosocysteine (SNOC), most likely through NO activation of soluble guanylyl cyclase (Ahmad *et al.*, *Neuron* 12:155, 1994). We have developed a culture system in which *Xenopus laevis* retinal ganglion cells extend neurites from explanted retina on polylysine- and laminin-coated glass coverslips. We show that bath application for several minutes of these nitroso-compounds (100 µM) in normal saline solution causes rapid growth cone collapse. Potassium ferricyanide, cysteine, sodium nitrite (each at 200 µM), and "exhausted" SNOC and SNP had no effect with similar application times. Cd^{2+} and nifedipine both caused collapse of these growth cones in a dose-dependent manner. However, this effect was not seen with 1mM 8-Br-cGMP application, similar to results with DRG neurites (Hess *et al.*, *Nature* 366:562, 1993). Peroxynitrite, a species formed by the reaction of NO with superoxide anion, is known to be cytotoxic to cortical cells in dissociated culture (Lipton *et al.*, *Nature* 364:626, 1993). However, application of SNOC with superoxide dismutase (50 U/mL) to scavenge free radicals and prevent peroxynitrite formation did not prevent the effect. Further investigations will study the mechanism of this nitroso-compound-induced effect on RGC growth cones and the effects of NO on these same axons after they contact cocultured tectal neurons. (R.C.R. is supported by a Howard Hughes Medical Institute Predoctoral Fellowship.)

447.8

RETINOGENICULATE PATHWAY IN THE NORMAL AND DEAFFERENTED MOUSE : A DiI STUDY. R. De Guevara[1], P. Clairambault[1], M. Diagne[1], M.T. Droy-Lefaix[2], G. Pinganaud[1], and L. Bueno*. [1] Neuroembryology Lab., University Paris 7, Paris ; [2] IPSEN Institute, Paris, * INRA, Toulouse, France.

We studied, after placing a cristal of DiI (fluorescent marker) in an experimental eye, the retinogeniculate pathway at 0, 3, 7 and 15 days of postnatal development in the C57BL/6J mouse. We used controls and animals which were monocularly deafferented at birth. Results show that, in the controls, the map of visual projections in the dorsolateral geniculate nucleus is present at D7. Deafferentation causes the loss of this map and an increase in ipsilateral visual projections. Until D7, the retinofugal fibres present many varicosities which thereafter start to regress and at D15 become sparse both in control and deafferented animals. Collateral fibres lead off perpendicularly from the superficial optic tract in the direction of the visual neuropil. They are numerous at D3 and D7 and scarce at D15. We do not observe any difference between controls and deafferented animals. We used the same methods on animals treated with a free radical scavenger (EGb 761, IPSEN). Its seems that at D7, the varicosities on the fibres are slightly more numerous ; the same for the perpendicular collateral fibres of the superficial optic tract.

447.9

TYROSINE PHOSPHORYLATION AT FILOPODIAL TIPS OF VERTEBRATE GROWTH CONES

D.-Y. Wu*, L.-C. Wang, D.J. Goldberg and C.A. Mason, Depts. of Pharmacology & Pathology, Ctr. for Neurobiology & Behavior, College Physicians & Surgeons, Columbia Univ., NY, NY 10032.

We have previously reported that phosphotyrosine is concentrated at filopodial tips of *Aplysia* growth cones, suggesting a role in regulating filopodial function. We have now used immunocytochemistry to show concentrations of phosphotyrosine at filopodial tips of the growth cones of several types of cultured vertebrate neurons: neonatal rat hippocampal neurons, embryonic chick retinal and sympathetic neurons and embryonic mouse retinal ganglion cells. Accumulation of phosphotyrosine at filopodial tips was sensitive to the culture substrate. Increasing the density of laminin on the substrate increased neurite outgrowth from mouse retinal ganglion cells, but decreased the percentage of filopodia displaying strong tip phosphorylation. Concentration of phosphotyrosine at filopodial tips and its sensitivity to the substrate suggest the potential importance of tyrosine phosphorylation in pathfinding decisions.

To identify proteins associated with phosphotyrosine in filopodial tips, we double-stained growth cones with antibodies to phosphotyrosine and each of several proteins known both to associate with actin filaments and to be substrates for tyrosine phosphorylation. Cortactin, integrin and ezrin (or a congener) frequently co-localized with phosphotyrosine. Tensin, vinculin and paxillin, actin-associated proteins found at focal contacts, were observed in lamellipodia around cell bodies, but not in growth cones.

447.10

RETINAL GROWTH CONE RESPONSES TO CELLULAR CUES FROM THE OPTIC CHIASM MIDLINE

L.-C. Wang*, D.-Y. Wu, J. Dani, D.J. Goldberg and C.A. Mason, Depts. Path. and Pharm., Cntr. Neurobiol. Behav., Columbia Univ., NY, NY 10032.

During divergence of retinal axons to both sides of the brain, both crossed and uncrossed fibers initially travel toward the midline, pause for several hours, and either cross the midline or turn back to ispilateral optic tract, respectively (Godement et al., J. Neurosci., in press). To investigate how growth cones detect cues and which cells in the midline provide those cues, we analyzed the behavior of retinal growth cones from dorsal or ventral temporal retina (source of crossed and uncrossed fibers) as they interact with chiasm cells *in vitro* by video microscopy and DIC optics. When axons grew on a polylysine/liminin substrate, they advanced rapidly and had long lamellopodial forms. Within 50 microns of a chiasm cell, growth cones slowed and became more filopodial. After filopodial contact, all growth cones paused in advance, with a single filopodium attached to the cell. Subsequently, the behavior of the two sets of growth cones differed. Uncrossed growth cones displayed a greater degree of collapse, did not grow onto the cells, and took longer to turn away from the cells. Retrospecive immunocytochemistry will indicate the extent to which neural and non-neural chiasm cells inhibit uncrossed growth cones.

To further characterize the intracellular machinery in detection of cues, we are investigating filopodia-specific phosphotyrosines by using retrospective immunocytochemistry of identified growth cones and antibodies to phosphotyrosine. These studies should elucidate the detection and sources of cues leading to retinal axon divergence.

447.11

FACTORS CONTROLLING DENDRITIC ARBORIZATION OF RETINAL GANGLION CELLS.

D. Troilo, M. Xiong , J. Crowley, and B.L. Finlay*Psychology, Cornell University, Ithaca, NY 14853.

The establishment of precise retinal connectivity during development is essential for the retina to transmit visual information. The factors that regulate convergence and divergence during development between bipolar, amacrine, and retinal ganglion cells (RGCs) are poorly understood. We manipulated RGC density and availability of pre-synaptic sites to examine their effects on the development of RGC dendritic arbor.

Visual form deprivation induces ocular enlargement, producing a larger than normal retinal area and a reduction in RGC density of 20-30%. Partial optic nerve section produces areas of RGC depletion with a mean reduction in density of 64%. In the former case, the ratio of pre-synaptic cells to RGCs remains constant, while in the latter the ratio is increased. This contrast allows us to separate the effects of RGC density from pre-synaptic input on RGC dendritic field area and arbor complexity.

In experimentally enlarged retinas, RGC dendritic arbors expand with increased area in a compensatory manner that is consistent with simple stretching; arbor area was about 30% larger than normal, branch density was decreased, and arbor length was about 14% longer than normal. In RGC-depleted retinas, dendritic fields of RGCs are larger than controls but do not fill the space available. Branch density of RGC dendrites in RGC-depleted areas is greater than in controls.

We conclude that both local RGC density and the availability of pre-synaptic terminals control RGC dendritic size and complexity. The limited growth of the RGC dendritic fields in areas with large space availability, together with the increase in arbor branches per unit area, suggest that pre-synaptic input is important in the shaping of RGC arbors postnatally.

(Supported by R01-NS19245 & T32-MH19389)

447.12

DYNAMICS OF DENDRITE DEVELOPMENT IN THE OPTIC TECTUM OF LIVE ZEBRAFISH EMBRYOS.

R.J. Kaethner* and C.A.O. Stuermer. University of Konstanz, D-78464 Konstanz, Germany.

To visualize the development of dendrites in the zebrafish optic tectum, 0.5 - 30pl of the fluorescent dye DiO (in solution) were pressure-injected into the cell rich layer of tecta in 50 - 78hr old embryos. These cells reside deep to the tectal neuropil into which retinal axons enter and where they form their terminal arbors (Stuermer, J Neurosci 8, 1988). Labeled cells and dendrites were viewed from above through the unopened skull of live embryos with low intensity light and a SIT camera (Hamamatsu), and monitored through a time lapse video recording system.

The tectal neurons were observed to send out efferent axons to subtectal regions. Simultaneously they begin to develop their dendrites which grow in a radial orientation towards the developing neuropil. While elongating, the dendrites form branches with growth cone like structures and up to 15μm long filopodia which radiate into all directions. As has previously been described for retinal axons (Kaethner & Stuermer, J Neurosci 12, 1992), dendrites develop through the emission and retraction of filopodia and primary branches over periods up to 17hr. With time some branches stabilize while others are entirely reabsorbed. Gradually, the dendritic arbors increase in diameter up to 30μm in 90hr old embryos.

Thus, developing dendrites of tectal neurons exhibit an exploratory growth behavior similar to that of retinal axon arbors in the developing tectal neuropil. Retraction and extension of processes of dendrites coincides with the dynamic modeling of retinal axon arbors, suggesting that processes of both may contact each other and influence each other's growth and shaping. Supported by DFG, SFB 156, TPC6.

PROCESS OUTGROWTH AND SECOND MESSENGERS

448.1

DEVELOPMENTAL EXPRESSION OF G PROTEINS IN THE EMBRYONIC NERVOUS SYSTEM OF MANDUCA SEXTA.

P.F. Copenhaver*, Michelle Rasmussen, and A.M. Horgan. Cell Biol. & Anat. L215, Oregon Health Sciences University, Portland, OR 97201.

We are investigating the developmental role of G proteins (heterotrimeric guanyl nucleotide binding proteins) during the formation of the CNS in Manduca. This highly conserved family of intracellular signalling molecules is widely distributed in the nervous systems of all organisms, but the potential role of G proteins in development remains poorly defined. Using affinity-purified antisera against different G_α subunits, we characterized the developmental expression of the major classes of G proteins (including proteins related to Go, Gi, Gs, & Gt) during the formation of the embryonic CNS. Whole-mount immunohistochemical staining and protein immunoblots of embryonic tissue revealed that the different G protein classes appeared in precise spatiotemporal patterns within the developing ganglia. Several G proteins were initially seen in subsets of undifferentiated precursors but underwent a subsequent phase of regulation as development progressed, suggesting that individual G proteins may serve a progression of functions during the formation of the nervous system. In particular, while several G proteins could be detected in the mature nerves of the CNS, only Go_α-related proteins could be detected in regions of axonal outgrowth. Preliminary experiments using aluminum fluoride to stimulate G protein activity caused a substantial inhibition of process outgrowth, suggesting that one function of Go may be to regulate the extent or duration of neuronal motility. These results can now be used to examine more specific effects of individual G proteins in the context of identified neurons within the embryonic nervous system. Supported by NSF BNS9010538.

448.2

SECOND MESSENGERS AND GROWTH CONE MORPHOLOGY.

Jane H. Viti, Cynthia Lance-Jones*, and Carl Lagenaur Dept. Neurobiol., Univ. Pittsburgh, Pittsburgh, PA 15261.

Multiple extracellular signals have been shown to contribute to neurite outgrowth and growth cone guidance. Such cues activate intracellular signal transduction pathways which in turn lead to changes in growth cone morphology. Additionally, second messenger systems are likely to influence the ability of the growth cone to interact with specific cell adhesion molecules. Protein kinases and phosphatases might play a crucial role as second messengers which regulate neurite growth and migration. For these studies, mouse cerebellar cells were cultured on three different substrates; L-1, NCAM, and P84. Various kinase and phosphatase inhibitors were then administered to the cells and the effect on growth cone morphology was assessed. Cells grown on L-1 or NCAM which were exposed to genistein, a tyrosine kinase inhibitor, had more filopodia, both at the growth cone and along the neurite. Treated cells also exhibited more side branching of the neurite. When the cells were grown on P84, the effect of genistein was less obvious and the cells resembled those in the control. Interestingly, growth cones on P84 normally have extensive filopodia. The lack of effect of genistein on cells grown on P84 may reflect a difference in the role played by tyrosine kinases in cells growing on different substrates. When cells growing on L-1 were treated with okadaic acid, a serine/threonine phosphatase inhibitor, the growth cones became more lamellopodial or sheetlike in appearance. Okadaic acid-treated cells grown on NCAM seemed to have larger growth cones than the control cells. Okadaic acid did not seem to have an effect on cells grown on P84. Each of the inhibitors affect the cells differently and the effect each drug was substrate dependent. These results support the idea that protein kinases and phosphatases are involved in the regulation of growth cone morphology.

Supported by NIH grant EY05308

448.3

DISTRIBUTION OF PROTEIN KINASE C (α, β, γ SUBTYPES) IN REGENERATING GROWTH CONES OF RAT SCIATIC NERVE. S. Okajima*, Y.Hirasawa, A. Mizoguchi and C. Ide. Dept. Orthopedic Surg., Kyoto Pref. Univ. Med., Kyoto 602 and Dept. Anatomy, Kobe Univ. Sch. Med., Kobe 650.

Protein kinase C (PKC) is an enzyme activated by diacylglycerol resulting from the receptor-mediated hydrolysis of inositol phospholipids and is involved in a variety of functions, including signal transduction, regulation of ion channels and neurotransmitter release, control of cell growth and differentiation, changes in cell morphology, and gene expression. In the present study, distribution of PKC (α, β, γ subtypes) was studied immunocytochemically in the normal nerve fibers and in the regenerating sprouts (growth cones) from nodes of Ranvier following crush injury in rat peripheral nervous system. Sciatic nerves of Sprague-Dawley male rats weighing 200-250 gms were ligated for 24 h and unligated 24 h prior to sacrifice. The animals were anesthetized with ether and perfused with 2% paraformaldehyde in PBS. The injured nerve segment was removed, embedded in OCT compound, quick-frozen, and cut on a cryostat. The sections were immunostained with anti-PKC α, β, γ subtype polyclonal antibodies (GIBCO/BRL) and after incubation in DAB solution containing 0.01% H_2O_2, the sections were observed by light microscopy. For electron microscopy, some selected sections were dehydrated in a graded series of ethanol, followed by QY-1, and embedded in Epon 812. Ultrathin sections were made and viewed on an electron microscope. In the normal nerve, PKC (α, β, γ) immunoreactivity (IR) was present in all myelinated and unmyelinated axons. Electron microscopy showed that IR was localized on the axolemma and in a part of axoplasm. In the injured nerves, growth cones extending through the crushed area exhibited intense and diffuse PKC (α, β, γ)-IR in the axoplasm. Thus, PKC-IR is strong in growth cones and demonstrates a pattern of distribution that differs from that of the parent axon, suggesting that PKC may have important functional roles in axonal sprouting and in the regulation of growth cone activity in the peripheral nervous system.

448.4

Ca^{++} SIGNALS AND NEURITE GROWTH INDUCED BY N-CADHERIN AND LAMININ IN CHICK NEURONS. J.L. Bixby*, G.B. Grunwald+, and R.J. Bookman. Dept. Pharmacology, University of Miami, Miami, FL 33101, & +Dept. Neurobiology, Thomas Jefferson Med. Coll., Philadelphia, PA.

We are interested in the intracellular signals underlying the induction of axon growth by substrate-bound growth-promoting factors. Previous studies indicate that cell adhesion molecules like N-cadherin promote neurite growth through the action of a PTX-sensitive G protein leading to the opening of voltage-sensitive Ca^{++} channels, while integrin-dependent growth does not involve these pathways (Williams et al., J. Cell Biol. 124:1029, 1994). We have examined this hypothesis using chick ciliary ganglion (CG) neurons responding to purified N-cadherin and laminin (LN). When these proteins were used as substrates, neurite growth was insensitive to PTX. However, fura-2 imaging of single cells revealed that soluble forms of N-cadherin were able to induce increases in $[Ca^{++}]_i$ when pressure-applied to neurons growing in vitro. Ca^{++} increases could be seen both in cell bodies and in growth cones. N-cadherin-induced $[Ca^{++}]_i$ increases were relatively insensitive to diltiazem and ω-conotoxin, but were very sensitive to $NiCl_2$. In addition, $NiCl_2$ but not diltiazem and ω-conotoxin, was able to inhibit neurite growth from CG neurons on N-cadherin or on LN. Although we have not yet obtained direct evidence for Ca^{++} increases induced by LN, $NiCl_2$ decreases "resting" intracellular Ca^{++} when added to neurons growing on LN, and we have seen spontaneous transient Ca^{++} elevations in the growth cones of these neurons. Our results are consistent with a role for Ca^{++} signals in neurite growth induced by N-cadherin and by LN, but suggest a revision of currently accepted models of signal transduction involving these proteins.

448.5

LOCALIZATION OF RYANODINE RECEPTORS AT GROWTH CONES OF CULTURED NEURONS **Y. Ouyang*[1], J. Airey[2], J.L. Sutko[2] and M.H. Ellisman[1] [1]San Diego Microscopy & Imaging Resource, Dept. Neurosciences, Univ.Calif.San Diego, La Jolla; [1]Dept. Pharmacology, Univ. Nevada, Reno**

In a previous investigation in chicken cerebellum, a developmental change in the distribution pattern of the beta or cardiac form of the ryanodine receptor (RyR) was observed (Ouyang et al., 1992, Soc.Nsci.). Immunolabeling for the beta or cardiac form of the RyR showed a increase in a staining pattern associated with parallel fibers in the cerebellar molecular layer between one to seven days posthatch. The change might be related to neurite extension in the molecular layer, where the increase may be required to regulate the intracellular calcium storage sites for the growth process. Also, since calcium release from caffeine-sensitive intracellular storage sites was reported to be crucial in NI-35-evoked growth cone collapse (Bandtlow et al., 1993, Science 259:80-3) and the RyR is a calcium release channel sensitive to caffeine, we decided to examine the RyR distribution within growth cones. Using a monoclonal antibody 34C, which recognizes all RyR isoforms in chicken, RyR-like immunoreactivity was observed within growth cones of chicken ciliary ganglion neurons and dorsal root ganglion neurons in primary cultures. Labeling was mainly concentrated in the lamellipodia of the growth cones, but not in the fine filopodia. The pattern of labeling was similar to that observed with alpha-tubulin in double-labeling experiments. This result suggests that the RyR gated calcium storage sites may be associated with the microtubule based cytoskeleton and/or transported into growth cones along microtubules. The IP3 receptor and cardiac SR Ca2+-ATPase, two other proteins involved in intracellular calcium regulation, also exhibited a similar distribution to the RyR. The presence of RyR and other calcium regulatory proteins in growth cones suggests that calcium released from intracellular storage sites may play an important role in the regulation of growth cone dynamics.

448.6

INTERCELLULAR ASSOCIATIONS STABILIZE MORPHOLOGICAL PLASTICITY AND CALCIUM RESPONSIVENESS IN HIPPOCAMPAL NEURONS. C.V. Williams*, P.J. Meberg, S.B. Kater. Dept Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523.

We have previously found that local changes in calcium can result in local morphological changes (i.e. filopodia induction) in isolated *Helisoma* neurons. The present study asked if: 1) local calcium rises can modify dendritic morphology of isolated hippocampal pyramidal neurons in culture and, 2) intercellular associations alter the magnitude of calcium rise and modify dendritic plasticity. Using a focal electric field, both local rises in intracellular calcium and filopodial extensions were induced from dendritic shafts of isolated hippocampal neurons. Since previous studies suggest that contact with glial cells alters calcium homeostasis, we asked if such intercellular associations would indeed alter calcium responsiveness and morphological plasticity in dendritic shafts. The average rise in intracellular calcium levels obtained in dendrites was nearly 2-fold lower in neurons contacting other cells than in isolated neurons. Irrespective of the type of contact (i.e., via axons or dendrites), neurons contacting other cells also had a significantly reduced frequency of filopodia induction in comparison with isolated neurons. These results indicate that factors which regulate calcium responsiveness may also regulate morphological plasticity. Local rises in calcium, such as induced in the present study could occur *in vivo* via presynaptic inputs or via the release of neurotransmitter from approaching axons during development. Subsequent morphological changes could lead to altered neuronal connectivity; in several cases, induced dendritic filopodia became new arbors in the dendritic tree. Thus tight regulation of intracellular calcium may be crucial for the establishment, maintenance and modification of neuronal architecture and the resultant neuronal circuitry.

448.7

INHIBITION OF PI 3-KINASE BLOCKS NERVE GROWTH FACTOR STIMULATED NEURITE OUTGROWTH AND MAINTENANCE IN PC12 CELLS. I. Blader*, T. Jackson^C. Burga, A. Wolf, L. Fraser, L. Hammonds-Odie, and A. Theibert. Neurobiology Research Center/Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294 and ^Babraham Institute, Cambridge UK CB2 4AT.

In response to NGF, PC12 cells undergo morphological and biochemical changes, extending processes, called neurites, and differentiating into sympathetic neuron-like cells. NGF activates tyrosine kinases, phospholipase Cs and arachidonic acid release, leading to activated PKC, ras and MAP kinases. NGF also stimulates phosphoinositide 3-kinase (PI3K), which phosphorylates PIP_2, resulting in rapid, sustained increases in phosphatidylinositol 3,4,5-trisphosphate (PIP_3). We have used an inhibitor of PI3K, wortmannin, to investigate the role of PI3K in NGF signalling. Wortmannin (WM) inhibited *vivo* NGF-stimulated increases in PIP_3 by direct inhibition of the catalytic activity without affecting recruitment to tyrosine phosphorylated complexes. At concentrations used (100nM), WM appears to specifically inhibit PI3K since calcium responses to NGF or ATP were unaffected. In morphological studies, treatment of cells with WM resulted in inhibition (>80%) in the number of cells extending neurites in response to NGF. Application of WM to cells with existing neurites resulted in a rapid loss of neurites. Our results indicate that activation of PI3K may be required for neurite extension and maintenance in PC12 cells. Investigations are underway to identify targets for PIP_3, specifically cytoskeletal elements, and determine roles for PI3K in axonal or dendritic growth in cultured neurons.

448.8

DIFFERENTIAL CONTROL OF NEURONAL GROWTH CONE FILOPODIA: NERVE CELL TYPE-SPECIFIC REGULATORY MECHANISMS. V. Rehder* and S.B. Kater†, *Dept. of Biology, Georgia State University, Atlanta, GA 30302, and †Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523

Neuronal filopodia serve as sensory extensions of neuronal growth cones as they navigate through a three-dimensional terrain towards their target. Given their function, their number and length critically determine the area a growth cone can actively survey. Changes in filopodial disposition were recently shown to be under physiological control, involving the concentration of the intracellular second messenger calcium in the snail *Helisoma* (Rehder 1992, J. Neurosci. 12:3175-3186) and the degree of protein tyrosine phosphorylation in *Aplysia* (Wu and Goldberg (1993) J. Cell Biol 123:654-664).

The present study tests treatments previously demonstrated to cause rapid filopodial elongation in several cell types *in vitro* to investigate whether the underlying mechanisms for filopodial elongation are general or neuron-type specific. With this comparative approach, including *Helisoma* neurons B5, chick DRGs, and chick ciliary ganglion neurons (CGN), we demonstrate that a defined stimulation such as raising the free intracellular calcium concentration, or inhibiting tyrosine kinase activity affects filopodial disposition (i.e. filopodial length and number) differently in different neuronal types. For example, KCl depolarization causes a decrease in filopodial number in *Helisoma*, an increase in chick ciliary ganglion neurons, and has no effect on chick DRGs. Tyrosine kinase inhibition leads to a dose-dependent increase in filopodial length in *Helisoma* neurons, while identical concentrations applied to CGN cause a spectrum of responses ranging from an increase to a decrease in filopodial length. Thus the filopodial parameters length and number which provide the growth cone with an optimal flow of information about its environment may be controlled in a neuron-type specific fashion. Such findings suggest that the implementation of a specific second messenger system for the control of a given growth cone function may depend upon the full spectrum of second messenger functions that must be served by a given neuronal cell type.

448.9

SECOND MESSENGER INVOLVEMENT IN PREGANGLIONIC GROWTH CONE COLLAPSE *IN VITRO*. James J. Walker * and Richard I. Hume. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Sympathetic preganglionic growth cones collapse and retract when they contact the processes of dorsal root ganglion neurons (DRG) *in vitro* (Moorman and Hume, 1990). The signals that trigger this behavior and the signal transduction mechanisms are unknown. In the present study we used time lapse video microscopy to determine whether inhibitors of specific second messenger systems prevent the collapse of preganglionic growth cones when they contact DRG neurites.

Preganglionic neurons were retrogradely labeled by DiI injections into the sympathetic chain of stage 30 chick embryos. Sixteen to 20 hours later the medial spinal cord containing the labeled preganglionic neurons was cut into small pieces and plated as explants on a laminin-coated substrate. After allowing several hours for these cells to extend neurites, DiO labeled DRG's were added to the dish and time lapse images were acquired., We examined preganglionic growth cone-DRG neurite interactions in the presence of pertussis toxin (100 ng/ml) or omega conotoxin GVIA (5 uM). Cultures were pre-incubated in pertussis toxin for at least 2 hours prior to observation. For each growth cone studied, both surface area and length measurements were made at regular time intervals.

Our results show that pertussis toxin, a blocker of G protein activation, and omega conotoxin, a blocker of N-type calcium channels, inhibit the collapse of preganglionic growth cones when contacting DRG neurites. These findings suggest that calcium and a G protein may both play a role in mediating growth cone collapse in preganglionic growth cones.

448.10

THE EFFECT OF cAMP/PROTEIN KINASE A AND TPA/PROTEIN KINASE C ON THE NEURITE OUTGROWTH OF MOUSE NEUROBLASTOMA N1E115 CELLS

H.Li, W.-H.Tsai*, and F.L.Huang, NICHD, NIH, Bethesda, MD

The neurite outgrowth (NOG) of N1E115, mouse neuroblastoma cell line derived from C1300 clones, can be induced by 1mM 8-Br-cAMP or cAMP-raising agents. The mechanism of 8-Br-cAMP induced NOG is not clear. Here the mode of N1E115 cell differentiation induced by 8-Br-cAMP and the crosstalk between cAMP/PKA and TPA/PKC signal pathways were investigated. Treatment of N1E115 with TPA, either simultaneously or briefly before the addition of cAMP, resulted in the inhibition of NOG induced by 8-Br-cAMP. However, such inhibition was released after prolonged TPA treatment. Most importantly, these phenomenon were not affected by the presence of 10μM cycloheximide. By immunoblot analysis, PKCβ1 and PKCζwere found to be present in N1E115 cells. While brief treatment of cells with TPA effectively translocated PKCβ1 from cytosol to membrane, long-term treatment nearly completely down regulated this PKC isoform. Similar treatments, however, had no effect on PKCζ. Furthermore, TPA enhanced the phosphorylation of several proteins especially that of a Mr=90K protein on SDS/PAGE. In comparison, cAMP greatly reduced the phosphorylation of this 90kDa protein. These results indicate that NOG is likely associated with the dephosphorylation of the 90kDa protein which is directly or indirectly promoted by the cAMP/PKA reaction(s). On the contrary, TPA/PKC (likely PKCβ1) may block the NOG through promoting its phosphorylation.

448.11

INTRACELLULAR Ca2+ OF SENSORY AXONS DURING GROWTH IN Ca-FREE MEDIA. Jun Fukuda, Kazuko Keino-Masu and Keiichi Torimitsu*, Laboratory of Molecular and Cellular Physiology, Department of Physiology, National Defense Medical College, Tokorozawa, Saitama 359, and NTT Basic Research Laboratories, Atsugi, Kanagawa 243-01, Japan.

Ca influx is essential for neurite growth and elongation in nerve cells. To examine the localization of Ca influx mediating axonal growth, we cultured newborn rat DRGs with their peripheral nerves in a specially designed 2-chamber dish, which allowed culture of the cell bodies and peripheral nerves in media of different Ca2+ concentrations. When the cut nerve ends were cultured in Ca-free medium, neurites whose length was 70-80 % of that in control cultures grew from the cut ends, as long as the cell bodies were in normal Ca medium. In contrast, neurites failed to grow from the cut ends that were bathed in normal Ca medium, when the cell bodies were cultured in Ca-free medium. We thus conclude that Ca influx at the cell body is crucial for regeneration of new axons, while Ca influx in the growing axon itself makes only a limited contribution. Measurement of intracellular Ca2+ level using fluo3 under a confocal laser microscope revealed that [Ca2+]i was reduced from 150 nM (control culture) to 50-70 nM during growth in the Ca-free medium. No centro-distal gradient of [Ca2+]i was observed in axons growing in Ca-free medium. As Ca influx is absent in the axon during growth in Ca-free medium, Ca2+ that enters at the cell body is apparently transported to the regenerating axon.

448.12

EXPOSURE TO VOLATILE ANESTHETICS AFFECTS PROTEIN PHOSPHORYLATION IN DEVELOPING NEURONAL TIPS. [1,2]S. Saito, [2]T. Fujita, [1]M. Igarashi*, [1]Department of Molecular and Cellular Neurobiology, and [2]Department of Anesthesiology and Reanimatology, Gunma University School of Medicine, Maebashi, Gunma 371, Japan.

The biochemical characteristics of developing neural tips (growth cones) was examined after exposure to anesthetics to elucidate molecular mechanisms of neuroteratogenicity by general anesthetics. Neonatal rats were exposed to an atmosphere containing inhalational anesthetics, 1% halothane or 75% nitrous oxide, or a control atmosphere for 6 hours at postnatal day 1. After this exposure, growth cone particles were isolated from forbrains using a subcellular fractionation method at postnatal day 2,3,4 and 5. Protein composition, phosphoprotein patterns and protein kinase C (PKC) activities of the isolated growth cones were compared between each group exposed to the anesthetics and the control group. Calcium-dependent protein phosphorylation of a 46 kDa protein and of an 80 kDa protein, which is reported to be mediated by PKC, were significantly reduced after exposure to the anesthetics. Direct assays of PKC activity in growth cone particles have indicated that PKC activity in the growth cone is decreased at 24 hours after exposure to 1% halothane, and after exposure to 75% nitrous oxide. Considering crucial roles of growth cones in the establishment of the neuronal network, interruption of signal transduction in growth cones at a critical time for axonal pathfinding, is possible to provoke long lasting alteration of neural network.

448.13

NEURONS AND GLIA USE PHOSPHOTYROSINE IN DIFFERENT ADHESIVE STRATEGIES *in vitro*. J.S. Deitch.* Cell Biol., UMDNJ-SOM, Stratford, NJ 08084

During development, neuronal processes are in constant contact with a substrate, other neurons, or glia. This study investigated the possibility that tyrosine kinases may transduce signals concerning adhesion in neurons and glia. Hippocampal neurons from E18 rat embryos or glia from P3 rats were plated on poly-L-lysine (1 mg/ml) -treated coverslips. A monoclonal anti-phosphotyrosine (PY) antibody (4G10, UBI), and α-mouse-Texas Red, labeled short (2-3μm) dashes located mostly at the periphery of glia cells, which coincided with the ends of actin cables, as determined by double-labeling with phalloidin-FITC (Sigma). Conversely, 4G10 labeling of hippocampal neurons (1-14 days in vitro) was very dim and diffuse (no dashes). Phalloidin intensely labeled the growth cones and filopodia of neurons at all ages examined, but glial-like actin cables were absent. Cultures were treated with 1 mM sodium vanadate (VO_4), a phosphotyrosine phosphatase inhibitor, to enhance a possible weak tyrosine kinase activity in neurons. Labeling remained dim up to 6 hrs in VO_4. By 9 hrs, 20% of the neurons were labeled more intensely, especially in patches on the surface contacting the substratum. Furthermore, axons and dendrites were flattened considerably and covered with filopodia. After 18 hrs in VO_4, labeled neurons possessed beaded axons: brightly labeled enlargements connected by thin strands of axon. Some neurons began to disintegrate. Neurons allowed to recover for 6 hr in VO_4-free media regained normal cylindrical processes, and 4G10 labeling returned to near control levels. Conversely, VO_4 treatment of glia resulted in a progressive loss of 4G10 staining, and some glia became more angular. With recovery the dashes reappeared. In both neurons and glia, actin labeling disappeared with time in VO_4 and returned with recovery. These observations suggest that glia utilize a tyrosine kinase in relation to actin filaments in their adhesion strategy, similar to fibroblasts. Neurons may also require PY for adhesion, but keep PY levels low in their processes, perhaps to enhance growth by limiting adhesion.

448.14

PROTEIN PHOSPHORYLATION REGULATES DENDRITE BRANCHING IN CULTURED RAT HIPPOCAMPAL NEURONS. G. Audesirk*, M. Kern, and L. Cabell. Biology Department, Univ. of Colorado at Denver, Denver, CO 80217-3364.

The microtubule-associated protein MAP2 is preferentially localized in dendrites. Friedrich and Aszodi (1991) have hypothesized that increased phosphorylation of MAP2 stimulates dendrite branching. We tested this hypothesis by culturing rat hippocampal neurons with selective inhibitors of protein kinases (calphostin C: inhibits PKC; KT5720: inhibits PKA; KN62: inhibits Ca^{2+}-calmodulin-dependent protein kinase) or phosphatases (okadaic acid). We found that dendrite branching was significantly inhibited by KN62, but not by calphostin C or KT5720 at comparable concentrations. Dendrite branching was enhanced by okadaic acid. Western blots immunostained for MAP2 show that culturing neurons with okadaic acid shifts the apparent molecular weight for MAP2c (the low molecular weight, juvenile form of MAP2) to higher values, consistent with increased phosphorylation. Culturing neurons with KN62 shifts the apparent molecular weight of MAP2c to lower values, consistent with decreased phosphorylation. These results suggest that increased protein phosphorylation enhances dendrite branching, whereas decreased phosphorylation, at least by CaM kinase, inhibits dendrite branching. Although there are undoubtedly many proteins whose phosphorylation is altered by inhibitors of protein kinases and phosphatases, our data are consistent with a specific role for phosphorylation of MAP2 in regulating dendrite branching.

448.15

MONOCLONAL ANTIBODY PHAGE DISPLAY LIBRARIES FROM MICE IMMUNIZED WITH THE INTRACELLULAR DOMAIN OF A LEECH RECEPTOR PROTEIN TYROSINE PHOSPHATASE. M.N. Nitabach* & E.R. Macagno Dept of Biological Sciences, Columbia University, New York, NY 10027.

We have previously identified a leukocyte antigen-related (LAR) receptor protein tyrosine phosphatase (rPTPase), *Hm*LAR2, which is localized to a set of identified growth cones in the embryonic medicinal leech, *Hirudo medicinalis*. We are interested in the role played by *Hm*LAR2 in the regulation of the motility and pathfinding ability of these growth cones. Like all rPTPases, those of the LAR subfamily possess an intracellular catalytic domain and an extracellular domain resembling a cell-adhesion molecule. Affinity-purified polyclonal antibodies were raised in rabbits against the intracellular domain of *Hm*LAR2, and used to detect *Hm*LAR2 protein both *in situ* in fixed embryos, and on Western blots of total embryonic protein. However, for use as function blocking reagents for intracellular injection, we desired monoclonal antibodies reactive to various *Hm*LAR2 intracellular epitopes.

In order to obtain such reagents, we have generated a monoclonal antibody phage display library from the spleen of a mouse hyperimmunized with the bacterially-expressed *Hm*LAR2 intracellular domain. PCRs were performed using spleen cDNA as template, and primers designed based on IgG heavy and light chain sequences. Fab fragments are expressed as a fusion with the filamentous phage gIII coat protein. Thus, upon superinfection with helper phage, the antibody is expressed on the surface of a phage particle which contains the genetic material which encodes that particular Fab fragment. This system allows for the affinity purification of phages on immobilized *Hm*LAR2, followed by amplification of the recovered phages in a suitable bacterial host. The affinity purification-amplification can then be iterated until the desired enrichment of *Hm*LAR2-binding phage clones has been achieved. Individual clones can then be isolated and assayed individually for *Hm*LAR2 reactivity. We are now in the process of analyzing clones from this library.

AXON GUIDANCE IN THE VISUAL SYSTEM

449.1

THE FUNCTION OF THE OPTIC DISC AND THE RETINAL PERIPHERY IN THE ONTOGENY OF THE CENTRIPETAL PATTERN OF OPTIC AXONS IN THE RETINA

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In the early embryonic retina optic axons grow toward the optic disc and form a centripetal axon pattern. To address the question whether the directed growth of axons results from an attraction by the optic disc or from repulsion by the retinal periphery pieces of donor retina were grafted to ectopic sites of E3 organ-cultured eyes. After 24-48 hrs the eyes were studied for alteration in the axonal pattern as a result from the transplants. 1. Peripheral donor retina grafted into the central position of the host are non-permissive for growing axons. 2. The grafting of an optic disc to an ectopic site of the host and the removal of the original optic disc caused a major rerouting of axons to the grafted optic disc. 2. Axons encountering the grafted optic disc left the optic fiber layer to exit the eye. The experiments demonstrate that the centripetal pattern of axons in the retina is caused by a repulsion of axons by the retinal periphery and by a local attraction by the optic disc. The optic disc operates as an exit for the axons from the eye into the optic nerve.

449.2

CHONDROITIN SULFATE INTERFERES WITH RETINAL GANGLION CELL AXON PATHFINDING IN THE VISUAL SYSTEM OF *XENOPUS*

A. Walz* and C.E. Holt Dept. of Biology, UCSD, La Jolla, CA 92093-0357.

The axons of developing retinal ganglion cells (RGC) navigate along a defined route to reach their target, the optic tectum. Pathfinding is mediated by specific interactions between the growth cones and the substrate presented by the local environment. We investigated the putative involvement of chondroitin sulfate (CS) in the development of the retinotectal projection.

Exposed brain preparations were used to investigate the influence of free CS on the developing optic projection during the period when RGC axons grow from the base of the optic tract to the tectum (stages 33/34 to 40), and axons were visualized with anterograde HRP at stage 40. In contrast to other bath applied glycosaminoglycans, CS (200μM) showed a dramatic effect on pathfinding. The tract was highly broadened with axons growing virtually over every area of the telen- and diencephalon. These widened tracts were not shorter and growth cone morphology did not appear to be different compared to controls. Immunolabeling revealed that normal CS expression in the neuropil overlapped with RGC axon staining showing that RGC axons grow on a CS rich substrate.

Explanted intact eyes grown in culture on a laminin (LN) substrate with CS (1mg/ml) added to the medium did not show any change in either neurite outgrowth or elongation. However, when explanted eyes were grown on a CS/LN substrate, a dose dependent inhibitory effect on neurite outgrowth was observed, suggesting that even though CS does not inhibit growth per se, it may direct growth toward lower CS concentrations in the embryo during pathfinding. Chondroitinase ABC and xylosides were used to create CS free embryos and the pathfinding behavior of RGC axons was examined. (Supported by NIH #NS23780 and PEW Scholars Award)

449.3

bFGF DISRUPTS TECTAL RECOGNITION IN THE DEVELOPING VISUAL SYSTEM OF *XENOPUS*. S. McFarlane* and C. E. Holt. Dept. Biology, Univ. of California San Diego, La Jolla, CA 92093.

We are investigating the molecular cues that developing retinal ganglion cell (RGC) axons of *Xenopus laevis* use to recognize and synapse with their tectal target. Since growth factors are known to affect neuronal differentiation, are spatially and temporally regulated in the developing brain, and are expressed in the target cells of some neurons, it is possible that they may be involved in these recognition events. The fibroblast growth factor (FGF) family and its receptors have been found in the visual system; therefore, we have investigated the role of one member, basic FGF (bFGF), in target recognition.

A recombinant form of *Xenopus* bFGF (XbFGF) was applied to exposed embryonic brain preparations during the period when RGC axons grow from the optic chiasm to the tectum (stage 33/34 to 40). Axons were visualized with HRP at stage 40. XbFGF (0.5-5nM) severely affected retinotectal targeting. In the majority of cases (94%, n=19), axons failed to enter the tectum, instead, they skirted around the diencephalon/tectal border; growing rostrally towards and occasionally over the dorsal midline, and ventrally towards the medulla. We performed timing experiments in an attempt to elucidate XbFGF's action. First, XbFGF was applied at stage 33/34 for 2 hours. This early pulse was sufficient to disrupt target recognition (90%, n=19). Concordantly, if XbFGF application was delayed until stage 37/38, when the first RGC axons usually reach the tectum, target recognition was essentially normal. These results indicate that XbFGF needs to be present early on, for a short period of time, to exert its effects. Immunostaining shows that exogenous XbFGF binds to nerve fibers in the developing brain, including RGC axons, raising the possibility that bFGF acts on the RGCs themselves. These findings suggest that bFGF may play a role in target recognition.

(Supported by NIH #NS23780, PEW Scholars Award and MRC of Canada)

449.4

PERTURBATION OF G-PROTEIN FUNCTION AND RETINAL GROWTH CONE BEHAVIOR. C.-B. Chien*, A.T. Benedict, and W.A. Harris. Dept. of Biology, UC San Diego, La Jolla, CA 92093-0357.

Heterotrimeric G-proteins are ubiquitous intermediates in signal transduction which are enriched in growth cone membranes. We are studying their possible function in the guidance of *Xenopus* retinal growth cones both *in vitro* and *in vivo*.

In culture, the wasp venom peptide mastoporan (MP), which directly activates G proteins, has been shown to cause growth cone collapse [Igarashi *et al.*, (1993) *Science* 259:77]. Furthermore, this collapse was prevented by pretreatment with pertussis toxin (PTX), which inactivates G proteins by covalent modification. We have obtained similar effects with *Xenopus* retinal growth cones in culture. 10 μM MP applied for 30 min increased the fraction of collapsed growth cones (lacking either filopodia or lamellipodia when viewed with Nomarski optics) from 27±2% (control, ±s.e.m..) to 69±3%. Preincubation for 2 h with 200 ng/ml PTX before MP treatment reduced the fraction of collapsed growth cones to 51±2%, while treatment with PTX alone gave 41±2% collapse. Viewed in timelapse, the effect of MP was quick and dramatic: within a few minutes of MP addition, growth cones first retracted their lamellipodia, then their filopodia, and eventually collapsed altogether.

Applying 10 μM MP for 2 h to retinal retinal growth cones *in vivo* using an exposed-brain preparation, we find similar effects: 53% of growth cones were collapsed, compared with 12% in control brains. We are currently investigating whether PTX can protect against collapse *in vivo*, and whether MP or PTX perturb retinal axon pathfinding or arborization, which would indicate a role for G-proteins in the signal transduction pathways used for normal growth cone guidance and target recognition.

Supported by grants from the NIH and the ACS.

449.5

CHICK RETINAL AXONS BRANCH PREFERENTIALLY ON MEMBRANE STRIPES DERIVED FROM TOPOGRAPHICALLY APPROPRIATE TECTUM. A.L. Roskies* and D.D.M. O'Leary, Molecular Neurobiology Lab, Salk Institute, La Jolla CA 92037.

Membrane-bound molecules with position-dependent distributions are hypothesized to guide or restrict growing retinal axons to topographically appropriate sites in their central targets. In vivo studies in developing chicks and rats indicate that retinal axons form topographically ordered arbors by the extension of collateral branches along their length. We have recently reported that E18 rat temporal retinal axons grown perpendicular to membrane lanes derived from rostral and caudal E18 superior colliculus (SC) branch preferentially on rostral SC membranes due to an inhibitory molecule PI-anchored to caudal SC membranes.

To determine whether chick retinal axons exhibit a branching specificity similar to that found for rat, we have grown E6 chick retinal explants on carpets of alternating lanes of membrane fragments from rostral and caudal E9 chick tecta. Temporal chick retinal axons show a consistent branching preference for their topographically appropriate rostral membrane lanes. Nasal axons show no consistent preference. Time-lapse videomicroscopy of the stripe assay cultures reveals that branches form interstitially along the axon shaft, as they seem to in vivo, as well as by growth cone bifurcation. Thus, in chick, as in rat, the topographic bias in retinal axon branching observed in vivo may be controlled by membrane bound molecules. Further time-lapse microscopy of branch formation will allow us to determine whether the observed branching preference is a result of substrate-influenced branch initiation or branch stabilization.

449.6

DEVELOPMENT OF CROSSED AND UNCROSSED VISUAL PATHWAYS IN PIGMENTED AND ALBINO MICE R.C. Marcus* and C.A. Mason, Dept of Path, Coll Phys Surg, Columbia Univ, NY, NY 10032.

In the mammalian visual system optic axons project to both sides of the brain. Compared to pigmented mice, in albino mice, a smaller number of retinal ganglion cells project ipsilaterally. To determine the site of action of the albino mutation, we studied the projection pattern of retinal ganglion cells and the environment they traverse in albino mice between E12.5 and E15.5. DiI was used to label either the entire eye or ventro-temporal retina which gives rise to the ipsilateral pathway. In pigmented mice, the retinal origin and pattern of growth of the earliest optic axons differs from the later, permanent optic projection (Marcus and Mason, Soc Neurosci Abstr, 1993). In albino mice, although decreased in size, the ipsilateral projection followed the normal pattern of growth at all ages. Thus, the reduction in the ipsilateral projection is not due to a defect in either the early or late pattern of growth. In addition, immunocytochemical labeling of the albino chiasm with MAb RC2 which recognizes radial glia, MAb F4/80 which recognizes microglia, and MAb 480-1.1 against the stage specific embyonic antigen was normal, suggesting that the albino mutation exerts its effects in the retina.

To further test this hypothesis we are studying "chimeric" cultures in which explants of pigmented retina are co-cultured with chiasm cells dissociated from albino animals at E14 - E15. In pigmented mice, crossed and uncrossed axons respond differentially to dissociated chiasm cells (Wang et al, Soc Neurosci Abstr, 1992). Preliminary results reveal that uncrossed axons from pigmented animals grown on chiasm cells from pigmented or albino animals behave similarly, i.e., neurites from uncrossed axons are shorter and more fasciculated compared to neurites from crossed axons. These results are consistent with the hypothesis that cells in the developing optic chiasm are unaffected by the albino mutation and that the mutation acts at the retinal level.

449.7

TOPOLOGICAL SPECIFICITY IN REINNERVATION OF THE SUPERIOR COLLICULUS BY REGENERATED RETINAL GANGLION CELL AXONS IN ADULT HAMSTERS. Yves Sauvé, Hajime Sawai, Michael Rasminsky*. McGill University Centre for Research in Neuroscience, Montreal General Hospital Research Institute, 1650 Cedar Ave., Montréal, QC, Canada, H3G 1A4.

In rodents, nasal and temporal retinal ganglion cells (RGCs) project their axons to caudal and rostral contralateral superior colliculus (SC) respectively. We have examined the projections of RGC axons regenerated through unbranched peripheral nerve grafts led from the eye to the contralateral SC in adult hamsters. Responses to visual stimulation were recorded from terminal arbors of individual regenerated RGC axons and the postsynaptic neurons with which they make synapses in the reinnervated SC. RGC positions in the retina corresponding to these terminal arbors were inferred from the location of their visual receptive fields on a tangent screen. 6 to 63 RGCs with projections to the SC were identified in 11 animals 30 to 60 weeks after grafting.

At a given site in the SC, recordings could be made from axon terminal arbors emanating from widely separated RGCs. Conversely, nearby RGCs could project to widely separated sites in the SC. The relative position of each possible pair (n=2695) of RGCs was determined with respect to the nasotemporal axis of the retina; relative positions of the axonal projections of these RGCs were inferred from the caudorostral displacement of corresponding sites of responses in the SC. Pairs were excluded as ambiguously oriented if the direction of displacement fell beyond ±45° from the nasotemporal axis in the retina or ±45° from the caudorostral axis in the SC. Of the 1016 evaluable pairs of RGC projections, 622 had appropriate and 394 inappropriate caudo-rostral displacements in the SC. Although normal retinotectal topology is not reproduced, this 3:2 preference for appropriate projection suggests that RGC axons may recognize caudorostral positional cues as they reinnervate the SC.

449.8

AN ANTIGEN IN THE OPTIC PATHWAY IS EXPRESSED IN EYELESS ANIMALS. Nielsen Fernandez and Sally Hoskins*, Department of Biology, City College of the City University of New York, New York, NY 10031.

To analyze the molecular basis of axon guidance in the developing optic nerve of the frog *Xenopus laevis*, we made monoclonal antibodies to optic tract membrane fragments and produced gro1, which stains the optic nerve projection to the optic tectum beginning at embryonic stages. As reported previously, the pathway to the thalamus and the thalamic target zones are not stained by gro1, raising the possibility that the gro1 antigen plays a role in optic axon target selection.

To examine whether all of the staining seen in the optic pathway was associated with the optic nerve, we deleted optic cups from early embryos, and assessed gro1 immunoreactivity in cryostat sections of eyeless brains at tadpole stages. Eyeless tadpoles showed gro1 immunoreactivity in the position normally occupied by the optic chiasm, as well as in a line of cells marking the position of the optic tract along the wall of the diencephalon, and in the optic tectum. We conclude that the gro1 antigen is associated both with the developing optic nerve and with its pathway, raising the possibility that early gro1 expression in the optic pathway marks the route taken by the subset of retinal ganglion cell axons that projects to the optic tectum. Experiments in progress will establish how early the pathway-associated gro1 appears.

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449.9

THE ORIGIN AND COURSE OF RETINOFUGAL AXONS DURING NORMAL DEVELOPMENT OF THE FERRET. G.E.Baker and R.J.Colello (SPON: Brain Research Association). Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.

Developing retinal ganglion cell axons grow into either the contralateral or ipsilateral optic tract. We have studied the course of these axons in fetal ferrets to examine their development and to determine if their prechiasmatic organization at any age might predispose them to a crossed or uncrossed course at the optic chiasm.

Pregnant jills were deeply anesthetized and fetuses were fixed with 4% para-formaldehyde. Retinal implants of the tracer DiI were made to label axons from one eye, and unilateral optic tract implants were made to label the uncrossed axons and ganglion cells of one eye, and the crossed population of the other.

Retinofugal axons were first observed in the optic tracts at E25. Between this age and E27 the distributions of ganglion cells giving rise to the first uncrossed and crossed axons overlap in the dorso-central retina, as previously found in mice and rats (Godement et al, Development 101, 515; Chan et al, JCN 336, 362). The ipsilaterally-projecting cells have no apparent preferred location within that region. Behind the eye, the uncrossed axons are widely dispersed but become increasingly concentrated laterally closer to the optic chiasm where few are found in medial locations. A narrow ventrotemporal (VT) crescent of ipsilaterally-projecting cells is first observed at E28 and broadens at later stages. The distribution of axons in the optic nerve at this and later ages contrasts with the earlier stages and resembles that of the adult; postoptically the majority of uncrossed axons are concentrated ventrolaterally, becoming more dispersed along the course of the nerve. At the chiasm many are found in a medial location. At no age are the uncrossed and crossed axons segregated prechiasmatically.

We conclude that spatial location in the prechiasmatic nerve may determine the uncrossed path of the earliest fibres but is not relevant for the later VT component.

Supported by the Wellcome Trust

449.10

A STUDY OF THE MICROENVIRONMENT IN THE EMBRYONIC MOUSE OPTIC CHIASM. S.J.Tavendale and L-A. Coleman*. Dept. of Human Anatomy, Oxford University, South Parks Rd, Oxford, UK, OX1 3QX.

In the developing visual system, retinal ganglion cell axons navigate through a range of environments to reach their final destination. We have studied the microenvironment in the developing mouse optic chiasm (OC) electron microscopically. Mouse embryos (at E16) were perfused and processed for em analysis. In a 100μm deep ventro-dorsal strip of the lateral OC, 150-200μm from the midline, axon and growth cone (GC) densities and GC shapes were plotted. Using serial sections, the relationships of GCs to glial cells at the ventral surface and in a region 50μm dorsally were studied and the relative frequencies of specialized membrane junctions were also compared. Axon density increased with distance from the pial surface, peaking at 60μm and remaining constant (at around 300-350 axons/100μm^2). GCs were found throughout the depth of the OC. Those close to the pial surface represented a much higher proportion of axon profiles and were larger and more bulbous compared with the thin, elongate and branched GCs seen dorsally. Serial section reconstructions confirmed these impressions. At the ventral surface, glial endfeet separated GCs from the basement membrane but not all GCs grew along endfeet and this area was not as rich with glial cell processes as the dorsal region, where portions of all GCs were in apposition to glial processes. Specialized membrane junctions occurred most frequently between axons and glial processes or GCs and glial processes and were characterized by an invagination where the glial cell membrane was darker, thicker and slightly fuzzy in appearance. Portions of axons or GCs often occupied this concavity and across the cleft between the two membranes fine filaments were seen. Dorsally these junctions were twice as frequent between axons and glial cell processes as between GCs and glial processes but were seen with equal frequency ventrally. We have shown that although GCs are present throughout the ventro-dorsal extent of the OC, both the environment and GC morphology varies. The nature of these environments may constrain or determine the range of GC morphologies in different regions of the OC.

449.11

SUBTRACTIVE cDNA LIBRARY SCREEN FOR GENES UPREGULATED OR DOWNREGULATED AFTER DEAFFERENTATION OF THE RAT SUPERIOR COLLICULUS. A. D. Crawford[1*], F. Bonhoeffer[1], and M. Bähr[1,2]. Max-Planck-Institut für Entwicklungsbiologie[1], and Neurologische Universitätsklinik[2], 72076 Tübingen, Germany.

During development of the vertebrate visual system an orderly projection of retinal ganglion cells onto the superior colliculus is established. The molecular mechanisms that might govern this process are still unknown, but probably include the coordinated interaction of target-specific axon guidance molecules and their corresponding growth cone receptors. Until recently, it was believed that in higher vertebrates, these molecules are only expressed during the actual formation of the retinocollicular projection. However, it was recently shown that cell-surface guidance activities that are not normally detectable in the superior colliculus (SC) of the adult rat reappear following deafferentation of the SC through axotomy of the optic nerve (Wizenmann *et al.*, 1993). We have initiated a subtractive cDNA library screen to clone the genes encoding these guidance molecules, or possibly those molecules that are responsible for masking them in the normal adult. The subtraction protocol involved multiple rounds of hybridization of cDNA from deafferented SC with an excess of biotinylated cDNA from normal SC, and vice versa, each round followed by extraction with streptavidin and PCR amplification of the remaining cDNA (Wang and Brown, 1991). The resulting subtracted cDNA libraries, one highly enriched for genes that are upregulated after deafferentation, the other for downregulated genes, were then differentially screened to identify these upregulated and downregulated clones. In an initial pilot screen of about 250 clones from each library, 16 upregulated and 7 downregulated clones were identified. These clones are currently being subjected to sequence, Northern, and *in situ* analysis. In addition, full-scale screens of about 5000 clones from each library are currently in progress. The differences in expression in normal versus deafferented SC of those clones isolated to date varied from 2-fold to at least 10-fold, with 2 upregulated clones showing no detectable expression in normal SC.

449.12

A MONOCLONAL ANTIBODY RECOGNIZING A PUTATIVE GUIDANCE MOLECULE REVEALS AN ANTERIOR-POSTERIOR GRADIENT IN THE EMBRYONIC TECTUM OF CHICK AND RAT. B.K. Müller * and F. Bonhoeffer, Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35, D-72076 Tübingen, Germany.

Gradients of directional cues have been proposed to be important for the guidance of retinal ganglion cell axons to their tectal target cells. *In vitro* temporal retinal axons can detect concentration differences of glycosylphosphatidyl-inositol (GPI) anchored guidance molecules (33/35 kD; Stahl et al., Neuron 5, 1990) expressed predominantly on membranes from the posterior part of the optic tectum. We have isolated a monoclonal antibody (F3D4) recognizing these molecules. The antigens of the F3D4 antibody are also found in fish and rat. On cryostat sections from embryonic chick tectum, antibody staining revealed a gradient in the outermost tectal layer, decreasing from the posterior to the anterior pole of the optic tectum. Strongest immuno-reactivity is observed in the stratum opticum (SO) and some immunoreactivity is also found in the stratum griseum et fibrosum superficiale (SGFS). In a preparation free of neuronal elements, F3D4 stained glial endfeets. F3D4 antigens are expressed in embryonic chick and rat tectum, before and during the time of retinal axon ingrowth and not thereafter. These observations suggest that the F3D4 antigens might be important for the construction of the retinotectal map.

449.13

PRECOCIOUS INVASION OF THE OPTIC STALK BY TRANSIENT CENTRIFUGAL AXONS IN THE FERRET. B.E. Reese* and S.F. Geller
Neuroscience Research Institute and Departments of Psychology and Biology, University of California at Santa Barbara, CA 93106-5060

The present study demonstrates 1) that the fetal optic nerve contains a conspicuous population of centrifugal axons; 2) that the cells of origin are situated in both the ventrolateral diencephalon and in the chiasmatic region; 3) that the projection is transient, and never invades the retina itself; and 4) that these centrifugal axons invade the optic stalk prior to the arrival of the retinofugal axons. Fetal ferrets (E-22 through E-36) were fixed with 4% paraformaldehyde and examined using DiI to trace the retinofugal and centrifugal pathways. In addition, we used ß-tubulin immunohistochemistry to detect the differentiation of the first axons within the retina and optic stalk.

Dye implants into the optic nerve head, but not the retinal periphery, retrogradely label somata in the ventrolateral diencephalon, provided the implants are made in fetuses before E-30. Likewise, dye implants into this region of the diencephalon, rostral to the optic tracts, anterogradely label centrifugal axons that course through the optic nerve but never enter the retina. The centrifugal axons course ventrally from their cells of origin as 2-5 fascicles and turn laterally to enter the optic nerve where it emerges from the hypothalamus. Some of the fascicles course extra-cerebrally for a short segment at this junction, as described by Guillery and Walsh (J. Comp. Neurol. 1987a, 265, 203; 1987b, 265, 218).

The optic nerve head and retinal fiber layer are immunoreactive for ß-tubulin on E-24 and thereafter, whereas on E-22, they are immunonegative. Yet immunopositive fascicles of axons course from the ventral diencephalon into the optic stalk on E-22, confirming the precocious nature of the centrifugal projection. These fascicles are identical in trajectory to the centrifugal axons which are still readily labelled by dye implants into the future optic nerve head on E-22. These same implants on E-22, and only on E-22, also label cells in the future chiasmatic region, situated immediately ipsilateral to the midline.

These centrifugal axons may serve a transient, guidance, function for later developing optic axons. The cells situated at the midline may additionally play a signalling function for optic axons as they invade the optic chiasm. The existence of these centrifugal axons complicates the identification of early-developing optic axons in otherwise unlabelled tissue.

FORMATION AND SPECIFICITY OF SYNAPSES III

450.1

FASCICLIN III AS A SYNAPTIC TARGET RECOGNITION MOLECULE IN DROSOPHILA. A Chiba[1*], P Snow[2] & Y Hotta[1]. [1]Dept. Physics, Univ. Tokyo, Tokyo, Japan, & [2]Dept. Biol., SUNY, Albany, NY.

Motoneuron RP3 consistently innervates muscles 6 and 7 in Drosophila embryos. During RP3 synaptogenesis, the cell surface glycoprotein fasciclin III appears in both the RP3 growth cone and muscles 6 and 7. As fasciclin III has been shown to be a homophilic adhesion molecule, such expression raises the possibility that it may function as a specific "target recognition molecule" for RP3. We have tested whether fasciclin III is necessary and/or sufficient for RP3 target selection in vivo by examining the effects of deletion or misexpression of this molecule as assessed by intracellular dye injection and immunocytochemistry. First we have found that in the existing *fasciclin III* null mutant RP3 reliably forms synapse with its normal targets. Next, we have generated transgenic flies which misexpresses fasciclin III ectopically on all skeletal muscles during neuromuscular synaptogenesis. This was accomplished by creating a construct which placed the *fasciclin III* gene under the control of the *Myosin heavy chain* promoter, and transforming this construct into flies by P element-mediated genomic transformation. In these transformant flies, we have observed that RP3 often innervates non-target muscle cells while other motoneurons (e.g. RP1) innervate their targets normally. Therefore, this provides single cell-level evidence supporting that fasciclin III functions as a specific "synaptic target recognition molecule" for a small set of neuronal growth cones. Furthermore, taken together with the analysis on the null mutation, the results also suggest that fasciclin III is one member of a set of functionally redundant recognition molecules which are involved in an "either/or" manner during target recognition by RP3. Controlled misexpression of such recognition molecules is an effective approach to determining the functional roles of these proteins in vivo, which may be difficult to confirm by analysis of null mutations alone.

450.2

MOTONEURONS DISCRIMINATE APPROPRIATE FROM NOVEL TARGETS DURING SYNAPSE FORMATION IN CULTURE. J. C. Poyer and M. J. Zoran*. Department of Biology, Texas A&M University, College Station, TX 77843.

Neuron B19 innervates the supralateral radular tensor (SLrT) muscle of the Helisoma buccal musculature. This neuromuscular synapse reforms in culture and its regeneration requires target-dependent induction of secretion capabilities. The presynaptic mechanism underlying this induction involves modifications in secretory function such that action potentials, uncoupled from secretion prior to contact, evoke neurotransmitter release following SLrT muscle contact.

Previous studies have shown that novel muscle targets, not typically innervated by B19, vary in their ability to induce secretory changes. For example, contact with supramedial radular tensor (SMrT) muscle causes an enhancement of secretory capabilities while contact with foot (POD) muscle fibers does not. Based on these results, we hypothesize that B19 has the capacity to recognize specific muscle fibers and that muscle contacts trigger different presynaptic secretory responses. Our hypothesis maintains that mechanisms responsible for these differential changes govern discriminate synapse formation. To further test the ability of B19 to discriminate between muscle targets, dual target-contact experiments were performed where single B19's were contacted in culture by two muscle partners. After 3 days of contact, rates of spontaneous synaptic potentials (SSPs) and the presence of evoked synaptic potentials were assayed. In B19-SLrT/SMrT preparations (n=24), SSP rates detected from SLrT- and SMrT-contacted neurites were 9.6 and 1.6 SSPs/min, respectively. Evoked release was detected from 20% of SLrT- and 13% of SMrT-contacted neurites. In B19-SLrT/POD preparations (n=15), SSP rates detected from SLrT- and POD-contacted neurites were 3.0 and 1.1 SSPs/min, respectively. Evoked release was detected from 50% of SLrT-contacted neurites and never in POD-contacted neurites. These data, taken together with mechanistic studies, suggest that specific neuron-muscle contacts can regulate secretory properties of motoneuron B19 in a cell-specific manner.

450.3

ACTIVITY REGULATES THE SEGREGATION OF PRESYNAPTIC INPUTS ON A COMMON POSTSYNAPTIC TARGET IN VITRO. Z. Sun* and S. Schacher. Ctr. Neurobiol. & Behav., Columbia Univ. College of P & S, NYSPI, New York, NY 10032.

Previous studies indicate that *Aplysia* sensory neurons may 'compete' with one another in establishing chemical connections with a single motor target *in vitro*. Although the sensory cells display no spontaneous action potential activity, they segregate their varicosities to different portions of the motor axons over time. The functional consequence is a cell number-dependent decrease in the average EPSP evoked by each sensory cell. We examined whether adding activity known to evoke a facilitatory response in mature connections--increase in cAMP levels via iontophoretic injection or tetanic stimulation (10 Hz for 4-5 sec)--during the early stages of synapse formation would modulate the intrinsic segregatory process. Two sensory cells were cocultured with one motor cell and examined on day 2 and day 4 for changes in synaptic efficacy and structure . There were no significant differences in the functional and structural changes for each sensory cell over time with control treatments or following tetanic stimulation of the motor cell. Injection of cAMP or tetanic stimulation of one senory cell significantly increased the amplitude of the evoked EPSP and varicosities of the treated cells compared to their respective control sensory cells in the same cultures. Increasing cAMP levels appeared to increase the rate of new varicosity formation by the injected cell while tetanus also evoked an increase in turnover of pre-existing varicosities of the control unstimulated cell. The transynaptic effect may be mediated by coincident depolarization in the motor cell. Paring tetanic stimulation in both the motor and one sensory cell resulted in a larger difference in the functional and structural development by the two sensory cells. These results suggest that different forms of activity may regulate specific aspects of the segregatory process--formation or elimination of synapses--associated with the fine tuning of connections formed by converging presynaptic inputs on a common target.

450.4

PRESYNAPTIC CALCIUM LEVELS AT THE CHEMICAL CONNECTION BETWEEN MOLLUSCAN NEURONES IN VITRO. A. Casadio, R. Levi#, L. Morando, G. Naretto and P.G. Montarolo*. Dept. of Hum. Anat. and Physiol., c.so Raffaello 30; #Dept. of Anim. Biol., via S. Croce 8, Univ. of Turin, Turin, Italy.

The studies on neuromuscular junction suggest that during synaptogenesis the muscle provide a retrograde signal which induced a twofold general increase of resting calcium level and a local action potential-evoked Ca^{2+} influx, dependent on cAMP, in the motoneurone (Funte and Haydon, Neuron 10:1069, 1993). The aim of our experiments was to study the Ca^{2+} homeostasis in functionally different neurones and in the presynaptic cell at the axon-axonal synapse. We have measured the resting level and action potential-evoked accumulation of Ca^{2+} in the motorneuron C3 of Helix pomatia, plated in culture alone, and in the multitarget metacerebral giant cell (MGC) either cultured alone or cocultured with the neurone B2, one of its target in the buccal ganglion, placed near the stump of the cerebro-buccal connective (CBC). The resting Ca^{2+} level in the C3 neurite terminals was found to be 126 ± 27 nM with a negligible increase to 156±22 nM after a train of action potentials. Instead, in the terminals of the CBC branch of MGC plated alone the Ca^{2+} level increased to 243±136 nM from a resting value of 129±11 after stimulation. This difference was not observed at the posterior lip nerve (PLN) branch (127.5± .71 nM and 147±2.25 respectively). After the functional synaptic contact was well established between CBC and neurone B2, the action potential evoked Ca^{2+} accumulation at the putative synaptic sites increased to 481±197 nM from a resting level of 123±24. This increase was significantly more robust than that measured at PLN (267±18 nM vs. a resting level of 129±29 nM). Our preliminary experiments suggest that in motorneuron C3, as preaviously reported., no significant Ca^{2+} increase can be observed in the absence of the target, while in the multifunctional MGC neurone a more complex pattern is present, depending both on the presence of a target and on which principal branch of the neurone is considered.

450.5

DIFFERENTIAL EXPRESSION OF G-PROTEINS IN IDENTIFIED *LYMNAEA* NEURONS MAY DETERMINE TARGET CELL SELECTION AND SPECIFICITY OF SYNAPTIC CONNECTIONS
Gaynor Spencer , N.I. Syed* and Lukowiak, K Departments of Anatomy and Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have recently developed an *in vitro* model system to study mechanisms underlying target cell selection and specificity of synaptic contacts in the snail *Lymnaea stagnalis*. An identified dopaminergic interneuron termed Right Pedal Dorsal 1 (RPeD1) makes monosynaptic connections with a number of target cells (Right Parietal A) *in vivo* and these connections are reestablished *in vitro*. RPeD1 does not however, synapse with non target cells (Left Pedal A) either *in vivo* or *in vitro*, despite the presence of functional dopamine receptors on these cells. In the present study, we therefore hypothesized that differential G-protein couplings to the dopamine receptor may determine target selection by RPeD1 *in vitro*. We first determined whether the effects of exogenous dopamine on both target and non target cells were mediated through different G-proteins. We used the bacterial toxin, Pertussis toxin (PTX), which is known to inhibit Gi and Go. Both target and non target cells were incubated in PTX(1µg/ml) for 18-24 hours. The inhibitory responses of the non target cells to dopamine were found to persist in PTX (n=7), whereas the similar inhibitory responses of the target cells were abolished (n=5). These data lend support to the hypothesis that G-proteins may play an important role in determining the specificity of target cell selection by *Lymnaea* neurons.

450.6

TIMING OF AFFERENT INGROWTH AND TARGET-DERIVED STOP SIGNALS IN DEVELOPING CEREBELLUM Q. Zhang, R. Blazeski, and C.A. Mason*, Dept. Pathology, Coll. Phys. Surg., Columbia Univ., NY, NY 10032.

Our previous work has demonstrated a "stop-growing signal" derived from target cells, based on the behavior of pontine mossy fibers and granule cells from mouse cerebellum *in vitro* (Baird et al., 1992, J. Neurosci. 12:619). To further investigate the nature of the stop signal and its specificity (Baird et al., 1992, J. Neurobiol. 23:579), we examined afferent-target interactions in two situations: when afferents arrive before target cell availability, and when the available target cells are more mature than usual.

First, vestibular mossy fiber ingrowth, position and contacts were studied by DiI labeling, immunocytochemistry with calbindin anitsera to label Purkinje cells, and electron microscopy of photoconverted DiI-labeled terminals. At E17, vestibular fibers extend well into the emerging cortical layers in lobule X. Vestibular fibers with small growing tips terminate amongst the Purkinje cell zone and on the underside of the external granule cell layer. Immature contacts are made primarily on Purkinje cells, an inappropriate target, but switch to granule cells after the first few postnatal days.

Second, explants of pontine nuclei were cocultured with purified granule cells, which were aged *in vitro*, or taken from different aged animals. After P11 (or the equivalent days *in vitro*), less than half of the explants exhibited short neurites, compared to about 90% at earlier ages, indicating lack of or waning of a stop signal. These results point to temporal and spatial orchestration of stop signals on target cells.
(Supported by NS 15961 to C.A.M.)

450.7

RESTORATION OF MOSSY FIBER PROJECTION IN CO-CULTURED SLICES OF DISLOCATED DENTATE GYRUS AND γ-IRRADIATED HIPPOCAMPUS. B. Heimrich* and J.L. Gaiarsa, Inst. Anat., Univ. Freiburg, P.O.B. 111, D-79001 Freiburg; INSERM U29, 75014 Paris, France

In rodent brains the excitatory input from the dentate gyrus to the hippocampus, the mossy fiber projection, shows a strong region and layer specific distribution. The granule cells of the fascia dentata which give rise to this projection are mainly generated postnatally. Thus, the major part of this pathway develops after birth, and neonatal irradiation of the hippocampus can prevent the formation of this pathway. In an in vitro system we have investigated if mossy fiber axons of ectopically positioned dentate granule cells can reach their target area in the irradiated hippocampus, form the characteristic type of synapse and may therefore compensate for the loss of intrinsic mossy fiber input. We co-cultivated a slice of dentate gyrus and irradiated hippocampus up to 16 days on Millipore membranes. The dentato-hippocampal pathway developed in vitro was analyzed using the anterograde tracer biocytin. Thin and varicose fibers originating from labeled hilar neurons invaded all parts of the co-cultured hippocampus and established symmetric as well asymmetric synapses. Biocytin labeled giant mossy fiber boutons were only found in the CA3 region of the co-cultured hippocampal slice. They often showed invaginations of spines and formed the characteristic multiple synaptic contacts on target cells.

From our data we conclude that the mossy fibers emerging from dislocated dentate gyrus explant form the correct termination pattern, develop specific connections, and even partially restore this excitatory pathway.
(Supported by INSERM and the DFG: SFB 325)

450.8

SYNAPSIN-LIKE IMMUNOREACTIVITY IN INVERTEBRATE NEURONS *IN VITRO*: REDISTRIBUTION FOLLLOWING THE ESTABLISHMENT OF SYNAPTIC CONTACTS. G. Cibelli[1], F. Benfenati[2*], M. Ghirardi[3], F. Vitiello[1] and P.G. Montarolo[3]. [1]Inst. of Human Physiology, University of Bari; [2]Dept. of Experimental Medicine, University of Rome "Tor Vergata"; [3]Dept. of Human Anatomy and Physiology, University of Turin, Italy.

The characterization of proteins involved in the storage and release of neurotransmitters has been a major step in understanding the synaptic function. Among them, the synapsins, a family of evolutionary conserved, neuron-specific phosphoproteins associated with mammalian small synaptic vesicles, appear to play a pivotal role in both mature and developing nerve terminals. Neuronal cultures from invertebrate nervous system represent a model particularly suitable to analyze the synaptic functions; in fact, in these cultures presynaptic and target neurons form active contacts which can undergo synaptic plasticity. We studied the redistribution of the synapsin-like immunoreactivity in identified *Aplysia* and *Helix* cultured neurons following the establishment of synaptic contacts. To this aim we cocultured a large serotonergic cell (GCN) from the cerebral ganglion and its target neuron (B2) from the buccal ganglion; these cells form *in vitro* reliable synaptic contacts that can be electrophysiologically tested. After different periods in culture we localized the synapsin-like immunoreactivity by means of a battery of anti-synapsin polyclonal antibodies. In the presence of the target neuron the synapsin-like immunoreactivity in the presynaptic cell substantially overlapped the serotonin immunostaining (used as a marker of active synapses). On the contrary, in the absence of the physiological target a diffuse labelling pattern along the outgrowing neurites was observed. This is in good agreement with the data showing that in cultures of hippocampal neurons of mammal synaptic vesicle proteins cluster in presynaptic terminals following contacts with dendrites of other neurons. Our results suggest that in invertebrate cultured neurons the sorting of synaptic vesicle proteins to the presynaptic ending is strongly affected by the presence of the target cell.

450.9

TARGETING OF FUNCTIONAL SUBSETS OF NEURONS MEDIATED VIA THEIR AXONAL CARBOHYDRATE MARKERS. B. Zipser* and J. Song.
Dept. Physiol., Michigan State University, East Lansing, MI, 48824

Sensory information is commonly channeled into multiple target regions in the CNS. Some of these target regions are exclusively innervated by just one sensory modality while other target regions are multimodal, receiving input from several sensory modalities. There is increasing evidence throughout phylogeny that neurons conveying different sensory modalities are chemically encoded with surface glycoconjugates. We investigated to what extent two such carbohydrate markers mediate the targeting of their respective axonal subsets in the CNS by molecularly perturbing cultured leech embryos. We found that the targeting of each axonal subset in the synaptic neuropil was perturbed only by Fab fragments binding to the subset's own carbohydrate marker. Characterizing the nature of the interactions mediated by these two carbohydrate markers with glycosidases and neoglycoproteins indicated that the targeting of putative mechanodetectors is mediated by a galactose-specific recognition; in contrast, the targeting of putative chemodetectors is mediated mostly by a glucose-specific recognition with some galactose component. The different interactions of the two neuronal subsets, one mediated exclusively by galactose and the other one mostly by glucose with a minor galactose component, are consistent with the observation that their projections show partial overlap in the synaptic neuropil. Thus, the intact-cultured leech embryo is a suitable model system to study the normal function of neuronal carbohydrate markers in synaptic connectivity.

450.10

DEVISING A MODEL SYSTEM TO STUDY THE ROLE OF LECTINS IN NEURAL CONNECTIVITY IN LEECH. M.-T. Tai, M. B. Rheuben* and B. Zipser.
Dept. of Physiology., Michigan State University, E. Lansing, MI 48824

We are investigating the role of carbohydrate-lectin interactions in neuronal connectivity. Previous work demonstrated the critical importance of carbohydrate markers for axonal targeting in the synaptic neuropil. We are now devising a system to study the role of leech galectins, LL35 and LL16, in the formation of an identified synapse. Several lines of evidence indicate that galectins are expressed by photodetectors in midbody sensilla and eyes. One to two cell bodies in midbody sensilla and their central projection into a distinct neuropil target region, the sensory midline tract, express galectins (Cole and Zipser, J. Neurochem. 64, 1994). We now showed that DiO-labeling photodetectors led to prominent axonal staining of the sensory midline tract with more lateral neuropil regions receiving only sparse projections. Immunostaining all sensory neurons and DiO-labeling photodetectors demonstrated that the sparse projections of photodetectors away from the sensory midline tract are into the more lateral target regions of mechano/chemo/heat detectors. To search for postsynaptic partners of photodetectors, we injected central neurons with Lucifer yellow after immunostaining sensory neurons. The axonneuritic projections of the AP cell demonstrated the strongest colocalization with the sensory midline tract, the target region of the photodetector. In preliminary experiments, illuminating leech eyes led to an increase in AP cell excitability. Thus, photodetectors appear to form a synapse with the AP cell that could serve as a model system for studying the role of lectin in target recognition.

450.11

MORPHOLOGICAL AND NEUROCHEMICAL TARGET PREFERENCES OF ADULT PHOTORECEPTORS REGENERATING *IN VITRO*. D.M. Sherry* and E. Townes-Anderson. Cornell U. Med. Coll., Dept. Physiology and Dyson Vision Research Institute, 1300 York Ave., NY, NY, 10021.

The specificity of regenerative synaptogenesis by adult vertebrate neurons is poorly understood. Contact formation between photoreceptors and second- and third-order retinal neurons with identified amino acid content was examined by culturing differentiated neurons isolated from salamander retina in a defined medium. In this system, neurons retain characteristic morphological features and regenerate synapses. Regenerating photo-receptors form presynaptic varicosities that contain synaptic vesicles and the transmitter glutamate. 134 varicosity contacts have been analyzed. Varicosities usually contacted cell bodies (82.3%) rather than processes. Photoreceptors had the opportunity to interact with all retinal cell types, but contacted more improper targets: multipolar cells (32.8%; includes amacrine, ganglion and interplexiform cells); than proper targets: bipolar (0.7%), horizontal (0.7%) or photoreceptor cells (5.2%). However, most contacts (60.4%) were onto cells that had lost processes during isolation and could not be identified unequivocally, but their morphology and growth were inconsistent with photoreceptor or horizontal cells. Single-label immunocytochemistry showed that less than 34% of target cells contained aspartate or glycine, but over 50% contained GABA or glutamate, consistent with a high proportion of amacrine cell targets. These data suggest that regenerating photoreceptors *in vitro* prefer inappropriate target cell types, however, the transmitter content of most target cells (GABA, glutamate) is similar to that of cells postsynaptic to photoreceptors in the intact retina.

450.12

SYNAPTOGENESIS AND SYNAPTIC PLASTICITY IN DISSOCIATED CHICK CEREBRAL NEURONS. T.Taguchi*, K.Kiyosue, S.Kudo, M.Kasai, Lab of Biomol. Eng., Osaka Nat'l. Res. Inst. AIST, Ikeda 563, Japan, and Lab. of Mol. Biol., Fac. of Eng. Sci., Osaka Univ., Toyonaka 560, Japan.

To elucidate mechanisms of formation, maturation and plasticity of central nervous system at single cell level, it is essential to analyze the synaptic transmission at cellular and molecular level. We developed a dissociated neuron culture system, in which it is possible to analyze the synapse between two particular neurons. The neurons from cerebral hemispheres of chick embryos were dissociated by trypsinization and cultured on polylysin-coated dish containing 50% of modified Earle's minimum essential medium, 10% of fetal calf serum and 40% artificial medium (GIT, Nihon seiyaku, Japan). Using whole cell patch clamp technique, we confirmed synapse formation in vitro. The synaptogenesis seemed to proceed in proportion to the embryonic equivalent days (E.E.days) which are the sum of the days in incubation and culture. The synapses were found to begin to form at E.E.days 15 and mature at 17. We also demonstrated the long-term increase of synaptic efficiency in the same culture. After the neurons were exposed in the recording solution without Mg^{2+}, the long-lasting (more than 40 min) increase was observed from two neurons showing synchronous excitatory postsynaptic current. This phenomenon was abolished by the addition of tetrodotoxin and glutamate receptor antagonists in the Mg^{2+}-free solution.

450.13

SYNAPTIC COMPETITION BETWEEN SUPERNUMERARY AND NORMAL SENSORY NEURONS IN THE COCKROACH IS MEDIATED THROUGH A CHANGE IN QUANTAL CONTENT AND NOT QUANTAL SIZE. M.A. Sosa* and J.M. Blagburn. Institute of Neurobiology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico 00901.

The cerci of the first instar cockroach (*Periplaneta americana*) bear two filiform hairs each, one lateral (L) and one medial (M), which are innervated by individual sensory neurons. These sensory neurons project to the CNS, where they synapse with giant interneurons (GIs) in the terminal ganglion. Mutant nymphs had been previously discovered which have an extra L hair on one or both cerci. The sensory neuron that innervates the extra L hair, known as Space Invader Neuron (SIN), projects to the same GIs as the normal L hair, in effect competing for these synaptic targets. The size of the L EPSP in GI3 is reduced in the presence of SIN. We wanted to determine whether this reduction in EPSP amplitude was the result of pre- or post-synaptic changes.

Intracellular recordings of ipsilateral GI3 EPSPs, in response to movement of the L hair, were made in normal cockroach saline and *[a]* 1 mM Ca^{2+}/10 mM Mg^{2+} (low Ca/high Mg) saline, or *[b]* normal saline + 1 μM tetrodotoxin (TTX). Quantal content was determined by the direct method (EPSP/quantal size). Measures of quantal size were obtained from the first peak of histograms of EPSPs recorded in low Ca/high Mg saline and/or from amplitudes of miniature EPSPs recorded following addition of 1 μM TTX. EPSP amplitude was significantly reduced from 2.42 ± 0.16 mV (mean ± s.e.; n=7) in wild-type nymphs to 1.74 ± 0.26 mV (n=6) in the SI mutants ($p<0.045$). Mean quantal size in wild-type and mutant nymphs was not significantly different (0.23 ± 0.02 mV (n=7) and 0.27 ± 0.04 mV (n=6), respectively). Quantal content, however, was significantly reduced from 10.99 ± 1.03 (n=6) in wild-type nymphs to 6.70 ± 0.79 (n=6) in the SI mutants ($p<0.008$). These results indicate that the reduction in synaptic efficacy observed in the presence of SIN is presynaptic in nature.

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450.14

INTERACTIONS BETWEEN PHOTODAMAGED AND INTACT TERMINALS AT POLYNEURONALLY INNERVATED FROG NEUROMUSCULAR JUNCTIONS. V. Pitaevski* and A.A. Herrera. Dept. of Biological Sciences, University of Southern California, L.A., CA 90089-2520.

Our goal is to study how changes induced in one motor nerve terminal can affect another in polyneuronally innervated neuromuscular junctions in frog. This approach can give important information about the role and nature of competition during synapse elimination in neuromuscular junctions.

Reinnervated sartorius muscle of *Rana pipiens* has a substantial percentage of polyneuronally innervated junctions 8 weeks after nerve crush. Our previous *in vivo* observations indicated that under normal conditions such junctions are morphologically stable over a period of several weeks which makes artificially induced changes easily detectable in this system. To induce changes in a small portion of a terminal *in vivo* we used the fluorescent dye 4-Di-2-Asp combined with local illumination by blue light at an intensity level shown to cause terminal damage *in vitro*. The vital fluorescent dye RH414 and low-light video microscopy was used for repeated *in vivo* observations of exposed and intact terminals. In cases where photodamage caused retraction of exposed branches, no changes were detected in intact branches in the same neuromuscular junction a week after photodamage.

We are continuing to make repeated observations of identified neuromuscular junctions over longer periods of time after selective photodamage. Our study also includes an electrophysiological investigation of how stimulation of one input in a doubly innervated junction affects the other input.

[Supported by NIH grant NS24805.]

450.15

A HIGH NUMBER OF GABA SYNAPSES TARGETING DISTAL PORTIONS OF CORTICAL NEURONS IS LOST AFTER PERIPHERAL DEPRIVATION. C. Beaulieu*, K. Micheva and C.Crevier. Dept Pathology and Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, (Qué) CANADA.

The numerical density (N_V) and proportion (%) of GABA synapses on dendritic spines, shafts, somata, and initial segments of the axon was estimated in the somatosensory barrelfield areas of 5 rats having their vibrissae from the right face continuously removed from birth.

For the entire cortical depth, no significant differences in the N_V and % of GABA synapses on these postsynaptic elements were found in the barrelfield cortex contralateral and ipsilateral to the deprivation. About 19.7 million GABA synapses per mm^3 of tissue were devoted to spines (representing 3.8% of all contacts on spines), 55.7 million were on dendritic shafts (38.1%), 5.6 million on somata (71.0%) and less than 0.01 million on the initial segments of the axon (100%).

In layer IV however, sensory deprivation induced a drop from 55.6 in the ipsi cortex to only 11.9 million/mm^3 of GABA synapses on spines in the contra cortex (a 4.7 times loss; $p<0.0001$) and from 82.2 to 33.8 million/mm^3 on shafts ($p<0.05$). Only 2.2% and 29.1% of all contacts on spines and on shafts respectively, were GABA in the contra compared to as many as 9.3% and 49.1% in the ipsi layer IV.

We conclude that the population of GABA synapses located on distal portions of layer IV neurons is the most plastic of all populations of cortical synapses. (Supported by MRC, FRSQ, and FCAR).

450.16

SELECTIVE DECREASE IN THE NUMBER OF GABA SYNAPSES IN LAYER IV AFTER SENSORY DEPRIVATION. K. Micheva*, C. Crevier and C. Beaulieu. Dept Pathology and C.R.S.N., Université de Montréal, (Qué) CANADA.

The effects of modified peripheral activity at cortical level were estimated by quantifying the overall and GABA synaptic populations in the somatosensory barrelfield cortex of 5 rats whose whiskers on the right face were continuously removed after birth.

The numerical density of GABA synapses was found to decrease more than twice ($p<0.01$) in layer IV contralateral to the deprivation (63 million/mm^3) compared to the ipsilateral layer IV (145 million/mm^3). Accordingly, GABA synapses represented only 9.4% of the overall synaptic population in the contralateral versus 18.7% in the ipsilateral layer IV. At the same time, GABA synapses in the deprived layer IV were bigger (0.48 µm in the contralateral layer IV vs 0.32 µm in the ipsilateral layer IV; $p<0.05$). No significant changes in the numerical density, proportion or size of GABA synapses were detected in any other cortical layer. No differences in these parameters were found for the total synaptic population either.

As the firing activity and receptive field properties of cortical neurons are under the control of GABA, such a selective reduction in the number of GABA synapses in layer IV, the major recepient of thalamocortical inputs, would undoubtly lead to profound functional alterations in the entire circuitry of the deprived somatosensory cortex.

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450.17

Development of iterated structures in the NMDA receptor deficient mice Yuqing Li*, Reha S. Erzurumlu+, Chong Chen, Sonal Jhaveri+ & Susumu Tonegawa HHMI at the Ctr. for Cancer Res., +Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA 02139.

Sensory pathways of the brain generally develop from crudely wired networks to precisely organized system and in some cases to iterated structures such as ocular dominance columns and barrels in the cerebral cortex. Several studies have implicated neural activity-dependent mechanisms including NMDA receptors, in this refinement process. We applied the gene targeting in mouse embryonic stem cells to the NMDAR1 gene and created a mutant mouse which lacks functional NMDA receptors. We analyzed the formation of iterated structures in the mutant mice. The development of glomeruli in the olfactory bulb of mutant mice is compared to that of wild type animals. The number of glomeruli in the mutant mice is the same as their normal littermates, suggesting that the development of glomeruli is not dependent on NMDA receptor activation. In contrast, whisker-related patterns in the trigeminal nuclei (barrelettes) failed to develop as judged by both cytochrome oxidase staining and immunohistochemistry, although pathfinding, initial targeting and crude topographic projection of trigeminal axons in the brainstem are unaffected. In addition, in the absence of functional NMDA receptors, the synaptic transmission from trigeminal ganglion to the second order neurons in the brainstem trigeminal complex is functional in mutant mice. These results suggest that NMDA receptor and/or activity is essential for the development of somatosensory maps in the mammalian brain. Funded by HHMI and the Shionogi Inst. for Medical Science (ST) and NS27678 (SJ).

450.18

NITRIC OXIDE SYNTHESIZED IN DEVELOPING CHICK TECTUM IS INVOLVED IN ELIMINATION OF THE IPSILATERAL RETINOTECTAL PROJECTION. H.H. Wu* and S.C. McLoon. Univ. Minnesota, Minneapolis, MN 55455.

The adult pattern of axonal connections from the eye to the brain arises during development by refinement of a roughly ordered pattern of connections in chick. One characteristic of the early pattern of connections is a transient ipsilateral retinotectal projection. Previous studies showed that administration of arginine analogs prevented the loss of this transient ipsilateral projection. The most likely interpretation of this result is that the drug inhibited nitric oxide (NO) synthesis in the visual system, and that NO is somehow involved in the refinement process. The present study examined whether administration of an arginine analog actually reduces NO synthesis and identified the most likely site of this action. L-NOARG was applied to the chorioallantoic membrane of embryos. Controls received saline. On E13, the middle of the period of refinement, nitric oxide synthase (NOS) activity was assayed by measuring the conversion of arginine to citrulline in homogenates of retina and tectum. In normal embryos, NOS activity was detected in the tectum but not in the retina. This was consistent with the histochemical detection of NOS in these tissues during the period of refinement. NOS activity in the tectum was inhibited in the L-NOARG treated group but not in the saline treated group. The effect of L-NOARG was dose-related. There was a correlation between the level of NO synthesis inhibition and the degree of preservation of the ipsilateral projection in the tectum. These results suggest that NO serves as a messenger from the tectal cells back to the retinal axons and that it is involved in the developmental refinement of the visual projection. (Supported by EY05371.)

450.19

HOMONYMOUS NEUROMUSCULAR PREPARATION USING THE CAMPENOT CHAMBER. A. Parfitt, E.A. Neale*, S.C. Fitzgerald and P.G. Nelson: Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

We have shown that mouse ventral horn motor neurons grown on mouse cortical astrocytes, in the side wells of Campenot chambers that have been sealed onto laminin-coated plastic culture dishes, will extend axons underneath the chamber barrier into the center compartment, there to make functional contact with mouse striated muscle fibers. These fibers will contract in response to electrical field stimulation across one or both of the Campenot barriers. Most field-induced contractions could be blocked by rhodaminated-α-bungarotoxin, which was used to label post synaptic cholinergic receptors, suggesting that they were neurally mediated and not the result of direct electrical stimulation of muscle fibers which had grown into or under the Campenot barrier. Neurites that were immunoreactive for ChAT could also be found in association with muscle fibers that had contracted in response to electric field stimulation. Cells and their processes in the side wells of the Campenot chamber could be stained with Fast DiI/DiO. Intramembrane diffusion of these dyes along the neurites that passed under the chamber barrier permits visualisation, and will facilitate the differential characterisation, of the neurite fields in the center compartment. Preliminary experimental data include evidence for activity-dependent synapse elimination.

450.20

ACTIVATION OF MOUSE SKELETAL MUSCLE *IN VITRO* PRODUCES INCREASED SECRETION OF THROMBIN. P. G. Nelson*, K. Yadav, S. Fitzgerald and D. E. Brenneman. Lab. Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892.

Thrombin, at nM concentrations, has been implicated in the process of activity-dependent synapse reduction (ADSR) in a mouse neuromuscular junction preparation *in vitro* (Liu et al., Soc. Neurosci Abs, this volume). This is based on the block of ADSR by nM concentrations of hirudin, a highly specific thrombin inhibitor. Also exogenous thrombin (~10 nM) causes synapse loss which is blocked by hirudin. We have, therefore, investigated the regulation of thrombin release from muscle cells. We compared the levels of thrombin in media conditioned by muscle cells treated with tetrodotoxin (TTX, 1 µM, to block activity) or acetylcholine (ACh, 10 µM, to activate the cells). Significantly more thrombin was released by the ACh treated cells (.056 nM ± 0.03 nM; S.D., n=18 with ACh vs .027 ± 0.02 nM, n=15 with TTX). (The medium with 5% horse serum contained 0.006 nM thrombin). We hypothesize that increased thrombin secretion produced by muscle activation could contribute to ADSR.

451.1

OVEREXPRESSION OF NGF IN TRANSGENIC MICE INDUCES NOVEL SYMPATHETIC PROJECTIONS TO PRIMARY SENSORY NEURONS. B.M. Davis*, D.M. Katz*, K.B. Seroogy and K.M. Albers#. Dept. of Anatomy & Neurobiology, and #Pathology, Univ. of Kentucky College of Medicine, Lexington, KY 40536; *Dept. of Neuroscience, Case Western Reserve Univ. School of Medicine, University Hospital of Cleveland, Cleveland, OH 44106.

Peripheral nerve crush induces novel projections from sympathetic neurons to sensory ganglia, and it has been suggested that these projections are the anatomical substrate for chronic pain syndromes. Our study demonstrates that novel sympathetic projections to sensory neurons are also induced in transgenic mice that overexpress NGF in the skin. Specifically, a large proportion of primary sensory neurons in NGF transgenic mice were innervated by tyrosine hydroxylase (TH)-positive pericellular arborizations that were seen only rarely in controls. Removal of the superior cervical ganglion abolished TH immunoreactive arborizations in the ipsilateral trigeminal ganglion confirming that these fibers were sympathetic axons. A two-site ELISA revealed that transgenic ganglia contained a ten fold increase in NGF peptide. Reverse transcriptase PCR analysis showed no apparent expression of transgene mRNA in sensory ganglia suggesting that the additional NGF was derived from increased NGF expression in the skin. These results indicate that NGF induces novel sympathetic projections to sensory neurons, and supports the hypothesis that increased NGF expression is a critical link in the development of sympathetic hyperalgesia. Supported by AR-40873 (KMA), NS-31826 (BMD), HL-42131 (DMK) and IBN-9221136 (KBS).

451.2

CHARACTERIZATION OF TRANSGENIC MICE THAT OVEREXPRESS NT-3 IN THE EPIDERMIS. K.M. Albers*, T. N. Perrone and B.M. Davis#. Depts. of Pathology and Anatomy & Neurobiology#, Univ. of Kentucky Sch. of Med., Lexington, KY, 40536.

During development of the mammalian skin neurotrophic molecules are expressed at times overlapping with sensory neuron outgrowth to the periphery. The production of neurotrophic compounds by the skin is thought to promote neuronal survival and differentiation and thus serve as a means to tailor the innervation pattern of the skin. To examine the role of neurotrophins in an *in vivo* system, we have isolated transgenic mouse lines that overexpress either NGF, NT-3 or BDNF in basal cell keratinocytes of the epidermis. NGF overexpression appears to block the naturally occuring program of neuronal cell death and leads to the formation of a dramatically hypertrophied peripheral nervous system. To isolate NT-3 expressing mice, the NT-3 gene sequence encoding the precursor and active peptide sequence was cloned from mouse genomic DNA using the polymerase chain reaction. The sequence was confirmed by DNA sequencing and then cloned into an expression cassette containing the human keratin K14 gene promoter and enhancer elements. Lines of NT-3 overexpressing mice have been characterized by Southern, northern, and *in situ* hybridization assays and show high levels of transgene expression. Mice appear normal in size and health. Gross and histological examination of the skin show it to contain a greater number of larger touch dome units, suggesting that NT-3 plays a role in the survival of the SA1 type neurons that innervate these structures. To begin to chemically characterize the sensory neurons of the dorsal root ganglion, the percentage of trk A, trk B, and trk C expressing neurons are being determined. Supported by AR40873 (KMA) and NS 31826 (BMD).

451.3

CHARACTERIZATION OF MICE OVEREXPRESSING BDNF IN THE EPIDERMIS. A.M. LeMaster*, B.M. Davis, T.N. Perrone, F. Davis, Z. Pang and K.M. Albers. Depts. of Anatomy and Neurobiology and Pathology, Univ. of Kentucky Sch. of Med., Lexington, KY 40536.

Neurotrophins, nerve growth factor (NGF), brain derived neurotrophic factor, (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), are a family of homologous proteins that affect neuronal cell survival and support neuronal growth and differentiation. During development neurotrophins are made by neuronal target tissues such as skin and the decline in their expression levels correlates with the onset of naturally occuring neuronal cell death. To examine the role of neurotrophins in the development of the peripheral nervous system, we isolated transgenic mice that express fusion gene constructs containing a human keratin (K14) gene promoter ligated to the various mouse neurotrophin cDNAs. Transgene expression begins in the basal cell layer of epidermis around embryonic day 14 (E14), coinciding with the normal decline in neurotrophin levels. Previously we have isolated and characterized mice containing a K14-NGF transgene. In the present study, mice expressing a K14-BDNF transgene have been isolated. BDNF is normally expressed in neuronal tissues of both the central and peripheral nervous system and in nonneuronal tissues such as heart , lung and skin. By overexpressing BDNF in the epidermis, its role in development and in the adult may be elucidated. Three lines of mice have been isolated that express the K14-BDNF transgene as shown by Southern and Northern hybridization. Mice appear normal in size and health. Initial histological examination of the skin and sensory ganglia show no major alterations in these structures. Supported by NS 31826 (BMD) and AR40873 (KMA).

451.4

NGF OVEREXPRESSION IN EPIDERMIS DISRUPTS SYMPATHETIC INNERVATION OF SWEAT GLANDS AND DIFFERENTIALLY ALTERS TRANSMITTER PROPERTIES. G. Guidry[1], B. M. Davis[2], S. C. Landis[1,*] and K. M. Albers[3]. [1]Dept. of Neurosci., Case Western Reserve Univ., Cleveland OH 44106, [2]Dept. of Anatomy and Neurobio. and [3]Dept. of Path., Univ. of Kentucky College of Medicine, Lexington KY 40536

We examined sympathetic innervation in transgenic mice carrying multiple copies of the NGF gene driven by an epidermal keratin promoter. In adult wild-type mice, cholinergic and noradrenergic sympathetic fibers and peptidergic sensory fibers innervated distinct targets in the footpad. Acetylcholinesterase (AChE) and vasoactive intestinal peptide (VIP) sympathetic axons were associated with sweat glands while catecholaminergic fibers innervated blood vessels. All sympathetic innervation was restricted to tissues in the pad interior. Calcitonin gene-related peptide (CGRP) and substance P (SP) sensory fibers traversed the pad and terminated at the interface between the epidermis and dermis. While the timing of innervation in footpads did not appear to be affected in transgenic mice, segregation of sympathetic and sensory innervation was altered. A dense plexus of AChE and catecholaminergic fibers was found beneath the epidermis and VIP fibers extended into the epidermis. The plexus was absent from footpads of mice treated as neonates with the adrenergic neurotoxin 6-OHDA, indicating its sympathetic origin. This same region was hyperinnervated by CGRP and SP sensory fibers. In addition, sweat gland innervation was disrupted. While some glands were innervated by AChE and VIP fibers, most lacked innervation. Despite the sparse gland innervation, secretory responsiveness appeared normal. Our findings suggest that over-expression of NGF in skin interfered with the normal segregation of sensory and sympathetic fibers. Further, the ectopic sympathetic fibers displayed an abnormal neurotransmitter phenotype, expressing both catecholamines and cholinergic properties.

451.5

ANALYSIS OF MICE CARRYING TARGETED MUTATIONS OF THE BDNF GENE. P. Carroll, G.R. Lewin, M. Koltzenburg§; K.V.Toyka§, E. Wolf#, G. Brem#, E. Castrén* and H. Thoenen Dept. Neurobiochemistry, Max-Planck Institute for Psychiatry D-82152, Martinsried. §Dept. Neurology, University of Würzburg, D-97080 Würzburg, #Dept. Molecular Animal Breeding, Veterinary School, Veterinärstr. 13, D-80539 Munich, Germany.

Brain derived neurotrophic factor is a member of the family of related molecules, which includes NGF, NT3, and NT 4/5. Neurotrophins effects on neuron survival and differentiation *in vitro* and *in vivo* suggest important roles for these molecules on essential developmental processes such as programmed cell death and determination of neuronal phenotype. As part of our efforts to clarify the *in vivo* role(s) of the neurotrophins we have generated, using gene targeting methodology, mice in which most of the sequence coding for BDNF was deleted and replaced with a neomycin resistance gene. Homozygous mutant mice (-/-) are reduced in size compared to their litter mates and most die within the first three postnatal weeks. Analysis of DRG cell numbers and size indicates that many neurons die in the (-/-) animals. Heterozygous animals (+/-) were viable but consistently intermediate in size. This prompted us to examine the functional properties of primary afferents innervating skin in adult (+/-) mice using an *in vitro* recording technique (Koltzenburg et al. Soc Neurosci., this meeting). Briefly, single primary afferents were recorded in the saphenous nerve using standard teased fiber techniques. All cutaneous primary afferent types were encountered and characterized in wild type and (+/-) animals (Aβ, Aδ and C-fibers), however, only one type was severely affected in the (+/-) animals. These fibers, slowly adapting mechanoreceptors conducting in the Aβ fiber range, had very elevated mechanical thresholds. These initial results suggest that BDNF is critically important for the regulation of this afferent type. It will be important to see if total absence of BDNF in (-/-) animals has more widespread effects on the functional properties of sensory neurons.

451.6

TRANSGENIC MICE OVEREXPRESSING BDNF IN NEURAL STEM CELLS. Torkel Falkenberg*[1,4] Thomas Ringstedt[1,2], Erik Nilsson[3], Urban Lendahl[3], Ron McKay[5], Håkan Persson[1] and Carlos F. Ibáñez[1]. [1]Molecular Neurobiology, Medical Biochemistry and Biophysics, [2]Department of Women and Child health, [3]Developmental Biology, Cell and Molecular Biology, [4]Department of Pharmacology and Physiology, Karolinska Institute, Stockholm, Sweden, [5]Molecular Biology, NIH, Bethesda, MD, USA.

Neural stem cells in the neuroepithelium of the neural tube are transiently present during embryonic life and later differentiate to form neurons and glial cells. The neural stem cell state is accompanied by expression of the intermediate filament nestin. Regulatory regions controlling the nestin expression in the stem cell have previously been characterized in transgenic mice using lacZ as a reporter gene. To adress the effects of overexpression of the neurotrophic factor BDNF (brain derived neurotrophic factor) in neural stem cells we have linked the regulatory regions of the nestin gene to the coding portion of the BDNF gene. This DNA construct was injected into fertilized mouse eggs with the attempt to generate stable transgenic mouse lines. With this particular construct we have observed a very low frequency of postnatal transgenic mice (6% of all offspring, compared to 20-40% for other constructs). Moreover, one of the founders did not express BDNF from the exogenous gene, while another one did not pass the gene further to its offspring. These data suggest that transient expression of BDNF in neural stem cells may be detrimental for the transgenic embryos. Ongoing experiments are aimed at analyzing phenotypes in transgenic embryos at different developmental stages.

451.7

CHARACTERIZATION OF DOPAMINE-ß-HYDROXYLAS: BDNF TRANSGENIC MICE. C.G. Causing*, S. Bamji, A. Gloster, A. Speelman, R. Varma, E. Chang, and F.D. Miller. Centre For Neuronal Survival, Montreal NeurologicalInstitute, Montreal, Quebec, H3A 2B4

Neurons secrete neurotrophins that may play a role in neuron:neuron interactions. Brain-derived neurotrophic factor (BDNF), is widely distributed in the mammalian CNS. In the PNS, sympathetic neurons of the superior cervical ganglia (SCG) express the mRNA encoding BDNF. Since sympathetic neurons do not respond to BDNF in culture, and since they do not express the BDNF receptor, trkB, we have hypothesized that sympathetic neuron-derived BDNF may provide neurotrophic support for their input neurons, the preganglionic sympathetic neurons. To examine this possibility, we have generated transgenic mice expressing BDNF from a 1.6 kb fragment of the dopamine-β-hydroxylase (DBH) promoter, which is sufficient to target gene expression to adrenergic and noradrenergic neurons (including sympathetic neurons) of transgenic mice. Based on Northern blot analyses, we have found that three of the lines generated express the BDNF transgene in the SCG, adrenals, and brainstem with varying levels of expression. There was a dramatic increase in ChAT staining within both the SCG and the thoracic spinal cord regions which contain the preganglionic neurons projecting to the SCGs (intermediolateral nucleus, the central autonomic area, and the intercalated region). The overexpression of BDNF also resulted in a hyperinnervation of the sympathetic neurons by the cholinergic preganglionic neurons. In addition, there was a 25% increase in SCG neuronal cross-sectional area, and possibly an increase in sympathetic innervation density within an SCG target tissue, the pineal gland. Thus, the BDNF induced hyperinnervation of the SCGs may produce an increase in SCG neuronal size and hyperinnervation of sympathetic target tissues. These studies provide support for the concept that sympathetic neuron-derived BDNF is a neuron-derived neurotrophic factor for their preganglionic input, and suggest that the density of afferent input may regulate the biology of the target neuron.

451.8

VISCERAL SENSORY NEURONS ARE SEVERELY DEPLETED IN MICE LACKING FUNCTIONAL trkB PROTEIN TYROSINE KINASE RECEPTORS. J.T. Erickson*[1], R.J. Smeyne[2], M. Barbacid[2] and D.M. Katz[1]. [1]Departments of Neurosciences and Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106 and [2]Department of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

Gene disruption targeting the catalytic domain of the trkB neurotrophin receptor results in deficits of somatosensory and somatomotor neuronal pools, and neonatal mortality, in mice homozygous for this mutation (Klein et al. Cell 75:113.1993). Recent findings from one of our laboratories (DMK) indicate that trkB ligands may also mediate peripheral target support of visceral afferent survival. In particular, BDNF mRNA is expressed in visceral receptor tissues *in situ*, and local application of BDNF can rescue visceral afferents following peripheral target removal *in vivo* (Hertzberg et al., this volume and submitted for publication). To further define the role of trkB ligands in visceral sensory development, the present study examined visceral afferent survival in the nodose and petrosal cranial sensory ganglia of trkB knockout mice. Ganglion volume, as well as cell number, were severely depleted in mice homozygous for the targeted allele. Furthermore, immunohistochemical detection of tyrosine hydroxylase, a marker of dopaminergic cardiorespiratory afferents in the rat petrosal ganglion (Katz and Black, 1986), revealed a striking reduction in catecholaminergic nodose-petrosal neurons. These results indicate that a large proportion of cranial visceral afferents, including many, but not all, of the catecholaminergic neurons in the nodose-petrosal complex, require trkB ligand(s) for survival during perinatal development. Nodose and petrosal neurons relay visceral sensory information from the cardiovascular, respiratory and gastrointestinal systems to the central nervous system, and thereby play a key role in regulating autonomic homeostasis. We hypothesize that deficits in these neuronal populations, and cardiorespiratory afferents in particular, may contribute to the lethality of the trkB mutant phenotype. Supported by HL-42131 (DMK) and training support from HL-07288 (JTE).

451.9

NEURON-SPECIFIC OVEREXPRESSION OF NEUROTROPHIN-3 IN TRANSGENIC MICE C.Helbig, M.Meyer*, E.Wolf[$], G.Brem[$] and H.Thoenen. Max-Planck Institute for Psychiatry, D-82152 Martinsried, Germany; [$]Ludwig-Maximilians University, D-80539 Munich, Germany

Neurotrophin-3 (NT3) is widely expressed in the central and peripheral nervous system as well as in various peripheral tissues. Although the expression pattern of NT3 is well known and *in vitro* effects on sensory, sympathetic and motoneurons have been described, little is known about the *in vivo* function of NT3.

To investigate the function of NT3, transgenic mice were generated. In these mice NT3 expression was driven by a 1.5 kb fragment of the PDGF-B chain promoter, previously shown to drive expression mainly to neurons.

Four founder animals were obtained by pronucleus injection. Two lines showed the same transgene expression pattern. By Northern blot analysis a 2-5 fold higher NT3 expression in cerebellum, cerebrum, lung, kidney, uterus and ovary was detected comparing F2 adult homozygous transgenic to wildtype littermates. Ectopic expression was detected in olfactory bulb, spinal cord, testes and vas deferens.

In situ hybridization was performed to examine the cellular localization of transgene expression. High expression was observed in mitral and periglomerular cells of the olfactory bulb, in granular neurons and Purkinje cells of the cerebellum. In addition to the endogenous hippocampal NT3 expression in dentate gyrus, CA2 and medial CA1, NT3 mRNA was detected in CA3 and CA4 neurons. Neurons of the neocortex also showed strong labelling, which was not seen in wildtype controls. Finally motoneurons in the spinal cord and more than 50% of DRG neurons were strongly labelled.

The transgenic animals showed no obvious phenotype with respect to weight, behaviour or gross morphology. Effects due to the overexpression or ectopic expression of NT3 on the sensory, sympathetic and motor nervous system are under investigation.

451.10

STRUCTURE AND TRANSGENIC EXPRESSION OF RAT NEUROTROPHIN-4 GENE. M. Metsis, T. Salin , Y. Hurd** and T. Timmusk. Lab. of Molecular Neurobiology, Karolinska Institute and *Dept. of Clinical Neuroscience, Karolinska Hospital, 17177 Stockholm, Sweden.

Neurotrophin-4 (NT-4) expression is very low in the adult organism, while higher levels are present during embryogenesis. Developmentally regulated expression of NT-4 in whisker pad has led to the hypothesis of its target derived action on the trigeminal ganglion. To study the regulation of the NT-4 gene and to evaluate the possible roles of this neurotrophin in developing and in adult nervous system we have characterized rat NT-4 gene. A cDNA library was made from P1 testis, where a high expression level was previously detected. Screening of this library resulted in 10 cDNA clones that were used to screen a rat genomic library. 10 overlapping genomic clones were isolated that covered 45 kb of the NT-4 locus. Mapping of the intron-exon structure revealed two upstream noncoding exons separated by an 800 bp intron. Transient expression analyses with bacterial chloramphenicol acetyl transferase reporter gene suggests an important role of intronic sequences in supporting the promoter activity. Rat NT-4 gene fragment containing upstream exons, promoter region and coding exon was introduced into transgenic mice. The expression pattern of the transgene recapitulates the endogenous gene expression pattern and regulation. Transgenic mice with high level of NT-4 expression have altered levels of other members of neurotrophin family in distinct brain regions.

451.11

Effects of Transgenic Over-expression of CNTF in the CNS. J.T. Henderson*, N.A. Seniuk, P.M. Richardson, J.C. Roder; Mount Sinai Hospital, Toronto, ONT M5G-1X5.

CNTF has been shown *in vitro* to mediate a wide variety of effects on neural cell. In order to more clearly delineate the developmental and regenerative effects of proteins using the CNTFRα/gp 130/LIFRβ receptor system, transgenic mice were created which over-express CNTF in the CNS. Both the endogenous (CNTF-ns) and secreted (CNTF-s) forms of rat CNTF were placed under the control of either the glial fibrillary acidic protein (GFAP) or the neuron specific enolase (NSE) promoters. Examination of the facial nucleus of transgenic mice which over-express GFAP/CNTF-ns show a developmental increase in the number of motoneuron (150% of non-transgenic siblings). In contrast, transgenic lines derived from the GFAP/CNTF-s transgene exhibit relatively normal numbers of facial motoneuron, however demonstrate a marked increase in motoneuron survival compared to non-transgenic siblings following axotomy of the facial nerve at postnatal day 3 (70% vs. 25%), concomitant with reactive gliosis at this site. Murine lines expressing NSE/CNTF-s exhibit alterations in oligodendrocyte gene expression, in proportion to the level of CNTF transgene expression. These results indicate CNTF can exert a variety of neural effects which are context dependent.

451.12

SYNAPSE WITHDRAWAL FROM DEVELOPING NEUROMUSCULAR JUNCTIONS OCCURS EARLIER IN LIF DEFICIENT TRANSGENIC MICE Young W. Kwon*[1], Susan J. Abbondanzo[2], Colin L. Stewart[2], and Mark E. Gurney[1]. [1]Dept. of Cell, Molecular and Structural Biology, Northwestern Univ., Chicago, IL. 60611 and [2]Dept. of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ. 07110.

We previously showed that leukemia inhibitory factor (LIF) mRNA expression occurs in both embryonic and neonatal muscle and that it is developmentally regulated. Furthermore, administration of exogenous LIF delays the timing of programmed synapse withdrawal without affecting its rate. Hence, endogenous LIF is a likely candidate for a cytokine that regulates this process during normal development. To provide additional evidence for this hypothesis, we examined the course of synapse withdrawal in mice whose LIF gene was disrupted by homologous recombination. Muscles from animals of various genotypes were stained by a combined silver and cholinesterase method and then were examined microscopically for the presence of multiply innervated fibers. The percentages of multiply innervated fibers in various animals are as follows (+/+, wild-type; +/-, heterozygote; -/-, homozygote):

Age (days)	+/+	+/-	-/-
5	75±3%	74±3%	75±2%
7	53±3%	52±3%	38±2% [a]
9	26±3%	19±2%	12±2% [a]

a) $p<0.001$, unpaired T-test.

Thus, synapse withdrawal occurs earlier in LIF deficient transgenic mice. This study, together with the previously mentioned results, provide evidence that endogenous LIF regulates the timing of programmed synapse withdrawal from the developing neuromuscular junction.

451.13

EVALUATION OF GROWTH FACTOR EFFECTS ON MESENCEPHALIC DOPAMINERGIC NEURONS USING A *FOS-LACZ* TRANSGENIC MOUSE LINE

J. Engele* and K. Schilling. Dept. Anatomy and Cell Biology, University of Ulm, 89069 Ulm, FRG.

One of the initial effects of many growth factors is the rapid and transient induction of immediate early genes such as c-*fos* in their target cells. In the present study, we have exploited this feature to probe the cellular target(s) of growth factors previously observed to support survival of cultured dopaminergic neurons. Specifically, we used low density cultures established from the dissociated mesencephalon of E14 *fos-lacZ* transgenic mice (generously provided by J.I. Morgan, Roche Institute, Nutley, N.J.) to monitor *c-fos* expression in immunocytochemically identified cellular phenotypes. NT-3 (10 ng/ml; 3 hrs), bFGF (25 ng/ml) or a combination of aFGF (50 ng/ml) and heparin (2.5 U/ml) induced transgene expression in subpopulations of tyrosine hydroxylase-immunoreactive dopaminergic neurons as well as in non-dopaminergic neurons and glial cells. The effects of these growth factors on dopaminergic neurons were not affected by cellular plating density or by eleminating synaptic communication. In contrast, TGFα (50 ng/ml) and PDGF-BB (30 ng/ml) induced transgene expression exclusively in glial cells but not in dopaminergic neurons.

These studies identify mesencephalic dopaminergic neurons as the primary target of NT-3 and FGF and further demonstrate that sensitivity for these growth factors is restricted to distinct subsets of dopaminergic neurons. Finally, our results suggest that the known survival-promoting effects of TGFα and PDGF-BB on dopaminergic neurons are mediated through mesencephalic glia.

451.14

EFFECTS OF GROWTH FACTORS AND CYTOKINES ON HIPPOCAMPAL CELLS DERIVED FROM THE H-2Kb-tsA58 TRANSGENIC MOUSE.

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A number of workers have made use of transfection and retroviral-mediated gene insertion using a variety of oncogenes in order to immortalise neural precursor cells. This study used a conditionally immortalised hippocampal cell population derived from the H-2Kb-tsA58 transgenic mouse. The mouse possesses an established integrated copy of the early region of the large tumour antigen from the temperature sensitive gene allowing immortality to be controlled at the permissive temperature of 33°C, the non-permissive temperature being 39.5°C.

We examined the effects of several growth factors including basic fibroblast growth factor (bFGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), the cytokines used were γ-interferon and ciliary neurotrophic factor (CNTF) on E15 transgenic mouse hippocampus progenitor cells.

Initial results showed that bFGF produced an elevation in both survival and proliferation in the hippocampal cells with maximal effects produced at a concentration of 10ng/ml. NGF produced minimal increase in cell number, but did however induce differentiation when kept at 39.5°C. The other 2 neurotrophins (BDNF and NT-3) as well as CNTF produced interesting results which will be shown in the poster. Cells treated with γ-interferon (γ-IFN) were lower in number and longevity at the higher concentrations.

Partially supported by the Wellcome Trust

451.15

ROLE OF ESTROGEN IN AFFECTING INCREASED ALCOHOL INTAKE IN TRANSGENIC TGFα MICE. Leena Hilakivi-Clarke*. Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007

Results with transgenic CD1 mice that overexpress transforming growth factor α (TGFα) suggest that this growth factor alters non-reproductive sex-related differences in behavior. Specifically, male TGFα mice exhibit a feminization of locomotor activity, immobility in the swim test, and sodium preference. These effects may be mediated through an interaction between TGFα and estrogen. The TGFα mice exhibit elevated plasma 17ß-estradiol (E2) levels. Castration reverses the feminized behavioral patterns in the male TGFα mice. The present study investigated the effect of orchiectomy, E2 and the antiestrogen tamoxifen on voluntary alcohol consumption in transgenic TGFα mice. Both the male and female transgenics consumed more 5% alcohol (expressed as a percentage of total fluid intake) than the non-transgenic mice ($F(1,39)=8.8$, $p<.005$). Orchiectomy did not have a significant effect on alcohol intake. In non-transgenic CD1 male mice, treatment with pellets releasing E2 increased and treatment with tamoxifen reduced alcohol intake, when compared with placebo-treated mice ($H(5)=25.2$, $p<.001$). In ovariectomized females, E2 reduced alcohol intake ($H(4)=12.6$, $p<.006$). These data suggest that overexpression of TGFα increases alcohol consumption in both sexes. Estrogen also increases alcohol intake in non-transgenic male mice, and this is reduced by the antiestrogen tamoxifen. However, since orchiectomy at adulthood did not reverse the increased alcohol intake patterns in the transgenic mice, the effects of TGFα on alcohol consumption may be mediated through other pathways than elevated E2. Alternatively, the effects of E2 occurred during the critical period of sexual differentiation.

451.16

Nerve growth factor (NGF) regulation of estrogen receptors in the developing nervous system. R.C. Miranda*, F. Sohrabji, C.D. Toran-Allerand. Columbia University, Dept. Anatomy and Cell Biology, 630 West 168th St., New York, NY 10032

We are interested in examining mechanisms regulating estrogen actions during neuronal differentiation in the central nervous system. Our research has focused on one possible mechanism, the developmental interactions of nerve growth factor (NGF) and estrogen receptors. Using a combination of steroid autoradiography, immunohistochemistry and *in situ* hybridization methodologies, we have shown that estrogen target neurons of the developing forebrain express the NGF receptors, $p75^{NGFR}$ (the pan-neurotrophin receptor) and *trk*A (the specific tyrosine kinase receptor). Moreover, NGF and estrogen reciprocally regulate their receptors in PC12 cells, a neurotrophin-responsive cell line. In this study, we examined the regulation of estrogen binding by NGF in the developing forebrain using organotypic explant cultures maintained in roller tube assemblies. Explant cultures of the postnatal day 2 rat cerebral cortex and basal forebrain were maintained 8 days *in vitro*, in the presence or absence of NGF (100 ng/ml). Our data indicate that estrogen receptors and receptor mRNA are expressed in these cultures, in patterns similar to those observed *in vivo*. Using a modified nuclear exchange assay to measure specific, intra-nuclear estrogen (^{3}H-moxestrol) binding, we found that NGF alters nuclear estrogen binding in a regionally specific manner. We are currently examining mechanisms underlying regionally-specific NGF regulation of estrogen binding. *(Supported by grants from the NIH, NIMH, NSF, AHAF and an ADAMHA-RSA to C.D.T-A)*

451.17

NEUROTROPHIC FACTORS FAIL TO PREVENT CASTRATION-INDUCED REGRESSIVE CHANGES IN AN ANDROGEN-SENSITIVE RAT SPINAL NUCLEUS. M.C. Clark-Phelps[1*], N.T. Neff[2], and D.R. Sengelaub[1]. [1]Program in Neural Science, Indiana University, Bloomington, IN 47405; [2]Cephalon, Inc., West Chester, PA 19380.

Motoneurons in the rat spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens. Castration of adults significantly reduces SNB dendritic length, soma size, and target muscle weight, and these changes can be prevented or reversed with androgen treatment. Using compounds demonstrated to have trophic effects on motoneurons in several models, we assessed maintenance of muscle weight and motoneuron morphology of the androgen-sensitive SNB system following castration in adulthood.

Adult male rats (Sprague-Dawley) at approximately 90 days of age were either castrated or left intact. Castrated rats received daily subcutaneous injections over the SNB target muscles of either IGF-I (1 mg/kg), CNTF (1 µg), or vehicle alone for six weeks. Following treatment, SNB target muscles (bulbocavernosus and levator ani; BC/LA) were weighed and motoneuron morphology was visualized histochemically after retrograde labeling with a cholera toxin-HRP conjugate. Castration significantly reduced SNB soma size and BC/LA muscle weight relative to intact males, and treatment with either IGF-I or CNTF had no effect. Thus, unlike androgen, IGF-I and CNTF failed to prevent castration-induced regressive changes in these aspects of SNB morphology, suggesting that the androgen-dependent trophic effects observed in this system are not mediated by these compounds in adulthood. (Supported by Cephalon)

452.1

NERVE GROWTH FACTOR-INDUCED PLASTICITY OF CEREBROVASCULAR INNERVATION. D.Ondris, C. Billieu, K. Schwenk, S.Page, and L.G. Isaacson* Dept of Zoology, Miami University, Oxford, OH 45056.

We have shown that a two week intracerebroventricular infusion of nerve growth factor (NGF) into a female adult rat results in a sprouting response by mature sympathetic perivascular axons associated with the intradural internal carotid artery (ICA). The purpose of the present study was to 1) use electron microscopy to examine the role of afferent input to the superior cervical ganglion (SCG) in the sprouting response, 2) carry out calcitonin gene related peptide (CGRP) immunohistochemistry with and without sympathectomy to examine whether sensory perivascular axons respond to NGF infusion 3) use High Performance Liquid Chromatography (HPLC) to determine whether increased catecholamines accompany the morphological response. The number of axons observed following deafferentation and subsequent NGF infusion was similar to that observed following NGF infusion only (1832 vs. 1829 axons). NGF infusion resulted in increased perivascular density of CGRP-positive axons compared to VEH infused animals but the most significant increase in CGRP-positive axons was observed following bilateral ganglionectomy and NGF infusion. In HPLC studies, ICA norepinephrine concentrations were significantly increased following NGF infusion (52 µg/g vs. 22 µg/g). These findings suggest that afferent input to the SCG is not a prerequisite to the NGF-induced sprouting response, that mature sensory perivascular axons respond to NGF infusion, and that increased catecholamines accompany the sprouting response.

452.2

THE ADULT RAT HIPPOCAMPUS CONTAINS NGF IN SOLUBLE AND BOUND FORMS. J. Costello, J. Conner, P. Burnham* and S. Varon. Dept of Biology 0601, Univ. of Calif., San Diego, San Diego, CA 92093.

The hippocampus is a primary site of neurotrophin mRNA expression in both developing and adult rats. Neonatal sympathetic ganglia transplanted into adult rat hippocampus grow neurites selectively into dentate gyrus and CA3 regions, which display NGF-like staining. The discrete boundaries of this staining suggest the occurrence of NGF, or a related neurotrophin, firmly bound to hippocampal structures. To test this possibility, supernatant and resuspended pellet fractions from centrifuged hippocampal sonicates were assayed on E8 DRG neurons, with one trophic unit (TU)/ml of NGF defined as the concentration required for half maximal survival.

Activity was found in both soluble (15 TU per hippocampus) and pellet (68 TU per hippocampus) fractions. The number of neurons supported by optimal pellet and soluble concentrations was the same as that supported by NGF, although the soluble fraction contained a toxic material which in some cases reduced maximal survival. No additional neuronal survival was achieved by combining optimal concentrations of soluble and pellet fractions or by combining either one with NGF. An amount of anti-NGF antibody that completely blocks the activity of 50 ng/ml of NGF blocked greater than 60% of the soluble fraction activity and fully blocked optimal concentrations of the pellet fraction. BDNF and NT-3 supported 70% and 55%, respectively, of the number of neurons as did NGF, but did not add further support when combined with NGF, and their activity was not blockable by the NGF antibody.

These data indicate that the adult rat hippocampus contains a bioactive neurotrophin--probably NGF--in both soluble and bound forms. The bound factor could be the material that displays NGF-immunoreactivity in the hippocampal sections and might be the signal that is responsible for the selective hippocampal innervation by NGF-sensitive fibers.

452.3

INTRAVENTRICULAR INJECTIONS OF NGF FAIL TO INCREASE THE HEMICHOLINIUM-3 BINDING IN THE HIPPOCAMPUS OF NEONATAL RATS. X. Tian, N. A.Bourjeily, J. Weingartner, K. A. Crutcher, and J. B. Suszkiw. Depts. of Physiology & Biophysics, and Neurosurgery, University of Cincinnati, Cincinnati, OH 45267-0576.

The effect of NGF on the development of cholinergic phenotypic markers in the septo-hippocampal pathway (SHP) was investigated in the rat at postnatal days 7 (PN7) and 14 (PN14). Relative to adult levels, the septa of PN7 and PN14 animals contained respectively, 31.3% and 84.1% NGF, and 24.6% and 92.9% cholineacetyltransferase (ChAT) activity. The densities of [^{3}H]hemicholinium-3 (HC-3) binding sites in the hippocampi of PN7 and PN14 animals were 7.1% and 11.8% of adult, respectively. Intraventricular administration of NGF three days prior to assays, increased septal ChAT to 70.8% at PN7 and 120% at PN14, but had no significant effect on the [^{3}H]HC-3 binding in the hippocampus. These results indicate that in contrast to ChAT activity, the developmental expression of the high affinity choline transport (HACU) in the terminals of septo-hippocampal neurons is not regulated by NGF. Furthermore, assuming that density of HACU sites provides an index of cholinergic innervation in the hippocampus, these data suggest that NGF probably has no effect on the extent of innervation of the hippocampal targets by the septal cholinergic neurons during development of SHP in the rat. *Supported by NIH grants ES06365 (JBS) and NS17131 (KAC).*

452.4

STAGE-SPECIFIC EFFECTS OF NEUROTROPHIN-4/5 AND BRAIN DERIVED NEUROTROPHIC FACTOR DURING CEREBELLAR GRANULE CELL NEUROGENESIS. J.L. Zheng, M. Karihaloo and W.-Q. Gao*. Department of Neuroscience, Genentech, Inc., South San Francisco, CA 94080

We have used purified, well-characterized granule cell cultures (Gao et al., 1991, Neuron *6*:705-715) to examine possible effects of the neurotrophins on four different stages of cerebellar granule cell neurogenesis. The four stages included neuronal proliferation, initiation of neuronal differentiation, neuronal maturation and neuronal maintenance. None of the neurotrophins stimulated proliferation of the granule cell precursors or rescued any phenotypic defects of the mutant *weaver* granule cells in the initiation of neuronal differentiation. However, neurotrophin-4/5 (NT-4/5) and BDNF, but not neurotrophin-3 (NT-3) or nerve growth factor (NGF), promoted neurite extension and survival of differentiated cerebellar granule cells. Moreover, NT-4/5 and BDNF enhanced neurite extension by *weaver* granule cells which were rescued by wild-type granule cells during differentiation. These findings suggest that NT-4/5 and BDNF promote the maturation and maintenance of differentiated granule cells, which are downstream to the weaver gene. In addition, no additive effects were seen with the combination of NT-4/5 and BDNF. The neurite-promoting and survival effects of NT-4/5 and BDNF could be completely blocked by the specific tyrosine kinase inhibitor K-252a. Thus, the two neurotrophins activate the same receptor trkB for signal transduction.

452.5

REGULATION OF SURVIVAL IN DEVELOPING NON-DOPAMINERGIC NEURONS BY GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF). A.Newsome, D.Prevette, J.Johnson*, S.Wang and R.Oppenheim. Dept of Neurobiology & Anatomy, Bowman Gray Sch of Med, Winston Salem, NC 27157.

GDNF is a potent neurotrophic factor that enhances the survival of cultured midbrain dopaminergic neurons. Although the highest levels of expression of GDNF are in developing brain cells and CNS regions that receive dopaminergic innervation (e.g., the striatum), it is also expressed in other areas including spinal cord. To examine the possible role of GDNF in the survival of non-dopaminergic neurons in brain and spinal cord, avian embryos were treated in ovo with GDNF during periods of naturally occurring (programmed) cell death. Treatment during a period of "early" cell death [embryonic day (E)6-9] greatly enhanced the number of surviving spinal motoneurons (MNs) and sympathetic ganglion (SG) cells but was without effect on sensory neurons in the dorsal root (DRG) and nodose ganglia (NG). GDNF also promoted survival in cultures enriched (60-80%) for MNs suggesting that it may promote survival in vivo by a direct action on neurons. Treatment at a later period of cell death (E9-E14) also rescued MNs and SG cells but was ineffective on cell death in the DRG, NG and ciliary ganglion. Several populations of neurons in the brain (sensory, motor, interneurons) that undergo late cell death between E9 and E15 were also examined following GDNF treatment from E9-E14. These included the III, IV, V, VI and VII cranial motor nuclei, the sensory mesencephalic nucleus of V, the auditory nuclei magnocellularis and laminaris, the accessory oculomotor nucleus (AON) and the isthmo-optic nucleus (ION). Preliminary cell counts indicate that only the AON, ION and n.III exhibit small, but statistically significant, increases in survival. Although additional studies are in progress, these data suggest that GDNF may be a trophic agent for non-dopaminergic populations of neurons.

452.6

TRKB LIGANDS REGULATE CARDIORESPIRATORY AFFERENT DEVELOPMENT T. Hertzberg[1,3], J.T Erickson[1], G. Fan[1], J.C.W. Finley[1,2]* and D.M. Katz[1]
[1]Depts. of Neurosciences and [2]Medicine, CWRU School of Medicine, Cleveland, OH 44106-4975 and [3] Dept. of Woman and Child Health, Div. of Neonatology, Karolinska Hospital, S-171 76 Stockholm, Sweden.

Carotid body afferent (CBA) neurons in the rat petrosal ganglion (PG) were used as a model system to define trophic requirements of cardiorespiratory sensory neurons *in vivo* and *in vitro*. CBA neurons are distinguished from other visceral afferents by expression of dopaminergic traits, including the catecholamine-synthesizing enzyme, tyrosine hydroxylase (TH; Finley, et al., 1993). *In vitro*, coculture of the PG and carotid body led to a 4-fold increase in TH neuron survival that was not seen in the presence of other tissues. The trophic effect of the carotid body was mimicked by treatment of PG explants with the trkB ligands brain-derived neurotrophin factor (BDNF) and neurotrophin-4 (NT-4), whereas nerve growth factor (NGF) and NT-3 had no effect. To determine whether similar mechanisms regulated development *in vivo*, we examined the effect of carotid body removal (glomectomy) on CBA survival in newborn rats. The majority (73%) of TH$^+$ CBA neurons died following glomectomy, indicating a predominant role of peripheral targets in regulating survival *in vivo*. However, at least half these cells could be rescued by replacing the carotid body with BDNF implants, indicating that BDNF could substitute for target-derived trophic support *in vivo*. Moreover, BDNF mRNA was detected in the newborn carotid body by RT-PCR, raising the possibility that BDNF normally plays a role in mediating carotid body influences on CBA development. CBA survival was unaffected, however, by glomectomy at three weeks of age, indicating that peripheral target dependence is limited to the immediate postnatal period. Our findings demonstrate that survival of this subset of cardiorespiratory afferents is critically dependent on peripheral target-derived support during a restricted period of perinatal development and suggest that trophic influences are mediated by trkB ligands, such as BDNF or NT-4. Supported by HL-42131 (DMK) and Wenner-Gren Foundation, Swedish Society of Medicine and Swedish MRC (TH).

452.7

INVOLVEMENT OF NEUROTROPHINS IN THE PHENOTYPIC SPECIFICATION OF CHICK CUTANEOUS AFFERENTS: G.R. Lewin*, M Koltzenburg§, K.V.Toyka§, and Y-A.. Barde Dept. Neurobiochemistry, Max-Planck Institute for Psychiatry D-82152, Martinsried. §Dept. Neurology, University of Würzburg, D-97080 Würzburg, Germany.

Neurotrophins may influence the phenotypic fate of sensory neurons during development. Here we have used an electrophysiological recording technique to study the functional properties of sensory neurons in the chick whose access to neurotrophins was manipulated *in ovo*. Monoclonal antibodies (anti-NGF and anti-NT3) were delivered by hybridoma cells placed on the choriollantoic membrane at E3, whilst NT3 was secreted by A-293 cells. Between E17 and hatching recordings were made from cutaneous sensory neurons as described (Koltzenburg et al. Soc. Neurosci. this meeting). After anti-NGF treatment fibers conducting in the C-Fiber range (<1 m/s) were still observed (n=11), however, none of these fibers responded to noxious heat, although all had mechanical thresholds similar to those in normal chicks (ie. they were C-M). This is in contrast to untreated control animals where nearly 50% of the fibers respond to noxious heat (C-MH). The functional properties of presumptive A-fibers appeared normal (conduction velocity >2 m/s). This is consistent with the idea that only C-MH fibers in the chick depend on NGF for survival. After NT3 treatment the conduction velocity range of recorded afferents was indistinguishable from controls. A-fibers also appeared unaffected by the treatment in terms of mechanical threshold. However, amongst the C-fiber population all the fibers studied (n=12) had unusually low mechanical thresholds, and none responded to noxious heat. Comparison of the NT3 treated C-fibers to control A-fibers revealed that their mechanical thresholds were very similar. Anti-NT3 treatment did not appear to affect the physiology of C-fiber afferents. The results indicate that the availability of neurotrophins can selectively effect the functional properties of developing sensory neurons *in vivo*.

452.8

SINGLE UNIT RECORDINGS OF CHICK CUTANEOUS SENSORY NEURONS *IN VITRO* M.Koltzenburg*, G.R.Lewin, Y.-A.Barde, K.V.Toyka *Dept. Neurology, University of Würzburg, D-97080 Würzburg, Dept. Neurobiochemistry, Max-Planck Institute for Psychiatry, D-81512 Martinsried, Germany.*

Chick sensory neurons have been commonly used as a model system to study the effects of neurotrophins on neuronal survival *in vitro*. As these DRG neurons are physiologically heterogenous, we have developed a technique to characterize functionally the different types during late embryonic development and early post-hatching period (E17-P21) *in vitro*. The cutaneous femoralis medialis nerve innervating medial thigh was dissected out together with the skin of its innervation territory and placed in an organ bath. Single unit recordings were obtained using standard teased fiber techniques and units were analyzed with controlled mechanical, thermal and chemical stimuli. A total of 91 neurons conducting between 0.31-6.6 m/s were studied. Units displayed no ongoing activity and mechanical thresholds (von Frey hairs) ranged from 1-64 mN. Of 44 units conducting ≤1 m/s many had probably nociceptive function, as 16/34 were excited by noxious heat (thresholds 39.5-47°C) and 4/33 by noxious cold. Application of algesic chemicals (mixture of 10μM bradykinin, 5-HT, histamine, PGE_2) activated 14/27 units all of which were also thermosensitive. Following chemical stimulation 10/14 units displayed lowered heat thresholds and stronger suprathreshold responses indicating sensitization. Heat-insensitive fibers did not respond to chemical stimulation. During the observed developmental interval mechanical thresholds tended to increase. At all ages units conducting > 2 m/s (presumptive A-fibres) could be classified on the basis of a slowly or rapidly responses to innocuous skin indentation, none responded to noxious heat or chemical stimuli. We conclude that the chick skin-nerve *in vitro* preparation is suitable for the study of functional properties of diverse cutaneous sensory neurons both during embryogenesis and the early post-hatching period.

452.9

INFLUENCE OF NEUROTROPHINS ON THE DEVELOPMENT OF PRIMARY AFFERENT PROJECTIONS IN THE CHICK SPINAL CORD. A.L. Eide*, G. R. Lewin and Y.-A. Barde. Inst. Basic Medical Sciences, University of Oslo, N-0317 Oslo, Norway, Dept. Neurobiochemistry, Max Planck Institute for Psychiatry, D-82152 Martinsried, Germany.

Primary afferent projections in the chick can be studied using the lipophillic tracer DiI in fixed embryos.In the present experiments we have examined the influence of neurotrophin treatment or deprivation with monoclonal antibodies to NT3 and NGF. NT-3 was delivered by NT-3 secreting A-293 cellspraced on the chorioallantoic membrane at E3, whilst antibodies were delivered by hybridoma cells. At E12 or E14 treated and control embryos were fixed and DiI was injected into the LS6 DRG, the LS6 spinal nerve or into an identified muscle or skin nerve. In normal embryos primary afferents have long ranging projections outside their segment of entry andhave collaterals at least 6 segments beyond their segment of entry(Eide & Glover J.Comp Neurol in press). In contrast LS6 afferents in anti-NT3 treated embryos projected primarily to their segment of entry. Furthermore, axons projecting ventrally to the motoneuron pools were largely absent although labelled collaterals were seen innervating all dorsal horn laminae. Interestingly, sensory neurons and their collaterals in the dorsal horn were labelled from muscle nerves, but few collaterals were seen in the ventral horn. After NT-3 application intersegmental projections appeared normal, although there appeared to be fewer collaterals in Lamina II within the segment of entry. This may be related to a change in the phenotype of sensory neurons treated with NT-3 (Lewin et al. Soc Neurosci Abst this volume). Finally, animals treated with anti-NGF showed little projection to laminae II which is consistent the idea that C-MH fibers are killed by this treatment. The results suggest that primary afferent projections may be molded by the availability of neurotrophins.

452.10

POSTNATAL AUDITORY NEURONS DEPEND UPON BRAIN DERIVED NEUROTROPHIC FACTOR FOR SURVIVAL AND BOTH NERVE GROWTH FACTOR AND NEUROTROPHIN-3 FOR THE MAINTENANCE OF PERIPHERAL NEURITES IN VITRO. H. Staecker[1,3], V. Galinovic-Schwartz[1], W. Liu[1], P.P. Lefebvre[1,3], B. Malgrange[3], G. Moonen[3], and T.R. Van De Water*[1,2,3]. Depts. of [1]Otolaryngology & [2]Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461; Dept. of [3]Human Physiology & Pathophysiology University of Liege, Liege, B4020 Belgium.

Both αtrkB and αtrkC immunolocalized over neurons while αtrkA staining associated with the supporting cells of the postnatal spiral ganglion. Using in situ RT-PCR of postnatal cochlea tissue sections, we identified products for NGF, BDNF and NT-3 mRNAs in this tissue. Organotypic explants of postnatal organ of Corti were cultured in the presence of either antisense or sense oligonucleotides (5μM) for NGF, BDNF or NT-3. ELISA testing showed a 90% reduction in NGF protein levels in response to NGF-AS oligo. Evaluation of the effects of neurotrophin antisense treatment in explants was accomplished by confocal microscopy of αNF66 immunostained whole mounts. BDNF-AS treatment resulted in neuronal cell death, NT-3-AS treatment affected synaptic contacts, and downregulation of NGF destabilized peripheral axons. These findings demonstrate that the neurotrophins studied play separate, unique but interrelated roles in postnatal organ of Corti explants. (Supported by grants from NIH, DC00088 to TRV,DRF to HS, NFSR of Belgium to PPL and GM).

452.11

HIGH-AFFINITY NEUROTROPHIN RECEPTOR EXPRESSION IN THE CAT RETINA DURING NORMAL DEVELOPMENT AND FOLLOWING NEONATAL VISUAL CORTEX DAMAGE. V.R. King, J.-T. Xue, and P.D. Spear*. Dept. of Psychology and Ctr. for Neuroscience, Univ. Wisconsin, Madison WI 53706.

We recently reported that normal 1-day to 4-week-old kittens show pericellular labeling of the low-affinity nerve growth factor receptor (LNGFr) in the retinal ganglion cell layer (GCL). In addition, kittens that receive a visual cortex (VC) lesion from 1 day to 18 weeks of age show an upregulation of this labeling in the hemiretinae that project to the damaged hemisphere. We examined retinal sections from these same cats to determine if similar changes are seen for the high-affinity tyrosine kinase (trk) receptors.

Sections were taken through the areas centralis and processed with rabbit polyclonal antibodies to the trkA, full length trkB, truncated trkB, and trkC receptors. Cats of all ages showed patterns of trkA and full length trkB labeling consistent with expression on Müller cells. Pericellular trkB labeling also was seen in the GCL of normal 1-day to 4-week-old kittens. Soma and dendrite labeling indicated that many of these cells are ganglion cells. Kittens that received VC damage on the day of birth had greater pericellular trkB labeling in the hemiretinae projecting to the damaged hemisphere than in the hemiretinae projecting to the intact hemisphere. This difference was seen in kittens that survived from 3 days to 8 weeks, but not for ≥ 12 weeks. It also was seen in cats that received a VC lesion at ≤ 18 weeks of age, but not ≥ 26 weeks of age. TrkC labeling was seen only on blood vessels. Truncated trkB labeling was light and limited to the nerve fiber layer.

The present study suggests that brain-derived neurotrophic factor (BDNF), which binds preferentially to trkB, is involved in retinal development and plasticity. In addition, both nerve growth factor, which binds preferentially to trkA, and BDNF may affect the retina via Müller cells.

452.12

NORADRENERGIC NEURONS IN THE LOCUS COERULEUS OF BIRDS EXPRESS TRKA AND P75 AND RESPOND TO NGF. C.S. von Barthold[1], A. Schober[1], Y. Kinoshita*[1], R. Williams[2], and M. Bothwell[1]. [1] Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195 & [2] Dept. of Developmental Biology, Biomedical Center, Uppsala Universitet, Uppsala, Sweden.

The noradrenergic neurons in the chicken locus coeruleus express the neurotrophin receptor p75 (von Barthold & Bothwell 1992, J. Comp. Neurol. 320:479-500). To determine which neurotrophin may regulate these neurons, expression of trk receptors was examined by in situ hybridization. A sub-population of neurons in the locus coeruleus expresses trkA receptors at 9 and 18 days of incubation (E9 & E18). To test if these neurons can bind and internalize NGF, retrograde transport of radio-iodinated (I-125) NGF was examined. Neurons in the locus coeruleus of E15 chick embryos accumulate I-125 NGF after injections into the basal forebrain. The retrograde transport of radio-iodinated NGF is restricted to the noradrenergic neuronal population as evidenced by double-labeling with an antibody against dopamine-beta-hydroxylase (DBH). To test if noradrenergic coeruleus neurons respond to NGF, explant cultures and dissociated neurons were treated with or without NGF. NGF did not significantly improve neurite outgrowth from E15 explants or the survival and morphology of DBH-labeled coeruleus neurons that were dissociated at E12 and labeled with DBH antibody two days later. In vivo, the size of DBH-labeled coeruleus neurons was significantly increased after injections of NGF into the telencephalon of E19-20 embryos, but NGF did not rescue these neurons from toxin-induced cell death after injections of 6-hydroxydopamine into the telencephalon. These results indicate that NGF may play a role in the development and physiology of the avian locus coeruleus. The data confirm major species differences between birds and mammals with regard to trophic regulation of presumptive homologous neuronal populations.

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452.13

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF), A POTENT SURVIVAL FACTOR FOR SPINAL MOTONEURONS, IS PRESENT IN DEVELOPING LIMB. C.E. Henderson*, R.A. Pollock, M. Armanini#, H.S. Phillips# and A. Rosenthal#. INSERM U.382, 34033 Montpellier, France; and #Dept. Neuroscience, Genentech, Inc., So. San Francisco, CA 94080.

Several neurotrophic factors enhance motoneuron survival *in vitro* and *in vivo*. The most potent of those reported to date, the neurotrophins BDNF, NT-3 and NT-4/5, act at picomolar concentrations. In mice in which the genes for trkB or trkC are inactivated, there is significant but not complete motoneuron loss, suggesting that the neurotrophins may not be the only factors required for physiological motoneuron survival.

GDNF was originally purified on the basis of its trophic actions on midbrain dopaminergic neurons. We tested recombinant rat GDNF on purified motoneurons from E14 rat spinal cord. After 3 days in culture, GDNF maintained nearly all (>90%) motoneurons that initially developed; similar efficacy was obtained for BDNF. However, dose response curves revealed that GDNF was 20- to 50-fold more potent than the neurotrophins; half-maximal survival was achieved at 10^{-14} M.

Using RT-PCR, GDNF mRNA was detected in E15 rat limb bud, cultured neonatal Schwann cells and embryonic myotubes; levels in skin were considerably lower. *In situ* hybridization on sections of E14.5 rat embryos showed signal over some muscle masses and some developing nerve tracts; labeling was essentially absent from the spinal cord and skin.

Taken together, these findings suggest that GDNF plays an important role in early motoneuron development. Its actions may be complementary to those of the neurotrophins (and perhaps other factors) both during development and in therapeutic approaches to human motoneuron diseases.

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452.14

EXPRESSION AND FUNCTION OF TGF-ß2 AND -ß3 IN THE EMBRYONIC CHICK SPINAL CORD, HINDBRAIN, AND SPINAL GANGLIA. K. Unsicker, C. Meier, and K.C. Flanders (SPONS.:ENA*). Dept. of Anat. & Cell Biol., Univ. Heidelberg, D-69120 Heidelberg, Germany.

TGF-ßs are multifunctional cytokines playing prominent roles in the control of cell proliferation, extracellular matrix composition, and inflammatory events. Established functions on neural cells include regulation of neuron survival as well as glial cell proliferation and gene expression. We have studied the distribution of TGF-ß3 mRNA by in situ hybridization and the localization of TGF-ß2 and -ß3 immunoreactivities (ir) with isoform-specific probes in the embryonic chick spinal cord, hindbrain, and dorsal root ganglia (DRG). At E3, TGF-ß3 mRNA as well as TGF-ß2 and -ß3-ir were prominent in the notochord, wall of the aorta, and dermomyotome, but absent to low in the neural tube and at sites, where DRG coalesce. At E5 and E7, strong TGF-ß2 and -ß3 ir was seen in radial glia of spinal cord and hindbrain as well as in fiber strands transversing the DRG and extending into spinal nerves, but not in neuronal cell bodies. Neuronal perikarya in DRG did not become ir for TGF-ß2 and -ß3 until E11, but even then the signals for TGF-ß3 mRNA could not specifically localized to the neuronal cell bodies. In the spinal cord, glial/glial progenitor cells were most strongly labeled by in situ hybridization for TGF-ß3 mRNA at this age. DRG explants from E8 embryos were used to investigate whether TGF-ß interfered with neurite growth. All TGF-ß isoforms augmented the number of neurites emerging from the explant rim, and increased average neurite length over a period of 24 hours, as compared to untreated cultures. At the concentration applied (20 ng/ml) TGF-ßs were, however clearly less potent than NGF. Although the site(s) of action of TGF-ß in the complex culture system employed remain to be explored, our data are consitent with the notion that TGF-ßs are present at sites where they can affect neuron migration, differentiation and axon outgrowth. DFG Un34/16-1

452.15

FETAL NGF AUGMENTATION AFFECTS WHISKERPAD INNERVATION DENSITY AND PATTERNS AT BIRTH. F.L. Rice*, T.A. Henderson, P.A. Osborne, E.M. Johnson & M.F. Jacquin. Pharmacology, Albany Med. Coll., Albany NY 12208; Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis MO 63104; Molecular Biology & Pharmacology, Neurology, Washington Univ. Sch. Med., St. Louis MO 63110.

Systemic NGF injections in fetal rats preserve excess trigeminal ganglion cells and interrupt whisker-related pattern formation in the brainstem (Henderson et al., *J. Neurosci. 14*, '94); primary afferents in the brainstem also lack somatotopic patterns and collaterals do not have exuberant arbors. Because these findings suggest mechanisms by which the periphery makes CNS patterns, it is important to understand how NGF alters pattern formation. One idea is that high levels of NGF preserve (or induce) a transient exuberant projection to inter-whisker surfaces that lessens disparity in the innervation densities of the whisker follicles and intervening skin, therein lessening the "digitized" nature of the receptor sheet and its resultant CNS map. A related idea is that NGF accelerates development of a late-arriving innervation of inter-whisker skin, leading to the same CNS effect. These hypotheses were tested by injecting rats systemically with NGF on embryonic days 15 and 18, sacrificing at birth, confirming that CNS patterns were absent, and examining the mystacial pad innervation with PGP 9.5 and RT97 immunofluorescence. Comparable infraorbital nerve fascicles in NGF-treated neonates were larger and contained more labeled axons than in normal neonates, although the innervation of whisker follicles seemed comparable in both distribution and maturation. However, preliminary observations indicate that the innervation of the intervibrissal epidermis is more mature in the NGF-treated cases. These data support both of the above hypotheses and suggest that neurotrophins control pattern formation by orchestrating innervation density patterns in the whiskerpad during a critical period in development. NIH DE07734, NS17763, NS24679.

452.16

bFGF INDUCES ASTROGLIOSIS IN ADULT MOUSE BRAIN. X.F. Zhang, E.J. Elliott*, D.A. Parks and P.S. Fishman. Neurology and Research Svc., Baltimore VAMC, and the Univ. of Md. Sch. of Med., Baltimore, MD 21201.

Basic fibroblast growth factor (bFGF) is a multipotential stimulant of many cell types including astrocytes. In culture bFGF stimulates both proliferation and protein synthesis in astrocytes. The effects of bFGF on astrocytes in situ are less clear. Some studies have found that bFGF increases the number of GFAP positive astrocytes while other investigations showed no increase in gliosis over controls. To resolve this question we evaluated mice treated with bFGF with both immunocytochemical and quantitative immunochemical techniques. Adult C57BL/6 mice were injected in the striatum with either saline, human recombinant bFGF (2 ul) or an equal amount of human recombinant albumin. Immunocyto-chemical studies showed pronounced gliosis in the entire injected striatum of bFGF injected mice. This response consisted not only of an increase in number and density of GFAP positive astrocytes, but also in a dramatic increase in astrocyte size. Increased gliosis was also seen after injection of human albumin compared to saline injected animals, but this increase was not as pronounced as with bFGF. This effect was maximal at 4 to 7 days. Immunoblot analysis for GFAP of injected hemispheres confirmed the observation of bFGF > albumin > saline > normal brain in stimulating GFAP accumulation.

452.17

IDENTIFICATION OF DIFFERENT ARIA SPLICE VARIANTS EXPRESSED BY CHICK CNS AND PNS NEURONS DURING DEVELOPMENT

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Many studies have shown that innervating motor neurons regulate the accumulation and enhanced synthesis of acetylcholine receptors at early nerve-muscle contacts. Two factors important to the clustering and enhanced synthesis of muscle AChRs have been identified (Agrin and Aria respectively; work of Scheller, McMahan, Fischbach and their colleagues).

Presynaptic input to CNS and PNS neurons can also regulate nAChR expression. For example, preganglionic input increases the ACh sensitivity, as well as the number, clustering and biophysical properties of surface nAChRs on sympathetic neurons from embryonic chicken. Conditioned medium (CM) from both preganglionic neurons (dorsal spinal cord) and motor neurons (ventral spinal cord) mimic the effects of innervation. These observations suggest that Agrin , ARIA and/or related molecule(s) might be expressed by preganglionic neurons and regulate the expression of neuronal nAChRs with the innervation of sympathetic neurons. To identify ARIA like molecules(s), an embryonic day 5-11 spinal cord cDNA library was constructed and screened with a PCR derived ARIA probe (~1.1kb). More than 10 distinct splice variants were cloned, including multiple "nARIA" like splice forms (Kuo et al.,Abstract, this volume). In situ hybridization reveals that ARIA, nARIA and Agrin are expressed in several regions of brain and spinal cord. In spinal cord, clear positive signals were detected with each probe in ventral motor neurons, with a full length nARIA probe yielding the strongest signal seen in the preganglionic (visceral) motor neurons at embryonic day 8. (supported by NS29071).

452.18

ISOLATION AND CHARACTERIZATION OF CHICK AND HUMAN nARIA, A NOVEL MEMBER OF THE ERBB2/HER LIGAND FAMILY WHICH LACKS THE IMMUNOGLOBULIN DOMAIN.

Y. Kuo*, X. Yang, and L. Role. Dept. Anat. & Cell Biol. in the Ctr. Neurobiol & Behav, Columbia Univ., P&S, 722 W. 168th St. NY, NY 10032.

ARIA is believed to mediate the increased transcription of muscle type nicotinic AChR subunits during innervation of the muscle by motor neurons (Falls et al. *Cell* 72, 801-815, 1993). The ARIA message is a splice variant of a gene that also encodes the glial growth factors, heregulins, and NDF's. Media conditioned by somatic or visceral motor neurons increase the number of new surface AChRs in sympathetic neurons (Gardette et al. *Devl. Biol.* 147, 83-95, 1991). We have begun to test the hypothesis that homologs of ARIA might be the active component released by visceral motoneurons by first identifying candidate regulators of neuronal nAChR expression.

Screening of an E13 chick brain cDNA and a human cerebellar cDNA library resulted in the isolation of a novel ARIA splice variant (nARIA). Unlike previously reported splice forms, the nARIA clone does not contain an extracellular Ig domain. An N-terminal domain unique to nARIA is spliced to the EGF-like domain of ARIA. The nucleotide sequence of the chick nARIA clone 3' to the splice junction is identical to the published ARIA sequence. Northern blotting shows that nARIA expression is nervous system specific, with particularly high levels detected in the cerebellum. *In situ* studies are underway to further localize expression of this splice form (See abstract by Yang et al). Members of the ARIA/NDF/GGF family have been shown to bind to erbB2/neu/her2 and her4, which are of 185kd and 180kd respectively. To test the role of the domain N-terminal to the EGF-like domain in receptor binding, mammary tumor cell lines which overexpress erbB2 were treated with conditioned media from nARIA transfected COS cells. Treatment results in a time-and dose-dependent tyrosine phosphorylation of an approximately 180kd protein. We hypothesize that the region N-terminal to the EGF-like domain is not necessary for receptor binding, but may restrict interaction between family members or modulate affinity for the receptor. (Supported by NS29071)

452.19

MICROCEPHALY AND DELAY OF DEVELOPMENTAL BEHAVIORS INDUCED BY PRENATAL BLOCKADE OF VIP.
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VIP has neurotrophic properties in CNS cultures (*PNAS* **83**:1159, 1986), influences neurobehavioral development (*Peptides* **12**:87, 1991) and stimulates *in vitro* embryonic growth during mid gestation through VIP receptors localized to the CNS (*Nature* **362**:155, 1993). In the present study, embryonic growth and neonatal behaviors were examined in the offspring of pregnant mice treated with a VIP antagonist during mid gestation. Treatment with a VIP antagonist resulted in a dose-dependent decrease in DNA (52% of controls), protein (43% of controls) in the heads of embryos which was prevented with cotreatment with VIP. In contrast, the DNA and protein content of the body was less affected (89% and 72% of the controls). Cortical wall thickness was reduced and BRDU incorporation demonstrated that the reduction was due to an inhibition of the G1-S transition of mitosis. Treatment later in development did not have growth-inhibiting effects. VIP antagonist treatment delayed both developmental milestones and the performance of neonatal behaviors in a dose-dependent manner. Co-treatment with VIP prevented antagonist-induced deficits. Milestones, including eye opening, ear twitch, and auditory startle, were delayed by about one day in treated rats. Motor behaviors such as surface righting, air righting, negative geotaxis, cliff aversion and rooting were slower developing in treated animals, with a delay of as much as 4 days in achieving normal performance. This *in vivo* evidence supports the importance of VIP in the growth and development of the nervous system.

452.20

Evidence for the role of DARP (Dopamine-Releasing Protein) during the development of rat dopaminergic neurons and the adrenal gland.
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Recent work from this laboratory suggests a role for DARP during development. DARP is a glycoprotein that stimulates dopamine release from the corpus striatum in vitro (Brain Res. 463: 335) and has been immunocytochemically located in the adrenal gland (Neuroendocrinology 58: 444) and in close association with catecholaminergic neurons in the rat CNS (Neuroendocrinology, in press). In addition, intrafetal administration of an anti-DARP monoclonal antibody (DARP mAb) at embryonic day 17 (E17) induces fetal resorption in a dose-dependent manner (Mol. Cell. Neurosci. 2: 410). In this study, ELISA analysis of crude E17 brain homogenates demonstrates the presence of a DARP-like protein. Purification of these crude homogenates with concanavalin A and immunoaffinity chromatography yielded a single 60 kDa protein that displayed dopamine-releasing activity in an in vitro superfusion assay. In addition, intrafetal administration of DARP mAb at E17 significantly elevated DA levels in the mesencephalon 24 and 48 hours post-injection, while decreasing these levels 72 hours post-injection. No changes in DA levels were detected in the diencephalon or in the telencephalon. We have shown that neonatal DARP mAb injections delay the development of hypothalamic and striatal dopamine levels and increases adrenal weight (Mol. Cell. Neurosci. 2: 410). Herein, we report that daily administration (Postnatal days 1-10) of a synthetic 36 amino acid peptide from the N-terminus of DARP causes adrenal atrophy in a dose-dependent manner at day P11. In addition, ELISA analysis indicates that DARP concentrations in whole brain extracts from E17 brains are two to three times higher than similar extracts from P5 rats. These findings demonstrate that DARP is present in the embryonic and early postnatal rat brain and may play a role in the prenatal development of dopaminergic neurons of the mesencephalon and the postnatal development of the adrenal gland.

NEUROTROPHIC FACTORS: BIOLOGICAL EFFECTS X

453.1

RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR-I (rhIGF-I) PREVENTS CISPLATIN-INDUCED NEUROPATHY
P. C. Contreras, C. Steffler, J. A. Gruner, A. Yee, J. Roberts-Lewis* and J.L. Vaught. Cephalon, Inc., West Chester, PA 19380.

rhIGF-I *in vitro* supports the survival of motor and sensory neurons and *in vivo* prevents development of the motor and sensory neuropathy induced by vincristine and paclitaxel, respectively. Cisplatin is an antitumor agent that produces a dose-limiting peripheral neuropathy. The purpose of this study was to evaluate whether rhIGF-I could also prevent the neuropathy induced by cisplatin. Male CD-1 mice were dosed once a week with cisplatin (10 mg/kg, ip) and daily with rhIGF-I (1 mg/kg, sc) for 16 weeks. Cisplatin treatment reduced body weight, decreased conduction velocity and amplitude of the compound action potential of caudal and tibial nerves, increased tail-flick and hot-plate latencies, altered parameters of gait and decreased ganglionic content of CGRP. The changes in neuronal conduction and tail-flick and hot-plate latencies were not due to changes in body, paw or tail temperatures. Co-treating cisplatin-treated mice with rhIGF-I prevented all of the effects of cisplatin except for the change in body weight. Since, rhIGF-I had no effect on the loss in body weight induced by cisplatin, rhIGF-I was not just simply a cisplatin antagonist. These results suggest the therapeutic utility of rhIGF-I in preventing the cisplatin-induced neuropathy.

453.2

NEUROTROPHIN-3 REDUCES DEPLETION OF STRIATAL CHAT AND GAD ACTIVITIES FOLLOWING QUINOLINIC ACID LESION.
T. M. Engber*, S. A. Dennis, S. L. Meyer and P. C. Contreras. Cephalon, Inc., West Chester, PA 19380.

Injection of the endogenous excitotoxin quinolinic acid into the rat striatum produces a lesion which is neurochemically similar to that seen in Huntington's disease. We examined whether neurotrophin-3 (NT-3), a member of the neurotrophin family of growth factors, is capable of reducing the damage caused by quinolinic acid in the rat striatum. NT-3 (0.15 or 0.5 μg/day in PBS) was infused via osmotic pump into the left striatum for 12 days, beginning immediately after injection of quinolinic acid (225 nmoles in 1 μl PBS); controls received PBS infusion following quinolinic acid injection. Rats were sacrificed on day 12 and both the lesioned and intact striatum dissected for measurement of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities, neurochemical markers for cholinergic and GABAergic neurons, respectively. Quinolinic acid injection depleted ChAT activity by 35% relative to that on the intact, uninjected side; NT-3 infusion at a dose of 0.15 μg/day attenuated the effect of quinolinic acid, with ChAT activity on the lesioned side reduced by only 12% ($p<.05$), while NT-3 at a dose of 0.5 μg/day did not have a significant effect. In the quinolinic acid-lesioned striatum, GAD activity was decreased by 62% relative to that on the intact side; following infusion of NT-3 at a dose of 0.15 μg/day, GAD activity was reduced by only 29% ($p<.05$), while NT-3 at 0.5 μg/day did not have a significant effect on the depletion of GAD activity. These findings suggest a possible therapeutic role for neurotrophic factors in reducing or reversing neuronal damage in Huntington's disease.

453.3

NEUROTROPHIN-4/5 AND TRANSFORMING GROWTH FACTOR-α PARTIALLY PROTECT STRIATAL CALBINDIN-CONTAINING NEURONS AFTER QUINOLINIC ACID LESION.
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Lesioning of the mammalian striatum by the excitotoxin quinolinic acid (QA) results in the degeneration of medium spiny neurons which contain γ-amino-butyric acid (GABA). Neurotrophic factors have been shown to rescue degenerating cells in a variety of lesion types, including chemical lesions. Several neurotrophic factors have either survival-promoting actions on striatal GABAergic neurons or are localized within the striatum. Of these factors, transforming growth factor α (TGFα) and neurotrophin-4/5 (NT4/5) were chosen for administration to rats lesioned acutely with QA.

Cannulated rats received a single unilateral intrastriatal injection of QA (150 nM). Trophic factors (1 μg TGFα, 0.8 μg NT4/5, or control protein cytochrome C) were administered for 7 days. The pattern of neuropathology was assessed by both in situ hybridization and immunolabelling. As expected, QA resulted in a preferential degeneration of medium spiny GABAergic neurons with a relative sparing of NADPH-diaphorase neurons, as well as neuronal fibers. GABA-expressing neurons were not rescued by either NT4/5 or TGFα. However, the degeneration of a subpopulation of cells which express calbindin was partially reversed by both factors, in an area limited to the medial aspect of the caudal striatum. The striatal patch-matrix pattern was disrupted by QA, such that the staining intensity of the matrix by both cholinesterase histochemistry and calbindin terminal staining was considerably decreased, except in the medial and ventral aspects of the striatum, where staining intensity approached control levels. Neither factor affected these staining patterns. These findings indicate that neurotrophic factors may provide protection for subpopulations of striatal cells.

453.4

FAILURE OF CONTINUOUS INTRATHECAL NGF INFUSION TO PREVENT TOXIC CHEMICAL-INDUCED DISTAL AXONOPATHY DESPITE REDUCING THE INJURY-INDUCED UPREGUATION OF C-JUN EXPRESSION IN DRG NEURONS.
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Clinicial trials of NGF for the treatment of peripheral neuropathies have begun. However, direct (morphological) evidence that NGF prevents axonal degeneration is lacking. In the present study, we asked whether supplementing the cell body's supply of retrogradely transported NGF prevents the development of distal axonal degeneration in a prototypic model of dying-back neuropathy: 3,4-dimethyl-2,5-hexanedione (DMHD), a potent derivative of the gamma-diketones. Rats were given daily intraperitoneal injections of DMHD (0.25 mM/kg/day) and NGF (125 ng/hr) was continuously infused into the subarachnoid space of the lumbar spinal cord via a osmotic minipump (Alzet); controls received cytochrome C. At 9 days, rats were perfused with 5% glutaraldehyde. Quantitation of the numbers of degenerating profiles and the total numbers of myelinated fibers was performed in the sciatic, tibial and sural nerves. To date, no significant differences have been found; in the soleus nerve, the percentage of degenerating fibers was 3.0 ± 0.22% (SEM) and 2.7 ± 0.31% in cytochrome C-infused (n=3) and NGF-infused (n=3) DMHD-treated rats, respectively. NGF also failed to prevent degeneration of fibers in the dorsal columns of the spinal cord (C1). In a separate immunocytochemical study, animals were perfused with 4% paraformaldehyde at 7 days. We previously reported (*Neurosci. Lett.* 154:129, 1993) that axotomy-induced induction of the protooncogene c-jun in DRG neurons is reduced by NGF infusion. Similarly, NGF markedly reduced (by 70%) the numbers of DRG neuronal nuclei demonstrating intense c-JUN-like protein immunoreactivity in DMHD-treated rats. Thus, NGF infusion to the cell body does not appear to prevent the development of DMHD axonopathy, but does decrease the injury-induced upregulation of c-jun expression. It remains possible, however, that delivery of NGF to the nerve itself could prevent axonal degeneration. *Supported by NS19611 and the Paralyzed Veterans of America Spinal Cord Reseach Foundation.*

453.5

BDNF PROTECTS CULTURED DENTATE GRANULE CELLS AGAINST HYPOGLYCEMIC DAMAGE

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We have previously shown (Lindvall et al., PNAS, 1992, 89, 648-652) that 1 and 30 min of insulin-induced hypoglycemic coma leads to a marked increase of BDNF and NGF mRNA levels in dentate granule cells. The aim of this study was to explore whether brain-derived neurotrophic factor (BDNF) can improve neuronal survival in cell cultures of rat dentate gyrus subjected to a hypoglycemic insult. Dentate gyrus was dissected from rat pups (P5-6), dissociated and cells were plated on chamber slides at 37° C in 5% CO_2 and with 95% humidity. After 24 h the serum-containing medium was switched to serum-free N2 medium. The hypoglycemic insult was induced after 7 days *in vitro* by glucose deprivation. The duration of hypogly-cemia was 15 h and cultures were fixed and processed for MAP-2 immuno-cytochemistry immediately thereafter. In control cultures 40 mM glucose was added. Glucose deprivation for 15 h caused severe neuronal loss (about 70%). BDNF added either 24 h before or 4 h after onset of hypoglycemia completely protected granule cells against the insult-induced damage. Nerve growth factor (NGF) had similar effects. These findings support the hypothesis that the rapid upregulation of BDNF and NGF mRNAs in dentate granule cells after brief periods of hypoglycemic coma and other insults is a local protective mechanism.

453.6

IMPORTANCE OF COLONY STIMULATING FACOTR-1(CSF-1) FOR NEURONAL SURVIVAL IN ISCHEMIC CORTICAL LESIONS. O. Berezovskaya, D. Maysinger and S. Fedoroff*. NCE NeuroScience Network at Department of Pharmacology and Therapeutics, McGill University, Montreal, Que., and Department of Anatomy, University of Saskatchewan, Saskatoon, Sask., CANADA S7N OWO.

In osteopetrotic op/op mice, deficient in CSF-1 (microglial survival and proliferation factor), microglia form in approximately normal numbers. However, their response to injured neurons is abnormal: initially there is some degree of microglial proliferation but the recruitment of microglia is only meager. The microglia initiate phenotypic transformation but do not transform to the extent seen in normal animals. The expression of CR3 and MHC-1 receptors in microglia seems to be unaffected as does astroglial hypertrophy. In ischemic cerebral cortical lesions in op/op mice, neuronal survival is significantly reduced as compared to that in normal CSF-1 producing animals. When CSF-1 producing astroglia are grafted in the vicinity of the ischemic lesion, or when encapsulated CSF-1 producing LM cells are implanted intraperitoneally into op/op mice, the microglial response to injured neurons is partially restored and neuronal survival in the ischemic lesion is significantly potentiated. We found a significantly greater number of surviving neurons in the area of ischemic cerebral lesions made in osteopetrotic op/op mice previously grafted with CSF-1 producing cells, as compared to the number of surviving neurons in similar lesions made in op/op mice or even in normal CSF-1 producing C_3H/HeJ mice. We conclude that CSF-1 secretion by astroglia is essential for normal function of microglia and for potentiation of survival of injured neurons.

Supported by a grant from Canadian NCE NeuroScience Network.

453.7

A QUANTITATIVE ANALYSIS OF ASTROGLIAL AND MICROGLIAL CELL REACTIONS IN PRIMARY SENSORY PROJECTION AREAS FOLLOWING SCIATIC NERVE INJURY AND TREATMENT WITH NERVE GROWTH FACTOR IN THE ADULT RAT.

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Previous quantitative immunohistochemical studies have shown that the microglial cell reaction in primary sensory projection areas after peripheral nerve injury follows a very rapid exponential growth phase which is succeeded by a considerably slower decline phase; the peak being approximately 7-10 days after injury (Eriksson et al, Exp. Brain Res., 1993; 96:19-27). Further, it has been shown that following peripheral nerve injury, astrocytes are activated via reactive microglial cells (Svensson et al, J. Neurosci. Res., 1993; 35: 373-381).

Using image analysis we have examined quantitatively the temporal appearance of the astroglial reaction to sciatic nerve injury as revealed by the area of glial fibrillary acidic protein (GFAP) immunoreactivity in the lumbar dorsal horn and the gracile nucleus. Concomitantly, the temporal appearance of GFAP-mRNA profiles were observed by in situ hybridization. Astroglial cells in the dorsal horn and the gracile nucleus were shown to follow similar growth and decline curves with an increase two days and a maximum two-three weeks following sciatic nerve transection. However, treatment with nerve growth factor (NGF) during four weeks following sciatic nerve transection did not result in any different glial expression, as investigated with our quantitative methods.

These results indicate that, even though promoting dorsal root ganglion (DRG) cell survival, NGF does not seem to have any influence on the glial cell reaction neither directly nor indirectly through the rescued DRG-neurons. The microglial cell reaction therefore does not seem to be triggered by the target loss of the sensory neurons.

453.8

THE MICROGLIA/MACROPHAGE RESPONSE FOLLOWING NGF INJECTION INTO THE RAT SPINAL CORD

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The response of macrophages and microglia during wallerian degeneration of axons is remarkably different in the peripheral [PNS] and central [CNS] nervous systems of adult mammals (Griffin et al, 1993; Perry et al, 1987). In the PNS, blood monocytes rapidly infiltrate degenerating nerve to clear axonal as well as myelin debris. In striking contrast, the infiltration of monocytes and response of microglia with clearance of cellular debris is much slower in the CNS. The mechanisms underlying this differential response of macrophages and microglia are unknown. Nerve Growth Factor [NGF] has been shown to be a possible chemotactic factor for leukocyte recruitment (Boyle et al, 1985). The low affinity [NGFr] and high affinity [Trk A] NGF receptor have been detected in certain types of monocytes and microglia (Ehrhard et al, 1993; Yan and Johnson, 1990; Morgan et al, 1989). In addition, a linear arrangement of macrophages was found immediately apposed to NGF-treated but not untreated nitrocellulose implants inserted into rat spinal cords (Houle, 1992). NGF may therefore play a role in modulating the response of macrophages and microglia.

To test this hypothesis, we performed local needle injections of NGF, vehicle (2% bovine serum albumin), or needle injections alone into rat spinal cords either with or without transection of an adjacent dorsal root (n= 28). We employed the ED1 monoclonal antibody which normally stains peripheral rat macrophages and monocytes but not CNS microglia. Immunohistochemistry was performed upon paraffin embedded parasagittal sections of spinal cords. Following simple transection of a dorsal root, we found ED1 labeled cells infiltrating the PNS portion of the cut dorsal root after a delay of 3 days that stopped abruptly at the PNS/CNS interface and did not extend into the spinal cord for up to 3 weeks. Needle injections into the spinal cord induced the local appearance of ED1 staining cells. The number of cells and volume of tissue staining with ED1 was greatest following injections of NGF, significantly less with injections of vehicle alone, and smallest following needle injection alone. Spinal cord injections of NGF combined with transection of an adjacent dorsal root resulted in ED1 labeled cells extending across the PNS/CNS interface of the cut dorsal root towards the injection site. These results suggest that NGF may activate microglia to express ED1 and/or induce the migration of peripheral ED1 monocytes/macrophages into the CNS. Supported by NIH and VA funds.

453.9

CHRONIC NGF TREATMENT RESTORES HIPPOCAMPAL MUSCARINIC, BUT NOT NICOTINIC RECEPTOR FUNCTION FOLLOWING FIMBRIAL TRANSECTIONS. P.A. Lapchak*, D.M. Araujo and F. Hefti. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

The effects of chronic NGF administration (icv, 1 μg qod for 21 days) on hippocampal muscarinic and nicotinic receptor densities and functions were determined in adult rats with partial fimbrial transections. First, the distribution of cholinergic receptors in the hippocampus was determined using autoradiography. Neither fimbrial transections nor NGF treament altered the density of muscarinic M1 [^{3}H-pirenzepine] or M2 [^{3}H-AF-DX 384] binding sites in the hippocampus. In addition, neither the lesion nor NGF treatment altered the distribution or density of nicotinic [^{3}H-cytisine] sites in the hippocampus. Second, we determined the effect of NGF treatment on muscarinic receptor-mediated second messenger production in rats with fimbrial transections. In lesioned rats, oxotremorine increased IP_3 synthesis by 81% ipsilateral to the lesion compared to the contralateral unlesioned side. This lesion-induced supersensitivity of M1 muscarinic receptor function was prevented by chronic NGF treatment. Third, we determined whether nicotinic receptor function was altered by fimbrial transections using nicotine-induced uptake of ^{3}H-tetraphenyphosphonium (TPP^+) into hippocampal synaptosomes as a marker. In the unlesioned control hippocampus, nicotine produced a characteristic 35% reduction of ^{3}H-TPP^+ uptake into synaptosomes. However, in rats with partial fimbrial transections there was a loss of nicotine-induced alterations of ^{3}H-TPP^+ uptake into synaptosomes. Chronic NGF treatment did not affect nicotine-induced ^{3}H-TPP^+ uptake. The present results indicate that NGF-induced increases of presynaptic hippocampal cholinergic functions involved in ACh turnover (Lapchak, Exp. Neurol. 124, 16-20, 1993) translate into an enhanced function of postsynaptic muscarinic M1 receptor function. However, chronic NGF treatment does not attenuate the lesion-induced loss of presynaptic nicotinic autoreceptor function.

453.10

THE EFFECT OF DOSE AND TIME ON THE EFFICACY OF NGF IN THE FIMBRIA FORNIX LESION MODEL

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We are interested in determining whether small brain-permeable molecules that upregulate endogenous NGF in the CNS can promote the survival of degenerating cholinergic neurons in a manner comparable to that demonstrated using exogenous NGF. Previous work has shown that treatment with various agents results in modest (1.5 to 2-fold) increases in NGF in the adult rat brain. The purpose of this study was to determine: (1) whether ICV administration of amounts of NGF approximating those obtained after NGF induction can prevent the cholinergic losses of fimbria fornix lesioning and (2) whether dose of NGF alters the time required to obtain neurotrophic effects. ICV injection of 1 μg NGF resulted in an approximate 4-fold transient increase in NGF levels in the septum, hippocampus and basal forebrain, as measured by a two-site immunoassay. In contrast, changes in NGF could not be detected in these brain regions when doses of 0.25 μg or less were used. Nonetheless, analysis of hippocampal ChAT activity in fimbria fornix lesioned rats 14 days after lesioning revealed that daily doses as low as 0.1 μg had maximal efficacy, suggesting that small increases in NGF can be therapeutically significant. The increase in ChAT activity using 1 μg/day occurred after 10, but not 7, days of treatment. The effects of dose on the time required to achieve efficacy will be discussed.

453.11

NT-3 ENHANCES GROWTH AND UPREGULATES GAP43 IN A SUBSET OF RETINAL GANGLION CELLS AFTER AXOTOMY. P. Kittlerova, D.E. Playford*, G.M. Bray and A.J. Aguayo. Centre for Research in Neuroscience, McGill University and Montreal General Hospital Research Institute, 1650 Cedar Ave., Montréal, Québec H3G 1A4.

Retinal ganglion cell (RGC) survival can be enhanced by the intraocular administration of BDNF or NT-4 (Mansour-Robaey et al., PNAS 91: 1632-1636, 1994; Clarke et al., Soc.Neurosci. Abstr. 19: 1104, 1993). We have now investigated the role of NT-3 on RGC survival and regrowth.

Intraocular injections of NT-3 (provided by Regeneron Pharmaceuticals Inc.) had only a minor effect on the overall survival of axotomized RGCs, consistent with the observation that immunocytochemistry for *TRK*C, the high affinity receptor for NT-3, revealed staining in only 5% of RGCs. However, in comparison with other neurotrophins, NT-3 increased intraretinal axonal growth, as revealed by staining with RT97, a monoclonal antibody to the heavy neurofilament subunit.

The effect of NT-3 on the expression of GAP43 mRNA was then investigated by *in situ* hybridization. Two weeks after axotomy, approximately 10% of the surviving RGCs expressed high levels of GAP43 mRNA. After NT-3 treatment, the incidence of these GAP43-expressing cells increased to 30% of surviving RGCs. Furthermore, GAP43 mRNA levels within these cells doubled, compared to operated controls. These findings suggest that NT-3 has a significant effect on GAP43 expression and on axonal growth of a discrete population of RGCs.

453.12

ROLE OF NT-3 IN INTACT & INJURED PRIMARY SENSORY NEURONS K.A. Gratto[1] B. Friedman[2] L. Liu[2] R.M. Lindsay[2] & V.M.K.Verge*[1] [1]Dept of Anatomy, Univ of Saskatchewan, Canada S7N 0W0 [2]Regeneron Pharmaceuticals Inc., Tarrytown NY 10591

The hypothesis that NT-3 plays a role in maintaining the differentiated state of responsive trkC-expressing neurons and may be responsible for reversing changes observed after injury, *in vivo* was tested. Adult rat right sciatic nerves were proximally cut. 14d after injury NT-3 was intrathecallly infused for an additional 7d in half of the rats. Cryostat sections of intact and injured DRG with and without NT-3 infusion were processed for *in situ* hybridization to detect mRNAs encoding trkC and peptides α-CGRP and NPY. Preliminary results indicate that in intact neurons, α-CGRP and trkC are abundantly and heterogenously expressed, whereas few if any neurons express detectable NPY mRNA. Two weeks after injury levels of α-CGRP and trkC mRNA are dramatically reduced, while, many neurons now express abundant NPY mRNA. Infusion of NT-3 counteracted injury-induced decreases in trkC mRNA and was also effective in upregulating expression of α-CGRP in many neurons, but the percentage of neurons appeared smaller when compared to injured ganglia infused with NGF. Finally, NT-3 infusion resulted in reduced expression of NPY mRNA in injured neurons, suggesting a role for NT-3 in gene suppression in intact neurons. The ability of exogenous NT-3 to regulate expression of its receptor trkC and differentially regulate peptide expression supports a role for it in maintaining aspects of the differentiated state of adult primary sensory neurons. *Canadian MRC supported.*

453.13

EFFECT OF NT-4/5 ON AXOTOMIZED RAT FACIAL MOTONEURONS. Karl Fernandes, Annie Bedard and Wolfram Tetzlaff*. Dept. of Physiology, University of Ottawa, Ottawa, Canada.

We have previously shown that axotomy of facial motoneurons induces the expression of GAP-43 and Tα1 tubulin mRNAs while the expression of neurofilament mRNA is decreased. In addition, we report here that the mRNA expression for AchE is reduced by about 35% consistent with a general downregulation of neurotransmitter related mRNAs after axotomy. In the present study we have tested the role of a presumed target derived neurotrophin, NT-4/5, in the regulation of these changes in gene expression. The facial nerve of male adult Sprague Dawley rats was transected at the stylomastoid foramen and the proximal nerve stump attached to the lumen of a silastic tubing. The latter was connected to an osmotic minipump which delivered 1 µl with 125 ng (low dose) or 500 ng (high dose) NT-4 per hour over a period of 7 days. The contralateral proximal nerve stump received vehicle only. *In situ* hybridization revealed that application of NT-4 sustained the expression of AChE mRNA at normal levels (202 % of vehicle, n=4) and further stimulated the expression of GAP-43 (211 % of vehicle, n=4) and Tα1-tubulin (170 % of vehicle, n=4) mRNA. The expression of neurofilament-M (NFM) mRNA was increased to 150% of vehicle (n=4), but was only significantly different from vehicle in 1 of 4 animals. NFM expression was still far below a normal level of expression, thus, not normalized by NT-4 application.

These data show that NT-4/5 enhances regeneration associated gene (GAP-43, Tα1-tubulin) expression and might be useful to stimulate peripheral nerve regeneration.

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453.14

NT-3 AND BDNF PREVENT AXOTOMY INDUCED DEATH OF CORTICOSPINAL NEURONS

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A major problem of regeneration in the CNS is atrophy and cell death of injured neurons. We have quantified the death of corticospinal neurons (CSN) induced by axotomy at the level of the internal capsule and tested the effect of survival promoting factors in this system. *In situ* hybridization combined with retrograde tracing revealed that CSN express mRNA for both trkB and trkC but not trkA receptors. This provided the rationale to test the survival effect of Neurotrophins in this model. Again, the CSN were positively identified by spinal cord injection of Fast Blue and a second spinal cord injection of Rhodamine Dextran was applied after internal capsule lesion to confirm the axotomy. The area of axotomized CSN extended in anterior posterior direction over 5-6mm. A mean of 49.6% (n=4) of the axotomized CSN died within 7 days after injury. Continuous application of saline and 0.5% rat serum albumin (vehicle) via an osmotic minipump over 7 days reduced this cell death to 30.4% (n=8), application of NGF (500ng/µl/h)resulted in a further reduction to 24.2% (n=4), however this was not significantly different from the vehicle. The application of NT-3 (500ng/µl/h) or BDNF (500ng/µl/h) prevented the axotomy induced death and only 3.7% (NT-3, n=7) and 10.2% (BDNF, n=4) of the CSN died within the first week after axotomy. These findings are the first demonstration for survival factors for CSN in vivo and may be important for traumatic brain injury and in neurodegenerative disorders which involve death of CSN like amyotrophic lateral sclerosis.

Supported by MRC and Network of Centres of Excellence for Neural Regeneration and Functional Recovery (Canada).

453.15

EFFECT OF CNTF DELIVERY METHODS TO RESCUE FACIAL MOTONEURONS FROM INJURY INDUCED CELL DEATH. S.A. Tan*, V. Padrun, A. Menoud, J. Hammang, E. Baetge, A.D. Zurn, P. Aebischer. Lausanne University Medical School, Switzerland; CytoTherapeutics, Providence, RI.

Ciliary Neurotrophic Factor (CNTF) has been shown to increase the survival of motoneurons *in vitro* and *in vivo*. For potential human application, the mode of delivery needs to be investigated. In the present study, local application of CNTF on the transected facial nerve of neonatal rats was compared to systemic delivery through subcutaneous injections of human recombinant (rhCNTF) and to transplantation of genetically engineered cells. Baby hamster kidney cells (BHK) were transfected with a pNUT vector containing either the gene for mouse CNTF (mCNTF) or human CNTF (hCNTF) and were encapsulated in polypropylene fibers to prevent tumor formation and immune rejection. Facial nerves of neonatal rats (P2) were transected and CNTF was delivered i) by direct application of CNTF on the nerve stump using gelfoam impregnated with rhCNTF (0.25mg/ml); ii) by repeated subcutaneous injections of rhCNTF (1mg/kg) 3 times a week; or iii) by subcutaneous implantation of $1x10^4$ encapsulated BHK cells releasing either mCNTF or hCNTF. Control animals received either bovine serum albumin or the parent BHK cell line. One week post-lesioning, the number of surviving motoneurons on the lesioned side was compared to the non-lesioned side. All three methods of CNTF application significantly improved motoneuron survival with the encapsulated method appearing to be the most efficient.

Mode of delivery	Control	CNTF treated	p values
hCNTF capsule	11%	40%	p<0.001
mCNTF capsule	11%	38%	p<0.001
repeated injection	18%	33%	p<0.02
gelfoam application	18%	31%	p<0.005

Encapsulated transfected cells offer a mean for the continuous slow release of trophic factors, as well as the potential for intrathecal delivery.

453.16

DOSE-DEPENDENT REVERSAL OF BDNF RESCUE EFFECTS ON MOTONEURONS. R. Vejsada, Y. Sagot and A.C. Kato*. Div. Clinical Neurophysiol. & Dept Pharmacol., CMU, University of Geneva, Geneva, Switzerland.

Brain-derived neurotrophic factor (BDNF) promotes the survival, differentiation and maintenance of motoneurons. We compared the effects of different BDNF doses on the rescue of axotomized sciatic and facial motoneurons in neonatal rats. Application of BDNF directly to the central nerve stump significantly enhanced motoneuron survival. Additional BDNF supplied with intermittent subcutaneous injections (1 mg/kg, at 3-day intervals) yielded a further small survival increase; however, injecting BDNF daily markedly reduced the motoneuron rescue at both 1 week and 2 weeks post-lesion. These results, corroborated by findings in motoneuron primary cultures, show that a dose-dependent reversal of BDNF-mediated motoneuron rescue may occur both *in vitro* and *in vivo*.

453.17

NERVE GROWTH FACTOR GENE TRANSFER TO THE ADULT AND AGED RAT BRAIN USING CNS DERIVED NEURAL PROGENITOR CELLS: CELLULAR EFFECTS AND BEHAVIORAL RECOVERY. A.Martínez-Serrano, W. Fischer, G. Nikkhah*, C. Lundberg and A.Björklund., Dept. of Medical Cell Research, University of Lund, S-223 62-Lund, Sweden.

Conditionally immortalized neural progenitors were infected with a retrovirus coding for mNGF, and clones were isolated and analysed for NGF production. Among them, one clone produced 20 ng of NGF /hour/10^6cells. The cells were characterised in vitro for their ability to produce NGF and for the expression of the retroviral vector, finding no differences between the proliferative and growth arrested cultures (permissive and non-permissive temperature). After grafting to the septum, striatum or nucleus basalis in young animal, the cells survive well and migrate away from the implantation site. Expression of the transgene in vivo and NGF production were studied in dissected pieces of nc basalis tissue 4 and 10 weeks after grafting. RT-PCR revealed moderate but stable expression of the transgene, and a bioassay for NGF demonstrated the presence of the neurotrophic activity (the NGF-grafted nc basalis showed as high neurotrophic activity on PC12 cells as the hippocampus tissue). Cells grafted to the ncbasalis induced a consistent 40% hypertrophy of the cholinergic cell bodies (1, 4 and 10 weeks post-grafting). When grafted to the septum in rats with fimbria-fornix lesion, over 90% of the cholinergic cells on the lesion side was rescued from axotomy-induced death. Thus, there seems to be a good correlation between cell survival, expression of the transgene, NGF production and cellular effects observed.

We next looked for behavioural effects of the NGF cells in vivo by grafting into the medial septum and/or nc. basalis of memory impaired aged animals. In the Morris water maze test, there was a progressive improvement of their performance (2 and 4 weeks after grafting) up to the point when they were not different from the control, non-impaired animals. Histological analyses revealed surviving grafted cells, as well as a clear hypertrophic effect in the host cholinergic neurons, that showed the same size as in young rats. Data on expression and recovery of NGF-like neurotrophic activity from grafted brains will also be presented.

453.18

THE EFFECTS OF NGF AND BDNF ON THE MORRIS WATER MAZE IN AGEI RATS. M.A. Pelleymounter*, M.J. Cullen and M.B. Baker. Dept. of Neurobiology, Amgen, Inc., Thousand Oaks, CA 91320.

Infusion of NGF into the lateral ventricle has been shown to improve water maze performance in aged rats, along with increasing septal cholinergic cell size and number. We have compared the effects of NGF with BDNF in acquisition and retention of the Morris maze in aged Long-Evans male rats. Prior to infusion of NGF or BDNF into the dorsal third ventricle, aged rats were separated into impaired or unimpaired subpopulations, based upon spatial bias demonstrated during the 12th training trial. Impaired rats were then equally distributed between PBS, NGF and BDNF groups. Infusions were conducted for a 4 week period, using Alzet mini-pumps (6 ug/day; 0.5 ul/hour). On the seventh day of infusion, rats continued their training on the Morris maze, where they were given three trials/day, with a probe trial inserted every 6 trials tc assess spatial bias. Young and unimpaired rats showed significantly lower latencies anc distances than their impaired PBS, NGF and BDNF-treated counterparts. Young, unimpaired and NGF-treated impaired rats performed significantly better than the PBS or BDNF-treated impaired rats on the spatial bias trials, however ($p<.05$). Seven weeks after all rats had reached a learning criterion based upon spatial bias, half were tested for retention of the task, and half were sacrificed for assessment of brain cholinergic activity and hypothalamic biogenic amine levels. Interestingly, once aged rats had met a spatial bias criterion, their retention of the task was similar to young rats, except that young rats made more annulus crossings in the retention probe trial ($p<.05$). Choline uptake was generally reduced in impaired old rats; NGF increased hippocampal choline uptake, whereas BDNF increased cortical choline uptake ($ps<.05$). Further, hypothalamic NE, 5-HIAA and 5-HT were all increased in old rats; BDNF, however, significantly reduced both 5-HIAA and 5-HT to the levels of young rats ($p<.01$). In conclusion, NGF improved the acquisition of the Morris maze in aged impaired rats, along with increasing hippocampal choline uptake. BDNF, however, did not improve any aspect of Morris maze learning, despite altering cortical cholinergic and hypothalmic serotonergic activity.

453.19

EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR ON DETERIORATED LEARNING AND MEMORY IN SENESCENCE ACCELERATED MICE. K. Sasaki[1], Y. Oomura[2], A. Li[3], H. Kimura[4], I. Tooyama[4], K. Hanai[4], Y. Nomura[5], Y. Kitamura[6], N. Yanaihara[6] and H. Yago[7]. [1]Fac. Eng., Toyama Univ., Toyama 930, [2]Inst. Bio-Active Sci., Nippon Zoki Pharm. Co., Hyogo 673-14, [3]Fac. Med., Kyushu Univ., Fukuoka 812, [4]Inst. Molec. Neurobiol., Shiga Univ. Med. Sci., Shiga 520-01, [5]Fac. Pharm. Sci., Hokkaido Univ., Sapporo 060, [6]Fac. of Pharm. Sci., Univ. Shizuoka, Shizuoka 422, Japan

Subcutaneous injection of acidic fibroblast growth factor (aFGF) into senescence accelerated mice (SAM-P/8) at one per week was started at 3 weeks after birth and continued for 9 months. When tested by passive avoidance and Morris' water maze tasks, leaning and memory performances deteriorated in the control group, while those of the aFGF-treated group did not. At the end of the 9 month, brain tissues of sacrificed animals were stained immunohistochemically using anti-choline acetyl-transferase (CAT) antibody. The number of cholinergic neurons in the medial septum (MS) decreased by 15% in the control group and was no change in the aFGF-treated group, as compared to SAM-R/1, a reference strain of SAM-P/8. The activity of CAT in individual cholinergic neurons in the MS also decreased significantly in the control group. Results suggest that aFGF has neurotrophic effects on cholinergic neurons in the MS of SAM-P/8 and ameliorate leaning and memory disorder in both tasks tested.

453.20

AIT-082 MODULATES NEURITOGENESIS THROUGH A CARBON MONOXIDE/GUANYLATE CYCLASE MECHANISM AND RESTORES AGE-INDUCED MEMORY DEFICITS. A.J. Glasky*[1,2], R.F. Ritzmann[1,2], C.L. Melchior[2], S. Hindley[3], J.W. Gysbers[3], P. Middlemiss and M.P. Rathbone[3] [1] Adv. ImmunoThera-peutics, Tustin, CA 92680, [2] Olive View/UCLA MC, Sylmar,CA 91342 and [3] Depts. Biomed. Sci. and Medicine, McMaster U., Hamilton, Ontario L8N 3Z5

Age-related memory loss is associated with loss of NGF-dependent basal forebrain neurons. Since guanosine and inosine (3-300μM) enhanced the neuritogenic effects of NGF on PC12 cells *in vitro*, we tested whether AIT-082, a purine analogue could promote neurite out-growth from PC-12 cells *in vitro* and determine the mechanism of this effect. AIT-082, when added to cultures of PC12 cells at concentrations of 0.1 to 10μM, promoted neuritogenesis and enhanced the effects of NGF. The neuritogenic effect of AIT-082 was reduced (a) by hemoglobin, which binds extracellular nitric oxide and carbon monoxide (CO), (b) by methylene blue, which inhibits soluble guanylate cyclase and (c) by Zn protoporphyrin IX, an inhibitor of heme oxygenase, which produces CO. AIT-082 (30 mg/kg) was able to increase the duration of the working memory trace utilizing the win-shift paradigm in mice (Pharmac. Biochem. Behav. 47:325,1994), in one year old Swiss Webster or C57BL/6 mice with mild or moderate memory deficits,. AIT-082 may be the first agent that restores working memory deficits through a mechanism that involves CO and soluble guanylate cyclase. Since AIT-082 is orally active and rapidly passes the blood-brain barrier, it may have therapeutic potential as a NGF-mimetic agent. (Supported by NIA AG09911 and Hospital for Sick Children Foundation)

NEUROTROPHIC FACTORS: BIOLOGICAL EFFECTS XI

454.1

EFFECTS OF BDNF IN ANIMAL MODELS OF PARKINSON'S DISEASE. C.W. Shults*+#, C. Shin#, and C.A. Altar◊.+VA Med. Ctr., San Diego, CA 92161; #Dept. of Neurosciences, Univ. Cal., San Diego; ◊Regeneron Pharm., Tarrytown, NY.

Groups of 8 naive rats received 3 unilateral, intrastriatal injections of either BDNF or cytochrome c (22.5 μg/injection) on consecutive days. Following the injection of BDNF or cytochrome c on the second day, the animals received an intrastriatal injection of 25 μg of 6-hydroxydopamine hydrobromide (6-OHDA). The animals were then tested weekly for amphetamine and apomorphine-induced rotation for 4 weeks. The BDNF treated animals had fewer apomorphine-induced contraversive and amphetamine-induced ipsiversive rotations than did the cytochrome c treated group. At the fourth testing session, the BDNF treated group had 0.8 ± 0.4 (mean ± SEM) apomorphine-induced and 0.7 ± 0.8 amphetamine-induced rotations/min, and the cytochrome c treated group had 3.1 ± 1.0 apomorphine-induced and 2.7 ± 0.6 amphetamine-induced rotations/min.

Two groups of rats with partial unilateral lesions of the mesostriatal dopaminergic system, which had been matched for apomorphine-induced rotation, received six injections of either BDNF or cytochrome c (22.5 μg/injection) into the partially denervated striatum over 2 weeks. After the sixth injection the BDNF-treated group had significantly fewer apomorphine-induced rotations (2.6 ± 0.4, n=10) than the cytochrome c treated group (5.4 ± 1.0, n=11).

454.2

EFFECTS OF BDNF-PRODUCING FIBROBLASTS ON SUBSTANTIA NIGRA DOPAMINERGIC NEURONS *IN VIVO*. W. R. Galpern,*[1,3] S. B. Tatter,[1,2] M. F. Beal,[2] D. M. Frim,[1,2] X. O. Breakefield,[2] and O. Isacson.[1,2] [1]Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02178; [2]Molecular Neurogenetics Unit, Neurosurgery and Neurology Services, Massachusetts General Hospital, Boston, MA 02114; [3]University of Massachusetts Medical Center, Worcester, MA 01655.

We have previously demonstrated that brain-derived neurotrophic factor (BDNF) is able to protect against dopaminergic (DA) cell loss in the substantia nigra pars compacta (SNc) following striatal infusion of the mitochondrial complex I inhibitor MPP+ in rats. To investigate the effects of BDNF on SNc neuronal function *in vivo*, DA metabolism in the SN was assessed by HPLC. Groups of animals received either genetically engineered BDNF-secreting or control fibroblast implants dorsal to the SNc. Half of the implanted rats received unilateral striatal infusion of MPP+ one week after grafting. For the non-lesioned animals, tissue was processed for tyrosine hydroxylase (TH) immunocytochemistry or for neurochemical analysis 1-2 weeks following implantation. Lesioned animals were sacrificed two weeks after implantation, and the effect of BDNF on the generation of free radicals using salicylate spin-trapping as well as thiobarbituric acid histological assays was evaluated. Initial neurochemical observations reveal an increase in DA content in SN neurons associated with BDNF administration. Preliminary assessment of the effects of BDNF on cellular morphology indicate there is no generalized effect on TH+ cell size in the SN. Further experiments aimed at determining the effect of BDNF on the metabolic and oxidative states of DA neurons in intact and MPP+ lesioned rats are in progress.

454.3

BDNF SECRETING ASTROCYTES GRAFTED INTO THE STRIATUM OF A PARTIALLY LESIONED RAT MODEL OF PARKINSON'S DISEASE AMELIORATE AMPHETAMINE-INDUCED ROTATIONAL BEHAVIOR. Q. Lin, Y.Yoshimoto, T.J. Collier*, D.M. Frim, X.O. Breakefield and M.C. Bohn, Dept. Neurobio. & Anat., Univ. of Rochester Med. Ctr., Roch., NY 14642 and Molec. Neurogenetics Unit, Mass. Gen. Hosp., Harvard Med. Sch., Boston, MA 02129.

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor for dopaminergic (DA) neurons, the cell type that degenerates in Parkinson's disease(PD) To examine the potential BDNF gene therapy for PD, astrocytes transduced with a BDNF cDNA or alkaline phosphatase (AP) cDNA (control) and prelabeled with DiI were grafted into the right rat striatum 15 days after partial lesioning of the right substantia nigra with 6-hydroxydopamine. Prior to grafting, BDNF mRNA was expressed by BDNF but not AP astrocytes. At 32 days, BDNF astrocytes, but not AP astrocytes, attenuated amphetamine-induced rotation by 45% ($p \leq 0.05$). Apomorphine-induced rotation was not significantly changed in either group. At 42 days, DiI labeled astrocytes were present in the graft site, however, BDNF mRNA positive cells were not detected with *in situ* hybridization. Analysis of the density of tyrosine hydroxylase (TH) immunoreactive fibers showed no effect of BDNF astrocytes on the area occupied by TH-positive fibers in the lesioned striatum. These findings suggest that BDNF gene therapy ameliorates Parkinsonian symptoms through a mechanism that does not involve regeneration or sprouting from DA neurons. Furthermore, expression of BDNF from this retroviral construct appears to be down-regulated in grafted astrocytes, a characteristic that needs to be addressed before this method can be utilized for long-term delivery of biologically produced BDNF. (Supported by Markey Charitable Trust & AG09016)

454.4

BDNF-TRANSFECTED OLIGODENDROCYTES INCREASE DOPAMINERGIC FETAL NIGRAL NEURON SURVIVAL AND ENHANCE NEURITIC EXTENSION *IN VITRO*. S.B. Schueler*, D.E. Bredesen[1], R. Anton[1], P.M. Carvey [2], and J.H. Kordower [2]. Dept. of Anatomy and Cell Biology, Univ. Illinois Sch. Med., Chicago, IL 60612; 1 U.C.L.A., Los Angeles, CA 90024; 2 Dept. Neurological Sciences, Rush Presbyterian Med. Ctr. Chicago, IL 60612.

Clonal lines of mouse oligodendroglia which have been genetically modified to secrete brain derived neurotrophic factor (BDNF) were examined for their ability to enhance the neuronal survival and neurite extension of embryonic rat dopaminergic neurons *in vitro*. Rostral mesencephalic tegmentum (RMT) of E15 rat embryos were harvested, dissociated, and cultured in a serum-containing medium. Shortly thereafter, three BDNF oligodendrocyte clones (A1, B6, and B12) and the non-BDNF secreting parental line (N20.1) were individually cocultured with RMT and placed in a 37°C incubator. The coculture medium was changed to a serum-free medium after 24 hours and placed back into the incubator for 4 days. The number of dopaminergic neurons as well as the number of neuritic extensions per neuron were then quantified in 7-8 replicates using tyrosine hydroxylase (TH) immunocytochemistry. An mean increase in TH-positive neurons of 3.5%, 41.0%, and 10.5% was observed in cocultures with clones A1, B6, and B12, respectively when compared to RMT alone or RMT cocultured with N20.1 oligodendrocytes. Thirty-40% of TH-immunoreactive neurons exhibited 3 or more processes when cultured alone or with N20.1 oligodendrocytes. In contrast, 60%, 80% and 84% of TH-immunoreactive neurons exhibited 3 or more processes when cocultured with the A1, B6, and B12 clones, respectively. BDNF-secreting oligodendrocytes enhance the viability and neuronal differentiation of embryonic dopaminergic ventral mesencephalic neurons suggesting that BDNF oligodendrocytes might enhance the structural and functional consequences of fetal nigral grafts in animal models of Parkinson's Disease.

454.5

INHIBITION OF BDNF EXPRESSION BY ANTISENSE OLIGONUCLEOTIDE AFFECTS DOPAMINERGIC NEURONAL FUNCTION IN ADULT RATS. Y.-S. Lau*, T. M. Anderson; Y.-K. Fung; J. F. Bishop, M. M. Mouradian. Dept. of Pharmacol., Creighton Univ., Omaha, NE 68178; Dept. of Oral Biol., Univ. of Nebraska, Lincoln, NE 68583; Exp. Ther. Branch, NINDS, Bethesda, MD 20892.

Brain-derived neurotrophic factor (BDNF) has trophic actions on developing dopaminergic neurons and alters the function of the nigrostriatal system in adult rats. In this investigation, we studied the effects of stereotaxic injections of a BDNF antisense oligodeoxy-nucleotide (ODN) on nigrostriatal dopaminergic transmission. The antisense molecule was an 18-mer phosphodiester ODN targeted to the translation start site of the rat BDNF mRNA. A nonsense ODN having the same base composition but in scrambled sequence was used as control. Two days following the injection of antisense ODN (0.5 μg/μl) into the right striatum, dopamine content in the substantia nigra increased by more than three fold bilaterally compared with nonsense or saline injected rats, while dopamine levels in the striatum were unaffected. Injections of antisense, nonsense or saline into the right substantia nigra did not produce significant changes in dopamine content in either side of the nigra or striatum. Seven days after striatal injection of the antisense ODN, nigral dopamine levels had returned to normal, while striatal levels had increased by two fold. These data add further support that BDNF has a critical function in modulating the function of the nigrostriatal dopamine system in adult rats. The neuronal and intracellular mechanisms responsible for such regulation remain to be determined.

454.6

PHYSICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF MICROENCAPSULATED BDNF AND BDNF-PRODUCING CELLS. D.Maysinger*, A.Cohen, K.Krieglstein and K.Unsicker . Dept. Pharmacology & Therapeutics, McGill University & MGH, Montreal H3G1Y6, Canada,and Dept. Anat. & Cell Biology, University of Heidelberg, D-69120 Heidelberg, Germany.

The delivery of neurotrophic factors to the CNS poses a major obstacle to the treatment of neurodegenerative disorders such as Parkinson's disease. We have developed a polymer encapsulation technology that provides a means for the delivery of BDNF (generously provided by Regeneron) for a prolonged period of time. Several different types of chitosans, alginates and co-polymers with various proportions of poly-l-lactic acid were used. Size distribution prophiles were determined by an image analysis system and surface characteristics were assessed by electron microscopy. The mean diameter of microspheres with BDNF was 1.2 + 0.05 μm whereas spheres with genetically transformed cells secreting BDNF (provided by X.O. Breakefield and M.P. Short) were much larger (300-500 μm). The total content of BDNF as well as the amounts released per day were determined by dot blot assays. The results from the release kinetics demonstrate that longterm secretion (30-60 days) of BDNF is achieved by chitosan microspheres. Alginate spheres provided only relatively short term release (2-7 days) and the type of macromolecules co-encapsulated with BDNF significantly influenced the rate of BDNF delivery. Neuron survival and neurite growth in cultures of rat retinal ganglion cells, DRG, and midbrain floor were supported by microencapsulated BDNF indicating biological stability of BDNF. BDNF-secreting cells survived for different periods of times depending on cell density within spheres, sphere sizes, and properties of the semipermeable membranes. These model systems can also be used for delivery of other trophic factors such as CNTF and GDNF. These studies provide a basis for future comparisons of microencapsulated genetically engineered cells and agents used in the treatments of neurodegenerative disorders.

454.7

REGULATION OF PLATELET-DERIVED GROWTH FACTOR (PDGF) AFTER NEURONAL LESIONS AND EFFECTS OF PDGF ON DOPAMINERGIC AND STRIATAL NEURONS IN VITRO. K. Pietz[1], K. Funa[2], N. Nakao[1], A.E. Ballagi[2], A. Tingström[1], A. Othberg[1], A. Åhgren[2], P. Brundin[1], O. Lindvall[1], and P. Odin[1]*. [1]Restorative Neurology Unit, University Hospital, S-221 85 Lund, Sweden. [2]Ludwig Institute for Cancer Research, Biomedical Centre, University of Uppsala, S-751 24 Uppsala, Sweden.

We have previously demonstrated an increased expression of platelet-derived growth factor (PDGF) around intrastriatal implants of fetal dopamine (DA)-rich mesencephalic tissue in a rat model of Parkinson´s disease. PDGF-BB but not PDGF-AA promotes survival and neurite outgrowth from rat and human DA neurons *in vitro* and influences expression of c-fos and tyrosine hydroxylase mRNA in mesencephalic cell cultures. These effects could be directly mediated via PDGF beta receptors expressed on the mesencephalic DA neurons. The objective of the present study was to further characterize the changes of PDGF expression following insults to the brain and to analyze the effects of PDGF-AA and -BB on striatal DARPP-32-positive neurons *in vitro*. Immunohistochemical, PCR and primary cell culture techniques were used. Following an ibotenic acid lesion to the rat striatum (model for Huntington´s disease), a strong increase in the expression of PDGF was observed in astrocytes in the striatal tissue. Also increased PDGF receptor expression was induced in the lesioned area. In cell cultures of fetal striatum PDGF-BB but not PDGF-AA promoted the survival of DARPP-32-positive neurons. Also neurite outgrowth from these striatal cells was significantly more pronounced in the presence of PDGF-BB. These findings indicate that PDGF could be of importance for the survival and function of CNS neurons after lesions and in neurodegenerative processes.

454.8

NEUROTROPHIC FACTORS AND CELL DEATH OF A CELL LINE DERIVED FROM RAT VENTRAL MESENCEPHALON. H. Takashima* Department of Neurology, Kawatana National Hospital, Kawatana, Nagasaki 859-36, Japan

Basic fibroblast growth factor (bFGF) has neurotrophic effects on dopaminergic neurons. Recently, reduced concentration of bFGF in substantia nigra in the Parkinson's disease brain has been reported. Reduction of this factor might be related to the cell death of dopamine neuron in Parkinson's disease. In this study, the effects of neurotrophic factors on the differentiation and cell death of a neural cell line derived from rat ventral mesencephalon were investigated.

A cell line was established from rat ventral mesencephalon using a retroviral vector containing the temperature-sensitive allele of the SV40T antigen. When cultured with serum-free medium at the permissive temperature, this cell line showed cell death accompanied with DNA laddering in gel electrophoresis and positive *in situ* staining for DNA 3'-OH ends. An addition of bFGF (10 ng/ml) prevented cell death, supported cell proliferation and increased the expression of neurofilament in serum-free medium.

NGF (10 ng/ml) and NT3 (10 ng/ml) did not support the survival of the cells in serum-free medium at the permissive temperature.

454.9

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) PROMOTES SURVIVAL AND REGROWTH OF MESENCEPHALIC DOPAMINE NEURON CULTURES DAMAGED BY MPP+. J.G. Hou* and C. Mytilineou, Dept. of Neurology, Mount Sinai Sch. of Medicine, New York, NY 10029

Astrocytic glia promotes neuronal growth and survival, but the mechanisms of the trophic effect are still not clear. It has been suggested that trophic substances secreted by astrocytes can modify the sensitivity of neurons to neurotoxins. GDNF, a selective dopaminergic neurotrophic factor secreted by a glial cell line, was purified and cloned (Lin et al., *Science* 260:1130-1132, 1993). We used human recombinant GDNF to determine whether it could protect dopamine (DA) neurons from the neurotoxins MPP^+ and 6-OHDA. Mesencephalic cultures prepared from E14.5 rats were maintained in serum-free medium. GDNF increased [^{3}H]DA uptake at concentration as low as 0.01 ng/ml, reached plateau at 1 ng/ml and reduced activity at 10 ng/ml. To test for neuroprotection, 100μM MPP^+ (60 min) or 30μM 6-OHDA (45 min) were applied at day 6 *in vitro* and the extent of neurotoxicity was assessed 24 hours later by [^{3}H]DA uptake. In the presence of 1 ng/ml GDNF, MPP^+ treatment caused a 44% decrease in uptake, compared with a 66% decrease in the controls. When the uptake was measured 5 days after MPP^+ treatment, control cultures showed a further reduction in uptake, which was prevented by treatment with GDNF. Furthermore, GDNF added only after the end of MPP^+ treatment also prevented the continuing decline in [^{3}H]DA uptake. GDNF, however, did not protect from 6-OHDA toxicity (63% reduction compared to 53% in controls). In contrast, bFGF, which increased the growth of glial cells in cultures, had a strong protective effect against 6-OHDA toxicity. These results indicate that the presence of glial cells is necessary for protection from oxidative damage by 6-OHDA; while GDNF, secreted by glial cells, supports DA neuron maintenance and regrowth. (Supported by NIH, NS-23017 and NS-30898)

454.10

THE NEUROPROTECTIVE EFFECTS OF GDNF ON SUBSTANTIA NIGRAL DOPAMINE NEURONS IN 6-OHDA LESIONED RATS C Kearns* and DM Gash, Dept. of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536

Glial-cell-derived neurotrophic factor (GDNF), a novel member of the TGF-B superfamily, has been shown to promote the survival and morphological differentiation of dopaminergic neurons *in vitro* (Lin et al, Science. Vol **260**, 1130, 1993). Recent interest has focused on the use of neurotrophic factors in preventing the degeneration of nigrostriatal dopaminergic neurons observed in Parkinson's disease. Since GDNF may specifically prevent this degeneration and enhance the functional activity of the remaining dopamine neurons, the present study was designed to investigate a possible neuroprotective role for GDNF in rats receiving 6-OHDA administered either into the ipsilateral substantia nigra or striatum. Prior to surgery, 32 young adult male Fischer 344 rats were divided into the following groups : 1)intranigral saline + intranigral 6-OHDA, 2) intranigral saline + intrastriatal 6-OHDA, 3) intranigral GDNF + intranigral 6-OHDA and 4) intranigral GDNF + intrastriatal 6-OHDA. The saline treated groups received a single 2ul intranigral injection of phosphate buffered saline (PBS) while the GDNF treated rats received 10ug/2ul GDNF in PBS. Twenty four hours later, the animals received a unilateral 2.5ug/ul 6-OHDA infusion either in the substantia nigra or striatum. The rats were sacrificed by intracardiac perfusion two weeks postsurgery and the brains processed for GFAP and TH immunocytochemistry. Cell counts of remaining dopamine neurons in the substantia nigra were evaluated in all groups. In the nigral lesioned groups,there was only a 50% loss of dopamine neurons within the substantia nigra of the GDNF treated rats as compared to the 90% loss of dopamine neurons in those treated with saline. In the striatal lesioned groups, there was a 75% loss of dopamine neurons in the substantia nigra of the GDNF treated rats, while a 90% loss of dopamine neurons was observed in those treated with saline. These results demonstrate a significant protective role of GDNF in the nigral lesioned animals and a moderate sparing of dopamine neurons in striatal lesioned rats.

454.11

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF): EFFECTS ON NIGROSTRIATAL DOPAMINE NEURONS AND BEHAVIOR IN MPTP-TREATED MICE. A. Tomac[1], E. Lindqvist[1], B. Hoffer[1], SO Ögren[1], L.-F. H. Lin[2] & L. Olson*[1]. [1]Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden. [2]Synergen, Inc., Boulder, CO 80301.

We examined the effects of a single intracranial injection of human recombinant GDNF 24 hours prior to or 7 days after MPTP exposure in C57/BL mice, monitoring neurochemical and behavioral changes. C57/BL mice, 11-12 weeks old, were exposed to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 2x40mg per kg body weight s.c., given 24 hr apart. Mice were unilaterally injected with 10 μg GDNF or vehicle in a volume of 2 μl, into striatum or over substantia nigra. Animals were monitored for either 5 or 18-20 days postoperatively, and were then sacrificed for HPLC analysis. Behavioral data indicated a significant increase in spontaneous motor activity (rearing, locomotion and motility) in GDNF-treated compared to vehicle-treated animals. HPLC data showed significant increases in dopamine levels in striatum (192%, $p=0.010$) and substantia nigra (187%, $p<0.003$) compared to vehicle-treated animals, when GDNF was given in striatum 24 hours prior to MPTP exposure. When GDNF was given into the striatum 7 days after MPTP exposure and mice sacrificed 18-20 days later, increases in dopamine levels were found in striatum (70%, $p=0.003$) and in substantia nigra (94%, $p<0.0001$) compared to vehicle-treated mice. Injection over substantia nigra also led to increases in dopamine levels, but only in substantia nigra (64%, $p=0.004$) compared to vehicle-treated animals. The findings reported here suggest that GDNF may play a crucial role for dopaminergic neurons, reversing effects of MPTP toxicity in this rodent model of Parkinson's disease.

454.12

BEHAVIORAL, NEUROCHEMICAL, AND HISTOLOGICAL CHANGES IN THE DOPAMINERGIC SYSTEM OF UNILATERALLY 6-OHDA LESIONED RATS FOLLOWING INTRANIGRAL ADMINISTRATION OF GLIAL CELL-DERIVED NEUROTROPHIC FACTOR (GDNF). K. E. Bowenkamp[1]*, A. F. Hoffman[1], A.-C. Granholm[3], M. A. Henry[3], P. Biddle[3], P. Huettl[2], L-F. H. Lin[4], D. Martin[4], J. L. Hudson[1], G. A. Gerhardt[1,2], and B. J. Hoffer[1,2]. Depts. of Pharmacology[1], Psychiatry[2], and Dentistry[3], University of Colorado Health Sciences Center, Denver, CO 80262 and Synergen, Inc.[4], Boulder, CO 80301.

Young adult male Fischer 344 rats were unilaterally injected into the medial forebrain bundle with 6-hydroxydopamine (6-OHDA, Sigma, 9μg/4μL in 0.02% ascorbate and 0.9% NaCl over 4 minutes). Apomorphine-induced rotational behavior (Sigma, 0.05 mg/kg in 0.02% ascorbate and 0.9% NaCl injected SC) was used to select animals whose average rotation exceeded 300 turns/hour, corresponding to greater than 99% dopamine (DA) depletion in the ipsilateral striatum. Six weeks later, 0.1 to 100μg of GDNF (Synergen, Inc.) or vehicle was injected intranigrally ipsilateral to the lesion. Apomorphine-induced rotational behavior was quantified weekly for up to five weeks post-GDNF injection. Rats were sacrificed at 1, 3, and 5 weeks post-GDNF, for tyrosine hydroxylase (TH) immunohistochemistry and HPLC-EC analysis for the presence of NE, DA, DOPAC, HVA, 5-HT, and 5-HIAA in the right and left substantia nigra (SN), ventral tegmenta, and striata. The highest dose of GDNF tested (100μg) produced a greater than 65% decrease in rotational behavior which persisted for 5 weeks. This dose also produced levels of DA and DOPAC in the ipsilateral SN which were not statistically different from the contralateral, unlesioned side. Vehicle-treated animals showed a marked DA depletion in the ipsilateral SN. In contrast, there was no change from the lesioned state in the ipsilateral striatum. Immunohistochemistry of nigral sections from the 100μg GDNF dose groups showed vacuoles associated with increased TH-immunoreactivity. These results demonstrate neurochemical and behavioral improvements in unilaterally-lesioned rats following intranigral administration of GDNF, suggesting that GDNF may be a useful therapy for Parkinson's Disease.

454.13

DISTRIBUTION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) DURING PRE- AND POSTNATAL DEVELOPMENT IN RAT CENTRAL AND PERIPHERAL TISSUES. E. Lindqvist,[1] A. Tomac,[1] J. Luthman,*[1] B. Hoffer,[1] S. Bektesh,[2] L. Olson.[1] [1]Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden, and [2]Synergen, Inc., Boulder, CO 80301.

Glial cell line-derived neurotrophic factor (GDNF), a recently described protein related to the transforming growth factor beta-superfamily, has dopaminotrophic activity. We have previously demonstrated by in situ hybridization that GDNF mRNA is expressed in the developing striatum but is not confined exclusively to brain areas with a rich dopamine innervation. We have now carried out a more detailed in situ hybridization mapping study of GDNF mRNA using two non-overlapping oligonucleotide probes. The striatal anlage signal is present from E17, maximal during the first week of life, and declines to low levels by postnatal week 4. The message is present in patches and with a stronger signal in dorsal striatum. Several other areas of the developing CNS express GDNF mRNA, such as sensory and motor trigeminal nuclei, the cerebellar anlage, hippocampus, pons, and the spinal cord where neurons of Clark's column are positive. In the olfactory tubercle, another dopamine-innervated area, GDNF mRNA persists into adulthood. Certain thalamic nuclei are strongly positive in P7 animals. The pineal gland and the subcommissural organ are both strongly positive in the neonate. Expression was also found in cingulate and piriform cortex postnatally, declining in 4-week-old animals. Interestingly, GDNF mRNA was also found in the periphery in sensory systems such as vibrissae, the developing tooth buds, as well as developing taste bud areas in the tongue. Furthermore, message was found in skeletal muscle tissue during development. We conclude that GDNF is expressed during development in dopamine-innervated areas of the brain as well as along neuronal systems associated with sensory information processing and proprioception.

454.14

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) AS A NIGRO-STRIATAL DOPAMINOTROPHIC FACTOR LINKED TO CORTICO-STRIATAL EXCITATION. R. Schmidt-Kastner*, A. Tomac, B. Hoffer, S. Bektesh[1], B. Rosenzweig [1] and L. Olson. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden; [1]Synergen Inc., Boulder, CO, USA.

Glial cell line-derived neurotrophic factor (GDNF) has recently been cloned and shown to have trophic effects on dopaminergic nigral neurons. GDNF mRNA was detected in the striatum of newborn rats, but not in adults. Enhanced neuronal excitation and depolarization in epileptic seizures has been shown to elevate the expression of several neurotrophins. Pilocarpine induces limbic motor status epilepticus, activating the limbic system and also basal ganglia circuits. Using this model, we have investigated GDNF mRNA production in forebrain of adult rats employing in situ hybridisation with two independent oligonucleotide probes. GDNF mRNA was undetectable in striatum, cortex or hippocampus of controls. After 6 h of seizures, hybrididsation for GNDF mRNA was detected in cells in the caudal striatum. Signals were positive in granule cells of the dentate gyrus at 3, 6 and 24 h, and in lateral CA3 and medial CA1 area of hippocampus at 24 h. Cellular labelling was also found in neocortex at 24 h. The positive findings in the striatum support the hypothesis that GDNF may be a target-derived, trophic factor in the nigro-striatal system. Excitatory cortical input onto striatal neurons might be responsible for the regulation of GDNF expression. This initial demonstration of GDNF mRNA expression in the adult brain is critical for postulates on the trophic interplay between substantia nigra and striatum in Parkinson's disease. Increases in GDNF mRNA after status epilepticus in hippocampus and neocortex may indicate additional roles for GDNF.

454.15

POSTNATAL SPINAL CORD GRAFTS IN OCULO: EFFECTS OF TREATMENT WITH GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR. K. Trok, B. Hoffer,* D. Russell,[1] L. Olson. Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden, and [1]Synergen Inc., Boulder, CO.

Glial cell line-derived neurotrophic factor (GDNF) is postulated to be a trophic factor for developing dopaminergic neurons. However, the distribution of mRNA for GNDF in the prenatal rat, including the spinal cord and skeletal muscle, suggests that GDNF has additional roles. Thus, we sought to determine if GDNF would also have a trophic effect on spinal cord tissue. The intraocular transplantation of neural tissue provides a unique approach to study the actions of trophic factors in vivo. In the case of spinal cord, although prenatal tissue matures well in the anterior chamber, grafts from newborn animals show limited survival and reduce their size. Grafts from two-week old animals usually do not survive at all. In our studies spinal cord tissue from newborn (P1) rats was transplanted to the anterior eye chamber of host rats after incubation in 100 μg/ml, 20 μg/ml, 10 μg/ml GDNF or vehicle. GDNF in doses of 0.5 μg, 0.1 μg, 0.05 μg or vehicle was then injected into the anterior eye chamber at days 5, 10, 15 and 20. There was a dose-dependent increase in growth of the transplants induced by GDNF. Indeed, grafts treated with 0.5 μg actually grew to larger than their initial size at grafting. We also transplanted P14 spinal cord to the anterior eye chamber and injected 0.5 μg GDNF or vehicle at days 5, 10, 15, 20, 25, 30 and 35. Grafts treated with GDNF also responded with increased growth compared with controls, but did not exceed their initial size at transplantation. We are currently investigating the immunohistochemical properties of these grafts.

454.16

Effects of Glial Derived Neurotrophic Factor (GDNF) on the Nigrostriatal Dopamine System in Non-human Primates. G.A. Gerhardt*, W.A. Cass[1], Z Zhang[1], A. Ovadia[1], M. Cheesman[1], B.J. Hoffer, P. Huettl, D. Russell[2], D. Martin,[2] and D.M. Gash[1]. Department of Psychiatry, Univ. of Colo. Health Sci. Ctr., Denver, CO, [1]Department of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY, and [2]Synergen, Inc., Boulder, CO.

In the present study, the effects of single injections of GDNF into the substantia nigra or striatum of normal female Macaque monkeys were investigated using immunohistochemical, neurochemical, and behavioral methods. GDNF was injected using MRI-guided sterile stereotaxic surgery in isoflurane anesthetized monkeys; 150 μg/18 μl was injected into the right substantia nigra or 450 μg/18 μl into the right caudate nucleus. A separate group of animals served as controls receiving equal volume injections of phosphate buffered saline. Monkeys were monitored behaviorally each day both pre- and post-GDNF infusions. GDNF was seen to produce some behavioral changes in the animals for up to three weeks following the injections. Some decrease in body weight, increase in activity and occasional sleep disturbances were seen in the GDNF injected group as compared to controls. *In vivo* electrochemical studies involving local application of potassium using Nafion-coated carbon fiber electrodes showed increased DA release in areas of the caudate nucleus and putamen, ipsilateral to the GDNF injections. HPLC-EC studies of tissue punches of caudate nucleus and putamen of GDNF injected animals showed an increase in DA content bilaterally in animals which had received GDNF as compared to controls. Immunohistochemical studies showed a proliferation of TH-positive neurites in the substantia nigra of the animals receiving intranigral GDNF injections. These studies demonstrate increases in the functional properties of DA neurons upon exposure to GDNF in monkeys and support the hypothesis that GDNF may be a useful therapy in Parkinson's disease.

454.17

EFFECT OF GLIAL CELLLINE-DERIVED NEUROTROPHIC FACTOR (GDNF) ON THE SURVIVAL OF CULTURED PERIPHERAL NERVOUS SYSTEM (PNS) NEURONS. T.J. Neuberger, D.J. Smith and L-F. H. Lin* Synergen Inc., Boulder, CO 80301

GDNF was known to enhance the survival and development of midbrain dopaminergic neurons with an EC_{50} of ~40 pg/ml (Lin et.al., Science, 260:1130, 1993). To further define the specificity of the neurotrophic activity of GDNF, we determined the EC_{50} for promoting the survival of several distinct classes of embryonic chick PNS neurons including sympathetic lumbar chain (SC) neurons, parasympathetic ciliary ganglion (CG) neurons and sensory dorsal root ganglion (DRG) neurons. In addition, we also examined GDNF as a neurotrophic factor for neonatal rat superior cervical ganglion (SCG) neurons. GDNF promoted the survival of SG, CG and DRG neurons with an EC_{50} of 9, 13 and 325 ng/ml, respectively. However, these values are several order of magnitude greater than that required for GDNF to function as dopaminergic neurotrophic factor in midbrain cultures. GDNF at all concentrations tested (100 pg/ml to 10 μg/ml) could not support the survival of rat SCG neurons in serum-free defined culture medium whereas NGF at 50 ng/ml promoted neuronal survival and neurite extension. Hence, although GDNF can promote the survival of some PNS neurons, GDNF appears to be most potent with cultured midbrain dopaminergic neurons.

454.18

INFLUENCE OF NEUROTROPHIC FACTORS ON BIOCHEMICAL CHANGES IN THE MESOLIMBIC DOPAMINE SYSTEM ASSOCIATED WITH DRUGS OF ABUSE. MT Berhow*, DS Russell, DW Self, RM Lindsay^, and EJ Nestler. Laboratory of Molecular Psychiatry, Yale Univ Sch of Med, New Haven, CT; ^Regeneron Pharmaceutical Inc., Tarrytown, NY

Chronic morphine and cocaine treatments have been shown to regulate levels of tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) in the ventral tegmental area (VTA) of the adult rat brain. Previous research demonstrated that the morphine-induced changes could be prevented by direct infusion of BDNF or NT4 (2.5μg/day) midline into the VTA. In contrast, CNTF (1.5μg/day) infusions mimicked these biochemical changes that are characteristic of chronic morphine treatment (Beitner-Johnson et al, Neurosci Abs 1993). In the present study, two alternative experimental paradigms were utilized. The first tested the ability of neurotrophins to reverse the biochemical changes in rats already pretreated with chronic morphine pellets. NT-4 infusion reversed the characteristic increases in TH and GFAP without any effect on these proteins when given alone. CNTF mimicked the morphine induced increase, but was not additive with morphine. The second paradigm tested the effect of concurrent cocaine (15mg/kg twice daily for 10 days) and growth factor treatment. As with morphine, BDNF abolished the cocaine-induced increases in TH levels.

A possible site of convergence for the intracellular actions of neurotrophins and drugs of abuse are the ERK's (extracellular-signal regulated kinases). We are infusing ERK1 and ERK2 antisense oligonucleotides into the VTA by osmotic minipumps. Initial studies suggest that these infusions can reduce ERK protein levels detected via immunoblotting. These studies, and related studies of other intracellular targets (Russell et al. Neurosci Abs, this volume), will help elucidate the molecular actions that may be common to the neurotrophins and drugs of abuse.

454.19

MODULATION OF NEUROTROPHIN RECEPTOR TYROSINE KINASE (RTK) PATHWAYS IN THE MESOLIMBIC DOPAMINE SYSTEM BY DRUGS OF ABUSE. D.S. Russell*, M.T. Berhow, K.L. Widnell, D.W. Self, and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts of Neurology and Psychiatry, Yale Univ Sch of Med, New Haven, CT 06508

The cAMP system is an important mediator of neuronal changes due to drugs of abuse in the mesolimbic dopamine system (*Neuron*, 11:995, 1993). Neurotrophins can modify or mitigate these changes (see accompanying abstract, Berhow, et al.). Various methods are being employed to investigate the role of the neurotrophin RTK pathways, and their regulation by the cAMP system, in the changes induced by morphine and cocaine. Chronic morphine treatment in rats increases PLC-γ immunoreactivity in the VTA by 20-30% as determined by western blotting. Morphine also appears to regulate ERK1 and 2 differentially, while significant changes in the levels of raf1, PI-3-kinase, and ras were not observed. Chronic cocaine, however, decreased PLC-γ by 10-20%. Several proteins that are immunogenically like insulin receptor substrate-1 (IRS-1) are detected in rat brain and some appear to be associated with trk receptor complexes. Levels of a 200 kDa IRS-1-like protein decrease in the VTA with morphine treatment, while levels of a 130 kDa form increase in the hippocampus. Studies of regulation of the phosphorylation states and activities of these proteins are ongoing. Several recent studies demonstrate interactions between the cAMP system and RTK pathways. Some proteins likely to mediate this include raf, ERK and CREB. Single injections of CREB antisense S-oligonucleotides into the nucleus accumbens lead to a 50% decrease in CREB levels, and attenuate Fos-like protein expression and result in rotation behavior in response to an acute amphetamine challenge. This approach should enable the study of CREB, and the various other proteins mentioned above, in mediating the converging actions of neurotrophins and drugs of abuse in specific brain regions.

454.20

CONDITIONED MEDIUM DERIVED FROM MESENCEPHALIC TYPE-1 ASTROCYTES EXERTS DIFFERENT EFFECTS ON DOPAMINERGIC NEURONS AND PC12 PHEOCHROMOCYTOMA CELLS.

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Previously, we have developed a primary neuronal culture of the ventral mesencephalic region of the E14 rat in which 20% of the neurons are dopaminergic (Shimoda *et al.*, Brain Res. 586: 319-331 1992). In the absence of serum, glial proliferation is suppressed and the percentage of dopaminergic neurons is reduced to <2% at five days in vitro (DIV 5). These cultures are thus good model systems for the bioassay of specific dopaminergic neurotrophic factors. In this study we use our model culture and a PC12 differentiation bioassay to demonstrate specific effects of conditioned medium derived from type-1 astrocytes (A-CM) on dopaminergic neuron suvival.

When cultured in defined medium, 3.8±1.6 x 100/cm² dopaminergic neurons were present at DIV 5. The addition of 10% serum, A-CM or conditioned medium derived from B49 glial cells (B49-CM) increased the number of dopaminergic neurons present to 42.4±15.9 x 100/cm², 30.6±26.7 x 100/cm² and 8.3±1.9 x 100/cm² respectively. Few PC12 cells express neurites in culture medium, however, 80% expressed neurites when cultured with 100ng/ml of NGF and 48% expressed neurites when cultured with B49-CM. A-CM had no significant effects on PC12 cells. In addition, two partially purified A-CM glycoproteins with molecular weights of 30 000 and 15 000 increased dopaminergic neuron survival but did not effect neurite expression in PC12 cells.

B49-CM increases the survival of dopaminergic neurons and promotes the differentiation of PC12 cells, however, the effects of A-CM are specific to the survival of dopaminergic neurons. We are presently further characterizing the active glycoproteins that we have identified. The *in vivo* significance of these factors remains to be elucidated.

455.1

A BIOASSAY METHOD FOR DOPAMINERGIC NEUROTROPHIC FACTORS BASED ON THE USE OF MICROCULTURES AND IMAGING METHOD:
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BDNF, GDNF, IL-6, bFGF, PDGF-ββ, EGF, TGF-α, type-1 astrocytes, type-2 astrocytes, O-2A progenitors, microglia, and injured striatum all promote the survival of dopaminergic neurons in culture directly or indirectly. Experimental conditions used to assess the survival of dopaminergic neurons differ widely among investigators; 1) cell density; 2) fetal age; 3) dissection technique; 4) the use of serum; 5) the age of the cultures when stained and 6) the methods selecting fields for analysis are among the most important variables: We have developed a method that, if used widely, could bring a degree of objectivity to the field, and allow for a comparison of data from different laboratories. In this method, the E14 fetus is used. Only 1.0 mm^3 of ventral mesencephalic tissue, which contain 90% of dopaminergic neurons, is dissected. The growth medium contains 0% of serum throughout. The cells are suspended at a density of 5.0 x 10^5/ml. Only 25 µl of the cell suspension is plated per chamber (area covered=6.2mm^2) as a microisland (MI), on a substrate of poly-D-lysine. The equivalent plating density is ~2.0 x 10^5 cells/cm^2. Cell attachment is completed in 4hr, and 375 µl of growth medium is added. The untreated culture contains approximately 20%, 5%, 1% and 0% of tyrosine hydroxylase positive (TH^+) neurons at 12 hr, DIV3, DIV5 and DIV7, respectively. The center of the MI is selected for analysis, using an imaging system with software capable of preparing a graphical montage. Four (2x2), or nine (3x3), or sixteen (4x4) adjacent fields are recorded (using a x20 objective), depending on the type of experiment, and especially the age of culture. Total neuronal counting is obtained by staining with anti-NSE or Neurotag. Double-staining is done for each neuronal phenotype of interest. The recorded image is displayed as a montage, saved as a .TIF file, and printed graphically. We have used this method successfully to compare the relative potency of most of putative dopaminergic neurotrophic factors listed above.

455.2

TRANSFORMING GROWTH FACTORS-β2 AND 3 ARE POTENT SURVIVAL FACTORS FOR EMBRYONIC MIDBRAIN DOPAMINERGIC NEURONS. K. Poulsen, M. Hynes, R. Klein, M. Armanini, H. Phillips, A. Rosenthal*. Dept. of Neuroscience, Genentech, Inc., South San Francisco, CA 94080.

The physiological factors that regulate survival of midbrain dopaminergic neurons have not yet been identified with certainty. Here we show that, transforming growth factors (TGF)-β2 and TGF-β3, at picomolar concentrations, prevent the death of embryonic dopaminergic neurons in culture, and that both factors are expressed in the environment of dopaminergic neurons. TGF-β2 & 3 could therefore be physiological trophic factors for midbrain dopaminergic neurons and may be useful as therapeutic agents for Parkinson's disease.

455.3

LONG-TERM SURVIVAL AND DEVELOPMENT OF DOPAMINERGIC NEURONS IN ORGANOTYPIC CULTURES OF POSTNATAL RAT VENTRAL MESENCEPHALON: DEPENDENCE ON BDNF FOR EXPRESSION OF NEUROTRANSMITTER SPECIFIC PHENOTYPE. C. Hyman* M. Juhasz, R.M. Lindsay Regeneron Pharmaceuticals Inc. Tarrytown, New York 10591.

Dissociated cultures of embryonic ventral mesencephalon have been succesfully used to establlsh survival and early differentiation effects of specific neurotrophins on nigral dopaminergic neurons (Hyman et al , 1994, Knüsel et al, 1991, Beck et al, 1993). It was of interest to determine whether the same neurotrophins are required for sustained survival and differentiation of postnatal dopaminergic neurons.

We have developed a method for the long-term maintenance of organotypic cultures of postnatal rat ventral mesencephalon under defined conditions. Viability and function of dopaminergic neurons were assessed by measurement of high affinity dopamine uptake activity, ^{3}H-mazindol binding, and immunocytochemical staining of tyrosine hydroxylase. Culture conditions were selected which resulted in stable neuronal survival over 2 to 7 weeks in vitro, as determined by measurement of neurofilament content. High affinity dopamine uptake activity increased to reach a maximum level at 4 weeks in vitro. At 3-5 weeks in vitro ^{3}H-mazindol binding was high but declined after 8 weeks. Exogenously added BDNF, included in culture medium at 50/ng/ml, did not influence the survival or function of dopaminergic neurons as assessed by dopamine uptake activity or ^{3}H-mazindol binding. In contrast, treatment of cultures with a neutralizing anti-BDNF antibody resulted in a 90% decrement in dopamine uptake activity after 3 weeks in vitro. This effect was reversible, indicating that postnatal dopaminergic neurons can survive in the absence of exogenous BDNF, but fail to express their appropriate transmitter-specific phenotype. Furthermore this suggests that locally synthesized BDNF may be required to maintain the phenotype of mature dopaminergic neurons.

455.4

REGULATION OF THE LOW-AFFINITY NGF RECEPTOR. S.M. Abelleira[1*], R.M. Carroll[1], R.L. Dean[1], R.J. Fundeklian[1], J.H. Kordower[2], V. Charles[2], R.T. Bartus[1]. [1]Alkermes, Inc., Cambridge, MA, 02139. [2]Rush Presbyterian/ St. Lukes Medical Center, Chicago, IL 60612.

It is known that while the cholinergic interneurons of the rat striatum express the low-affinity NGF receptor (LNGFR) during development, the immunoreactivity of these neurons to monoclonal antibody against this receptor (p75) is lost in adults. Previous findings [Gage et al., Neuron, 1989] indicate that the cholinergic neurons of the adult striatum will resume their p75 immunoreactivity in response to striatal damage and that this phenomenon is apparently modulated by endogenous NGF.

Because immunoreactivity of the LNGFR following tissue damage may provide fundamental information on the modulation and function of this receptor, we studied various parameters which might affect immunoreactivity. Injury was administered in one group by implantation of a cannula unilaterally into the striatum (AP+0.7, L-2.5, DV-5.0), and attached to an Alzet 2002 osmotic pump. In addition to injury, this group also received a 14 day continuous infusion of either NGF (300 ng/day) or ACSF. Injury was administered to a second group via injection of quinolinic acid (90 or 120 nM) into the striatum. In both cases animals were sacrificed and the tissue was processed for p75 and ChAT immunohistochemistry. Results indicate that contrary to expectations, in neither group was p75 immunoreactivity of striatal cholinergic neurons observed, despite clear immunoreactivity of septal-diagonal band cholinergic neurons in the same sections. Additionally, pronounced hypertrophy of ChAT immunoreactive striatal cholinergic neurons was seen in the NGF infusion group, demonstrating a biologically effective NGF response in these neurons.

In summary, the lack of striatal p75 immunoreactivity in either the damaged or NGF infused animals, in the face of clear p75 immunoreactivity in the septum, as well as NGF-induced ChAT(+) striatal hypertrophy , raises more questions than answers. While regulation and function of the LNGFR remains an area of interest and intrigue, these results suggest that a final analysis of this phenomenon will not likely be simple.

455.5

NGF CONJUGATE AMELIORATES BASAL FOREBRAIN CHOLINERGIC HYPOFUNCTION INDUCED BY AF64A. R.L. Dean*[1], R. Stackman[3], S.M. Abelleira[1], R.M. Carroll[1], J.H. Kordower[2], T. Walsh[3], and R.T. Bartus[1]. [1]Alkermes, Inc., Cambridge, MA 02139. [2]Rush Presbyterian, Chicago, IL [3]Rutgers University, New Brunswick, N.J.

To date, two key pieces of evidence are lacking to substantiate the hypothesis that NGF may provide an effective means to treat neurodegenerative diseases: (1) better evidence that NGF enhances activity or function of basal forebrain cholinergic neurons under conditions of neurodegeneration (as opposed to atrophy); and (2) an effective and convenient means of delivering NGF to the target cells in the brain. The present studies were intended to address these issues. The neurotoxin AF64A (infused ICV, bilaterally) was used to induce degeneration of the basal forebrain cholinergic neurons. In this first study NGF was continuously infused into the lateral ventricles via an Alzet pump. The results demonstrated a reliable decrease in high affinity choline uptake (HACU) due to AF64A neurotoxicity and nearly complete protection via ICV NGF infusions. Thus, these data demonstrate that NGF is able to provide a protective and/or a compensatory response against neurotoxic-induced degeneration of the basal forebrain cholinergic system.

In a second study, we attempted to determine whether similar effects could be achieved through peripheral (IV) administration of NGF conjugated to the transferrin receptor antibody, OX26. This conjugate has been shown previously to transport NGF across the BBB. As in the first study, substantial degeneration of basal forebrain neurons was accomplished via bilateral ICV injections of AF64A. While no difference between HACU in NGF or vehicle-treated groups was observed (i.e., both showed significant decreases due to AF64A), HACU in rats administered the NGF/OX26 conjugate was increased by 50% compared to AF64A/vehicle-treated rats. However, immunocytochemistry directed against ChAT and the low affinity NGFr (p75) indicated that no increase in cell number was achieved with the NGF conjugate. Thus, under the conditions employed in this study, the NGF conjugate apparently increased HACU by enhancing the activity (and presumably the vitality and function) of surviving neurons while not necessarily protecting against the AF64A cytotoxicity, per se.

455.6

INTRAVENOUS ADMINISTRATION OF AN NGF CONJUGATE PREVENTS THE DEGENERATION OF CHOLINERGIC STRIATAL NEURONS IN A MODEL OF HUNTINGTON'S DISEASE V.Charles[1], P.M. Friden[2], R.T. Bartus[2], C. Anderson[3*], S. Putney[2], L.R. Walus[2], and J.H. Kordower[1]. [1]Dept. Neurological Sciences, Rush Presbyterian Med. Ctr., Chicago, IL 60612; [2]Alkermes Inc. , Cambridge Ma. 02139., [3]Dept. Anatomy and Cell Biology, Univ. Illinois School of Medicine, Chicago Ill. 60612.

We have previously demonstrated effects on CNS cholinergic neurons following intravenous (iv) administration of an NGF-anti-transferrin receptor antibody conjugate (OX26-NGF). The present study investigated whether iv infusions of the OX26-NGF conjugate could cross the blood brain barrier and protect cholinergic striatal neurons against excitotoxicity in a manner similar to that seen previously with central NGF. All rats received an intrastriatal infusion of quinolinic acid (120 nmol). Rats received iv injections of vehicle (n=9), a nonconjugated mixture of OX26 and NGF (n=8) or the OX26-NGF conjugate (n=12). Injections were made for two consecutive days prior to the lesion, the day of the lesion, and every other day thereafter for 14 days. The dose of NGF was 20 µg per injection for both the NGF conjugate and NGF mixture groups. Rats receiving the vehicle or the nonconjugated mixture of NGF and OX26 displayed similar reductions in striatal ChAT-immunoreactive neurons (38.7% & 42.8%). The loss was significantly attenuated in rats receiving the OX26-NGF conjugate (24%; $p<.01$). The protective effect was specific for cholinergic neurons since similar losses of somatostatin or diaphorase-containing striatal neurons was observed regardless of treatment. Neurochemical analyses from additional animals demonstrated that there was a dose-dependent increase in the amount of NGF delivered to the brain following a single iv injection of the OX26-NGF conjugate (1-20µg). These data demonstrate that systemic administration of a NGF-antibody conjugate can cross the blood-brain barrier and prevent the degeneration of central NGF responsive neurons.

455.7

NGF-TRANFERRIN FUSION PROTEIN RESCUES CHOLINERGIC MEDIAL SEPTUM NEURONS IN FIMBRIA-FORNIX TRANSECTED RATS E.-C. Park*, A.J. Schutz, P. Lynch, P. Jin, T. Lee, R.M. Starzyk, M. Percoskie, R. Dean, Y. Lee, D. Carriero, S. Abelleira, R. Carroll, R. Bartus and S. Putney. Alkermes, Inc., Cambridge, MA 02139

We have constructed plasmids that were used to express human nerve growth factor (NGF)-human transferrin fusion proteins in chinese hamster ovary (CHO) cells. To facilitate dimerization of NGF molecule, the hinge region from human IGG3 sequence was used to link the NGF moiety with the transferrin moiety in the fusion protein. The fusion protein present in tissue culture supernatant was purified using anti-NGF antibody affinity column chromatography to approximately 90% purity. When the N-terminus of the fusion protein was sequenced, it was identical to the mature human NGF protein sequence, showing that the fusion protein is processed correctly in CHO cells. The fusion protein migrated as a dimer in non-reducing condition as expected from molecules containing IGG3 hinge.

The fusion protein retained full NGF activity *in vitro* as shown by its ability to stimulate neurite formation in 6-24 cells, a derivative of PC12 cell line transfected with trkA (provided by D. Kaplan and L. Parada, NCI) and approximately 1/4- 1/2 of native transferrin binding affinity as measured by its ability to compete 125I-transferrin binding to purified soluble human transferrin receptor (cell line producing soluble human transferrin receptor was provided by S. Harrison, Harvard University).

To address whether the NGF-transferrin fusion protein retains NGF activity in vivo, we tested the fusion molecule in fimbria fornix transected rats. Rats that received the fusion protein icv at 300 ng NGF equivalent/day show significantly higher ChAT positive neurons in medial septum than those that received vehicle alone. This result confirms the *in vitro* 6-24 cell assay results and shows that the fusion protein retains NGF function *in vivo*.

Our ability to generate biologically active NGF molecule as a part of a fusion protein opens a new way to deliver NGF to specific target tissues by selecting an appropriate carrier molecule for a given target tissue. Furthermore, the approach can be applied to other neurotropic agents.

455.8

DIFFERENTIAL EXPRESSION OF P75 IN SEPTAL CHOLINERGIC NEURONS IN RESPONSE TO DIFFERENT TYPES OF DEGENERATIVE PERTURBATIONS. P.M. Friden*[1], S.M. Abelleira[1], R. Carroll[1], R.L. Dean[1], R.W. Stackman[2], J.H. Kordower[3], T.J. Walsh[2] and R.T. Bartus[1]. Alkermes, Inc., 64 Sidney Street, Cambridge, MA 02139[1], Dept. of Psych., Rutgers Univ., NJ[2] and Dept. Neurol. Sci., Rush Med. Ctr., IL[3].

The regulation and function of the low affinity NGF receptor (p75) are issues of current interest and controversy. To better understand the role of this receptor in basal forebrain cholinergic neurons, we analyzed tissue for changes in immunoreactivity to the p75 protein in two different models of neurodegeneration: AF64A toxicity and fimbria-fornix (FFX) transections. In the first model, 10 days following the bilateral administration of AF64A, we detected a significant reduction in the levels of hippocampal high-affinity choline uptake, as expected. Additionally, the number of septal choline acetyltransferase (ChAT)-immunoreactive neurons was reduced. However, enlarged fibers intensely immunoreactive for p75 were observed in the septum. These fibers were most prevalent in the area dorsal to the anterior commissure, were organized in a dorsal-ventral orientation and were never associated with an obvious cell body.

Brain sections from animals subjected to FFX lesions were characterized by a loss of acetylcholinesterase activity in the target tissue and both ChAT and p75-immunoreactive neurons in the septum. In contrast to the AF64A lesion, there was no evidence of thick $p75^{+}$ fibers as a result of the FFX lesion. While it remains unclear whether the presence of these p75-positive fibers represents an aborted regenerative response (i.e. sprouting) or evidence of a degenerative response (i.e. tombstone marker), it is likely that an understanding of this phenomenon may provide insight into the regulation and/or function of the p75 low affinity NGF receptor.

455.9

ADMINISTRATION OF DIFFERENT NEUROTROPHINS PRODUCES DISTINCT AS WELL AS OVERLAPPING CHANGES IN STRIATAL NEUROPEPTIDE MRNA EXPRESSION H. Sauer[1], S.J. Wiegand[2], V. Wong[2], R.M. Lindsay*[2], and A. Björklund[1]. Dept. of Medical Cell Research, Biskopsgatan 5, S-223 62 Lund, Sweden; [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

In order to determine whether administration of exogenous neurotrophins can modulate the expression of neurotransmitter-related genes in the striatum we injected the neurotrophins NGF, BDNF, NT-3 and NT-4/5, as well as vehicle alone, into the dopamine-depleted striatum of unilaterally 6-hydroxydopamine-lesioned rats at a dose of 4 µg/4µl/day for 8 consecutive days (n=5/group). The brains were processed for in situ hybridization histochemistry for preprotachykinin (PPT)-, preproenkephalin (PPE)-, GAP-43, choline acetyltransferase (ChAT)- and glutamic acid decarboxylase (GAD)-mRNA. Dissected nigral tissue samples were assayed for levels of substance P protein.

Vehicle-,NGF- and NT-3-injected animals displayed a 6-OHDA-lesioned-induced down-regulation of striatal PPT-mRNA to 56-57% of contralateral. This decrease was significantly attenuated in BDNF and NT-4/5-injected animals, which displayed on average 92% of contralateral. The lesion-induced overexpression of PPE mRNA amounted to 209% of contralateral in vehicle-177% in NGF-, 258% in BDNF-, 245% in NT-3-, and 251% in NT-4/5- injected animals. Injection of NT-4/5 caused a significant 94% increase in the expression of GAP-43 mRNA, while NGF, BDNF, and NT-3 caused a moderate and non-significant elevation of 39-44% of contralateral. Nigral levels of substance P, as determined by radioimmunoassay, were found to be unaffected by neurotrophin administration. Data on ChAT-and GAD-mRNA expression will be presented. These data suggest that neurotrophins can have neuromodulatory effects in the basal ganglia, and that it is possible to ameliorate some of the effects of striatal dopamine denervation in the continued absence of a dopaminergic innervation.

455.10

EXPRESSION OF BIOLOGICALLY ACTIVE NERVE GROWTH FACTOR (NGF) MEDIATED BY RECOMBINANT HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) GENE TRANSFER VECTORS.

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In order to study the effects of neurotrophic factors in chronic neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, we have constructed replication compromised and replication defective (mutant d120: DeLuca et. al., 1985, *J. Virol.* 56:558) Herpes simplex virus (HSV-1) gene transfer vectors that express the beta subunit of nerve growth factor (ß-NGF). A cassette containing the human cytomegalovirus immediate early promoter (HCMV-IEp) driving transcription of the murine ß-NGF cDNA was incorporated into the HSV-1 thymidine kinase (tk) gene locus. Acute infection of non-neuronal cells with the NGF vectors resulted in NGF specific transcript production as detected by Northern blot analysis. *In vivo* expression of the NGF transcript was observed by *in situ* hybridization two days following stereotactic injection of the expression vectors into the hippocampus of rat brains. These results suggest that the NGF vectors are transcriptionally active *in vitro* and *in vivo*. Cell culture supernatants displayed immunoreactive NGF protein production as detected by ELISA. This same culture supernatant also induced PC-12 cells to differentiate as observed by neurite sprouting. These two results indicate that the NGF vector infected cells produce and secrete mature, biologically active ß-NGF. We are currently testing the NGF vectors for the length and level of bioactive ß-NGF expression in the CNS *in vivo*. Thus, we are poised to use these vectors in studies of recovery from fimbria-fornix transections and excitotoxic lesioning of striatum in rat brain as models of chronic neurodegenerative conditions.

455.11

NGF MICROINJECTION INTO THE ROSTRO-DORSAL PONTINE RETICULAR FORMATION OF THE CAT INDUCES ACTIVE SLEEP. J. Yamuy*, F.R. Morales and M.H. Chase. *Department of Physiology, Department of Anatomy and Cell Biology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, 90024-1746.*

Neurons that produce neurotrophins and neurons which express neurotrophin receptors are present in pontine regions involved in the generation of active sleep (*Neuron*, 5: 511, 1990; *J. Neurosci.*, 8: 2312, 1986; *Neuroscience*, 34: 89, 1990). We were therefore interested in testing the hypothesis that neurotrophins enhance or induce active sleep.

Three adult cats were prepared for monitoring sleep and wakefulness and for drug microinjection into the brainstem. Microinjections of a solution of NGFβ (Sigma, 0.5-4 µg diluted in 1 µl of vehicle) or a solution of vehicle [1 µl of phosphate buffered saline (PBS) with 0.1-1% of bovine serum albumine (BSA, Sigma)] were delivered to the pontine reticular formation [(PRF) (P 3, L 2, H -3.5)].

NGF injections evoked active sleep with a mean latency of 10 min (range, 3.5 to 20 min.). The percentage of time spent in active sleep during the first hour following NGF injection was significantly greater than that following vehicle injection (44% ± 2.8, n=30 vs. 7.4% ± 2.0, n=16, respectively, means ± SEMs, $p<0.0002$); this increase was accompanied by a significant reduction in the percentage of time spent in wakefulness and quiet sleep.

This finding indicates that PRF neurons in areas involved in the generation of active sleep are responsive to NGF. The relatively short latency for the response to NGF indicates that the signal transduction mechanisms responsible for active sleep induction were other than the transcriptional processes that have been postulated for NGF effects, which have latencies of more than 30 minutes (*Ann. Rev. Neurosci.*, 14: 421, 1991). It is well established that injections of muscarinic agonists into the PRF induce an active sleep-like state (*Brain Res.*, 414: 245, 1987) and it has been recently reported that NGF, probably by acting on low-affinity receptors, excites putative cholinergic neurons (*Exp. Brain Res.*, 93: 226, 1993). The present data indicate that NGF induces changes in neuronal excitability and raise the intriguing possibility that neurotrophins are implicated in mechanisms of behavioral state control. This work was supported by USPHS Grants NS 23426 and MH 43362.

455.12

ASPHYXIA DURING BIRTH MAY PRIME THE CNS. Induction of a long-lasting increase in bFGF gene expression concomitantly with increased number of dopamine cell bodies in the substantia nigra.

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Asphyxia was induced during birth to male Sprague-Dawley rat pups. A quantitative analysis of the number of tyrosine hydroxylase immunoreactive (TH-IR) and thionin-stained nerve cell bodies as well as measurement of basic Fibroblast Growth Factor (bFGF) mRNA levels was carried out in the substantia nigra and the hippocampus on male rats at 3-4 weeks of age. Asphyxia produced an increase in bFGF mRNA levels and a concomitant increase in the number of dopamine nerve cell bodies of the substantia nigra. Asphyxia reduced the number of cell bodies in the CA1 and CA3 regions of the hippocampus while bFGF mRNA levels remained unchanged.

In conclusion, the asphyxia-induced long-lasting increase in bFGF gene expression may cause the increase in dopamine cell body number in the substantia nigra. The present results indicate that asphyxia during birth can prime the development of the central nervous system in a long-term perspective which may be of importantance in the development of neurodegenerative disorders later in life.

455.13

EVIDENCE FOR NOVEL NERVE GROWTH FACTOR (NGF)-RESPONDING NEURONAL POPULATIONS IN THE MAMMALIAN BRAIN. MH Cayouette[4], RR Sukhov[4], MJ Radeke[5], DL Price[1-4] and VE Koliatsos*[1-4]. Departments of [1]Pathology, [2]Neurology, and [3]Neuroscience, [4]Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; [5]Neuroscience Research Institute, University of California, Santa Barbara, CA 93106.

In the CNS, only striatal and basal forebrain cholinergic neurons have been shown to respond to NGF. Both of these populations are also known to express the high-affinity NGF receptor, trkA. This study was designed to delineate novel NGF-responsive neuronal populations in the rat brain. Using in situ hybridization histochemistry and RT-PCR, trkA mRNA was found in the nucleus prepositus hypoglossi and ventral cochlear nucleus. TrkA protein was also detected by immunocytochemistry in the nucleus prepositus and in the dorsal and ventral cochlear nuclei. In addition, trkA immunoreactivity was seen in the spinal vestibular nucleus, neurons within the medial longitudinal fasciculus, and the gigantocellular nucleus of the reticular formation. NGF mRNA was present throughout the vestibular and auditory systems with high levels of expression in the cerebellum. These results suggested that NGF might play a role in cerebellar afferent systems. Radiolabeled NGF injected into various sites within the cerebellar cortex was retrogradely transported to the nucleus prepositus and spinal vestibular nucleus. The trkA and NGF expression patterns and retrograde transport data together identify novel trophic targets for NGF in the brain and raise the possibility that NGF may be used as a therapeutic agent for diseases of the vestibular and auditory systems.

455.14

IMPLANTATION OF GENETICALLY-MODIFIED CELL LINES PRODUCING NEUROTROPHIC FACTORS REVEAL TROPHIC SUPPORT TO ADULT NORADRENERGIC NEURONS OF THE LOCUS COERULEUS IN VIVO. Ernest Arenas* Isabelle Neveu and Miles Trupp. Lab. of Molecular Neurobiology, Karolinska Institute, S-17177 Stockholm, Sweden.

Neurotrophins and the Glial cell line-derived neurotrophic factors (GDNF) are two families of trophic factors that support neuronal survival and/or regulate their phenotype. In order to investigate the role of neurotrophic factors *in vivo*, stable cell lines secreting high levels of recombinant neurotrophins or GDNF have been produced and transplanted into the brain. We have previously reported that NT3, but not any other neurotrophin produced by the cell lines, is able to promote the survival of the noradrenergic neurons of the locus coeruleus in a 6-hydroxydopamine lesion model that resembles the pattern of cell death found in Alzheimer's disease. It is now being examined whether GDNF may promote the survival of noradrenergic neurons *in vivo* and what receptors are involved in mediating the survival-promoting effects on locus coeruleus neurons. We are also studying whether these neurotrophic factors are able to modify the phenotype of central noradrenergic neurons *in vivo*, as shown by changes in the levels of the specific marker, tyrosine hydroxylase. Finally, since NT3 supported the survival of adult central noradrenergic neurons *in vivo* and it is expressed in their target fields, we are analyzing whether NT3 could act as a target derived neurotrophic factor for the neurons of the locus coeruleus. This question is being addressed by measuring the levels of expression of neurotrophic factors in the target tissues both after selective noradrenergic lesions and after noradrenergic activation.

455.15

COORDINATE EXPRESSION OF NEUROTROPHIN-3 AND TRK C LINKED TO A MORE FAVORABLE PROGNOSIS IN MEDULLOBLASTOMA. S.L. Pomeroy*, L.C. Goumnerova, Y.K. Kwon, C.D. Stiles, R.A. Segal. Depts. Neurol. and Neurosurg., Children's Hospital, Dept. Cell. and Mol. Biol., Dana-Farber Cancer Inst., Harvard Medical School, Boston, MA 02115.

We have examined the expression of neurotrophins in medulloblastoma, a malignant brain tumor believed to be derived from the external germinal layer of the cerebellum. Northern and western analyses were performed on tumor samples (n = 11) which were snap frozen in the operating room to preserve RNA and protein integrity.

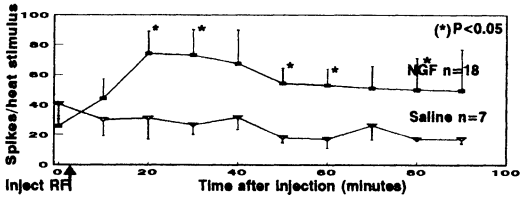
All of the tumors were found to express mRNA encoding neurotrophin-3 (NT-3) and its cognate receptor, trk C. Due to limited tissue, 3 tumor samples were tested and found to have NT-3 protein and Trk immunoreactivity. Patients with tumors expressing high levels of trk C mRNA had significantly longer intervals without disease progression than those with low levels (Kaplan-Meier, P = .02). Expression of trk A, trk B, or p75 was not associated with favorable disease outcome.

Coordinate expression of ligand and receptor implies the constitutive activation of Trk C receptors in patients with a more favorable outcome. Conceivably, this activation promotes the differentiation of medulloblastoma and may be relevant to the development of new therapeutic strategies for the tumor. At minimum, our findings indicate that trk C expression is a marker of prognostic value.

455.16

NERVE GROWTH FACTOR ACUTELY ENHANCES THE RESPONSIVENESS OF DORSAL HORN NEURONES TO NOXIOUS HEAT. N.Andreev*, M. Inuishin, and S.B. McMahon. Dept. Physiology, UMDS, London, SE1 7EH, UK.

The responses of single convergent dorsal horn neurones to a standard heat ramp (c.1 ^{o}C/sec to 52°C) were measured before and after a s.c. dose of human recombinant NGF (0.5 µg in 50 µl) or, as a control, 50 µl saline, into the centre of the neurone's receptive field (RF), in adult anaesthetised rats.



The rapid enhancement of dorsal horn neurone responsiveness, see figure, indicates that this dose of NGF can sensitise primary afferent nociceptors. Supported by the MRC and Royal Society.

455.17

NT-3 ATTENUATES REDUCTION IN H-REFLEX PRODUCED BY PYRIDOXINE TOXICITY TO LARGE PRIMARY AFFERENT NEURONS IN RATS. K.D. Cliffer*, M.E. Helgren, C.C. Cavnor, P.S. DiStefano, S. J. Wiegand, and R.M. Lindsay. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

High doses of pyridoxine (vitamin B6) are toxic to large primary afferent neurons, producing a peripheral neuropathy in experimental animals that can serve as a model for toxic large-fiber neuropathies in humans. Affected neurons are part of the circuitry that mediates the monosynaptic stretch reflex and a neurophysiological correlate, the H-reflex. Sprague-Dawley rats were treated with pyridoxine (800 mg/kg/d), with or without additional NT-3 treatment. When half the animals receiving either of these two treatments developed severe deficits on a precise locomotor task, all were tested electrophysiologically. Animals receiving NT-3 without pyridoxine and non-injected control animals were also tested. We recorded electromyographic activity in the plantar muscles, evoked by stimulation of the tibial nerve. Amplitudes of directly evoked motor (M-) and reflex-evoked (H-) waves were recorded. M-wave amplitudes did not differ among groups. H-reflexes (amplitudes normalized to the M-wave amplitude as H/M ratio) were absent or greatly diminished in nearly all animals receiving pyridoxine without NT-3. The reduction was significantly attenuated in animals receiving NT-3, resulting in substantial H-reflexes in nearly all animals so treated. H-reflex amplitudes were correlated with performance on the locomotor task. The results are consistent with protection by NT-3 of large sensory fibers from neurotoxic damage, potentially including damage caused by chemotherapeutic agents.

455.18

THE UPREGULATION OF NGF DURING INFLAMMATION PRODUCES PAIN HYPERSENSITIVITY AND AN INCREASED GROWTH CAPACITY IN PRIMARY SENSORY NEURONS. C.J. Woolf, B. Safieh-Garabedian, T. Leslie, J. Winter. SPON: Brain Research Association. Dept. of Anatomy, University College London, London WC1E 6BT, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN

The inflammation produced by an intraplantar injection of complete Freunds adjuvant results in a significant elevation in NGF levels, first in the inflamed tissues, then in the innervating nerves. This is accompanied by the development of substantial thermal and mechanical hyperalgesia, and an upregulation in DRG neurons of the mRNA neuropeptides substance P, and CGRP and the growth associated protein GAP-43. Systemic pretreatment with a neutralizing anti-NGF antibody which does not recognize BDNF or NT_3, prevents the development of the abnormal sensitivity and the changes in chemical phenotype, without reducing local edema or erythema. The production of increased levels of NGF in the periphery during inflammation may have a key role in generating inflammatory pain hypersensitivity by; increasing transduction sensitivity, amplifying the input to the dorsal horn, sensitizing dorsal horn neurons and promoting a hyperinnervation of the inflamed area by inducing collateral sprouting.

Supported by Sandoz and the Sir Jules Thorn Trust.

455.19

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) PRODUCES AN ANTI-DEPRESSANT LIKE EFFECT IN TWO ANIMAL MODELS OF DEPRESSION. Judith A. Siuciak*, Dacie Lewis, Stanley J. Wiegand and Ronald M. Lindsay, Regeneron Pharmaceuticals, Tarrytown, NY. 10591

We have previously reported that midbrain infusion of the BDNF, a member of the NGF/neurotrophin family, produced analgesia and increased serotonergic activity within the brain and spinal cord (Siuciak et al., 1994). Alterations in serotonergic function are involved in the pathogenesis and treatment of depression. The present study examined the ability of BDNF to produce antidepressant-like effects in two animal models of depression, the forced swim test and the learned helplessness paradigm. Adult rats were infused with BDNF (24 ug/day) or PBS vehicle into the midbrain, near the PAG and dorsal raphe, as previously described (Siuciak et al., 1994). In the forced swim test, BDNF infusion (day 5) produced a 70% decrease in immobility time in the 5 min post-test as compared to vehicle-infused controls (46.6 ± 16.8 vs. 155.5 ± 27.8 sec) In the learned helplessness model, rats pre-exposed to inescapable shock showed severe impairments in escape behavior during subsequent conditioned avoidance trials (51% decrease in the number of escapes, 5 fold increase in escape latency). Midbrain BDNF infusion reversed this escape deficit to levels similar to that obtained from non-shocked control rats. No changes in locomotor activity as measured by the total number of 60° partial rotations or grid activity was seen. These data demonstrate an anti-depressant-like action of midbrain-infused BDNF. Furthermore, these results suggest a possible role for neurotrophins in the etiology and treatment of neuropsychiatric disorders.

455.20

NT-3 ATTENUATES PROPRIOCEPTIVE DEFICITS IN THE ADULT RAT WITH A LARGE FIBER NEUROPATHY. M.E. Helgren*, K.Torrento, P.S. DiStefano, R. Curtis, R.M. Lindsay and S.J. Wiegand. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

The spatial and temporal pattern of expression of neurotrophins and their cognate receptors provide insight into the role of trophic factors in establishing the mammalian nervous system's structure-function relationship. For example, NT-3 and its receptor, TrkC, are prevalent in the proprioceptive system where connections of muscle and joint afferents with their central and peripheral targets are critical for feedback during movement. Early in development NT-3 and TrkC mRNA are expressed at high levels in skeletal muscle (peripheral target) and large (proprioceptive) DRG neurons, respectively. Both messages decrease in the adult suggesting that low levels are sufficient for maintaining homeostasis, but may be inadequate for providing support following injury. Pyridoxine (vitamin B6) is a selective neurotoxin for large DRG cells and at 800mg/kg/d for 8-14 days produces a distinct sensory-motor neuropathy in which axonal retrograde transport of ^{125}I-NT-3 to DRGs is greatly reduced, reflecting the loss of TrkC expressing sensory neurons. Using behavioral and morphometric analyses we examined the effect of NT-3 in this neuropathy model. We developed a quantitative assay to test proprioceptive function. Trained rats cross a 6' beam and a motor score is calculated based on paw placement: scale 1 (normal) - 4 (severely impaired). Rats receiving pyridoxine + NT-3 performed significantly better on this task than vehicle control rats. Taken together with cell counts and area measurements our results indicate that NT-3 can overcome the functional and anatomical deficits produced by pyridoxine neurotoxicity. These data may be relevant to the treatment of human large fiber peripheral neuropathies.

DEVELOPMENT OF THE AUDITORY AND VESTIBULAR SYSTEMS

456.1

DEVELOPMENT OF SOMATOSTATIN AND LEU-ENKEPHALIN IN THE AUDITORY BRAINSTEM OF THE RAT. M. Kungel* and E. Friauf Univ. Tübingen, Animal Physiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany.

During early ontogeny, the neuropeptides somatostatin and leu-enkephalin are prominent in several brain areas and are therefore thought to be involved in developmental events. This study was focused on the developmentally related expression of these peptides in the auditory brainstem of the rat by means of PAP-immunocytochemistry between the ages of embryonic day 17 and adulthood. During fetal development somatostatin immunoreactive (SIR) somata appeared in the nuclei of the lateral lemniscus (NLL), the cochlear nucleus complex (CN), and the inferior colliculus (IC), whereas SIR fibers were predominantly found in the superior olivary complex (SOC) and in the IC. In all these auditory brainstem nuclei, the immunoreactivity increased dramatically until postnatal day (P) 7. At this age the immunoreactivity in the brainstem was nearly restricted to auditory nuclei, which showed a heavy labeling. The most dense SIR fiber network was found in the SOC, which most probably originated from neurons of the CN, thus providing a system-immanent innervation. Subsequently SIR decreased dramatically until adulthood. In contrast to the transient expression of somatostatin, the number and labeling intensity of leu-enkephalin immunoreactive (LIR) structures increased progressively with age. LIR fibers in the fetal brain appeared in the SOC, NLL, CN and IC. LIR somata were found only after P12 in the CN and in the IC. Our results demonstrate a strong and transient expression of somatostatin and a progressive increase of the leu-enkephalin expression during development. Both neuropeptides are expressed during a period when synapse stabilization occurs in the rat auditory brainstem, suggesting that they may play a role in synaptic refinement. Supported by the DFG (Fr 772/1-3) and the GKN Tübingen.

456.2

DEVELOPMENT OF PRIMARY VESTIBULAR AFFERENTS AND VESTIBULOSPINAL PROJECTIONS IN THE OPOSSUM, *MONODELPHIS DOMESTICA*. J.-F. Pflieger* and T. Cabana. Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. centre-ville, Montréal, Canada, H3C 3J7.

The opossum *Monodelphis domestica* is born after only 14 gestational days. It is then very immature (eyes and ears closed, budlike hindlimbs, etc.) but has the capacity to locomote on the mother's belly with its forelimbs, from the genital aperture to a nipple where it attaches and stays until about postnatal day 28. This behavior of the newborn suggests that some sensory systems must be present to guide it. One such system may be the vestibular system, which is known to mediate sensory clues about linear and rotational head movements, and which has a strong influence on posture and balance during locomotion in adult mammals. We have looked at the development of the primary afferents to the vestibular nuclei and the vestibular projections to the spinal cord using the neuroanatomical tracing of DiI and WGA-HRP. We have found that VIIIth nerve fibers have attained the vestibular nuclear complex at birth and that some of the fibers project to the contralateral vestibular ganglion, a projection which was not found in the adult opossum. Projections from the lateral vestibular nucleus, and, to a much lesser extent, parts of the median and the inferior vestibular nuclei, to the brachial and lumbosacral enlargements of the spinal cord are also present on the day of birth. Without excluding the possibility of olfactory and/or tactile guidance for directing the "climbing" behavior of the newborn opossum, the vestibular system is likely involved.

456.3

POSTNATAL DEVELOPMENT OF THREE ISOFORMS OF PROTEIN KINASE C IN CENTRAL AUDITORY PATHWAYS OF THE RAT BRAIN. M.M. Garcia* and R.E. Harlan. Depts. of Otolaryngology and Anatomy and Neuroscience Training Program, Tulane University School of Medicine, New Orleans, LA 70112.

Although several protein kinase C (PKC) isoforms are highly expressed in brain, little is known about patterns of expression of PKC during development of the CNS. We used immunocytochemistry to study the ontogeny of PKC-βI,-βII and -γ in central auditory pathways of the rat brain, at postnatal (P) days 5, 11, 15, 20, and adult. With the βI antibody, labeled fibers were found in primary afferents of the VIIIth nerve in all ages. Weakly labeled terminals were found in the ventral cochlear nucleus (VCN) at P5 only; no terminals were found in the dorsal cochlear nucleus (DCN), suggesting that the labeled afferents are primarily vestibular. Labeled cells were found in the VCN at all ages, with a few labeled cells in the DCN in adults only. Labeled terminals were found in the superior olive (SO) beginning at P15. Cell body labeling was found in the SO and trapezoid body (TZ) beginning at P11. Labeled fibers were found in the lateral lemniscus (LL) at all ages, with labeled terminals on neurons of the nucleus of the LL (NLL) beginning at P20. Labeled terminals and a few labeled cells were found in the inferior colliculus (IC) at all ages, and in the medial geniculate (MG) at P20 and adult. With the βII antibody, a few labeled fibers were found in the VIIIth nerve as early as P11, corresponding to a few labeled terminals in the DCN beginning at the same age. Labeled cells were found consistently in the VCN beginning at P15. Cells decorated with labeled terminals were found in the SO beginning at P11. Cytoplasmic staining of SO cells was also noted beginning at P15. Staining of TZ cells was found beginning at P11. Decorated cells were found in the NLL and IC beginning at P11. Labeled cells and terminals were found in the IC and MG at most ages. With the γ antibody, labeled cells were first observed in the VCN and DCN at P11. Labeled terminals were found in the SO beginning at P11. Labeled terminals were found in the central core of the IC at all ages, and in the MG beginning at P15. These results suggest that these isoforms of protein kinase C may play roles in the development and function of central auditory pathways. (Supported by the Deafness Research Fnd.)

456.4

POSTNATAL DEVELOPMENT OF THREE ISOFORMS OF PROTEIN KINASE C IN CENTRAL VESTIBULAR PATHWAYS AND CEREBELLUM OF THE RAT. R.E. Harlan* and M.M. Garcia. Depts. of Anatomy and Otolaryngology and Neuroscience Training Program, Tulane Medical School, New Orleans, LA 70112.

The protein kinases C are a family of enzymes which are enriched in brain, yet little is known about their abundance during development of the CNS. We used Immunocytochemistry to study the ontogeny of the βI, βII and γ isoforms of protein kinase C in the vestibular system and cerebellum of the rat brain, at postnatal (P) days 5, 11, 15, 20, and adult. With the βI antibody, immunoreactivity (ir) was visible in primary afferents of the VIIIth cranial nerve at all ages. Labeled terminals were found in the medial vestibular nucleus (MeVN) from P11 through adult. Cell bodies in all vestibular nuclei were labeled at P5, but this labeling persisted only in the MeVN. Labeled cells were found in the deep cerebellar nuclei at all ages. Strongly-ir fibers were found in the white matter layer of the cerebellum, with development in the hemispheres proceeding faster than in the vermis. Large labeled cells, possibly Golgi neurons, were found in the internal granular layer at P5-P15. At P20 and adult, many granule cells were labeled. With the βII antibody, ir was not seen in the VIIIth nerve at any age, although some punctate ir was found in all vestibular and deep cerebellar nuclei from P5 to P20. Labeled cell bodies in all vestibular and deep cerebellar nuclei were seen at P20 and adult. In the cerebellum, strongly-ir cells were found in the external granular layer at P5, and to some extent at P11. Internal granular cells were labeled at P5, P11 and adult. Apparent labeled terminals were found on Purkinje cells beginning at P11. With the γ antibody, strongly-ir fibers were found innervating the vestibular and deep cerebellar nuclei at all ages. Strong ir was found in Purkinje cells, with projections to deep nuclei, at all ages. These results suggest that these isoforms of protein kinase C, especially the γ isoform, may play roles in the development and function of the vestibular system and cerebellum. (Supported by a grant from the Deafness Research Fnd [to MMG]).

456.5

FIBER OUTGROWTH AND PATHFINDING IN THE DEVELOPING AUDITORY BRAINSTEM M. Niblock[2], J.K. Brunso-Bechtold*[1,2], and C.K. Henkel[1,2]. Dept. of Neurobiology and Anatomy[1] and Program in Neuroscience[2], Bowman Gray Medical School, Wake Forest Univ., Winston-Salem, NC 27157

The trapezoid body decussation carries information that is essential to the localization of sound by the mammalian auditory system. The trapezoid body decussation consists of fibers from the ventral cochlear nucleus that terminate in the contralateral superior olivary complex. The purpose of this study was to establish the developmental time course of the decussation and to examine the relationship of the fibers and their growth cones to potential cues during pathfinding. The carbocyanine dye DiI was placed in the area of the presumptive cochlear nucleus in rats aged E13, E15, and E17 in order to label the efferent fibers and their growth cones. At E13, although cochlear nucleus neurons were just beginning to migrate away from the rhombic lip ventricular zone, they were already sending axons along the marginal edge of the hindbrain towards the midline. By E15, many fibers had entered the midline region and some appeared to contact glial cells at the midline. In addition, by this time some of the fibers had crossed the midline and had reached the vicinity of the presumptive superior olivary complex on the contralateral side. At E17, the presumptive cochlear nucleus could be clearly distinguished on the lateral surface of the hindbrain and there was a noticeable increase in the number of fibers in the trapezoid body decussation. Based on growth cone morphology and axon trajectory, it does not appear that all trapezoid body fibers depend on the basal lamina for guidance during pathfinding. In addition, growth cone complexity did not show any consistent increase in the floorplate region. Growth cone contact of glial cells at the midline is consistent with these cells playing a role in directing fibers across the midline to their targets. Supported by DC00335

456.6

A TRANSIENT DEVELOPMENTAL GRADIENT OF GLYCINERGIC ENDINGS IN THE FERRET SUPERIOR OLIVARY NUCLEI. C.K. Henkel* and J. K. Brunso-Bechtold, Department of Neurobiology and Anatomy, Neuroscience Program, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27157.

The development of inhibitory connections from the medial nucleus of the trapezoid body (MNTB) to the lateral (LSO) and medial (MSO) superior olivary nuclei was investigated in ferrets using immunocytochemistry with antibody for glycine (courtesy of Dr. R. Wenthold,). We were particularly interested in whether or not the glycinergic inputs develop along a tonotopic gradient. Videoimages of LSO and MSO in selected immunostained sections at 7 postnatal ages were digitized. Samples of the neuropil were analyzed and averaged to determine the relative density of glycine immunostaining. Only a diffuse background matrix is immunostained in the neuropil at postnatal day 7, but by the end of the second postnatal week immunostaining in the neuropil is more granular. Between the third and fourth postnatal weeks, the neuropil has the characteristic puncta of immunostained endings in the adult. A gradient is present across the tonotopic axis of both nuclei during the earliest period of diffuse immunostaining. At this time, there is nearly a two-fold increase in density of immunostaining from low to high frequency regions. By postnatal day 28 when puncta are evident, glycine immunostaining is homogeneous all along the tonotopic axis. Glycine-immunopositive cells in the MNTB appear progressively along a spatial gradient during the first postnatal month as this maturation of glycinergic terminals in LSO and MSO is taking place.

Supported in part by NIH grant DC00335.

456.7

FREQUENCY-DEPENDENT ADJUSTMENT OF BINAURAL TUNING PROPERTIES IN THE BARN OWL'S OPTIC TECTUM AS A RESULT OF ALTERED AUDITORY EXPERIENCE. J. I. Gold* and E. I. Knudsen. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Barn owls localize sounds by combining auditory cues, including interaural differences in timing (ITD) and level (ILD), which are computed across a broad range of frequencies. The auditory space map in the optic tectum (OT) contains neurons responsive to broadband noise stimuli from restricted receptive fields. Previous studies have shown that, in the OT, neuronal tuning to ITD and ILD for narrowband noise stimuli reflects the frequency-dependence of the cues corresponding to the unit's receptive field. We examined the extent to which such frequency-dependent tuning is shaped by experience by testing the ability of neurons in the OT to adjust to novel, frequency-dependent localization cues.

An ear plug-like device ("twisted tube") was developed and shown via cochlear microphonic and probe tube microphone measurements to affect the timing and level of sounds in a frequency-dependent manner. Juvenile owls were raised for 60 d with a twisted tube in one ear. The resulting adjustments of tectal ITD and ILD tuning were assayed in two ways. First, neuronal tuning to dichotically-presented, narrowband stimuli was characterized at a variety of sites. The frequency-dependence of both ITD and ILD tuning was shown to reflect the frequency-dependent changes in acoustic cues caused by the twisted tube. Second, with the twisted tube removed, the auditory receptive fields measured with narrowband stimuli of different frequencies were shown to be misaligned in both azimuth and elevation, corresponding to the frequency-dependent changes in ITD and ILD tuning, respectively. Re-insertion of the device realigned the receptive fields, thereby demonstrating the compensatory nature of the adaptive adjustments. Thus, experience-dependent plasticity shapes the frequency-dependence of unit tuning to ITD and ILD of neurons in the barn owl's optic tectum.

Supported by NIH: 5T32 MH17047 and R01 DC00155-14.

456.8

TOPOGRAPHY OF THE PROJECTION FROM THE CENTRAL TO THE EXTERNAL NUCLEUS OF THE INFERIOR COLLICULUS IN NORMAL AND PRISM-REARED BARN OWLS. DE Feldman* and EI Knudsen. Dept. of Neurobiology, Stanford University, Stanford CA 94305-5401.

The central nucleus of the inferior colliculus (ICc) contains neurons sensitive to interaural timing difference (ITD), the primary cue used by the barn owl to localize sounds in azimuth. ICc neurons are organized into a map of ITD which is relayed to the external nucleus of the inferior colliculus (ICx) via a topographic projection from ICc to ICx. Rearing owls with laterally displacing prismatic spectacles systematically alters ITD tuning in the ICx but not in the ICc, suggesting that the projection from ICc to ICx is altered by prism-rearing. This hypothesis was tested by making small injections of biotinylated dextran amine (BDA) at known locations in the ICx of normal and prism-reared owls. Retrogradely labeled ICc neurons were visualized by avidin-biotin-peroxidase histochemistry. The positions of labeled somata were measured along the rostrocaudal axis of the ICc (the axis of varying ITD), relative to calbindin immunostaining of the ICc core.

Injections (n=4) made at the representation of 0 μsec ITD in the ICx of normal owls labeled ICc neurons (n=149) in the rostral one-third of the lateral shell of the ICc, consistent with the known projection from the ICc to the ICx. Injections (n=2) made in the ICx of prism-reared owls at the same anatomical location, which in these birds was tuned to 40-50 μsec contra-ear leading ITD, labeled ICc neurons (n=243) in a significantly different distribution. About half of the labeled neurons were situated caudally in the lateral shell at locations representing ITDs corresponding to the prismatically induced shift in the auditory map. The remaining neurons were situated in the rostral third of the lateral shell, as in normal birds. These preliminary data suggest that the adaptive alteration in ITD tuning observed in the ICx of prism-reared owls results at least in part from an alteration in the projection from ICc to ICx.

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456.9

RETINAL PROJECTIONS INDUCED INTO AUDITORY THALAMUS IN FERRETS: DIFFERENTIAL TERMINAL DISTRIBUTION AND EYE-SPECIFIC ZONES. A. Angelucci*, F. Clascá and M. Sur. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Retinal axons can be induced to innervate the auditory thalamus followin neonatal lesions in ferrets. We have now shown that induction of retinal projections into the medial geniculate nucleus (MGN) critically depends on extensive ablation of its ascending inputs including those from both inferior colliculi, as well as from other auditory brainstem nuclei..

To examine the pattern of retinal innervation to the MGN, adult "rewired ferrets received binocular injections of cholera toxin B subunit (CTB). The procedure stains axons and terminals in detail, thus allowing reconstruction of single axons. Retinal fibers can invade all the subdivisions of the MGN, but consistently avoid its caudal part. The ventral and the medial divisions are the most densely innervated. In general, axons innervating the ventral division enter the MGN directly from the optic tract. These axons are thick (2-4 μm diameter), are more highly branched, and have large clustered boutons. Retinal axons innervating the medial division enter the MGN through the lateral posterior nucleus (LP), the pretectal nuclei (PT) and the lateral terminal nucleus. They are thin (0.3-0.6 μm diameter), have smaller boutons and sparser terminal arborizations. Axons entering the MGN through the LP and PT can also arborize in the dorsal division and in the posterior nucleus (Po).

By injecting WGA-HRP into one eye and CTB into the other, we find that both eyes project to the same areas of MGN and the LP, the ipsilateral projection being less dense. The patches of projections formed by one eye are adjacent to, but clearly segregated, from those formed by the other eye. In LP, the projection from each eye appears band-like. These data suggest that while the site of retinal arborization into the MGN depends on factors intrinsic to the target, retinal axons play a significant role in forming specific patterns of terminal distribution.

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456.10

CHANGES OF AUDITORY EVOKED POTENTIALS IN RESPONSE TO BEHAVIORALLY MEANINGFUL TONES INDUCED BY ACUTE ETHANOL INTAKE IN ALTRICIAL NESTLINGS AT THE STAGE OF FORMATION OF NATURAL BEHAVIOR. L.I.Alexandrov, Y.I.Alexandrov* Inst. of Higher Nervous Activity & Neurophysiology, and Inst. of Psychology, Russian Acad. of Sci. Moscow, Russia.

Acute etanol's influence on fild L auditory evoked potentials (AEP) was studied in 4-7-day-old altricial nestlings of the pied flycatcher. Nestlings were presented with behaviorally meaningful tone pips (2.0 and 5.0 kHz) and the control tone pips (3.0 kHz). Ethanol ingestion was found to reduce the N1 amplitude and maturity index (MI) of the AEP in response to "behavioral" but not to control frequencies. This effect was first observed on day 5, when the nestlings' behavior became more complex: their eyes opened and defence behavior appeared, and when previously formed feeding behavior was undergoing modifications. The MI increase during the early postembryonic ontogeny was probably due to the selsctive involvement of neurons with newly formed behavioral specializations into the subserving of new behavioral patterns while the decrease of MI under alcohol was due to the depression of activity in these neurones.

456.11

SYNAPTOPHYSIN EXPRESSION IN THE DEVELOPING INNER EAR OF THE CHICK. A. M. Cunningham* and B. H. A. Sokolowski, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia and University of South Florida, Tampa, FL 33612.

Synaptophysin is the major integral membrane protein of presynaptic vesicles and is a useful marker for synaptic terminals. The glycoprotein may be involved in presynaptic functions such as vesicle-membrane fusion and has been implicated as having a specific role in neurotransmitter release [Adler et al., 1992]. The expression of synaptophysin was examined during synaptogenesis using a monoclonal antibody (SBI 20.10) which recognises the avian homologue of synaptophysin [Cunningham & Jeffrey, 1990, Soc. Neurosci. Abst. 16, 1291].

We examined the developing inner ear of the chick from embryonic day (ED) 6 to posthatch using an immunoperoxidase technique. In the cochlea, synaptophysin immunoreactivity was detected as early as ED 8 in the proximal region of the sensory epithelium. In this area, immunoreactivity was restricted to the regions containing the tall and intermediate hair cells. As development progressed, by ED 10, immunoreactivity also appeared in the distal regions of the cochlea, consistent with later maturation of this region. In the vestibular endorgans, the saccule, utricle and cristae ampullares, immunoreactivity was observed at ED 14, the earliest age studied to date.

Previous studies in mammals have suggested that synaptophysin is expressed primarily in the nerve fibres innervating the cochlea as well as the afferents of the peripheral vestibular system [Gil-Loyzaga & Pujol, 1988; Scafone et al., 1991; Nadol et al., 1993]. Our studies in developing cochlea show a pattern of synaptophysin immunoreactivity concentrated in areas which primarily receive afferent innervation and would be consistent with localization to the base of the hair cells. ImmunoEM studies are needed to verify these findings and to determine the relationship between the appearance of synaptophysin immunoreactivity and the events of synapse formation and maturation. *Supported by grants from The Medical Foundation, Uni. of Syd. to AMC & NIH/NIDCD R29 DC01923-01 to BHAS.*

456.12

DEVELOPMENTAL PLASTICITY OF THE PROJECTION FROM THE MEDIAL SUPERIOR OLIVE TO THE INFERIOR COLLICULUS IN THE RAT. S. Okoyama, T. Moriizumi, Y. Kitao, J. Kawano and M. Kudo*, Dept. of Anatomy, Sch. of Med., Kanazawa Univ., Kanazawa 920, Japan.

In one set of experiments, normal development of projection from the medial superior olive (MSO) to the inferior colliculus (IC) was examined by injecting Fluoro-Gold into the IC unilaterally at postnatal days 0 (P0), 3 (P3), 7 (P7) and adult. They were killed 1 day after injections. Retrogradely labeled neurons in the MSO appeared on the ipsilateral side only in any cases. The labeled frequency of MSO neurons was increased stepwise (from 35% to 90%) with postnatal stages, suggesting differential growth of early- and late-developing axons.

In another set of experiments, cell counting in the MSO was performed in rats, of which IC had been unilaterally ablated between P0 and maturity. Upon reaching adulthood, the rats received injections of Fluoro-Ruby into the contralateral IC so that aberrant crossed projections to the intact IC could be examined. These rats were sacrificed 2 days after injections.

1)No exuberant projections to the contralateral IC are found in the normal development. 2)When the IC is ablated unilaterally, many neurons die in the ipsilateral MSO as a result of axotomy. 3)An aberrant crossed projection occurs in a few of the survived neurons only in the P3 ablation cases. 4)Growth of late-developing axons is a major factor of plasticity in this system.

456.13

POSSIBLE ROLE OF VISUAL INPUTS TO THE SUPERFICIAL LAYERS OF THE SUPERIOR COLLICULUS IN THE DEVELOPMENT OF THE MAP OF AUDITORY SPACE. A.J. King*, J.W.H. Schnupp and I.D. Thompson. Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK.

Visual signals appear to play an instructive role in the development of the map of auditory space in the deeper layers of the superior colliculus (SC), although the site at which this visual-auditory interaction occurs has yet to be identified. We have examined the contribution to the development of the auditory space map of the visual representation in the overlying superficial layers of the SC in the ferret.

The caudal region of the superficial layers of the right SC was removed by aspiration on the day of birth. Recordings were made bilaterally when the animals were fully grown. On the left, unoperated side, both the visual representation in the superficial layers and the auditory representation in the deeper layers appeared to be normal. On the right side, the visual responses recorded from units in the remaining superficial layers in rostral SC had normal receptive field locations that covered a restricted region of anterior visual space. Auditory units recorded in the deeper layers of the same electrode tracks were tuned to corresponding locations, suggesting that the auditory representation in this part of the SC was unaltered. However, the preferred sound directions of auditory units recorded in more caudal regions of the SC, where the superficial layers were missing, were much more scattered, even though many of these units were also visually responsive.

These data suggest that visual responses in the superficial layers of the SC may contribute to the development of the auditory topography in the deeper layers.

456.14

CHANGES IN OTOLITHIC FUNCTION IN INFANTS LEARNING TO WALK DEMONSTRATED WITH OFF VERTICAL AXIS ROTATION (OVAR). S. R. Wiener-Vacher*, B. Bril, A. Ledebt ,Study Center of Postural and Motor Control in Children, ORL Department, Robert Debre Pediatric Hospital, Paris, 75019, France.

We applied vestibular otolithic stimulation by off vertical axis rotation in normal children aged from 7 months to 3 years. Our apparatus permits rotational stimulation at variable angular accelerations as well as at constant angular velocity around the vertical axis or at an inclination up to 15 deg with respect to vertical. Children are seated on the lap of their mother and the axis of the chair is adjustable so that child's cephalic axis is in the axis of the rotation. The test is performed in total darkness. VOR is recorded with skin ENG electrodes. We recorded the horizontal and vertical components of canalar and otolithic VOR. We found that 13 deg of tilt for the off vertical rotation gave an optimal signal/noise ratio for otolithic VOR, without altering tolerance to the test.

On a group of 30 normal children aged from 7 months to 3 y.o. we found a clear change in the characteristics of the otolithic VOR at the onset of independent walking while canalar VOR did not change during this same period. The modulation in amplitude of the slow phase velocity of the otolithic VOR increased when the standing position and the first independent steps were obtained.

Preliminary results of a longitudinal study conducted on 5 children since the age of 7 months confirm these findings. Every month we record the canalar and otolithic VOR as well as the parameters of posture and locomotion by means of a 3D video system synchronized with gait data recorded with a large force plate. This study demonstrates that the evolution of the otolithic VOR parallels that of the control of equilibrium during the first phase of independent walking as described by Brenière Y & Bril B (1989). The otolithic information may be of greater importance than canalar information at this period when the child must learn how to control disequilibrium situations in the erect position.

DEVELOPMENT OF VISUAL CORTEX III

457.1

Effect of neonatal ablation of layer 1 on the development of corticocortical projections in the cat's visual cortex. D. Car ić* and D. J. Price, Dept. of Physiology, Univ. Med. Sch., Edinburgh, EH8 9AG, U.K.

We are interested in factors that control the formation of visual corticocortical connections from area 17 to area 18 and the reciprocal projections to area 17. We examined the role of layer 1 in these processes. From the earliest postnatal ages, apical dendrites of pyramidal corticocortical cells project to layer 1, where they connect with long horizontal fibres that cross several cortical areas.

We lesioned layer 1 in area 18 in newborn kittens, let them develop to 1 month of age and examined cortical projections after labelling them with carbocyanine dyes (DiI and DiA) in fixed brains. In normal kittens, by the end of the first postnatal month, fibres projecting from area 17 to area 18 terminate in patches in the cortex of area 18 and cells projecting back from area 18 are grouped in clusters, mainly in superficial layers with a few in deep layers. In lesioned animals, very few projections interconnected areas 17 and 18 although in Nissl-stained sections the cortex appeared normal below the lesions in layer 1. Thus, the lesions produced a disproportionate loss of corticocortical connections, that is not easily explained on the basis of generalised cortical damage. We postulate that intercellular signaling via layer 1 is important for the development of corticocortical connections in the visual cortex.

457.2

DEVELOPMENT OF LONG-RANGE HORIZONTAL CONNECTIONS IN FERRET PRIMARY VISUAL CORTEX Edward S. Ruthazer* and Michael P. Stryker W.M. Keck Center for Integrative Neuroscience and Neuroscience Program, UCSF, San Francisco, CA 94143-0444.

The specificity of long-range tangential connections emerges during development from initially diffuse connections. In the cat, manipulation of visual experience influences only the late portion of this refinement. To examine the activity-dependence of the early component we have studied the ferret.

Focal injections of either the B subunit of cholera toxin (CTB) or CTB-gold conjugate were made into area 17 of ferrets ranging in age from PND 20 to adulthood. Following two to four days post-injection survival, animals were perfused and their occipital cortex flattened for sectioning parallel to the pial surface. In all but the youngest ferrets studied (PND 27 and older), small injections of CTB into superficial cortex resulted in clusters of retrogradely labeled neurons, the axons of which can be traced back to the injection site, and also in many cases to nearby sites of dense axonal arborization. These areas rich in labeled axons coincided generally, but not always, with clusters of labeled cells. At all ages studied, several clusters of labeled cells were also found in areas 18, 19, and suprasylvian cortex.

In adult animals, the clusters in area 17 were most prominent in layer III where they formed discrete groups of cells, generally within 3 mm of the injection site, separated by regions of relatively low density of labelling. In an animal injected on PND 20 a halo of labeled axons radiating from the injection site was present at all depths, however labeled somata outside of the injection site were almost exclusively found in layer VI and the underlying white matter. Supragranular labeled somata were first seen following injection on PND 24, but the earliest discrete clusters of labeled somata were observed following injection on PND 29. The degree of clustering of labeled somata appeared greater with increasing injection age studied. By PND 46 the adult-like degree of clustering was still not attained.

Activity blockade by intracortical TTX infusion from PND 23 until sacrifice two weeks later appeared to disrupt normal cluster formation in area 17.

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457.3

DEVELOPMENT OF CLUSTERED LATERAL CONNECTIVITY IN MACAQUE VISUAL CORTEX. T. Yoshioka*. Krieger Mind/Brain Institute, Johns Hopkins University, Baltimore, MD 21218

The clustered lateral connection network in the visual cortex is thought to be a substrate for the parallel information processing underlying visual perception, and is mainly present in layers 2-3 and 5. To examine the development of this cortical wiring, and to identify whether the visual experience influences cluster formation, the anatomical organization of striate cortex (area V1) in the fetal and infant macaques was studied using DiI as a tracer. Following the placement of DiI crystals at various depths of gray matter, lateral spread of axons and densely branched dendrites of pyramidal neurons were seen in both supra- and infragranular layers at E140days, approximately 3 weeks before birth. In the supragranular layer, labeled axon terminals and somata formed clusters, whereas, in the infragranular layer, a continuous lateral spread of labeled axons was visible without noticeable clusters. These data suggest that the patches seen in layer 5 of adult V1 develop by elimination of axonal branches in later stage. Cytochrome oxidase (CO) histochemistry revealed no evidence of stained blobs despite the presence of clustered terminal patches in supragranular layers. From these observations, it was concluded that the visual experience is not necessary to form clustered horizontal connections in macaque V1, and that CO blobs do not guide axons to the proper termination site of intrinsic neurons, but rather blobs are expressed after the time of the formation of patchy connection system. (Supported by NIH grant EY06432).

457.4

DEVELOPMENT OF HORIZONTAL PROJECTIONS IN LAYER 2/3 OF FERRET VISUAL CORTEX. J. C. Durack and L.C. Katz.* Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Pyramidal cells in cat striate cortex extend long axons within layer 2/3 that form clustered projections linking iso-orientation columns (Gilbert and Wiesel, *J. Neurosci.* 1989, 9:2432). We examined the sequence of events that occur during the formation of these projections in the ferret to determine 1) whether horizontal projections exhibit similar patterns of development in the ferret and the cat; 2) whether selective axon elaboration and refinement play a role in establishing column-to-column connectivity and 3) the time course of horizontal projection formation relative to the onset of patterned visual experience and orientation selectivity. Extracellular injections of biocytin in tangential slices of layer 2/3 visual cortex were used to label axon collaterals of single neurons and small groups of cells. At the first appearance of axon collaterals in layer 2/3, around postnatal day 22, (P22) pyramidal cell axons are uniformly distributed and unbranched up to 1mm from the cell body. By P26, axons begin to form secondary branches 1-2mm from the cell body, with little evidence for clusters. The first evidence of selective elaboration of secondary branches and retraction of unbranched collaterals occurs around P28. By P34, patchy regions of axon branches emerge, although unbranched collaterals are still present. Distinct, adult-like clusters are observed by P45. Since eye opening in ferrets occurs between postnatal days 30 and 32, this system of orientation specific patches begins to develop in the absence of patterned visual input. The first signs of clustered connections are in fact apparent when most cortical cells are not yet orientation selective (Chapman and Stryker, *J. Neurosci.* 1993, *13*:5251). Although the general pattern of horizontal projection formation closely resembles that seen in the cat (Callaway and Katz, *J. Neurosci.* 1990, *10*:1134), the ferret circuitry matures earlier than the cat's relative to the time of eye opening. Supported by NIH grant EY07690 (L.C.K.) and Howard Hughes Undergraduate Research Forum grant (J.C.D.).

457.5

SYSTEMIC YOHIMBINE PREVENTS THE EXUBERANT CALLOSAL CELL DISTRIBUTION IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING MONOCULAR ENUCLEATION. A. M. Grigonis,*[1] Yuechu Wang,[2] and E. H. Murphy.[2] [1]Dept. of Anatomy, Hahnemann Univ., Philadelphia, PA 19102-1192, and [2]Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

We have previously shown that, following neonatal monocular enucleation (ME), the adult callosal cell (CC) distribution is exuberant, extending into areas of visual cortex which do not normally contain callosal cells, similar to the CC distribution in the neonate. We have also shown that levels of noradrenaline (NA) influence the normal development of the CC distribution. There is a decreased tangential extent of the CC distribution in the adult rabbit visual cortex following administration of an alpha-2 receptor antagonist, yohimbine, during the critical period. We presently examine the effects of yohimbine administration on the CC distribution following neonatal ME. Rabbits (N=4) were deeply anesthetized and had ME on the day of birth. Each animal received injections of yohimbine HCl (2.5 mg/kg, IP) every day from postnatal day 5 through 12. A control group (N=3) had ME and received equal volumes of IP injections of saline. Rabbits were raised until adult, at which time multiple injections of HRP (Boehringer, 20% in H_2O) were made (total volume injected: 7 µl) throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. ME plus saline animals had a significantly increased tangential extent of the CC distribution, similar to previous results. ME plus yohimbine animals had a restricted tangential extent of the CC distribution in area 17 similar to the normal adult pattern. Yohimbine administration during the critical period prevents ME induced CC exuberance. Results indicate that NA influences developmental plasticity of visual callosal cells.
Supported by NIH-NS26989 and DA06871.

457.6

QUANTITATIVE ANALYSIS OF THE VISUAL CALLOSAL PATTERN IN NORMAL AND MONOCULARLY ENUCLEATED RATS
C.A. Gilson[1], C.S. Hernit-Grant[2], B. Chou[1], S.A. Langer[1], K.M. Murphy[3] & R.C. Van Sluyters*[1]. School of Optometry[1], University of California, Berkeley, CA 94720, Department of Neurology[2], Harvard Medical School, Boston, MA 02115 and Department of Psychology[3], McGill University, Montreal PQ H3A 1B1.

Previously we reported that in neonatal rats the pattern of visual callosal cells resembles a dense, compact version of the mature pattern. The present study extends this analysis to include quantitative data on the callosal cell distribution in normally reared animals and in animals monocularly enucleated (ME) on PND 1. Pups, aged 6 to 21 days, received multiple injections of retrograde fluorescent tracer (Fluorogold or green latex beads) into the occipital cortex of one hemisphere and the density of labelled cells in the supragranular layers of the opposite hemisphere were analyzed at various survival times (2 to17 days).

In neonatal animals, labelled cells appear to be more densely aggregated near the 17/18a border than in the body of area 17, and this difference becomes more pronounced with age. However, the present data indicate that the ratio between the callosal cell density in the body of area 17 to that in the 17/18a border region actually does not change over the first 21 postnatal days. Thus, the "sharpening" of the callosal labeling pattern with age results from a relative decrease in the intensity of labeling for callosal cells in the body of area 17, as opposed to a selective decrease in their overall number. Similarly, the density of cells in the "extra band" of callosal neurons that runs rostrocaudally through the center of area 17 in ME animals actually is no greater than that found in adjacent regions. Once again, it is the intensity with which these callosal cells are labeled, and not their overall number, that is increased. The present data indicate that postnatal maturation of the callosal cell distribution occurs over a longer time course than previously thought, and they argue against a topographically selective withdrawal of immature callosal axons as the mechanism for producing a mature visual callosal pattern.

457.7

DEVELOPMENTAL CHANGES IN DENDRITIC ARBORS OF RAT VISUAL CORPUS CALLLOSUM CELLS OF ORIGIN: A CONFOCAL LASER SCANNING MICROSCOPE (CLSM) ANALYSIS. A.J. Elberger* and D.L. Dodson. Dept. of Anatomy & Neurobiology, University of Tennessee, Memphis TN 38163.

The carbocyanine dye DiI placed in rat corpus callosum (CC) labels many transitory CC axons extending throughout visual cortex, which gradually disappear during postnatal month (PNM) 1 (Elberger, 1994). At least some of these transitory CC projections form synapses, providing an opportunity for the CC to influence visual cortical development (Elberger et al., 1992). To determine if other features of the callosum display transitory components, the present study examined the morphology of dendritic arbors of CC cells in rat visual cortex. Multiple crystals of either DiI or another carbocyanine dye, DiA, were placed in the corpus callosum of sagittally-bisected, aldehyde-fixed rat brains of various ages during PNM 1. Tissue was sectioned after 5 to 12 months, depending upon the age of the animal.

The cell body and dendritic arbor of individual CC cells in vibratomed coronal sections were imaged using CLSM. Cells were randomly selected from all layers of cortex and cells with a partially sectioned apical dendrite were excluded. The length and complexity of the apical dendritic arbor in areas 17 and 18a were morphologically analyzed. In addition, cell body size was measured as well. The length and complexity of the dendritic arbor changed with age. Just as the CC axon was shown to undergo major changes during PNM 1, the CC apical dendritic arbor and cell body size also underwent major changes. The elimination of portions of the apical dendritic arbor with age indicates that not only the CC axon but the CC dendrite has transitory components, the function of which is as yet unknown. Supported by NIH grants EY08466 (AJE), NS07323 (STK), and The University of Tennessee Neuroscience Center of Excellence.

457.8

NEUROCHEMICAL HETEROGENEITY OF DEVELOPING CORPUS CALLOSUM PROJECTIONS IN THE RAT AND CAT. S.-L. Ding* and A.J. Elberger. Dept. Anatomy & Neurobiology, Univ. of Tennessee, Memphis, TN 38163.

Corpus Callosum (CC) projections in adult mammals are often reported to be excitatory and to use glutamate and/or aspartate as their transmitters. However, neurochemicals used by developing CC projections are not well known. The present study investigates, using immunocytochemistry (ICC), several presumed neurochemicls used by developing CC axons in rats ranging in age from newborn to adult and in neonatal cats. The cells of origin of CC axons were examined using a combination method of DiI retrograde tracing and ICC with epifluorescence and confocal laser scanning microscopy.

In rats during postnatal week (PNW) 1, a large and subsequently increasing number of neuropeptide Y (NPY)-immunoreactive (ir) axons was found in the CC bundle, some of which displayed growth cones. The peak number of NPY-ir CC axons was reached during PNW 2. By the end of PNW 3, the number of NPY-ir CC axons had decreased to adult level, i.e., only a few NPY-ir CC axons were observed. During PNW 1-2 many somatostatin (SS)-ir CC axons were seen; much fewer SS-ir CC axons were present at PNW 3 - adult. Some GABA-ir and a few VIP-ir CC axons were found during PNW 1. In neonatal cat, a small number of SS-ir and GABA-ir CC axons was also observed in addition to many NPY-ir CC axons described previously (Ding and Elberger, 1992,1994). Double labeling study in both species showed the existence of NPY-ir and SS-ir CC cell bodies in cerebral cortex. The present results indicate that multiple neurochemicals exist in developing CC projections and most of them are transiently expressed. These results also suggest that developing CC projections may be inhibitory or modulatory in nature, in addition to excitatory as generally thought. Supported by NIH grant EY08466 (AJE) and Univ. of Tennessee Neuroscience Center of Excellence.

457.9

CHANGES IN CYTOCHROME OXIDASE-RICH PATCHES IN STRIATE CORTEX OF HUMANS WITH RETINAL LESIONS.
M. Marcondes, R. Gattass*, L.F. Pary, and M.F. Farias.
IBCCF/ UFRJ, Rio de Janeiro, RJ, 21941-900, Brazil.
We studied the tangential distribution of cytochrome oxidase (CytOx)-rich patches in striate cortex of two humans with retinal lesions. Patch spatial density and patch cross-sectional area were analyzed in CytOx-reacted tangential sections of flat-mounted preparations of V1. Rows of patches are more conspicuous in the cortical representation of the retinal lesions than in normal areas. The spatial density of patches remains constant in the binocular field representation in V1, although patch size changes drastically in the region of representation of the retinal lesion. In this region, patches are larger and darker above and below the ocular dominance stripes of the normal eye than in the alternate stripes. After long-term lesions, the patches corresponding to the normal eye columns appeared larger than in normal controls. In contrast, patches corresponding to the columns in the retinal lesion representation appeared smaller than in normal controls. These results suggest the existence of competitive interactions which modify the cortical intrinsic organization even in adult humans.
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457.10

PLASMINOGEN ACTIVATOR AND S-100β IN THE REGULATION OF OCULAR DOMINANCE PLASTICITY.
K. Imamura*[1,2], H. Morii[1,3], K. Muguruma[1,2], T. Shiomitsu[1,2], and Y. Watanabe[1,2]. Dept. of Neurosci., Osaka Bioscience Institute[1], SFBR project JRDC[2], PRESTO[3], Suita, Osaka 565, Japan.

Previously, we have shown that the reduction of glial functions by a gliotoxin retarded the usual process of shift in ocular dominance (OD) of cortical cells following monocular deprivation. One possibility for a glial involvement in cortical plasticity is via biochemical signals released from astrocytes. In the present study, we examined roles of plasminogen activator (PA) and S-100β, both of which are considered to be released from astrocytes. Reactive center hexapeptide of PA inhibitor-1, (PAI-1, 10-100μM) was infused directly into kitten (5-7week-old) visual cortex, concomitantly with one week of monocular deprivation. Electiophysiological recordings revealed a retardation of OD shift in the hemisphere infused with PAI-1 (binoculanity, B～50%). Next, we examined a role of S-100β on OD plasticity by synthesizing and directly infusing *anti-sense* DNA into kitten visual cortex. The shift in OD was found to be suppressed (B～50%) in the hemisphere infused with antisense DNA, while normal shift was consistently confirmed (B～13%)in control cortex infused with *sense* DNA. These results suggest that proteinases and trophic factors released from astrocytes, including PA and S-100β play roles in the regulation of ocular dominance plasticity.

TRANSPLANT- AND PROSTHESIS-ASSISTED REGENERATION

458.1

ELECTROPHYSIOLOGICAL RECOVERY AFTER SPINAL CORD INJURY USING CARBON FILAMENT IMPLANTS AND ORG 2766
S. Sayers*, T. Khan, M. Dauzvardis, L. Liu, and C. Trausch. Rehabilitation R&D Center, Hines VA Hospital, Hines, IL 60141.

Our previous studies have shown that carbon filaments are capable of supporting neurite growth by providing a favorable attachment surface and directionality to regrowing axons. Melanocortins have been found to exert a trophic influence on both the central and peripheral nervous systems. The purpose of this study was to determine whether the implantation of carbon filaments and/or the administration of Org 2766 (a tri-substituted ACTH 4-9 analog) would have any beneficial effect after spinal cord injury.

Rats were anesthetized and a total transection of the spinal cord was performed at the T8-T9 level. For animals receiving carbon filament implants, a bundle of approximately 10,000 carbon filaments of 5 μm diameter (AMOCO Thornel™) were placed into the transection gap. For animals receiving Org 2766 (N.V. Organon, The Netherlands), a 10 μg subcutaneous injection was given after surgery and 1 μg subcutaneous injections at 48 h intervals for a two-week period. At the end of the eight-week survival period, the return of sensory function was evaluated by somatosensory evoked potentials (SSEPs) and the return of motor function by motor evoked potentials (MEPs) using an electromagnetic stimulator.

All of the five spinal cord transected animals, which received carbon filament implants and Org 2766, showed SSEPs recorded across the transection site, as well as MEPs. Two of the three spinal cord transected animals which received Org 2766 without carbon filament implants showed SSEPs recorded across the lesion site and one animal also exhibited MEPs. None of the four spinal cord transected animals without Org 2766 showed any SSEPs recorded across the lesion site.

This preliminary study demonstrated that Org 2766 had a beneficial effect after spinal cord injury, as evaluated by electrophysiological methods.

This research was supported by funds from the Veterans Administration, Rehabilitation R&D Center.

458.2

CARBON FILAMENT IMPLANTS ENHANCED WITH GENETICALLY MODIFIED NERVE GROWTH FACTOR SECRETING FIBROBLASTS PROMOTE REGROWTH OF INJURED SPINAL CORD FIBERS.
T. Khan*, M. Dauzvardis, C. Trausch, and S. Sayers. Rehabilitation R&D Center, VA Hines Hospital, Hines, IL 60141.

Neurotrophins are proteins which are essential for the survival and maintenance of different populations of neurons. Among the neurotrophins, nerve growth factor (NGF) is the most well characterized. NGF infusion into certain types of neural tissue results in a dramatic outgrowth of nerve fibers. Recently, studies have shown that NGF has an effect on a broader spectrum of neuronal populations than was originally believed. Carbon filaments have been shown to provide a good substrate for the regrowth of injured spinal axons. The goal of this study was to determine whether the implantation of NGF-secreting fibroblasts, grown on carbon filaments and implanted into the contused spinal cord, would promote translesion growth of injured spinal cord fibers.

Fibroblasts which were genetically modified to secrete NGF were cultured on carbon filaments attached to the bottoms of petri dishes. After 24 hrs, the fibroblast-coated carbon filaments were implanted into the lesion site of spinal cord contused Fischer 344 rats. All animals were cared for according to AAALAC guidelines. After a ten-week survival period, all animals received injections of 2% WGA-HRP in the cervical spinal cord, above the lesion. Retrogradely labeled neurons were observed below the lesion, indicating that a small number of neurons were able to cross the lesion site and reach at least the mid-cervical level of the spinal cord. Immunocytochemical labeling, using anti-neurofilament antibodies showed robust growth of fibers into the lesion site. Anti-CGRP and anti-ChAT-labeled fibers were found in and around the carbon filament implants. These results indicate that manipulation of the substrate with trophic factors can substantially influence the regrowth process.

Supported by funds from the Veterans Administration, Rehabilitation R&D Service and the AMOCO Foundation.

458.3

NERVE GUIDES OR NERVE GRAFTS RESULT IN FASTER RECOVERY RATES OF MEDIAN NERVE FUNCTION COMPARED TO DIRECT SUTURE.
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Adult male macaques received bi-lateral median nerve section at the wrist and removal of a 5mm nerve segment, repaired by:A) Graft (n=5), B) Collagen nerve guide (n=5), or C) Direct suture (n= 4). Serial evoked EMGs from the abductor pollicis brevis and sensory nerve conduction studies were performed up to 1400 days after repair.

The recovery rates were faster for the Compound Motor Action Potentials (CMAP) and the Compound Sensory Action Potentials (CSAP) following nerve guide or graft repair compared to direct suture ($p=0.001$, Tukey). The average CMAP amplitude in all cases eventually recovered to the normal range. The average CSAP amplitudes and motor and sensory conduction velocities were all lower than normal control values ($P< 0.01$, Scheffé) with no significant differences between the procedures. Reinnervation of digital sensory receptors was suggested by tactile evoked potentials and was confirmed by EM analysis of the Pacinian corpuscles.

A greater number of regenerated myelinated axons were maintained in the distal stump following nerve guide repair compared to normal ($P< 0.05$, Scheffé) and the average fiber diameter in the distal stump following graft repair was significantly smaller compared to normal ($P< 0.05$, Scheffé).

The measures of regeneration did not show significant differences between graft and nerve guide, whereas direct suture impeded the CMAP and CSAP recovery rates. This indicates minimal suture line tension can adversely effect the rate of regeneration.

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458.4

DISSOCIATED, CULTURED SCHWANN CELLS SUPPORT AXONAL GROWTH WHEN IMPLANTED INTO THE SPINAL CORDS OF ADULT RATS.
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The ability of cultured Schwann cells to support axonal growth in the spinal cord has been investigated using adult rats. Schwann cells were cultured from the sciatic nerves of newborn rat pups. They were then harvested and injected into small tubes (>1mm diameter; 5-7mm long) made of polycarbonate film. Following a laminectomy, a lesion was made in the dorsal half of the thoracic spinal cord (total length 5-7mm) and a tube was implanted. In other animals empty tubes were implanted as controls. 1-5 weeks later the animals were perfused and longitudinal sections were cut through the tube and the adjacent spinal cord. Sections were then immunolabeled with antibodies specific for axons, astrocytes and basement membranes. After implantation of 11 days or longer the tubes are filled with cells, many of which have a spindle shape typical of cultured Schwann cells. Neovascularization is evident. Axons can be seen streaming into both ends of the tubes. With short survival times (<2 weeks) these axons extend 2-3mm toward the center. With longer survivals axons are seen throughout the full length of the tubes. Many are grouped in fascicles while others appear as single fibers. Astrocyte labeling reveals a sharp border between the host tissue and the ends of the tubes. These results provide additional evidence that cultured Schwann cells can support axonal growth in the adult central nervous system.

458.5

Cellular growth in optic nerve regeneration chambers. R.J.Podhajsky, A.R.Blight, B.B.Walters* and D.J.Bidanset. Divisions of Neurosurgery and Orthopaedic Surgery, University of North Carolina, Chapel Hill, NC 27599-7060.

Experiments in female Long-Evans rats tested the suitability of a mixed terrain of CNS and PNS components to support axon elongation. A transected optic nerve stump was inserted into the end of a silicone tube and a peripheral nerve graft (PNG) was sutured in the opposite end forming a 3-mm chamber gap. The chambers were resected after various time periods post implantation and the contents of the chambers were studied in serial cross sections to assay the growth across the gap. Early events in these chambers are similar to the analogous PNS chambers with fluid filling the chamber within one day; macrophages, erythrocytes and other vascular components exude from the stumps. A fibrin matrix forms a suspended, coaxial spindle-shaped bridge across the gap. During the second week, there was growth of fibroblasts, capillaries and glial cells into the gap. Although regenerating axons elongate readily across the gap terrain in silicone tubes implanted on peripheral nerves (Lundborg et al, Exp. Neurol. 76: 36-1, 1982), axons did not elongate into the mixed gap terrain. Numerous axons grew retrogradely through the PNG but advanced no further than the end of the graft inserted into the tube, suggesting that this mixed PNS and CNS terrain does not support axonal elongation. Supported by the Canadian Spinal Research Organization.

458.6

METHYLPREDNISOLONE ADMINISTRATION IMPROVES AXONAL REGENERATION INTO SCHWANN CELL (SC) GRAFTS IN THORACIC RAT SPINAL CORD. A. Chen, X.M. Xu, N. Kleitman, M.B. Bunge* The Miami Project & Depts. Neurol. Surg. & Cell Biol. & Anat., University of Miami School of Medicine, Miami, FL 33136

SCs support regeneration of axons from spinal cord neurons into guidance channels grafted into transected adult rat spinal cord (Xu et al. '92,'93). We investigated if axonal regeneration is improved if the neuroprotective agent, methylprednisolone (MP), is administered when SC grafts are implanted. SCs were purified in culture from adult rat sciatic nerves, suspended in Matrigel:DMEM (30:70), and seeded into semipermeable PAN/PVC channels at a density of 120 X 10^6 cell/ml. MP (30 mg/kg) or vehicle (control) was injected i.v. at 5m, 2h, and 4h after transection at T8 and removal of 1-3 caudal cord segments. Either the rostral stump was inserted 1mm into a channel capped at the caudal end, or both stumps were inserted into an open-ended channel. One mo later, the MP + SC/capped channel group, compared with controls, had larger tissue cables, improved blending of host cord and graft tissue, more myelinated axons (n=6; x=1159 vs. 500) in the graft, and labelling of more spinal cord neurons (x, 1128 vs. 280) after injection of Fast Blue into the graft (n=4). Also, in contrast to the control, 5-HT+ and DBH+ axons were detected in the graft, and brainstem neurons (x=34) extended axons into the graft as determined by Fast Blue tracing. When the channel was open at both ends, 45-60 d later the mean of myelinated axons in the graft was 3237 (n=5; control, 1217). Retrograde tracing showed labelled cord (rostrally to C4 and caudally to S4) and brainstem neurons. Additional tracing is underway to assess axonal growth from graft into caudal host cord. In sum, MP improves axonal regeneration from both cord and brainstem neurons into SC grafts, possibly by reducing secondary injury of cord adjacent to graft. (NIH NS09923, NS28059 and The Miami Project; XMX is Heumann Int. Scholar)

458.7

AXONAL OUTGROWTH FROM AN INTRASPINAL PERIPHERAL NERVE (PN) GRAFT CAN BE PROMOTED BY SUBSTRATE BOUND NEUROTROPHIC FACTORS (NTFs). J-H. Ye and J.D. Houlé*, Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

One major obstacle to the use of PN grafts to repair injured central nervous system (CNS) pathways has been the reluctance of regenerated axons to extend beyond the distal end of the graft. Substrate bound NTFs (BDNF; NT-3; or CNTF; Regeneron Pharmaceutics, Inc.) were used to test whether the CNS environment adjacent to a PN graft insertion site could be made more favorable for the ingrowth of axons. Following hemisection lesion of the mid-cervical spinal cord of adult rats, a segment of autologous PN was apposed to the rostral cavity surface. The distal end was ligated and left unapposed for 2 weeks, then apposed to a strip of NTF-treated nitrocellulose membrane implanted into the spinal cord 5 mm caudal to the lesion. Untreated nitrocellulose served as a control. Five weeks later the PN graft was cut at midpoint and the caudal end exposed to HRP and the rostral end exposed to True Blue to label neurons contributing to the graft.

CNTF-treated implants were lined by bundles of labeled axons that had grown down to the ventral edge of the implant, with some fibers extending into the adjacent intermediate gray matter. Outgrowth associated with BDNF- or NT-3-treated implants was less robust in terms of length and number of regrowing axons than with CNTF, yet was greater than with untreated nitrocellulose implants where very few axons extended beyond the PN graft. True Blue-labeled neurons were prominent in the spinal cord, raphe area, reticular formation and vestibular nuclei, the distribution of which was the same with the different NTFs used. These results indicate that regenerating axons of supraspinal neurons can be induced to extend beyond a PN graft, back into the spinal cord, by a variety of substrate bound NTFs, thereby increasing the potential for establishing contact with host neurons. Supported by NIH grant NS 26380.

458.8

BDNF AND NT-3 PROMOTE AXONAL REGENERATION OF BRAINSTEM NEURONS INTO SCHWANN CELL GRAFTS IN MID-THORACIC SPINAL CORD OF ADULT RATS. X.M. Xu*, V. Guénard, N. Kleitman, and M.B. Bunge. The Miami Project and Depts of Neurol. Surg. and Cell Biol., Univ. of Miami Sch. of Med., Miami, FL 33136.

We previously demonstrated that Schwann cells (SCs) in semipermeable guidance channels promote regeneration of propriospinal but not supraspinal axons in adult rat spinal cord transected at T8. Here, we tested whether exogenous brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) could stimulate supraspinal axonal regeneration in this model. SCs were purified in culture from adult rat sciatic nerves, suspended with Matrigel and seeded into PAN/PVC channels (Soc. Neurosci. Abstr., 18:1479, 1992). BDNF and NT-3 were delivered together into the capped caudal end of the channel by an Alzet mini-pump (12 μg/day/trophin) for the first 14 of 30 days tested. Controls received vehicle solution. The spinal cord was transected at T-8 and T9-11 segments were removed. The rostral cord stump was inserted 1 mm into the channel. One month after grafting, a mean of 1523 myelinated axons was present in SC/trophin grafts, twice as many as in SC/vehicle grafts. Brainstem serotonergic axons regenerated into the SC/trophin grafts for at least 5 mm, but not into control grafts. When Fast Blue was injected at the cable midpoint, on average, 92 retrogradely labeled neurons were found in 10 specific regions of the brainstem with the highest labeling (67%) in vestibular nuclei. Labeled cells were also present throughout the rostral cord. Thus, regeneration of some neural populations distant from a spinal cord transection can be recruited by combinations of trophic factors and a favorable cellular substrate. [Regeneron Pharmaceuticals generously provided neurotrophins and Dr. P. Aebischer (Lausanne), channels; supported by NIH NS 28059 and NS 09923 and The Miami Project. XMX is the Heumann International Scholar.]

458.9

HUMAN SCHWANN CELLS CAN ENHANCE AXONAL REGENERATION AND MYELINATION IN THE NUDE RAT SPINAL CORD. J.D. Guest , R.P Bunge*The Miami Project and Dept. of Neurological Surgery, Univ. of Miami School of Medicine, Miami, FL 33136

Human Schwann cells (HSC) can be purified from donor peripheral nerves; such human cells have been shown to enhance regeneration and to provide myelination for regenerating axons when seeded into semipermeable guidance channels in combination with Matrigel and then placed into gaps spanning sciatic nerve transections in immune-compromised rodents (Levi et al, J. Neurosci 14:1309-1319,1994). Furthermore it is known that syngeneic Schwann cells transplanted within semipermeable guidance channels spanning a thoracic spinal cord transection promote axonal regeneration from locally axotomized axons with cell bodies up to 9 segments rostral and provide myelination for many regenerating axons (X.M Xu et al Soc.Neurosci.Abstr.19:681,1993). We have now evaluated whether human Schwann cells can have similar effects on the regeneration and myelination of CNS axons. In this series of experiments we have placed closed ended (caudal) guidance channels containing a cable of HSC (120 x 10^6 cells/ml) mixed with Matrigel (30:70 vol/vol) into contact with the T8 spinal segment of adult female Nude rats. We provide evidence that the HSC survive in contact with the the Nude rat spinal cord and promote regeneration of axons from several neuronal populations (including brainstem DBH positive neurons) into the cable and provide myelination to large numbers of regenerating axons within the closed ended channel when assessed at 30 days after surgery. Because numbers of HSC obtained from human donors are limited methods to expand HSCs to large numbers at high purity have been developed (Levi et al. see abstract this meeting). After 3 passages with mitogens the HSC used within guidance channels appear as competent to recruit regenerating fibres and provide myelination as non-expanded HSC. Dr. Guest is a Fellow of the American Association of Neurological Surgeons; support from NS09923

458.10

EMBRYONIC CNS TRANSPLANTS ASSIST ADULT DORSAL ROOTS TO FORM SYNAPSES IN HOST SPINAL CORD. Y. Itoh[1,2]* F. Mori[1,2], T. Sugawara[1], M. Kowada[1], and A. Tessler[2] [1]Dept. of Neurosurg., Akita Univ. Sch. of Med., Akita 010, Japan; [2]Dept. of Anat. and Neurobiol., Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

Adult dorsal root axons regenerate into embryonic spinal cord transplants and form contacts with transplant neurons that are synaptically driven in response to electrical stimulation of regenerated dorsal root axons. It is unknown whether embryonic CNS transplants also assist synapse formation by adult dorsal roots regenerated into host spinal cord. Adult Sprague-Dawley rats received intraspinal transplants of E14 spinal cord or E18 occipital cortex into left dorsal quadrant cavities in the lumbar enlargement. The severed host L4 or L5 dorsal root was sandwiched between the transplant and host spinal cord. Three to 12 months later sagittal sections were processed for calcitonin gene-related peptide (CGRP) immunohistochemistry and examined by LM and EM. CGRP-labeled axons regenerated into host spinal cord and some extended into the motoneuron pool. The area fraction occupied by regenerated axons in transplanted rats was significantly larger than in control rats without transplants. CGRP-labeled axons formed synaptic terminals within host spinal cord; most were axo-dendritic. Embryonic CNS transplants therefore mediate permanent synapse formation by adult dorsal root axons in host spinal cord and may provide a milieu that promotes reconstruction of interrupted spinal reflex arcs.

458.11

INTERSPINAL WIRING OF THE TRANSECTED RAT SPINAL COLUMN. Henrich Cheng, Fong-Lee Huang*[1] and Lars Olson. Department of Neuroscience, Karolinska Institute, Stockholm, Sweden, and [1]Institute of Neuroscience, National Yang-Ming Medical College, Taipei, Taiwan.

Shortening of the spinal column has been regarded as one possible method to obtain cord-to-cord apposition after total transection of the spinal column. However, to further improve regenerative possiblities, the problems of inconsistent bony fusion and cyst formations within the junctions must be resolved. To modify the method of de Medinaceli on the rat thoracic spine, we attempted several strategies to achieve better interspinal fixation after spondylectomy and transection, including transpedicular miniscrews, wiring of the transverse processes, and wiring of the posterior spinal processes. A dynamic model, based on retracting and compressing the cut ends of the spinal cord by means of adjustable fixation devices to permit swelling and shrinkage of the stumps was also attempted to better compensate pathophysiologic changes of the transected cord. Preliminary results support that the best regeneration, as indicated by regrowth of 5-HT fibers below the level of transection, was obtained following compressive wiring of posterior spinal processes. In this group, the distance between two spinal cord stumps lacking anti-GFAP immunoreactivity was also the shortest. With better approximation, the numbers of regenerated 5-HT fibers seemed to improve remarkably, suggesting that perhaps also other descending or ascending fiber systems might be able to regenerate under these conditions.

458.12

CHANGES IN GAP JUNCTIONAL COMMUNICATION COORDINATE SCHWANN CELL RESPONSES TO INJURY. K. J. Chandross*, D. C. Spray, R. Dermietzel, and J. A. Kessler. Department of Neuroscience, Albert Einstein College Medicine, Bronx, NY 10461

To elucidate the mechanisms underlying coordinated, phasic Schwann cell responses to nerve injury, we examined whether the onset of de-differentiation and proliferation is temporally related to changes in the strength of intercellular communication between these cells. Previously, we have shown that cultured neonatal rat Schwann cells are weakly coupled through gap junction channels and that coupling strength is increased by conditions mimicking injury-induced de-differentiation and proliferation. To determine whether nerve injury resulted in changes in coupling *in situ*, we enzymatically (1hr, 1mg/ml type IV collagenase) separated adult rat sciatic nerve into single fibers and compared coupling strength between the Schwann cells surrounding intact and crush-injured axons. Individual Schwann cells were either microinjected with Lucifer yellow or the preparation was first loaded with acetoxy esters of indicators (Fura2, Indo1) and then Ca^{+2} was microinjected into one Schwann cell. Scanning confocal imaging (Nikon RCM 8000) determined that in intact nerve, Ca^{+2} and Lucifer yellow spread longitudinally from one internodal region to the next. Spread of the dye appeared to be enhanced after nerve injury. Further, PCR analysis of gap junction expression (using primers against a conserved transmembrane region) and immunocytochemical detection (using antibody probes) showed different patterns of connexin expression between intact and crushed sciatic nerve. These observations suggest that gap junctions between Schwann cells may be important for both normal function of peripheral nerves and their responses to injury.

458.13

REPAIR OF PARTIAL NERVE DEFECT BY THE SILICONE CHAMBER TECHNIQUE. T. Urabe, Q. Zhao, G. Lundborg and N. Danielsen*. Dept. Hand Surg., Malmö Gen. Hosp., Univ. Lund, S-214 01 Malmö, Sweden.

Partial nerve defects are a challenging clinical problem. In this study we examined the regeneration process in partially transected rat sciatic nerve repaired with the previously established silicone chamber model. The tibial nerve fascicle was transected and a 10 mm segment was removed. The tibial proximal and distal stumps together with the intact peroneal fascicle were enwrapped into a silicone tube leaving a 10 mm defect between the two tibial stumps. Nerve regeneration was examined by immunocytochemistry and light microscopy after 7, 16, 28 or 42 days. A cellular fibrin matrix, spanning the proximal and distal stumps of the tibial fascicle and surrounding the intact peroneal fascicle, was formed within one week. This cellular matrix was then invaded by non-neuronal cells and regenerating axons. Regeneration in nerves with a partial defect was more advanced with respect to ingrowing vasculature, Schwann cells, axons and myelination as compared to a totally transected nerve with a 10 mm gap repaired with a silicone chamber. The results suggest that a partial nerve defect could be repaired using the silicone chamber technique. It opens a perspective to solve the clinical problem of repairing a partial nerve defect when neither direct suture nor nerve grafting is applicable.

458.14

TUBULAR REPAIR OF ULNAR AND MEDIAN NERVES IN THE HUMAN FOREARM. CASE REPORTS. L. Dahlin*, B. Rosén, N. Danielsen and G. Lundborg. Dept. Hand Surg., Malmö Gen. Hosp., Univ. Lund, S-214 01 Malmö, Sweden.

The silicone tube technique was used to repair transected median (two cases) or ulnar nerves (one case) at the distal forearm level in three patients. In all three cases the nerve ends were enclosed in a silicone tube of such a dimension that would not cause compression of the nerve and leaving a gap of 3-5 mm between the two nerve ends. Three years after nerve repair the patients were examined including sensory evaluation and assessment of muscle contraction force. In two cases the tubes had to be removed two years (median nerve) or three years (ulnar nerve) after nerve repair because of minor local discomfort. At the time of exploration the former gap was bridged by a smooth continuous nerve structure of the same diameter as the adjacent nerve trunk. There were no signs of neuroma formation. In the median nerve cases there were excellent motor recovery of the thenar muscles. Two point discrimination (2PD) was < 6 mm (12 year old patient) and 8-10 mm (21 year old patient) respectively. In the ulnar nerve case (21 year old patient) the first dorsal interosseus muscle was almost normal in size and the strength remarkably good. 2PD was 6 mm. These three cases have demonstrated that the principle of using the tube technique in humans is feasible. The results have inspired us to conduct a randomized, prospective clinical study of the tube technique versus the conventional technique for repair of median or ulnar nerves at the distal forearm level.

NEUROGLIA AND MYELIN III

459.1

REDUCTION IN AMMONIA INDUCED ASTROCYTE HYPERTROPHY BY GLUTAMINE SYNTHETASE INHIBITION. C. Willard-Mack, T. Hirata, R.C. Koehler, L.C. Cork*, R.J. Traystman and S.W. Brusilow. The Johns Hopkins Medical Institutions, Baltimore, MD 21287.

Glutamine synthetase localized in astrocytes utilizes glutamate and ammonia. We tested the hypothesis that astrocyte enlargement associated with hyperammonemia can be reduced by inhibition of glutamine synthetase with methionine sulfoximine (MSO). Pentobarbital-anesthetized rats were pretreated with either vehicle, MSO (150 mg/kg, i.p.), or buthionine sulfoximine (BSO; 888 mg/kg, i.p.), an analogue of MSO that does not inhibit glutamine synthetase. Infusion of ammonium acetate for 6 hours increased plasma ammonia levels from 58 to 533 μM. Cortical astrocytes had marked enlargement of all cytoplasmic areas with increased numbers of mitochondria, rough and smooth endoplasmic reticulum, and glycogen. Enlargement of small astrocytic processes in neuropil and perivascular endfeet was reduced by MSO but not BSO pretreatment despite elevated ammonia levels. Nuclear diameter in hyperammonemic rats treated with vehicle (7.98 ± 0.73 μm; $\pm$SD) was greater than in those pretreated with MSO (7.34 ± 0.94 μm) or in controls receiving sodium acetate (6.79 ± 0.81 μm). We conclude that some of the astrocytic hypertrophy that occurs during acute hyperammonemia is related to glutamine accumulation and synthesis localized in astrocytes. (Supported by NS25275).

459.2

Na^+/H^+ EXCHANGE CHARACTERIZED AS NHE-1 IN RAT HIPPOCAMPAL ASTROCYTES. John H. Pizzonia*[1] and Christopher A. Pappas.[2] Departments of Internal Medicine[1] and Neurology[2], Yale University School of Medicine, New Haven, CT 06510.

Precise regulation of intracellular pH (pH_i) in astrocytes is essential for proper function and modulation of the surrounding environment. The Na^+/H^+ exchanger (NHE) is an integral membrane protein which mediates 1:1 electroneutral countertransport of Na^+ for H^+. Four distinct isoforms (NHE's 1-4) have been reported with differing localizations and pharmacological properties. NHE-1, an amiloride sensitive (IC50~10μM) isoform mediating various cell functions such as growth and proliferation, has been found to be expressed in all mammalian cell types examined. NHE's 2-4 are less well characterized with a more restricted pattern of localization, which includes brain for NHE's 2 & 4, while NHE's 2 & 3 are known to be amiloride resistant (IC50 > 50μM). We examined Na^+/H^+ exchange activity in primary cultures of rat hippocampal astrocytes. pH_i recovery from an acid load in the absence of CO_2/HCO_3^- was assessed using BCECF. Rate of recovery from an acid load measured at a pH_i of 6.7 was 0.193 ± 0.03 pH units/min. and was completely inhibited by 50μM of amiloride (IC50 = $3.18 \pm 0.47\mu$M). Northern blot performed under low stringency, using full-length NHE-1 showed expression of a single isoform (~4.8 kb) consistent with NHE-1. Interestingly, application 50μM EIPA, a highly potent amiloride analog, did not inhibit recovery from an acid load and caused a reversible alkalinization when applied to resting cells. (Supported by NS 15589 and CT Heart Association).

459.3

RAT HIPPOCAMPAL ASTROCYTES EXHIBIT ELECTROGENIC SODIUM-BICARBONATE COTRANSPORT

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Using whole-cell patch-clamp recordings we studied expression of electrogenic Na^+/HCO_3^- cotransport in mammalian astrocytes. Primary cultures of hippocampal astrocytes derived from newborn rats were studied after 10 days in culture. Application of 25 mM HCO_3^-, at a constant pH_o, to astrocytes bathed in a nominally HCO_3^-- free solution, produced a reversible change in membrane potential ranging from +3 to -30 mV (Ave ± S.D. = -11.8±9.34 mV). The size of the HCO_3^--induced hyperpolarization was strongly related to the cell's initial resting membrane potential; cells with more negative resting potentials had smaller responses. The HCO_3^- induced change in membrane potential was dependent on extracellular Na^+, blocked by the stilbene derivative DIDS, and independent of extracellular chloride. Voltage-clamp recording demonstrated that HCO_3^--induced hyperpolarization was caused by outward currents averaging 335±104 pA (holding potential = -60 mV). The reversal potential of the HCO_3^--induced current was between -80 to -90 mV. Based on the reversal potential of the HCO_3^- -induced response, and knowledge of the transmembrane gradients for HCO_3^- and Na^+, it was calculated that the cotransporter has an apparent HCO_3^-:Na^+ stoichiometry of 2:1. These findings indicate that hippocampal astrocytes express electrogenic Na^+/HCO_3^- cotransport. This transporter may play an important role in regulation of intracellular pH, depolarization-induced alkalinization and intracellular Na^+ homeostasis. (Supported by NIH grants NS 09542 to ERO and 15589 to BRR).

459.4

ION CHANNELS AND GLIAL PROLIFERATION: I. Role of K^+ channels, $[Ca^{2+}]_i$, and $[pH]_i$.

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Astrocyte proliferation was studied in primary cultures of rat spinal cord to search for factors that might influence glial proliferation. Proliferation was assayed quantitatively by determining relative ^{3}H-Thymidine incorporation. Astrocyte proliferation could be largely reduced by culturing cells for 6-24 h in the presence of K^+ channel blockers, namely Cs^+ (1 mM, 43%), Ba^{2+} (1 mM, 46%), 4-AP (2 mM, 61%) and TEA (5 mM, 35%). Proliferation was also reduced by nifedipine (100 µM, 33%), but was unaltered by blocking Na^+ channels with TTX. The phorbol ester PMA (100 µM), a known mitogen, increased proliferation by 33% whereas the mitogenic inhibitor ARA-C (5 µM) completely inhibited proliferation. To address whether these effects could have been mediated by changes in resting potential, $[Ca^{2+}]_i$ or $[pH]_i$, we measured membrane potential electrophysiologically, and ratiometrically measured $[Ca^{2+}]_i$ and $[pH]_i$ using Fura-2 and BCECF respectively. While Ba^{2+} and TEA depolarized the cells by 37 and 10 mV respectively, both Cs^+ and 4-AP, which had the largest effects on proliferation did not change resting potential. $[Ca^{2+}]_i$ effects were similarly heterogeneous: Ba^{2+} increase $[Ca^{2+}]_i$ by 8%, Cs^+ and 4-AP reduced it by 3 and 17% respectively, and TEA and ARA-C had no effect. However, all drugs effective in inhibiting proliferation also resulted in an alkaline shift in $[pH]_i$ ranging from 0.05 (ARA-C) to 0.22 (4-AP) pH units. To further substantiate the role of $[pH]_i$ on cell proliferation, cells were cultured under conditions that alter $[pH]_i$ in a defined way. Proliferation showed a strong dependence on $[pH]_i$ with an optimum ~pH 6.7. Interestingly, we observed that resting pH of proliferating astrocytes was consistently more acidic than that of sister cultures growth inhibited by ARA-C. These observations suggest that astrocyte proliferation is sensitive to changes in $[pH]_i$ and that K^+ channel blockers may have excerpted their anti-proliferative effects through changes in $[pH]_i$

459.5

ION CHANNELS AND GLIAL PROLIFERATION: II. Comparison of normal and neoplastic glia.

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Unlike neurons, glial cells retain the ability to proliferate postnatally and can divide rapidly to form astrocytomas under neoplastic conditions. The precise control of factors involved in proliferation is poorly understood. We studied possible roles of K^+ channels in glial cell proliferation by comparing properties of spinal cord astrocytes, rat C6 glioma cells, and human astrocytoma cells in culture. To determine whether chronic exposure to ion channel blockers alters proliferation, control and sister cultures were grown in the presence or absence of channel blocking agents, and cell proliferation determined using ^{3}H-thymidine incorporation as a quantitative measure of DNA synthesis. Application of 4-aminopyridine (2mM), Ba^{2+}(1mM), Cs^+(1mM), and TEA (5mM), at concentrations which block glial K^+ channels, significantly reduced ^{3}H-thymidine incorporation in astrocytes, but failed to alter proliferation of neoplastic cells. The phorbol ester PMA (100µM), a known mitogen, increased proliferation in normal astrocytes by 33%, but reduced proliferation in neoplastic cells by up to 50%. As K^+ channel block should result in depolarization, we examined the potential interrelationship of resting potential and proliferation. Astrocytes in which proliferation was inhibited by cytosine arabinoside (ARA-C) exhibited 20-30 mV more hyperpolarized resting potentials than untreated, proliferating, sister cultures. Similar treatment of neoplastic cells resulted in changes in V_m of the same magnitude, but towards more depolarized potentials. These differences suggest that proliferation is regulated differently in normal and neoplastic glial cells. We are presently investigating the possibility that differences in steady-state pH_i between control and growth-inhibited cells play a role in linking ion channel expression, membrane potential, and glial proliferation.

459.6

EXPRESSION OF VOLTAGE-ACTIVATED Na^+ AND K^+ CHANNELS IN HUMAN ASTROCYTES

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Primary astrocyte cultures were derived from lateral temporal neocortex (normal cortex) and the hippocampus/parahippocampus (seizure focus) of patients with Temporal Lobe Epilepsy associated with mesial temporal sclerosis. These cultures contained primarily stellate, process-bearing, GFAP positive astrocytes. Na^+ and K^+ channel expression was studied using whole cell patch-clamp recording techniques. Astrocytes from both regions expressed Na^+ channels and up to three forms of K^+ channels. Astrocytes from the seizure focus displayed Na^+ currents at approximately 25 fold higher density (80.2±28.5 pA/pf) than normal astrocytes (3.2±1.9 pA/pf), but were indistinguishable in their biophysical features. In seizure focus astrocytes K^+ conductance equaled that of peak Na^+ conductance whereas in control cells K^+ conductance exceeded that of Na^+ by 20 fold. Biophysically, and pharmacologically K^+ channels could be classified as delayed rectifier (K_d), transient "A" type (K_a), and inward rectifier (K_{ir}). Interestingly, K_{ir} currents were only observed in normal cortical astrocytes and were absent in astrocytes from seizure foci. The resting membrane potential of seizure focus astrocytes was significantly more positive (-55±10.2 mV) than that of normal astrocytes (-80±7.8 mV). The lack of K_{ir} currents may be causal of the more depolarized potential of seizure focus astrocytes. Since expression of K_{ir} has previously been linked to cell proliferation, we hypothesize that the above observations are reflective of different proliferative states of normal and seizure focus astrocytes. This work was supported by NS 27081.

459.7

K^+ INDUCED RELEASE OF [^{3}H]-D-ASPARTATE FROM ASTROCYTES BY A SWELLING MEDIATED PROCESS AND REVERSAL OF THE TRANSPORTER.

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In pathological conditions astrocytes are known to swell markedly and one of the effectors thought to be responsible is an increase in $[K^+]_o$. Astrocytes are well-known, both in culture and in vivo, to take up the excitatory amino acids, glutamate and aspartate, on a specific transporter. Mechanisms of release involving K^+-dependent reversal of this transporter and/or cell swelling were studied. Primary astrocyte cultures, from P 1 rat cerebral cortex, were exposed to high K^+ and the release of preloaded [^{3}H]-D-Aspartate was measured. A twenty min. exposure to 100mM K^+resulted in a biphasic release of [^{3}H]-D-Aspartate; an initial transient release response followed by a slower sustained release. L-644,711, an anion transport blocker, blocked the slower release component but had no affect on the initial release component. The initial release component can be enhanced by increasing $[Na^+]_i$ by treating cells with ouabain; which had no effect on the second release component.The initial component seemed more sensitive to increased $[K^+]_o$, while the second component required >50mM K^+. We propose that the initial release is caused by reversal of the uptake transporter and the second caused by a Na^+ insensitive swelling induced anion transport process. (Suppored by NS 30303)

459.8

CALCIUM-DEPENDENT RELEASE OF EXCITATORY AMINO ACIDS FROM SCHWANN CELLS

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The regulation of the extracellular levels of excitatory amino acids (EAAs) is a complex interplay between EAA release from neurons and uptake into neurons and glia. This study demonstrates an additional process in the regulation of external EAAs through the neuroligand-activated, calcium-dependent release of EAAs from Schwann cells.

The release of EAA from Schwann cell cultures derived from dorsal root ganglia (DRG) was assayed using high-performance liquid chromatography. The neuroligand bradykinin (BK) caused a receptor-mediated, dose-dependent increase in release of the EAAs (glutamate and aspartate) from Schwann cell cultures.

To ask whether calcium might play a role in BK-induced EAA release, we used fura-2 AM (4 µM) to monitor Schwann cells calcium levels. BK increased the cytoplasmic level of Schwann cells free calcium. The calcium mobilizing action of BK was impaired by thapsigargin (1 µM), but not by caffeine (10 mM), ryanodine (10 µM) nor by removal of extracellular calcium.

To determine whether the BK-induced calcium mobilization stimulates EAA release, cells were loaded with BAPTA-AM (50 µM). Addition of this calcium chelator blocked BK-induced EAA release. Addition of ionomycin (5 µM) caused calcium-dependent EAA release. Taken together these data demonstrate that calcium is both necessary and sufficient for stimulating the release of EAA from Schwann cells cultures.

459.9

GLUTAMATE MEDIATES ASTROCYTE-NEURON SIGNALING IN CORTICAL CULTURES V. Parpura[1]*, T. A. Basarsky[1], F. Liu[2], K. Jeftinija[2], S. Jeftinija[2] and P. G. Haydon[1]. Signal Transduction Training Group, Dept. of Zoology and Genetics [1], Dept. of Vet. Anatomy [2], Iowa State Univ., Ames, IA 50011.

Bradykinin (BK) caused a receptor-mediated calcium-dependent release of the excitatory amino acids (EAAs), glutamate and aspartate, from astrocyte cultures obtained from cerebral cortex together with an increase in the cytoplasmic level of astrocytic free calcium.

When applied to mixed astrocyte-neuronal cultures BK elevated calcium levels in neurons only when they contacted astrocytes. The general glutamate receptor antagonist D-glutamylglycine (DGG) and N-methyl-D-aspartate receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5) prevented BK-induced neuronal calcium elevation. These data indicate that BK elevates neuronal calcium levels through the action of glutamate that is released from astrocytes.

Direct photo-stimulation of astrocytes can increase their calcium levels. Exposure of a portion of an astrocyte to focal application of UV light reliably caused an elevation in internal calcium. Since elevated calcium is sufficient to stimulate the release of glutamate from astrocytes, we asked whether astrocyte photo-stimulation caused a glutamate-dependent elevation of neuronal calcium. Photo-stimulation of astrocytes led to an elevation in calcium levels of adjacent unstimulated neurons that was reduced 83 % by DGG. Thus, the mobilization of calcium in astrocytes causes the release of glutamate which signals to adjacent neurons.

459.10

Regional specificity in astrocyte gap junction coupling

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Astrocytes are coupled to each other via gap-junctions both *in vivo* and *in vitro*. Gap-junction coupling is essential to a number of astrocyte functions including the spatial buffering of extracellular K^+ and the propagation of Ca^{2+} waves. Using fluorescence recovery after photo-bleach (FRAP), we quantitatively assayed and compared the coupling of astrocytes cultured from six different central nervous system (CNS) regions in the rat: spinal cord, cortex, hypothalamus, hippocampus, optic nerve and cerebellum. The degree of fluorescence recovery (%recovery) and time constant of recovery (τ) served as quantitative indicators of coupling strength. Gap-junction coupling differed markedly between CNS regions. Coupling was weakest in astrocytes derived from spinal cord (43% recovery, τ~400 s) and strongest in astrocytes from optic nerve (91% recovery, τ~226 s) and cerebellum (95% recovery, τ~100 s). As indicated by the degree of recovery, coupling strength among CNS regions could be ranked as follows: spinal cord < cortex < hypothalamus < hippocampus = optic nerve = cerebellum. Gap-junction coupling also differed between CNS regions with respect to its sensitivity to inhibition by the uncoupling agent octanol. Kd values for 50% inhibition by octanol ranged from 188 μM in spinal cord astrocytes to 654 μM in hippocampal astrocytes. Sensitivity of gap-junctions to octanol could be ranked as follows: spinal cord = cortex = hypothalamus > cerebellum > optic nerve > hippocampus. Regional differences in gap juction coupling and octanol sensitivity observed in cultured astrocytes may reflect differences in the *in vivo* rat CNS. These variations may be significant evolutionary adaptation of astrocytes to varying functional requirements in different CNS regions.

459.11

RESPONSE OF ASTROCYTIC GAP JUNCTIONS FOLLOWING NEURONAL DAMAGE IN RAT BRAIN. J.I. Nagy, M.Z. Hossain*, P.A.Y. Ochalski, L.J. Murphy, E.L. Hertzberg and M.A. Sawchuk. Department of Physiology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, CANADA R3E 0W3

Gap junctional communication in astrocytes is postulated to be critical for metabolic homeostasis and K^+ spatial buffering following neuronal activity. Neuronal dependence of astrocytic gap junctional regulation was investigated by examining immunohistochemically the distribution of the astrocytic gap junction protein connexin43 (Cx43) in two model systems in rat brain which cause extensive neuronal damage, namely kainic acid (KA) lesion and ischemia. With an antibody against amino acids 346-363 of Cx43, we observed (i) increased Cx43 immunolabelling at early time points after KA-treatment or ischemic insult, (ii) complete disappearance of Cx43 immunoreactivity at later time points in areas with severe neuronal damage and (iii) an increased Cx43 labelling in areas surrounding the lesion. Western blot analysis showed no detectable changes in either the normally present phosphorylated 43 kD form of Cx43 or in the post-mortem dephosphorylated 41 kD form of Cx43 at any time post-injury. Detailed analyses of KA-lesion sites with antibodies against other sequences of Cx43 revealed not only a presence but in some cases an increased density of Cx43 immunoreactivity. Ultrastructural studies in ischemic tissues, where neuronal damage was less severe than in KA-lesioned tissue, revealed increased Cx43 immunoreactivity at sites containing injured neurons. Immunoelectron microscopy of KA-lesioned tissue demonstrated a time-dependent internalization of astrocytic gap junctions and relocalization of Cx43 in multivesicular clusters which culminated in a virtual absence of astrocytic gap junctions by 7 days post-lesion. These results suggest that, depending on the degree of neuronal injury, astrocytic gap junctions undergo molecular reorganization that results in redistribution or disappearance of junctional plaques and an altered antibody recognition of Cx43.

459.12

THE GAP JUNCTIONAL UNCOUPLING AGENTS OCTANOL AND HEPTANOL ALTER pH_i AND $Ca^{2+}{}_i$ IN RAT HIPPOCAMPAL ASTROCYTES Christopher A. Pappas* and Bruce R. Ransom. Department. of Neurology, Yale University School of Medicine, New Haven, CT 06510.

Astrocytes are typically linked together by intercellular channels called gap junctions. Octanol and heptanol uncouple cells by blocking gap junction channels, but their mechanism of action is not known. Decrease in intracellular pH (pH_i) or increase in intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) also uncouples cells. We tested the possibility that octanol and heptanol induce changes in pHi and/or $[Ca^{2+}]_i$, which may be relevant to their uncoupling action. We studied cultured rat hippocampal astrocytes using the ion-sensitive dyes BCECF (for pH) and Fura-2 (for Ca^{2+}).

Application of 1mM octanol to confluent astrocytes in HCO_3^--buffered solutions resulted in a biphasic change in pH_i. pH_i initially declined by -0.14 pH units (range: -0.44 to -0.06) in 87% of cells, followed in 8-10 minutes by a rapid pH_i increase of 0.36 (98% of cells). In HEPES-buffered solutions, octanol caused a -0.16 pH_i decline (93% of the cells), followed by an increase of 0.19 pH units (90% of cells) at ~20 min. Octanol's effect on pH_i was partially reversible after brief application (2 min), but not after longer application (8 min). $[Ca^{2+}]_i$ also showed a biphasic response to octanol (HCO_3^- buffer), with an initial decrease of 21% followed by an increase at 9-11 minutes to 140% of initial levels. Removal of bath Ca^{2+} (HEPES) caused a slight decrease in pH_i (-0.07) and blocked the late pH_i increase caused by octanol; the drug still elicited an early decrease in pH_i (-0.36) and $[Ca^{2+}]_i$. Isolated astrocytes exposed to octanol also exhibited biphasic pH_i shifts of -0.23 and 0.18, respectively. Heptanol (1mM) had similar effects as octanol. Astrocytes from rat spinal cord responded to octanol in a similar manner as hippocampal astrocytes, but hepatocyte pH_i did not change with octanol. The changes in pH_i and $[Ca^{2+}]_i$ induced by octanol and heptanol are complex and could contribute to their actions as uncoupling agents in glia but not hepatocytes. (Supported by grant NS 15589 to B.R.R.).

459.13

ASTROCYTES *IN SITU* RESPOND TO GLUTAMATERGIC AND PURINERGIC NEUROLIGANDS AND DEPOLARIZATION J.T. Porter and K.D. McCarthy* Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599

In culture, astrocytes express many of the same receptors as neurons. *In vivo*, it is likely that these receptors are important for the modulation of astrocytic functions such as the uptake of neurotransmitters and ions. Currently, however, very little is known about the expression or stimulation of astrocytic receptors *in vivo*. Using confocal microscopy and calcium sensitive fluorescent dyes we obtained data indicating that hippocampal astrocytes *in situ* (identified by glial fibrillary acidic protein immunostaining) respond to glutamate, 1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD), ATP, adenosine, and high K+ with increases in intracellular calcium ([Ca2+]i). The responses exhibited various temporal patterns which included both oscillations and sustained increases. Increases in [Ca2+]i occurred both in the cell bodies and in the processes and were unaffected by 1 uM tetrodotoxin. N-methyl-D-aspartate (NMDA) caused increases in astrocytic [Ca2+]i which were sensitive to tetrodotoxin suggesting that neuronal signals were necessary for these responses. Often a single astrocyte responded to more than one neuroligand and to depolarization. These findings suggest that astrocytes *in vivo* contain receptors coupled to increases in [Ca2+]i and are able to respond to neuronally released neurotransmitters. The presence of multiple pathways for increasing [Ca2+]i within a single astrocyte suggests the possibility of integration of multiple stimuli.

459.14

NEUROTRANSMITTERS EVOKE Ca^{2+}-RESPONSES IN GFAP (+) CORTICAL ASTROCYTES ACUTELY ISOLATED FROM POSTNATAL 3 TO 16 DAY ($P_{3\text{-}16}$) RATS. H.K. Kimelberg*, V. Dave and C. Charniga. Division of Neurosurgery, Albany Medical College, Albany, NY 12208.

Although many neurotransmitters evoke Ca^{2+}-responses in astrocytes in primary culture it is more difficult to determine whether the same responses occur in astrocytes *in vivo*. One approach to this question is by examining cells soon after their isolation from brain tissue. In the present study, P_3, $P_{6\text{-}9}$ and $P_{14\text{-}16}$ rat cortical cells were plated onto coverslips following dissociation of enzyme treated slices, and fura-2 loaded cells were studied individually for their Ca^{2+}-responses to a panel of 9 transmitters. So far, 7 GFAP(+) astrocytes have been examined within 8 hours of isolation (Grp 1), and 11 astrocytes were cultured and studied from 9-80 hours post-isolation (Grp 2). The percentages of Grp1 vs Grp 2 astrocytes that responded were 43 vs 55 to ATP, 29 vs 73 to norepinephrine, 14 vs 64 to bradykinin, 50 vs 0 to histamine and 17 vs 10 to adenosine. This could represent heterogeneity in receptor expression, receptor up- or down- regulation, or recovery from the effects of enzyme treatment. Responses to acetylcholine, glutamate and serotonin were elicited in ≤ 10% of astrocytes in both groups. Responses to endothelin-1, -2 and -3 were seen in 1/3, 3/5 and 2/7 astrocytes respectively. Almost half the astrocytes responded to any 3 of the 9 neurotransmitters tested and the remainder generally responded to less than 3 neurotransmitters. Incubation with enzyme prior to dissociation could have affected cell responsiveness, but astrocytes examined within 8 hrs of isolation using enzyme-free dissociation showed comparable patterns of responses. These results lend additional support to the idea that rat cortical astrocytes *in vivo* normally express Ca^{2+}-mobilizing neurotransmitter receptors. (Supported by NINCDS 19492 to H.K.K.)

459.15

"*IN VITRO* ISCHEMIA" INCREASES THE $[Ca^{2+}]_i$ OF ASTROCYTES WITHIN HIPPOCAMPAL SLICES. S.N. Duffy* and B.A. MacVicar Dept. of Neuroscience, University of Calgary, CANADA T2N-4N1

Increased $[Ca^{2+}]_i$ contributes to neuronal death following ischemia, but it is not known what factors trigger the concomitant transformation of astrocytes to the reactive form. Many aspects of astroglial physiology are regulated by $[Ca^{2+}]_i$, so we tested whether simultaneous hypoxia and hypoglycaemia ("*in vitro* ischemia") can induce $[Ca^{2+}]_i$ elevations in astrocytes within hippocampal slices. Astrocytes, identified by high resting membrane potentials (E_m= 75 ± 3 mV, n=41) and passive responses to injected currents, were iontophoretically loaded with the Ca^{2+}-sensitive dye Calcium Orange. Hypoxia-hypoglycaemia triggered rapid elevations in the $[Ca^{2+}]_i$ of dye-coupled astrocyte networks (11 of 14 slices). The duration of hypoxia-hypoglycaemia required to elicit such increases ranged from 3 to 7 min (averaged 5.4 min). We have shown previously that depolarization (by high $[K^+]_o$) triggers Ca^{2+} influx in these cells by activating voltage-gated Ca channels. Hypoxia-hypoglycaemia for 3.5-7 min also depolarized astrocytes (range 30-70 mV, n=7), suggesting that the $[Ca^{2+}]_i$ increases associated with this treatment are mediated, at least in part, by voltage-dependent Ca^{2+} influx. Increased astroglial $[Ca^{2+}]_i$ may trigger many of the changes (hypertrophy, alterations in gene expression, proliferation) characteristic of reactive gliosis.

459.16

ELEVATION OF EXTRACELLULAR $[K^+]$ CAN TRIGGER AUTOREGENERATIVE CALCIUM WAVES IN CULTURED MAMMALIAN BRAIN CELLS. Maiken Nedergaard,* Aitken Neurosurgery Laboratory, Division of Neurosurgery, Department of Surgery, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

Increments in intracellular calcium that propagate among cells ("calcium waves") have been described in a wide variety of cell types. These calcium waves may propagate from cell to cell by gap junction-mediated diffusion of a second messenger, as cell to cell propagation is dependent upon expression of the gap junction protein connexin43. In cultures of mammalian brain, calcium waves are carried by astrocytes. Neurons in contact with astrocytes participating in the wave respond to glial stimulation with increases in their cytosolic calcium levels (Science: 263, 1768-71, 1994). Signaling from astrocytes to neurons is unidirectional, since stimulated neurons are unable to evoke astrocytic calcium increases. Wave propagation is resistant to both sodium and calcium channel blockers: Glutamate receptor antagonists, L-type calcium channel blockers, and tetrodotoxin affect neither the velocity vector nor the maximal radius of propagation. Thapsigargin, on the other hand, is an effective inhibitor of wave propagation. Thus, calcium wave propagation appears to be relatively resistant to changes of the extracellular environment. The present study examined consequences of elevation of extracellular potassium $[K^+]$ from 5 to 60 mM on propagation of the calcium waves elicited by electrical field stimulation. High $[K^+]$ was an effective promoter of wave propagation. In fact, self-regenerating calcium waves, often lasting for several minutes after initiation, were regularly evoked by high potassium; these included both astrocytes and neurons. Thus, potassium-induced elevation of resting cytosolic calcium might decrease the threshold for propagation of the calcium wave among glia and neurons. (Supported by grants from the American Heart Association, the Migraine Trust, and NINDS R29 NS30007.)

CYTOSKELETON TRANSPORT AND MEMBRANE TARGETING: AXON AND DENDRITE CYTOSKELETON

460.1

MULTIPLE CALCIUM-DEPENDENT KINASES MAY REGULATE THE INTERACTION OF PEA-15 WITH MICROTUBULES IN ASTROCYTES. H. Chneiweiss, M. Kubes, M. Yokoyama*, J. Cordier, A. Estelles and J. Glowinski. INSERM U114/ Collège de France, Paris, France.

Neurotransmitters, growth factors and cytokines have been shown to mediate neuron-astrocyte communication. Astrocytes are known to exhibit receptors for most of these mediators. Activation of receptors by agonists results in a cascade of intracellular events, including the stimulation of protein kinases and subsequent phosphorylation of substrate proteins. To study the regulation of astrocytic functions , we analyzed intracellular target phosphoproteins of extracellular signals, in cultured astrocytes using 2-dimensional polyacrylamide gel electrophoresis. Previously, we described a novel substrate of protein kinase C (PKC) enriched in astrocytes, PEA-15 (J. Biol. Chem. 268:5911-5920). More recently, phosphorylation studies performed both in vitro and in vivo, followed by microsequencing, suggested that PEA-15 is also a substrate for calcium-calmodulin-dependent protein kinase II (CamKII), at a seryl residue different from the PKC site. Indirect immunofluorescent studies using antibodies raised against synthetic peptides corresponding to the two phosphorylated sites, demonstrated that PEA-15 is colocalized with microtubules in cultured astrocytes. To determine which form(s) of the protein associate(s) preferentially with tubulins, polymerized tubulin was separated from unpolymerized tubulin by extracting soluble proteins using triton-X 100. Analysis of the microtubule-associated fraction suggests that both PKC and CamKII phosphorylation of PEA-15 may be involved in regulating the binding of the protein to microtubules.

460.2

REGIONAL AND FOCAL ALTERATIONS IN NEUROFILAMENT AND TUBULIN IMMUNOREACTIVITY IN INJURED RAT BRAIN K.E. Saatman and T.K. McIntosh* Dept. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104.

Axonal injury resulting from rapid deformation of the brain is often characterized by the presence of discontinuous axons with swollen club-like endings (terminal clubs) and axons with regions of enlarged diameter. These pathological changes in axonal morphology have been described clinically, in cases of diffuse axonal injury, and in animal models of acceleration and fluid percussion brain injury. We have used antibodies to tubulin, tyrosinated tubulin, and both 68 kD and 160 kD neurofilament (NF) subunits to examine axonal swelling and terminal clubbing in the rat brain following lateral fluid percussion injury. In addition, regional alterations in the immunoreactivity (IR) of these cytoskeletal proteins were investigated. Adult male Sprague-Dawley rats were anesthetized (sodium pentobarbital, 50 mg/kg) and subjected to lateral fluid percussion brain injury of moderate severity (2.4 atm). Animals were sacrificed at 2, 3, or 7 days post-injury. Cortical neurons surrounding the injury site showed decreased tubulin and NF IR and disruption of their normal radial alignment when compared to the contralateral cortex and to sham-injured animals. Decreased tubulin IR was also seen in the CA3 region at all time points. At 3 days, typical axonal swellings and terminal clubs were readily visible in injured rats, using NF antibody labelling. Populations of these damaged axons were seen in the ipsilateral subcortical white matter, cortex, and thalamus. While these focal axonal swellings could be easily located due to their intense NF IR, neither tubulin antibody used in this study showed intense labelling at the regions of axonal damage. (Supported, in part, by NS26818 and NS08803)

460.3

MEC-12 ENCODES THE ACETYLATED ALPHA TUBULIN TBA-3 WHICH MEDIATES THE 15 PROTOFILAMENT MICROTUBULE FORMATION AND TOUCH SENSITIVITY IN THE NEMATODE C. ELEGANS. Shahid S. Siddiqui* and Tetsunari Fukushige. Lab. of Molecular Biology, Dept. Ecological Engg. Toyohashi Univ. of Tech. Toyohashi 441, JAPAN

In Caenorhabditis elegans, of the four alpha tubulin genes (tba-1 - tba-4), only the tba-3 gene encodes the alpha tubulin with a lysine residue at position 40,typical of the acetylated alpha tubulins. The McAb 6-11B-1 which is specific for the acetylated alpha tubulins (Piperno and Fuller, 1985) cytochemically stains the set of 15 pf (proto filament) containing MTs in the six touch receptor neurons and the axonal processes from many other neurons in the head, tail ganglia and the ventral nerve cord. In the mec-12 (u76) mutants that are touch insensitive and lack the 15 pf MTs in the six touch cells (Chalfie & Au, 1989), the 6-11B-1 staining is abnormal. However the mec-12(u76) mutants can be rescued by the tba-3 gene in the germline transformants, regaining the staining of touch neurons and touch sensitive behavior. The mec-12(u76) allele also harbors a RFLP change when its genomic DNA is probed with the tba-3 specific probe. A tba-3::lacZ fusion gene is expressed in the set of six touch cells, ventral cord motor neurons, and a few more neurons in the head and tail ganglia, consistent with the pattern of immunocytochemical staining with 6-11B-1 McAb.

We thank M. Hamelin and J. Culotti for cooperation and M. Chalfie for ideas and strains. This work was supported by the Ministry of Education and Science, Japan grant to SS.

460.4

A MICROTITER PLATE FORMAT FOR THE MEASUREMENT OF TAXOID-INDUCED POLYMERIZATION OF TUBULIN. E.D. Macdonald, S.Blumenthal,Z-P. Gu,K. Nyiredy and B.S. Glaeser*,PHYTOpharmaceuticals, Inc., San Carlos, CA 94070 and S. Mudumba Department of Pharmacology, University of the Pacific, Stockton, CA 95211.

We describe a 96-well plate assay for the measurement of tubulin polymerization based on the method described by Gaskin *et al.* (1974). Tubulin was purified from fresh bovine brain by two cycles of assembly and disassembly induced by heat, glycerol and GTP, followed by a third cycle with assembly induced by DMSO. For the assay (200ul), tubulin is diluted in buffer (0.1M PIPES, 1mM EGTA, 1mM $MgSO_4$). Prior to tubulin addition, each microtiter well receives 100ul of sample or reference compound in 10%DMSO/buffer. Optimal tubulin concentration is 0.75mg/ml. Polymerization is monitored kinetically at 340nm and at 37°C using a Thermomax plate reader (Molecular Devices Corp., Menlo Park, CA). The OD(340nm) correlates with compound concentration until saturation of binding sites. Compound potency is defined as the concentration of compound causing a response equal to 50% of that observed at saturation (SC_{50}). Results in the Table show good reproducibility. Compounds promoting the disassembly of tubulin (for example colchicine -10 to 200uM), inhibit taxol-induced polymerization in a dose-dependent manner. This assay may be useful for the characterization of compounds that affect tubulin assembly and disassembly such as specific MAPs and tau proteins.

COMPOUND	n	SC_{50} (uM)
Taxol	12	2.33 +/- 0.23
Cephalomannine	3	4.73 +/- 0.47
7-epi-10-deacetyltaxol	3	4.67 +/- 0.39

460.5

PHOSPHORYLATION AND CONFORMATIONAL CHANGES OF MAP2 DURING CAT BRAIN DEVELOPMENT B.M. Riederer*, E. Dráberová, P. Dráber, V. Viklický. *Institute of Anatomy, University of Lausanne, Lausanne, Switzerland; Institute of Molecular Genetics, Prague, Czech Republic.

Microtubule-associated protein 2 (MAP2) is essential for the stability of microtubules and for dendritic growth. In this study five monoclonal antibodies were used to analyze MAP2 during cat brain development. Antibody AP18 was the only one directed against a phosphorylation-dependent epitope on MAP2b and c. Dephosphorylation with alkaline phosphatase abolished most immunoreactivity with AP18 and indicated that MAP2 is phosphorylated throughout postnatal development, possibly by a cAMP-dependent kinase. The AP18 epitope was less susceptible to dephosphorylation in larger apical dendrites of cortical pyramidal cells and in some neurons of the upper layers of visual cortex. It is suggested that the AP18 epitope is less accessible to alkaline phosphatase, and that MAP2 conformation must differ within parts of neurons. Three antibodies, AP14, MT01, MT02, were directed against the central region of MAP2b. An immunocytochemical comparison revealed differences in the cellular distribution during early postnatal development of cat cerebellum, while at later ages, most antibodies stained similar neuronal elements. Antibody MT02 stained at early stages cell bodies and dendrites in cerebral cortex and cerebellum. Within the first postnatal month immunoreactivity became restricted to the distal parts of apical dendrites of pyramidal cells but staining was absent from perikarya and finer basal dendrites, and no immunostaining was found in cerebellum. MT02 immunoreactivity is independent of phosphorylation. It is concluded that immunohistochemical detection of MAP2 depends on its conformation, posttranslational modifications such as phosphorylation, or masking of sites by other proteins. Supported by grants of the Swiss National Science Foundation 31-33447.92 and 7TRPJ038608.

460.6

REGULATION OF NEURONAL CYTOSKELETAL PHOSPHORYLATION BY CATION FLUX. M. Mata*, , and D.J. Fink, Department of Neurology and GRECC, VAMC, University of. Michigan, Ann Arbor, MI 48105 and University of Lausanne, Lausanne, Switzerland.

We studied the state of carboxy terminal phosphorylation of neurofilament (NF) proteins, and of the microtubule associated tau protein in fetal brain aggregates in culture. Cultures containing rat telencephalic neurons and glia in defined medium were grown for 4 weeks until myelination occurred. The cultures were then treated with 20 μm veratridine for different times in order to trigger Na^+ influx. We used Western blot with antibodies SMI-31 (Sternberger Meyer) and N-14 (Boehringer Mannheim) to detect phosphorylated NFs, and AB 1991 (Chemicon) to determine total NF content independent of phosphorylation. Antibody tau-1 (Boehringer-Mannheim) was used to detect tau.

Beginning at 2 hrs of veratridine treatment, there was a marked decrease in phosphorylated NFs, with no change in total NF content, although a shift in mobility of total NFs detected by AB1991, consistent with dephosphorylation of NF-H. Veratridine treatment also resulted in multiple bands with more rapid electrophoretic mobility detected with the tau-1 antibody, consistent with dephosphorylation of tau.

Prolonged treatment with veratridine led to a decrease in total content of a variety of calpain substrates (NF, tau, GAP-43 and calcineurin) without affecting the level of other proteins that are not calpain substrates (membrane Na,K-ATPase α1 subunit).

These results suggest that sodium entry through voltage gated channels may regulate the dephosphorylation of cytoskeletal proteins while prolonged treatment may result in Ca^{++} entry and activation of calpains.

Supported by grants from the Zyma Foundation and the NIH.

460.7

PROPOFOL INDUCES NO CHANGES IN ACTIN IN THE CYTOSKELETON WHEN INTRACELLULAR CALCIUM IS DEPLETED BY MAPTA/AM. C. Eintrei*, A. G. Jensen, A. Sjölander. Departments of Anaesthesiology and Cell Biology, Faculty of Health Sciences, University Hospital, S-581 85 Linköping, Sweden.

The site of action of the intravenous anesthetic drug propofol (Diprivan®) is still uncertain. The intracellular action of propofol was studied on primary cultures of neurons from rat. Cytosolic free calcium changes in neurons were studied using Fura2 and a single-cell micro fluorometric method. Fluorescence microscopy was used to study the organisation of actin. An increase in the cytosolic free calcium concentration (ΔCa^{2+}) amounting to 244±83nM was seen shortly after addition of 3μg x ml^{-1} of propfol. When neurons were depleted of intracellular calcium by the presence of MAPTA/AM and stimulated by 3μg propofol no changes were seen in the actin organisation of cytoskeleton, as revealed by a fluorescence microscopy. Only 18 of 306 cells exposed to 3μg. ml^{-1} of propofol exhibited changes in the morphology. Of 250 untreated cells that served as controls, 21 showed morphological changes.

Propofol in concentration exceeding or equivalent to clinical relevant concentration induces changes in the morphology of cultured neurons from the rat. These changes are a concequence of the increase in cytosolic free calcium concentration seen after stimulation with propofol. Evidence of this is that no changes in actin organisation were observed when intracellular calcium was depleted.

460.8

CORTICAL INTERNEURONS EXPRESSING CALCIUM BINDING PROTEINS HAVE AN ERYTHROCYTE-RELATED MEMBRANE CYTOSKELETON. M. R. Celio*[1], B. M. Riederer[2], S. Lambert[3]. [1]Institute of Histology and general Embryology, University of Fribourg, CH-1705 Fribourg; [2]Institute of Anatomy, University of Lausanne, Lausanne; [3]Howard Hughes Medical Institute and Dept. of Biochemistry, Duke University, North Carolina USA.

Cortical interneurons have a non-pyramidal shape, receive their inputs mainly on the cell body and proximal dendrites, express a calcium-binding protein of the EF-hand family and are often surrounded by the "perineuronal net", a specialized extracellular matrix containing e.g. restrictin/janusin. We have found by immunohistochemistry that cortical interneurons immunoreactive for the calcium-binding proteins parvalbumin, calbindin D-28k and calretinin express the red blood cell isoform of spectrin and ankyrin ($ß_R$ spectrin [brain spectrin 240/235 B or α+β Splb] and ankyrin$_R$). The immunolabelling for the two cytoskeletal proteins in each nerve cell is restricted to the cortical portion of the cytoplasm, whereas the calcium-binding proteins occur dispersed in the whole cytoplasm. Ankyrin$_R$ and $ß_R$ spectrin can be found not only in the cell body but also underlying the membrane of the main dendrites, in decreasing concentration at least until the second dendritic ramification. In thicker sections the $ß_R$ spectrin-immunolabelling is reticulated, whereas the ankyrin$_R$ staining is punctuated. The study of the distribution of ankyrin$_R$ and $ß_R$ spectrin in relationship to restrictin/janusin reveals that the membrane cytoskeletal proteins are in register with the extracellular matrix protein. We suspect that calcium-binding proteins interact with the proteins of the membrane cytoskeleton, thus modulating the lateral mobility of integral membrane proteins and the adhesion of interneurons to the extracellular matrix.

460.9

Vilip-a 22 kD neuronal EF-hand Ca2+-binding protein from chick brain: regulation of its interaction with intracellular target molecules. **K.-H. Braunewell, S. E. Lenz and E. D. Gundelfinger*. Federal Institute for Neurobiology (IfN), Dept. of Neurochem./Mol.biology, P.O. Box 1860, D-39008 Magdeburg, F.R.G.**

Vilip (visinin-like-protein) (Lenz et al., 1992, Mol. Brain Res., 15, 133-140) is a member of a new family of neuronal Ca2+-binding proteins, such as visinin, recoverin/s-modulin or frequenin. The function of these proteins remains still unclear.

Vilip is expressed by a subset of neurons in the chicken brain and retina. The molecule is localized in the cellbody, dendrites and axons and seems to be associated with the cortical cytoskeleton. At least one of the four EF-hand structures of Vilip is able to bind Ca2+-ions, as revealed by mobility shift assays. The molecule is able to interact with membrane- and cytoskeletal cellular fractions in a Ca2+-dependent manner. In overlay assays several potential binding partners for Vilip have been identified. One of them is actin, a main component of the cortical cytoskeleton. Several potential phosphorylation sites exist in the EF-hand structures of Vilip. This raises the possibility that the ability of Vilip to bind Ca2+-ions and therefore to interact with intracellular target molecules in a Ca2+-dependent manner is regulated by phosphorylation. These results suggest that Vilip may act as a Ca2+-effectormolecule. It may transmit Ca2+-signals to the cytoskeleton, thereby affecting processes, such as neurotransmitter release, neurite outgrowth or synaptic plasticity, which are dependent on the dynamics of the cytoskeleton. *This work is supported by DFG*

460.10

SORTING SIGNALS FOR TRANSFERRIN RECEPTOR AND RETROVIRUS PROTEINS IN AXONAL AND DENDRITIC TRANSPORT: K. Kristensson*, K. Weclewicz and H. Garoff. Karolinska Institutet, Dept. of Neuroscience, S-17177 Stockholm, Sweden, Dept. of Molecular Biology, Karolinska Institutet, Huddinge, Sweden.

Rat embryonic dorsal root ganglia, spinal cord and hippocampal neurons in culture were infected with recombinant Semliki forest virus (SFV) containing the genes for the human transferrin receptor, the envelope or nucleocapsid proteins of human immunodeficiency virus type 1 (HIV-1) or Moloney murine leukemia (Mo-MLV) virus. All nerve cell types expressed the human transferrin receptor in their cell bodies, but not in their axons. In spinal cord and hippocampal neurons the receptor also occurred in the dendrites. A mutant form of the receptor, in which the amino acids 6-53 in the cytoplasmic tail had been deleted, appeared both in axons and dendrites. HIV-1 envelope protein was also exclusively localized to the dendrites, while the gag protein appeared both in axons and in dendrites. Co-expression of the envelope and the gag proteins resulted in a dendritic localization of the latter, indicating that the envelope protein can interact with and determine the intracellular sorting of the gag protein. Similarly, the Mo-MLV gag appeared in both axons and dendrites, but only in dendrites when co-expressed with the envelope protein. Heterologous co-expression of HIV-1 envelope and Mo-MLV gag proteins and vice versa did not influence the distribution of the gag proteins.

460.11

DENDRITIC TRANSPORT OF NEURAL BC1 RNA IN SYMPATHETIC NEURONS IN CULTURE. I. Muslimov[1], S. Perini[2], D. Higgins[2], H. Tiedge[1*]. [1]Fishberg Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029. [2]Department of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214.

Extrasomatic protein synthesis in postsynaptic dendritic domains may contribute significantly to long-term synaptic plasticity of nerve cells [see Steward and Banker (1992), Trends Neurosci. 15, 180-186, for review]. In this model, selected RNAs would have to be specifically sorted and targeted to dendritic microdomains. However, the mechanism of dendritic RNA targeting and transport has remained elusive.

Here we analyse the dendritic targeting competence of neural BC1 RNA. This short, non-translatable RNA has been identified in somatodendritic domains of neurons [Tiedge et al. (1991), Proc. Natl. Acad. Sci. USA 88, 2093-2097] where it may participate in transport- and/or translation-related processes. We have generated various radiolabeled BC1 sequences, including full length BC1 RNA as well as 5′ and 3′ segments, and microinjected them into somata of rat sympathetic neurons in primary culture. We observed a significant and specific accumulation of full length BC1 RNA in dendrites. In contrast, injected small nuclear RNAs, such as U4 and U6 RNAs, distributed to somata and nuclei. Random irrelevant RNA sequences did not exit somata to any significant extent. Transport of BC1 RNA was rapid: distal dendritic tips were reached in less than 2 h. The various structural subdomains in BC1 RNA showed significant differences in their dendritic targeting potentials. These results support the notion that BC1 RNA contains specific signaling elements that direct its sorting and targeting to dendritic domains. (Supported by NSF grant IBN-9210149 to H. T.)

SYNAPTIC STRUCTURE AND FUNCTION III

461.1

CODISTRIBUTION OF SYNTROPHIN-1 WITH DYSTROPHIN AND SYNTROPHIN-2 WITH UTROPHIN IN SKELETAL MUSCLE. M.F. Peters, N.R. Kramarcy, R. Sealock and S.C. Froehner*. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

Syntrophin is associated with different members of the dystrophin family of proteins, including dystrophin, short forms of dystrophin containing only the cysteine-rich and carboxy terminal domain, and utrophin (a postsynaptic dystrophin homolog at the neuromuscular junction (NMJ)). Two forms of syntrophin which share approximately 50% amino acid identity and are encoded by separate genes have been found in mouse tissues. The tissue expression pattern of syntrophin-1 mRNA mimics that of dystrophin, while the distribution of syntrophin-2 follows that of utrophin. Thus, Adams *et al.* (*Neuron* **11**:531) suggested that syntrophin-1 is associated with dystrophin and syntrophin-2 is associated with utrophin. To test this idea, we have prepared isoform-specific polyclonal antibodies using peptides unique to each isoform. In immunofluorescence on cryosections of rat diaphragm, anti-syntrophin-1 labeled both the NMJ and the non-junctional sarcolemma. In contrast, the anti-syntrophin-2 antibody labeled only the NMJ; labeling of the non-junctional sarcolemma was undetectable. Double labeling for syntrophins and dystrophin or utrophin revealed codistributions consistent with the predicted syntrophin-1/dystrophin and syntrophin-2/utrophin associations. We also examined the distribution of syntrophin-1 in the dystrophin-negative *mdx* mouse. Syntrophin-1 staining was absent from the sarcolemma of *mdx* muscle, presumably because its association with the membrane is dependent on the presence of dystrophin. However, the NMJ in *mdx* muscle retained syntrophin-1 staining. Thus, at the neuromuscular synapse, syntrophin-1 may be associated with some, yet to be identified member of the dystrophin family.

461.2

NEURAL REGULATION OF ACETYLCHOLINESTERASE GENE EXPRESSION WITHIN RAT MUSCLE FIBER COMPARTMENTS. C.Q. Vu, R.N. Michel, W. Tetzlaff and B.J. Jasmin.* Department of Physiology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

Nerve electrical activity and release of trophic factors are implicated in the accumulation of mRNAs encoding AChR subunits within the postsynaptic sarcoplasm (Witzemann et al, *J. Cell Biol.* 114: 125-141, 1991). We therefore studied the influence of these two components of neural function on the regulation of acetylcholinesterase (AChE) gene expression in mammalian skeletal muscle fibers. This was achieved by comparing the expression of AChE mRNA in adult rat muscle fibers after denervation or after selective blockage of the propagation of sciatic nerve action potentials with the sodium channel blocker tetrodotoxin (TTX). Chronic TTX superfusion of the sciatic using an osmotic pump and cuff delivery system causes a prolonged and reversible paralysis of target muscle fibers while maintaining the integrity of nerve-muscle contacts. Initially, the preferential localization of AChE mRNA within innervated versus non-innervated regions of skeletal muscle fibers was verified in microdissected fiber bundles of rat extensor digitorium longus (EDL) and diaphragm muscles using quantitative RT-PCR. In addition, *in situ* hybridization experiments using a synthetic oligonucleotide showed that AChE transcripts in junctional regions were confined to the area immediately beneath the postsynaptic membrane. These molecular procedures were then used to compare the expression of AChE mRNA in control EDL, plantaris and soleus muscles with that observed 10 days after sciatic nerve section or chronic inactivation with TTX. AChE mRNAs were substantially down-regulated after disuse in these three functionally distinct muscles. The extent of this down-regulation was similar after denervation and TTX paralysis. Our results suggest that the selective accumulation of AChE transcripts within the postsynaptic sarcoplasm is largely regulated by muscle electrical activity *per se* and not by the release of trophic factors (see abstract; Michel et al, 1994).

Supported by MRC and MDA of Canada to BJJ

461.3

INTERACTIVE EFFECTS OF CILIARY NEUROTROPHIC FACTOR AND DENERVATION ON ACETYLCHOLINESTERASE GENE EXPRESSION IN RAT SOLEUS MUSCLES. R.N. Michel, H. Sveistrup*, D.J. Parry and B.J. Jasmin. Department of Physiology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada.

Ciliary neurotrophic factor (CNTF) has recently been identified as a potential neurotrophic agent having an effect upon mammalian skeletal muscle fibers. For instance, daily injections of CNTF have been shown to partially counteract muscle wasting associated with denervation of the rat hindlimb musculature (Helgren et al, *Cell* 76: 493-504, 1994). Because CNTF enhances neuronal survival, axonal regeneration, and collateral sprouting of intact nerves, the possibility also exists that this particular trophic agent may promote reinnervation of muscle fibers by acting specifically upon molecular control mechanisms localized at the neuromuscular junction. We have addressed this issue by measuring acetylcholinesterase (AChE) transcript levels in control (right) and denervated (left) soleus muscles of animals treated with daily injections of CNTF (0.3 mg/kg s.c.). CNTF treatment attenuated the relative loss in muscle mass observed 7 days post-denervation thereby confirming previous findings (Helgren et al, 1994). Intra-animal comparisons of left and right soleus muscles in vehicle-treated animals showed a drastic reduction in AChE mRNA levels after denervation as also observed in untreated animals (see accompanying abstract; Vu et al, 1994). In contrast, intra-animal comparisons revealed an interactive effect of CNTF and denervation. In these animals, daily CNTF injections appeared to attenuate the reduction in AChE transcripts after denervation. These results suggest that CNTF may have subtle effects on the expression of genes encoding synaptic proteins, and as such, may contribute to maintaining muscle fibers in a condition that may be less refractory to reinnervation.

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CNTF was generously provided by Regeneron Pharmaceuticals Inc.

461.4

TROPHIC- AND ACTIVITY-DEPENDENT REGULATION OF SUCCINATE DEHYDROGENASE IN JUNCTIONAL & EXTRAJUNCTIONAL REGIONS OF RAT MUSCLE FIBERS. R.J. Campbell, B.J. Jasmin and R.N. Michel* Laurentian University, Sudbury; and University of Ottawa, Ottawa, Canada.

Information concerning the density and biochemical composition of mitochondria located in the postsynaptic sarcoplasm of skeletal muscle fibers is still rudimentary and conflicting. We therefore studied the distribution of succinate dehydrogenase (SDH) within this specialized, as well as other distinct compartments of soleus muscle fibers. We also examined the influence of the motor nerve on the regulation of this enzyme at the endplate using two models of short-term muscle inactivity namely, tetrodotoxin (TTX)-induced neural inactivation and denervation. Using quantitative microphotometric imaging techniques, we showed that the endplate region of soleus fibers displays SDH activity that is two- and three-fold higher than in subsarcolemmal (SS) and intermyofibrillar (IM) compartments, respectively, and that essentially all endplate SDH activity is of postsynaptic origin. Both denervation and TTX paralysis reduced SDH activity to a comparable extent (30%) in SS and IM compartments suggesting that expression of this enzyme is co-regulated in these two regions. Alternatively, denervation and TTX-inactivation led to distinct alterations at the endplate level. SDH activity at denervated endplates was reduced dramatically (60%) in comparison to controls whereas at endplates of TTX-inactivated counterparts this reduction was significantly less (35%). These findings suggest that SDH activity is regulated by distinct factors in innervated vs non-innervated regions of muscle fibers. The data are coherent with the view that activity is the key factor regulating expression of SDH in non-innervated regions of muscle fibers and that accumulation of SDH within the postsynaptic sarcoplasm is also subjected to local mechanisms involving neurotrophic factors.

Supported by NSERC (RNM); MRC and MDAC (BJJ); FICU and Bickell Foundation (collaborative grants).

461.5

PSD-95, A BRAIN-SPECIFIC RAT HOMOLOGUE OF THE DROSOPHILA *DLG* PROTEIN ASSOCIATES WITH POSTSYNAPTIC DENSITIES IN FOREBRAIN SYNAPTOSOMES. M.B. Kennedy*, C.A. Hunt, L.S. Schenker, and J. L. Rawlings Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

PSD-95 is a prominent, highly-enriched protein in postsynaptic density fractions prepared from rat forebrain. We recently cloned a cDNA encoding PSD-95 (Cho et al. (1992), Neuron **9**, p.929). The deduced sequence identified it as a homologue of the Drosophila tumor suppressor protein "discs-large," (*dlg*) a component of septate junctions which bind together developing epithelial cells in insect embryos and larvae. We have used affinity-purified antiserum against PSD-95 to confirm its association with the postsynaptic density in forebrain synaptosomes examined in the electron microscope. Synaptosomes were purified from rat forebrain homogenates under non-lytic or lytic conditions to preserve or rupture the presynaptic compartment, respectively. They were lightly fixed and embedded in thin agarose slabs as previously described (DeCamilli et al. (1983), J. Cell Biol **96**, p1355). The slabs were incubated with α-PSD-95 antiserum, followed by gold-labeled secondary Abs. Controls were incubated with non-immune IgG or with α-PSD-95 preabsorbed with recombinant PSD-95 protein. Prominent labeling of the PSD was observed in synaptosomes labeled with α-PSD-95. 92% of PSDs were labeled, with a median density of 10-15 particles per μm PSD. In contrast, 81% of non-immune IgG-incubated synaptosomes and 88% of preabsorbed α-PSD-95-incubated synaptosomes showed no labeling of the PSD. Even in lysed synaptosomes, no labeling of the presynaptic terminal or of the cytoplasmic face of the presynaptic membrane was observed. Lysed presynaptic terminals were labeled by Abs against synapsin I, a synaptic vesicle protein, indicating that serum was able to penetrate into the terminal. These data confirm the association of PSD-95 with forebrain postsynaptic densities.

461.6

A NEWLY-IDENTIFIED PROTEIN FROM THE RAT POSTSYNAPTIC DENSITY FRACTION, PSD-UP180, IS A MEMBER OF THE FAMILY OF LEUCINE-RICH REPEAT-CONTAINING PROTEINS. M.A. Apperson*, M.B. Kennedy, Division of Biology 216-76, Caltech, Pasadena, CA 91125.

We have identified a new protein, termed PSD-UP180, from the postsynaptic density (PSD) fraction of rat brain. PSD-UP180 has an apparent molecular weight of 180 KDal, and is enriched in the PSD fraction where it comprises about 1% of total protein. It was purified from the postsynaptic density fraction, and trypsinized. Five sequences of purified tryptic fragments were used to design degenerate oligonucleotide primers. One pair of primers amplified a brain-specific 1.1 kb PCR product from rat brain cDNA. Additional complementary clones were isolated from a rat brain cDNA library and the coding region was sequenced. It encodes a 195 kDal protein of 1495 residues. The cognate mRNA was detected only in the forebrain and not in the cerebellum or other tissues. The deduced a. a. sequence reveals a 354 residue domain with homology to the leucine-rich repeat (LRR)-containing proteins. The predicted N-terminus of PSD-UP180 contains 15 LRRs of23 a. a.'s each. The LRR motif has been identified in a wide variety of proteins most of which are membrane-bound. PSD-UP180 also has a cysteine-rich region spanning amino acids 486-546 and mucin-like threonine/proline-rich repeats spanning amino acids 825 to 940. These three domains are also found in the LRR-containing platelet receptor, glycoprotein Ibα (GP Ibα), which is a transmembrane protein that forms a complex responsible for platelet adhesion to blood vessels. Finally, PSD-UP180 also contains a single "GLGF" repeat at the C-terminus, spanning amino acids 1403 to 1490. GLGF repeats were first identified in another PSD protein, PSD-95. Other GLGF-containing proteins include several associated with intercellular junctions. The enrichment of PSD-UP180 in the PSD fraction and its forebrain-specific expression suggest a role for this LRR-containing protein in signal transduction or adhesion at forebrain synapses.

461.7

PROTEIN TYROSINE KINASES IN THE POSTSYNAPTIC DENSITY AND THE CTK-LIKE PROTEIN. S.Y. Lin*[1,2], K. Wu[1,2], J.B. Bolen[3], R.C. Penhallow[3], Y. Huang[4,5], J.L. Xu[1] and J.B. Black[1,2]. [1]Dept. Neurosci. and Cell Biol., UMDNJ/RWJ Med. Sch. and [2]Grad. Program in Physiol. and Neurobiol., Rutgers Univ., Piscataway, NJ 08854; [3]Dept. Mol. Biol., Bristol-Myers Squibb Pharm. Res. Inst., Princeton, NJ 08543; [4]Div. Neurosci., NYSPI and [5]Dept. Psychi., Columbia Univ., New York, NY 10032.

Recent evidence showing high levels of protein tyrosine kinase (PTK) activity in adult rat brain suggests that these enzymes, known to regulate cell growth and differentiation, are also functionally important during maturity. Despite their potential roles, however, little is known about specific PTKs and their substrates in the CNS. Since chemical synapses play pivotal roles in brain function, and since the postsynaptic density (PSD) is crucial for synaptic function, we examined this structure for PTKs. Our pilot studies revealed that there were 4 PTKs (M.W.= 166, 90, 66, 50 kDa) present in the cortical PSD of adult rat brain. Results of phosphoamino acid analyses confirmed tyrosine phosphorylation of these kinases. Recently, Ctk, designated as Csk-type kinase, was found to be predominantly expressed in the brain. Similarity in molecular weight and unique localization in the brain suggest that Ctk and the 50 kDa PTK in the PSD may be similar or identical. Indeed, we found that the 50 kDa PTK crossreacted with antisera to Ctk, suggesting that the two proteins are related. Consequently, we termed the 50 kDa PTK in the PSD Ctk-like PTK, and have employed the antisera to Ctk to begin characterizing the enzyme in synapses. Our results, obtained by Western blot analysis, suggest that the Ctk-like PTK was selectively localized to the PSD. Further, the protein was differentially expressed in the PSD isolated from hippocampus, cerebral cortex, cerebellum and olfactory bulb, implying region-specific function. Among the 4 brain regions examined, the PTK was expressed most abundantly in the hippocampus, an area of crucial importance for learning and memory.The identity of the Ctk-like PTK remains to be determined. Nonetheless, our present findings suggest that the enzyme may play important roles in synaptic function at the postsynaptic site.

461.8

A MULTITUDE OF SYNAPTOSOMAL PROTEINS POSSESS O-LINKED *N*-ACETYLGLUCOSAMINE. R.N. Cole* and G.W. Hart. Dept. of Biochem. and Molecular Genetics, Univ. of Alabama, Birmingham, AL 35294

Glycosylation of Ser/Thr residues with a single *N*-acetylglucosamine (O-GlcNAc) is a dynamic post-translational modification on a variety o nuclear and cytoplasmic proteins. This abundant form of glycosylation often occurs at sites of proline-directed protein kinases and appears to have a reciprocal relationship with protein phosphorylation. A current hypothesis views O-GlcNAc glycosylation as a mediator of reversible association/disassociation of multi-protein complexes.

Recent studies have demonstrated that the neuron-specific phosphoproteins, synapsins, neurofilaments, and now, tau proteins (Arnold et al., 1994 Soc. Neurosci. Abstr.), also possess the O-GlcNAc modification. As a first step towards elucidating a role of O-GlcNAc glycosylation in the nervous system, we assayed for the presence of O-GlcNAc in synaptosomes. Galactosyltransferase, a highly specific probe for terminal GlcNAc residues, labeled numerous synaptosomal proteins, ranging from 30-200 kD. More than 60% of the label associated with synaptosomal proteins was resistant to PNGase F (removes N-linked sugars), but sensitive to alkaline β-elimination (removes O-linked sugars). Analysis of these β-elimination products by gel filtration and Dionex HPLC confirmed that the O-linked sugars originated from single GlcNAc modifications. Taken together these data indicate that O-GlcNAc is abundantly present on synaptosomal proteins.

We also detected O-GlcNAc transferase activity in synaptosomal preparations. Thus, this dynamic form of glycosylation could play a role in the association/disassociation of multi-protein complexes regulating neurotransmitter release.

461.9

LIPASES AND PHOSPHOLIPASES IN SYNAPTONEUROSOMES FROM RAT, MOUSE, AND PIG BRAIN. L.A. Horrocks*, H.-C. Yang, and A.A. Farooqui. Dept. Med. Biochem., The Ohio State Univ., Columbus, Ohio 43210

Lipases (EC 3.1.1.34) and phospholipases (EC 3.1.1.4) are involved in signal transduction and neurodegeneration. Compared to homogenates, synaptoneurosomes from cerebral cortex of rat, mouse, and pig contain 20 to 30% of the diacylglycerol and monoacylglycerol lipase activities and 15 to 20% of the Ca^{2+}-independent phospholipase A_2 activity. Specific activities of the lipases were higher in pig and mouse brain synaptoneurosomes than in rat brain synaptoneurosomes. Ca^{2+}-independent phospholipase A_2 activities of synaptoneurosomal fractions from various animal species were similar. However, the specific activity of the Ca^{2+}-independent phospholipase A_2 acting on 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine was higher than that of the Ca^{2+}-independent phospholipase A_2 acting on 1-alk-1-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine. The occurrence of lipases and phospholipases A_2 in synaptoneurosomes from rat, mouse, and pig brain suggests that these organelles provide a useful model for studying signal transduction systems.

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461.10

A NOVEL INTERACTION BETWEEN SYNAPTOBREVIN AND SYNAPTOPHYSIN. L. Edelmann*, E. Chapman and R. Jahn, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510

Synaptobrevin and synaptophysin are integral membrane proteins of synaptic vesicles. It has recently been established that synaptobrevin is a key component of the exocytotic fusion complex. In contrast, the function of synaptophysin is still unclear but it has been proposed to form a channel which may play a role in transmitter release.

Here we report about a specific interaction between synaptobrevin and synaptophysin. Using a novel monoclonal antibody for synaptobrevin II, we performed immunoprecipitation experiments from synaptosomal detergent extracts. With this antibody, synaptophysin co-precipitated together with syntaxin and SNAP-25, the members of the putative fusion complex. When a monoclonal antibody for synaptophysin was used, synaptobrevin but none of the other proteins co-precipitated. The interaction between synaptobrevin and synaptophysin was further investigated using recombinant synaptobrevin-GST fusion proteins immobilized to glutathione beads. Synaptophysin bound to all isoforms of synaptobrevin, including cellubrevin. Our findings indicate that synaptobrevin participates in at least two independent sets of protein-protein interactions. Thus it is possible that the availability of the v-SNARE synaptobrevin for membrane fusion may be regulated by synaptophysin or synaptophysin-like proteins indicating that synaptophysin may function as an important regulator of exocytosis.

461.11

PROTEIN-PROTEIN INTERACTIONS BETWEEN ACTIN AND ACONITASE-LIKE PROTEINS IN THE PHOTORECEPTOR TERMINAL.

E.A. Estrella, G.W. Balkema*. Department of Biology, Boston College, Chestnut Hill, MA 02167.

Actin binding proteins are important in the dynamic role the actin molecule plays in the architecture of the cellular cytoskeleton. It is thought that many of these actin binding proteins interact with actin by mimicking certain amino acid sequences found within the actin molecule. Certain actin binding proteins are found concentrated at the presynaptic terminal of neurons. These proteins function in part in synaptic vesicle exocytosis. We have generated a monoclonal antibody, B16, that labels the ribbon structure of photoreceptors. Biochemical analysis of tissue homogenates shows that B16 also recognizes four proteins; 31, 49, 88, and 102kD on western blots. Sequencing of the 88kD protein revealed homology to aconitase. Aconitase is found in two forms; mitochondrial, a TCA cycle enzyme that catalyzes the conversion of citrate to isocitrate and cytoplasmic, an iron responsive element for translational regulation. Both forms of the enzyme have the citrate to isocitrate activity.

We decided to investigate the relationship between aconitase and actin. Amino acid sequence comparison of aconitase, actin and other actin binding proteins showed that aconitase contained two motifs implicated in the molecular mimicry of other actin binding proteins. We found that the putative actin binding sites were active by probing western blots of aconitase and aconitase containing homogenates with biotinylated actin (far-western). Next to determine if this actin-aconitase interaction was functional we found that aconitase contributes to the polymerization of actin into a filamentous network in low Ca^{2+}. Aconitase is also recognized by actin poly and monoclonal antibodies. These results suggest there maybe a functional as well as a physical similarity between aconitase and other actin binding proteins. (Supported by Boston College REG)

461.12

NEUROCHEMICAL CHARACTERISTICS OF A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM BOVINE RETINA.

Y. Huang, J.E. Adler* and K. Wu. Div. of Neuroscience, New York State Psych. Inst. and Dept. Psych., Columbia Univ., New York, NY 10032;

Considerable evidence suggests that the PSD, a disc-shaped proteinaceous structure attached to the inner surface of postsynaptic membrane, play a crucial role in synaptic function. We have previously demonstrated that the molecular characteristics of the PSD differ among the rodent cerebral cortex (CTX), cerebellum (CBL), hippocampus (HI) and olfactory bulb (OB). To further characterize the regional differences in PSD characteristics throughout the brain, we examined the adult bovine retina, a structure richly innervated by both peripheral and central afferents. Bovine CTX-PSD was studied in parallel for comparison. Morphologically, both the CTX-PSD and retina-PSD exhibited a typical disc-like structure. On average, the retina-PSD was shorter in length than the CTX-PSD and appeared to be more sensitive to Triton X-100, the nonionic detergent employed for isolation of the PSD. SDS-PAGE profiles of the retina-PSD and CTX-PSD were similar qualitatively, but were different quantitatively. Both bovine retina- and CTX-PSD contained several intrinsic protein kinase activities, similar to those found in rodent and canine PSDs. We conclude that bovine retinal PSD may provide a useful model to study the regulation of synaptic molecular architecture in the visual system in response to environmental stimuli.

PRESYNAPTIC MECHANISMS III

462.1

DIRECT RECORDING FROM IDENTIFIED SYNAPTIC TERMINALS IN THE RAT AUDITORY PATHWAY: EXAMINATION OF A GLUTAMATE AUTORECEPTOR.

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We are interested in the mechanism by which presynaptic glutamate receptors modulate transmission at excitatory synapses. A quantal analysis can give indirect information on changes in the probability of transmitter release, but to address the mechanism of such phenomena, direct presynaptic recording is desirable. The small size of most synaptic terminals in the mammalian CNS makes direct recording impossible, so we have developed a slice preparation of the rat brainstem, containing the superior olivary complex, within which a large synaptic terminal called the calyx of Held is localised.

Transverse slices were prepared from 6-12 day-old Lister Hooded rats. Whole-cell patch recordings were made from the principal neurones of the medial nucleus of the trapezoid body (MNTB). Dual component monosynaptic EPSCs were evoked by stimulation of the presynaptic axon. A fast component is mediated by AMPA receptors and a slow EPSC is mediated by NMDA receptors. In the presence of NMDA-receptor antagonists, perfusion of 10-50μM 1S3S ACPD or L-AP4 depressed the EPSC by up to 80% as has been observed in the hippocampus (Forsythe & Clements, J. Physiol. 429: 1-16, 1990). Analysis of the coefficient of variance for the evoked EPSCs showed that both drugs act at a presynaptic site to depress transmitter release. The putative mGluR antagonist α-methyl-4-carboxy-phenylglycine (1 mM) had no effect on the presynaptic depression.

Direct patch recordings from these presynaptic terminals have been achieved, using Lucifer yellow to confirm the identity of the recording site. Under current-clamp the action potential possessed a profound after-hyperpolarization and responded to depolarising current injection with a train of APs at up to 200 Hz (25°C). In voltage-clamp a tetrodotoxin-sensitive inward current was followed by a rapidly activating outward current which was sensitive to micromolar concentrations of 4-aminopyridine.

Supported by the Wellcome Trust.

462.2

$GABA_B$ AND METABOTROPIC RECEPTOR AGONISTS ATTENUATE EVOKED EXCITATORY POSTSYNAPTIC CURRENTS IN LATERAL PARABRACHIAL NUCLEUS (LBPN) NEURONS *IN VITRO*.

J.A. Zidichouski*, J.C. Easaw and J.H. Jhamandas. Department of Medicine (Neurology), University of Alberta, Edmonton, Canada.

The LBPN is a major recipient of autonomic information from more caudal regions of the brainstem. Previously, we have reported that postsynaptic excitatory (NMDA & non-NMDA) and inhibitory ($GABA_A$) amino acid receptors mediate neurotransmission within the LPBN. The present study shows that excitatory postsynaptic currents observed in the LBPN are attenuated by $GABA_B$ and metabotropic receptor agonists. These actions are independent of any apparent postsynaptic effect and suggest that the principal transmitters released in the LBPN can negatively influence transmitter liberation via a presynaptic mechanism of action.

LPBN neurons were recorded from 400 μm thick coronal brainstem slices using the whole-cell patch clamp technique. Monosynaptic excitatory postsynaptic currents (EPSC) were evoked via bipolar electrodes (@ 0.2Hz; duration 100μs; 10-50V) placed across the dorsal medial aspect of the LBPN. Bath application of the metabotropic receptor agonist, trans-1-aminocyclopentane-1,3-dicarboxylate (t-ACPD; 10 μM) reduced EPSC amplitude by 49.9±6.9% (n=9). No concomitant change to the resting current (V_{hold} = -60mV) or the current observed during voltage ramp (-120 to -40mV; 20sec) was observed. The $GABA_B$ agonist, baclofen (BAC; 1μM), similarly reduced the amplitude of the EPSC by 61.3±9.3% (n=2). Bath application of BAC at this dose did not activate postsynaptic $GABA_B$ receptors as no change was observed in resting current or voltage ramp-generated currents. These results provide evidence for the existence of two receptor-mediated presynaptic inhibitory mechanisms that reduce evoked transmitter release from terminals and thereby influence the excitability of LPBN neurons.

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462.3

PRESYNAPTIC METABOTROPIC GLUTAMATE RECEPTORS REDUCE SYNAPTIC TRANSMISSION IN THE NEONATAL RAT HIPPOCAMPUS

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Presynaptic modification of synaptic transmission occurs in the developing rat hippocampus. Developmental increases in population and unitary EPSPs are accompanied by decreased paired-pulse facilitation suggesting an increase in the probability of transmitter release. Facilitation changes may reflect developmental regulation of autoreceptors. Previous research has shown that the metabotropic glutamate receptor (mGluR) agonist, trans-ACPD, depresses CA3-CA1 EPSPs in voltage-clamped CA1 pyramidal cells from neonatal rats. This experiment investigates possible presynaptic effects of mGluR activation on synaptic function. Evoked field EPSPs (~1mV ampl.) were recorded from area CA1 in hippocampal slices taken from five week old rats. Paired-pulse stimulation (50ms ISI) was applied (0.1Hz) in order to measure changes in facilitation before and after iontophoresis (5min) of trans-ACPD. Recording was performed for 10min before and continued for at least 30min after drug application. EPSP amplitudes and paired-pulse facilitation ratios were averaged in ten minute epochs. Application of trans-ACPD (25mM) resulted in rapid decay of the EPSP to approximately 20% of baseline values and slowly recovered over the following half hour. ANOVAs revealed that the EPSP was significantly reduced ($p<0.01$) and paired-pulse facilitation was increased relative to baseline ($p<0.01$) suggesting a decrease in the probability of transmitter release. Post-hoc Scheffe tests showed that the reduction in the EPSP amplitude (predrug vs. 20-30min postdrug, $p<0.05$) outlasted the increase in facilitation, suggesting either increased duration or sensitivity of the postsynaptic effects. Experiments are underway to examine possible developmental shifts in the effects of presynaptic mGluR activation on CA3-CA1 synaptic transmission. Supported by NS31830 to TCF.

462.4

PRESYNAPTIC MODULATION OF GLUTAMATERGIC NEUROTRANSMISSION BY $GABA_A$ AND METABOTROPIC RECEPTORS IN THE HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA (hDBB) *IN VITRO*.

J.C. Easaw* and J.H. Jhamandas, Dept. of Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada T6G 2B7

The DBB, a basal forebrain cholinergic nucleus, participates in central autonomic regulation, theta rhythm generation, and memory mechanisms. Previously, we reported that postsynaptic NMDA and AMPA/kainate receptors mediate excitatory transmission in hDBB neurons. In the present study, we examined the mechanism whereby $GABA_B$ and metabotropic receptors modulate glutamatergic neurotransmission.

Whole cell patch clamp recordings were obtained from coronal slices (400μm) of the rat forebrain containing the hDBB. Bipolar stimulation electrodes (200 μs duration; 2Hz; range 10-70V) placed in the hDBB evoked excitatory postsynaptic currents (EPSCs). Bath application of baclofen (5μM), a $GABA_B$ receptor agonist, attentuated evoked EPSCs in the hDBB with no changes in resting current or input resistance measured during voltage ramps (-140 to -40 mV; 20 sec) (n=12). NBQX (1μM), an AMPA receptor-selective antagonist, also attenuated the EPSC. The metabotropic receptor agonist trans-ACPD (10 μM) also reduced evoked EPSCs with no concomitant changes in resting current or input resistance (n=13). Furthermore, both baclofen and trans-ACPD did not alter postsynaptic AMPA-mediated inward current, thereby confirming their presynaptic locus of action. These data suggest that glutamatergic synaptic transmission, which may mediate physiologically important inputs to the hDBB, can be modulated presynaptically by $GABA_B$ and metabotropic receptors.

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462.5

A NEW TECHNIQUE FOR COMPARING EVOKED AND SPONTANEOUS SYNAPTIC TRANSMISSION AT CORTICOSTRIATAL SYNAPSES. E. C. Tyler* and D. M. Lovinger. Dept. of Mol. Physiol. & Biophys., Vanderbilt Med. Sch., Nashville, TN 37232.

We would like to compare the effects of presynaptic receptor activation on evoked and spontaneous excitatory transmission at corticostriatal synapses. However, we have found that spontaneous EPSCs (sEPSCs) recorded in striatal slices are very small and infrequent. Thus, we have developed a new preparation in which we grow cortical explants in culture with dissociated striatal neurons. The cortical explants (~500 x 500 μm) extend processes and form synapses with the neighboring striatal cells. In this preparation we can record sEPSCs using whole-cell recordings in the striatal cells. In the presence of 200 nM TTX we can also record miniature EPSCs (mEPSCs). To record evoke EPSCs in the same striatal cells a bipolar metal electrode placed on the cortical explant is used to stimulate the tissue. 5 μM DNQX blocks mEPSCs, sEPSCs and EPSCs indicating that synaptic transmission in this system is glutamatergic. The amplitude of evoked EPSCs is inhibited by mGluR activation by *t*-ACPD and by adenosine. 5 μM *t*-ACPD produces a 9.8 ± 3.6% (n=9) potentiation, 50 μM *t*-ACPD produces a 31.3 ± 5.2% (n=7) inhibition, 75 μM *t*-ACPD, 46.9 ± 5.2% (n=3) inhibition, and 50 μM adenosine, 43.8 ± 8.6% (n=3) inhibition. Spontaneous transmission is also affected by mGluR activation with *t*-ACPD. 5 μM *t*-ACPD has no effect on the mean amplitude of sEPSCs and decreases the time between events by 21.8% (n=2), 50 μM *t*-ACPD produces a 12.3 ± 10.0% (n=7) inhibition of the mean amplitude and increases the time between events by 100.8±49.3%. 50 μM *t*-ACPD appears to have no effect on the mean amplitude of mEPSCs (n=3) and increases the time between events by 0 - 35%. We have successfully used this preparation to look at evoked and spontaneous transmission at the same synapse and have shown that mGluR activation affects both. (Supported by NS 30470)

462.6

RECEPTOR-EFFECTOR COUPLING MECHANISM UNDERLYING THE DESENSITIZING PRESYNAPTIC GABAb RECEPTOR-MEDIATED INHIBITION OF EXCITATORY SYNAPTIC TRANSMISSION. K. D. Phelan*. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

We previously proposed the existence of a desensitizing presynaptic GABAb receptor (DS-GABAb) mediating inhibition of glutamate release at a functionally distinct subset of excitatory terminals in which the postsynaptic membrane only expresses N-methyl-D-aspartate (NMDA) receptors (Phelan et al., Soc. Neurosci. Abstr. 18:792). In the present study, whole-cell patch clamp recordings of cultured embryonic hippocampal neurons were used to investigate and compare the receptor-effector coupling mechanisms utilized by this DS-GABAb receptor and the non-desensitizing (NDS-GABAb) receptor regulating transmission at conventional excitatory synapses which express postsynaptic NMDA and non-NMDA receptors.

Superfusion of Ba^{2+} (1mM) failed to block baclofen-induced activation of DS-GABAb or NDS-GABAb inhibition of spontaneous NMDA or non-NMDA receptor-mediated synaptic currents, respectively, indicating that neither receptor is coupled to a K^+ channel. Pretreatment of cell cultures with pertussis toxin (1.0 μg/ml, 24-48 hrs) selectively blocked DS-GABAb but not NDS-GABAb inhibition in some cultures suggesting receptor coupling to separate G-proteins. Pretreatment and co-superfusion of phorbol 12,13-dibutyrate (1 μM) blocked both DS-GABAb and NDS-GABAb receptor-mediated inhibition. Pretreatment with dioctanoylgycerol (1 μM) blocked NDS-GABAb but not DS-GABAb inhibition though receptor desensitization was blocked. These results suggest that presynaptic DS-GABAb and NDS-GABAb receptors may regulate the synaptic release of glutamate through distinct receptor-effector coupling mechanisms. This raises the possibility for selective modulation of transmission at functionally distinct glutamatergic synapses.

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462.7

PRESYNAPTIC CHARACTERISTICS OF TWO $GABA_A$ IPSCS IN RAT HIPPOCAMPAL CA1 NEURONS. R.A. Pearce* and S.D. Grunder. Departments of Anesthesiology and Anatomy, Univ. Wisconsin, Madison, WI 53706.

Acting via a $GABA_B$ autoreceptor, GABA limits its own release during trains of synaptic excitation, thus permitting induction of LTP and imparting a filter characteristic to the hippocampal circuit. It is not known to what extent these effects reflect modulation of the fast somatic or the slow dendritic $GABA_A$ components, nor even whether the two components are modulated similarly. In the present study we have characterized the frequency-dependent and $GABA_B$-mediated depression of the two components of $GABA_A$-mediated inhibition.

Monosynaptic IPSCs were recorded from adult rat hippocampal CA1 cells in the presence of APV (40 μM) and CNQX (20 μM). Sharp electrodes filled with CsAcetate (3M) or CsCl (3M) and QX-314 were used to record $GABA_{A,fast}$ and $GABA_{A,slow}$ in response to electrical stimulation of stratum pyramidale and stratum lacunosum-moleulare, as described previously (Neuron 10:189-200, 1993). It was found that paired-pulse depression of $GABA_{A,slow}$ follows the time course described previously for stratum radiatum responses, with a slow onset and maximal depression of 60% at approximately 160 ms. In contrast, $GABA_{A,fast}$ was depressed maximally by 40% at 5 ms, and declined at later time points, so that only 10% depression remained at 160 ms. $GABA_{A,fast}$ was also relatively resistant to the action of (-)baclofen, being maximally depressed by only 50% at 10-100μM, with no effect at 1 μM, whereas $GABA_{A,slow}$ was depressed by 50% at 0.3 μM, and completely eliminated at 1 μM. CGP 35348 only partially reversed the paired-pulse depression of $GABA_{A,fast}$ but eliminated paired-pulse depression of $GABA_{A,slow}$.

These experiments demonstrate significant differences between the presynaptic characteristics of the two components of $GABA_A$ inhibition, suggesting that different mechanisms may underly their use-dependent depression, and further supporting distinct functional roles for the two systems.

462.8

DOES MUSCARINE ACTIVATE K^+ CHANNELS AT PRESYNAPTIC TERMINALS IN HIPPOCAMPAL AREA CA1?
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Presynaptic inhibition of glutamate release in hippocampal area CA1 can be mediated by the activation of several different receptors, including $GABA_B$, Adenosine A_1, Neuropeptide Y_2, and a muscarinic acetylcholine receptor. The mechanism whereby these receptors act at presynaptic terminals cannot be recorded directly. Nevertheless, if they all act via an identical mechanism, their actions should be equally affected by manipulations that affect transmitter release. 4-Aminopyridine (30 μM) reduces inhibition mediated by the Y_2, A_1 and $GABA_B$ receptors, but not that caused by muscarine. Tetraethylammonium (1 mM) reduced the inhibition caused by muscarine. Here, we tested the hypothesis that muscarinic receptors activate presynaptic K^+ channels, by examining its actions when some K^+ channels are blocked by applying Ba^{2+}.

Extracellular recordings were made of population EPSP's evoked in area CA1 by stimulation of stratum radiatum in hippocampal slices (400 - 500 μm, 21 - 30 d.o. ♂ S.-D. rats). Dose-response curves to muscarine were performed in the absence and presence of 100 μM Ba^{2+}. Surprisingly, Ba^{2+} potentiated the inhibitory actions of muscarine throughout the dose-response curve. At the same time, in the same preparations, Ba^{2+} modestly reduced the effects of both baclofen and adenosine.

The observations are not consistent with muscarinic receptors inhibiting glutamate release by the same mechanism as A_1 or $GABA_B$ receptors. Indeed, the observed response is consistent with the activation by muscarine of a presynaptic K^+ channel. If this were the case, the block of presynaptic K^+ channels by Ba^{2+} would increase the resistance of the terminal, and activation of another K^+ channel by muscarine would bring the membrane potential closer toward E_K, thus further from threshold than under control conditions.

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462.9

CHARACTERIZATION OF AN ATP RECEPTOR ON A CHOLINERGIC PRESYNAPTIC NERVE TERMINAL. X.P. Sun and E.F. Stanley* Synaptic Mechanisms Section, NINDS, Bldg.36, Rm.5A27, NIH Bethesda, MD 20892.

ATP activates a cation channel on the presynaptic nerve terminal of the cholinergic calyx-type synapse in chick ciliary ganglion (Soc. N.S. Abst. 19:901). We have examined the pharmacology of this receptor using purinergic agonist and antagonists.

Calyx nerve terminals and their postsynaptic ciliary neurons were isolated and patch clamped (J Neurosci, 11:985). ATP induced fast-activating currents in both the calyx and neuron with reversal potentials of about 0 mV. The nerve terminal ATP current inactivated slowly whereas the neuron exhibited both fast and slow inactivating current components. The P_2-agonist, ATPγS and 2 MeS-ATP mimicked ATP while the P_1 and P_{2x} agonists, adenosine and β,γ-methylene ATP were ineffective. The P_2-blockers, reactive blue and suramin, were ineffective on the nerve terminal and eliminated only the fast-inactivating current in the neuron.

Our results suggest that the slowly inactivating ATP conductance on this presynaptic nerve terminal is mediated by neither P_{2y} nor P_{2x}-type receptors but by an as yet unidentified receptor type. Since ATP is stored with ACh in secretory vesicles, this presynaptic ATP receptor may play a role in the feed-back modulation of transmitter release.

462.10

NICOTINE FACILITATES AND DEPRESSES GLUTAMATERGIC SYNAPTIC TRANSMISSION AT A CNS SYNAPSE *IN VITRO* D.S. McGehee*& L.W. Role Dept of Anatomy & Cell Biology in the Center for Neurobiology and Behavior, Columbia University, 722 W 168th St, NY, NY 10032.

Although habenular neurons from both chick and rat express nicotinic ACh receptors (nAChRs). Physiological studies demonstrate that these receptors are not involved in synaptic transmission. Autoradiographic studies localize many CNS nAChRs to terminal fields, including that of the habenula-IPN projection. We tested whether habenular nAChRs could play a role in modifying synaptic transmission *in vitro*. Explants of embryonic chick habenula were cultured with dissociated IPN neurons, a principle target of habenula neurons *in vivo*. Whole cell recording from IPN neurons located near habenula explants revealed robust, spontaneous and evoked synaptic activity that is blocked by a mixture of the glutamatergic antagonists, CNQX and APV, and not by the nicotinic antagonist, mecamylamine, demonstrating glutamatergic synapses at these habenula-IPN synapses. Stimulation of the habenular neurons via an extracellular stimulating electrode evokes postsynaptic currents (EPSCs). Application of a low concentration of nicotine (0.5μM) increases the amplitude of EPSCs, returning to baseline within 30-90sec, even in the continued presence of the nicotine. This concentration of nicotine also caused an increase in the frequency of spontaneous miniature EPSCs recorded in the presence of 5μM TTX, without affecting the amplitude of these currents. This supports the idea that the receptors responsible for nicotine-induced synaptic facilitation are located on the *presynaptic* cell.

In contrast, application of nicotine at 10-20μM increases the percent failure of the evoked EPSCs, possibly due to shunting of the invading action potential. The mechanisms and nAChR subtypes responsible for these nAChR-mediated modifications in synaptic transmission are not yet known. Supported by a grant from the Ctr for Tobacco Res. & NIH grants NS22061 to LR and NS09395 to DM

462.11

α-LATROTOXIN ENHANCES SYNAPTIC TRANSMISSION BUT DOES NOT PREVENT PRESYNAPTIC INHIBITION INDUCED BY ADENOSINE, BACLOFEN AND OPIOID PEPTIDES IN HIPPOCAMPUS. M. Capogna *, B. H. Gähwiler and S. M. Thompson. Brain Research Institute, University of Zurich, CH-8029 Zurich Switzerland

The black widow spider venom component α-latrotoxin (α-LTX) binds to the α-LTX receptor that is associated with presynaptic proteins, synaptotagmin, syntaxin, and Ca^{2+} channels. These proteins control the docking of synaptic vesicles at the active zones and regulate exocytosis. Spontaneous mEPSCs (in the presence of TTX and bicuculline) and mIPSCs (in the presence of TTX, CNQX, and AP5) were recorded by means of whole-cell voltage clamp in CA3 pyramidal cells of rat hippocampal slice cultures. Focal application of α-LTX (< 1nM) produced a 10-100 fold increase in the frequency of both basal mEPSCs and mIPCSs which persisted for at least 1 h. The α-LTX-induced activity was characterized by intermittent high-frequency bursts, and was only slightly affected by 100 μM Cd^{2+}. Furthermore, α-LTX (with or without Cd^{2+}) did not occlude the reduction in the frequency of miniature currents induced by 50 μM adenosine, 10 μM baclofen, and by 1 μM of the μ-opioid agonist FK 33-824. Therefore, these agents inhibit transmitter release by a mechanism that does not involve the modulation of the proposed α-latrotoxin/synaptotagmin /syntaxin/Ca^{2+} channel complex.

462.12

ACETYLCHOLINE RELEASE EVOKED BY ACTION POTENTIALS IN THE ABSENCE OF CALCIUM ENTRY IN FROG MOTOR NERVE; ANTAGONISM BY ADENOSINE. M.Watanabe, E.M. Silinsky*, R.S. Redman, J.K. Hirsh, R. Qiu, J.M. Hunt, S. Alford and R.C.MacDonald. Depts of Physiol., Pharmacol. and Biolog. Chem. Northwestern U. Chicago, IL 60611 and Evanston, IL 60208.

While the essential need for Ca entry through Ca channels is indisputable for normal secretion, the importance of voltage-sensitive Ca binding proteins as mediators of secretion in vertebrate nerve endings has been a subject of considerable controversy. The target site for adenosine, which has been implicated as a presynaptic mediator of neuromuscular depression, is likewise controversial. We decided to test the possibilities that: i) a voltage-sensitive Ca binding protein promotes phasic acetylcholine (ACh) release and ii) that the action of adenosine might be to impair the functioning of Ca binding proteins associated with the secretory apparatus. We delivered Ca via Ca-containing lipid vesicles (Ca liposomes) to motor nerve terminals in frog cutaneous pectoris muscle under conditions in which Ca entry cannot occur. Neurally-evoked ACh release (recorded as multiquantal end-plate potentials) was generated by Ca liposomes suspended in solutions with no added Ca and containing either a) Mg (1-3 mM), b) Mg (1-3 mM)+ Co(1 mM) or c) Mg (1 mM) + EGTA (1 mM). Adenosine (10-100 μM) inhibited ACh release generated by nerve impulses in the absence of Ca entry. Both electrophysiological controls and morphological studies using confocal laser microscopy suggest that the liposomes are interacting with the nerve terminal membrane and delivering their entrapped contents to the cytoplasmic milieu without leakage to the exterior of the cell. The results suggest that action potentials in vertebrate motor nerve endings can promote the synchronous, physiologically-functional form of ACh release in the absence of Ca entry provided the cytoplasmic Ca is elevated by Ca liposomes. Such evoked ACh release in the absence of Ca entry is antagonized by adenosine. (Supported by NIH Grants NS12782 and NS30795).

462.13

GENETIC EVIDENCE THAT MODULATION OF BOTH K AND Ca CURRENTS CONTRIBUTE TO α_2-ADRENOCEPTOR-MEDIATED INHIBITION OF HORMONE SECRETION. P.P. Lakhlani, D.M. Lovinger and L.E. Limbird*, Department of Pharmacology and Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232.

The α_2-adrenoceptor (α_2-AR), a member of the G-protein-coupled receptor family, inhibits neurotransmitter and hormone release in neuronal and non-neuronal systems. Point mutation of the highly conserved aspartate residue in the putative second transmembrane segment of the receptor to asparagine (D79N α_2-AR) results in loss of coupling of the receptor to potassium (K) current without any profound effect on the receptor-mediated inhibition of adenylate cyclase (AC) and calcium (Ca) current (*Science*, 257:977, 1992). We have utilized this unique D79N α_2-AR as a tool to examine the precise involvement of these effector systems in the inhibitory action of the α_2-AR. The effect of activation of stably transfected wild-type (D79 WT) and D79N α_2-AR on secretogogue-induced adrenocorticotropic hormone (ACTH) secretion from mouse anterior pituitary cells (AtT20 cells) was examined. The basal ACTH secretion was not significantly different in AtT20 cells expressing D79 WT and D79N α_2-AR. Treatment with the β-adrenoceptor agonist isoproterenol resulted in concentration-dependent stimulation of ACTH secretion which, again, was not significantly different in the two cell lines. However, activation of D79N α_2-AR by the maximal concentration of the α_2-AR agonist UK 14,304 resulted in significantly less inhibition of isoproterenol-stimulated ACTH secretion compared to that observed with D79 WT (39±9% vs 77±14%). Next the possible involvement of AC in the α_2-AR-mediated inhibition was examined. When ACTH secretion was stimulated by the cAMP analogue 8-Br-cAMP, D79 WT and D79N α_2-AR activation was still able to suppress the stimulated secretion. Together these results suggest that (1) only a part of the α_2-AR-induced inhibition is mediated by K current activation, and (2) modulation of AC plays a minor role in the α_2-AR-induced inhibition, implying that suppression of Ca current may be responsible for the remaining α_2-AR inhibitory component. (Funded by HL 25182 and NS 30470)

462.14

PRESYNAPTIC ENHANCEMENT OF EXCITATORY SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS BY β-ADRENERGIC RECEPTOR ACTIVATION. P.J. Conn* and R.W. Gereau IV. Department of Pharmacology and Program in Neuroscience. Emory Univ. Sch. of Med. Atlanta, GA 30322.

β-adrenergic receptor activation has effects in the hippocampus that might influence synaptic transmission, including potentiation of voltage-gated calcium channels and phosphorylation of synapsins. While direct activation of adenylyl cyclase with forskolin has been shown to potentiate transmission in area CA1, it is not known if the β-adrenergic receptor-induced increase in cAMP has similar effects. We now report that application of isoproterenol to hippocampal slices potentiated evoked excitatory postsynaptic currents (EPSCs) in CA1 pyramidal cells, and this response was potentiated in the presence of a phosphodiesterase inhibitor. Isoproterenol also resulted in the appearance of a late inward current that likely represents polysynaptic EPSCs. The potentiation of EPSC in response to isoproterenol was blocked by H89, an inhibitor of cAMP-dependent protein kinase. In addition, isoproterenol induced an increase in the frequency of spontaneous miniature EPSCs, but did not affect the amplitude of mEPSCs or currents elicited by direct application of AMPA. These results suggest that presynaptic β-adrenergic receptors may act to enhance synaptic transmission in area CA1 via activation of cAMP-dependent protein kinase.

462.15

NORADRENALINE PRESYNAPTICALLY INHIBITS THE EPSC OF SYMPATHETIC PREGANGLIONIC NEURONS. T.MIYAZAKI, H.KOBAYASHI, S.MOCHIDA* and T.TOSAKA Dept. of Physiol., Tokyo Medical College, Tokyo 160, Japan

Sympathetic preganglionic neurons (SPNs) in spinal cord thin slices from P7 to P12 rats were identified by DiI preinjection to the superior cervical ganglion. Under the whole-cell configuration of slice-patch method, we recorded a monosynaptic EPSC in the SPNs evoked by stimulation at the nucleus intercalatus, in the solution containing strychnine (5μM) and bicuculline (10μM). The EPSCs were abolished by kynurenate (2mM) or CNQX (10μM). Noradrenaline (NA) inhibited reversibly the EPSCs in about 80 % neurons tested. The inhibitory effect was dose-dependent, reaching maximum at 10μM. However, at a higher dose of NA (500μM), about 50 % of EPSC was remaining. NA did not induce any appreciable shift of the reversal potential of the EPSCs, which was about zero mV. Glutamate current generated by pressure application to the soma was not affected by NA, though the simultaneously recorded EPSC was inhibited. NA effect completely disappeared in the phentolamine (10μM) solution. The results suggest that NA affected the presynaptic terminal to inhibit the release of glutamate through activation of the alpha-adrenergic receptors.

462.16

EFFECT OF PERGOLIDE ON ENDOGENOUS AND EXOGENOUS L-DOPA METABOLISM IN THE RAT STRIATUM: A MICRODIALYSIS STUDY. S. Dethy, M.A. Laute, A. Luxen, J. Hildebrand and S. Goldman*, Service de Neurologie and PET/Biomedical Cyclotron Unit, ULB-Hôpital Erasme, Brussels, Belgium.

Previous studies have shown that dopamine (DA) receptor agonists and antagonists may modulate release and metabolism of endogenous DA. We used a model of intrastriatal microdialysis in freely moving rats to study the effect of pergolide, a D_1 and D_2 DA receptor agonist, on the biotransformation of endogenous and exogenous L-DOPA.

Levels of L-DOPA, DA, DOPAC and HVA were measured by high-performance liquid chromatography. Pergolide (50 mg/kg, i.p.) caused a 47 % decrease in basal striatal extracellular (EC) levels of DOPAC and a 65 % decrease in basal striatal EC levels of HVA, 60 minutes and 120 minutes after injection, respectively. EC DA became undetectable 60 minutes after injection of pergolide. L-DOPA (100 mg/kg, i.p.), injected 2 hours after carbidopa, produced significant increase in EC levels of L-DOPA, DOPAC, HVA and DA in rats with and without local perfusion of 10^{-4} M pergolide. The DOPAC peak value was lower and was reached 60 minutes later in the group with pergolide.

This study demonstrates inhibitory effects of pergolide on release and metabolism of endogenous DA and on biotransformation of exogenous L-DOPA. These effects, probably mediated by DA autoreceptors, might have important pharmacological implications.

463.1

Cloning and expression of the P2X receptor reveals a new class of ligand-gated ion channel

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A cDNA encoding an ion channel gated by extracellular ATP (P2X receptor) was isolated from the rat vas deferens by expression cloning in *Xenopus* oocytes. When expressed in oocytes or in the HEK-293 cell line, this clone produces a receptor activated by nM concentrations of ATP, which shows strong desensitization. It is non-selective for cations and highly permeable to Ca^{2+}. It exhibits a P2X-type pharmacology, being activated by 2-methyl-S-ATP > ATP > αβ-methylene-ATP, and inhibited by suramin and pyridoxalphosphate-6-axophenyl-2',4'-disulphonic acid (PPADS). In outside-out patches excised from oocytes expressing the P2X clone, single channels of about 11 pS are activated by ATP. The cDNA consists of 1837 bp with an open reading frame predicting a protein of 399 amino acids. The protein sequence bears no homology to other ligand-gated ion channels and appears to have only two hydrophobic segments long enough to span the membrane. This receptor with novel structure probably represents the first member of a new class of ligand-gated ionotropic receptors.

463.2

ZN^{2+} POTENTIATES STEADY-STATE ATP ACTIVATED CURRENTS IN RAT NODOSE GANGLION NEURONS: A WHOLE-CELL AND SINGLE-CHANNEL STUDY. Jerry M. Wright* and Chaoying Li. LMCN, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD 20892

Zn^{2+} and ATP are normally present in serum at low levels and are released from some neurons during synaptic activity. In whole-cell recordings, 10 μM Zn^{2+} potentiated steady-state currents in rat nodose ganglion nerve cells by 18% in 0.5 μM ATP and 120% in 2 μM ATP. Fluctuation analysis of whole-cell currents suggested the mechanism was increased burst-duration. Single channel recording indicated the presence of several channel conductances. There was a primary conductance of 25-35 pS plus other low conductances present in the baseline noise. 10 μM Zn^{2+} did not alter the conductance of the 25-35 pS ATP-activated channel but did increase the probability of opening. Kinetics of the 25-35 pS channel were complex with fast openings and closings in addition to substate transitions and bursting. The response of the low conductance channels to Zn^{2+} was highly variable, suggesting multiple types of low conductance ATP-activated channels.

463.3

BIPHASIC MODULATION OF THE GLYCINE RECEPTOR BY Zn^{2+}. A.B. Bloomenthal, E. Goldwater, D.B. Pritchett+ and N.L. Harrison*.
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Zn^{2+} exerts a subunit-dependent inhibitory action on the $GABA_A$ receptor (Draguhn et al., *Neuron*, 5: 781-8). We have studied the effects of Zn^{2+} on the structurally related glycine receptor (GLY-R). In whole-cell patch clamp experiments on cultured rat spinal neurons, Zn^{2+} potentiated GLY-activated Cl^- currents at a concentration of 500nM, but blocked the current at a concentration of 50μM. The native GLY-R is an $\alpha_3\beta_2$ heteropentamer (Langosch et al., *PNAS*, 85:7394-8). Further experiments were conducted by transient expression of receptor in HEK 293 cells by transfection with cDNAs encoding the α2 and β subunits of the human GLY-R. When the α2 subunit alone was expressed, GLY-activated Cl^- currents were blocked by strychnine (100nM) and inhibited by picrotoxin (PTX) concentrations as low as 1μM, potentiated by Zn^{2+} concentrations between 0.2 and 2μM, and blocked by concentrations > 5μM. When the α2 and β subunits were expressed together, PTX sensitivity was abolished, as in spinal neurons. However, the addition of the β subunit had little effect on Zn^{2+} sensitivity. It seems likely that Zn^{2+} acts on the α subunit, while the β subunit has little effect on the Zn^{2+} interaction. The dual action of Zn^{2+} in modulating GLY-Rs suggests that two distinct binding sites may be present. The biphasic action of Zn^{2+} on the GLY-R is interesting in view of other reports of dual Zn^{2+} modulation of ligand-gated ion channels (Hollmann et al., *Neuron*, 10:943-954). The possible conservation of Zn^{2+} modulatory sites may prove significant in the case of the NMDA receptor, which also carries a binding site for glycine as an essential co-agonist.

We thank Heinrich Betz for the β subunit cDNA.

463.4

EFFECTS OF NICOTINIC ANTAGONISTS ON GLYCINE RECEPTORS. V.E. Wotring* and K.-W. Yoon. Department of Pharmacological and Physiological Science and Division of Neurosurgery, Saint Louis University Health Sciences Center, St. Louis, MO 63104.

Since certain nicotinic antagonists can inhibit GABA induced currents (Wotring and Yoon, Soc. Neurosci. Abs., 19, 1993), we pursued the possibility that these same agents might also inhibit glycine induced currents. Measurements of glycine evoked currents were made using the whole cell patch clamp technique with rat hippocampal cells grown in culture for 10 - 15 days; drug applications were made with a U-tube. In these experiments, GABA currents and glycine currents could be distinguished with the use of bicuculline and strychnine. Strychnine (1μM) blocked 63% ($p<0.001$, $n=4$) of the control glycine (100 μM) current while it did not alter a 30 μM GABA current ($p>0.2$, $n=4$). Correspondingly, bicuculline (1μM) decreased the GABA current but did not affect the glycine current.

Application of the nicotinic antagonist, d-tubocurarine at a concentration of 100 μM reduced glycine currents by 57% ($p<0.001$, $n=5$). 100 μM trimethaphan camsylate diminished glycine currents by 63% ($p<0.001$, $n=8$). Nicotinic antagonists that we have previously found to not significantly affect GABA currents had unexpected effects on glycine currents. 100 μM dihydro-β-erythroidine blocked 51% ($p<0.001$, $n=5$) of the glycine current. Application of 1 mM mecamylamine resulted in a nearly complete blockade (89%, $p<0.001$, $n=6$) of glycine current. However, 1 mM hexamethonium did not alter the control glycine current ($p>0.2$, $n=6$).

The ability of these nicotinic antagonists to block glycine and GABA currents foreshadows structural similarities among these ligand-gated channels. However, the differential effect of mecamylamine on GABA and glycine currents suggests complex variances in the mechanisms of antagonism.

463.5

CLONING OF A cDNA FOR A GLYCINE-BINDING PROTEIN. K. N. Kumar*, K. K. Babcock, P. S. Johnson, X. Chen, A. Riegel, and E. K. Michaelis. Department of Pharmacology and Toxicology and The Higuchi Biosciences Center, Univ. of Kansas, Lawrence, KS 66045

Four proteins isolated from rat brain synaptic membranes have molecular sizes of 67-70, 53-58, 41-46, and 31-36 kDa and contain binding sites for NMDA, glutamate, 2-AP5, TCP, and glycine (Kumar et.al., *Nature, 354*, 70, 1991). A 40-42 kDa glycine-binding protein has been isolated by affinity chromatography on 5,7-dichlorokynurenate (5,7-DCKA) columns (Babcock et. al. *Neurosci. Abs., 20*, 1994). Polyclonal antibodies raised against the 40-42 kDa proteins labeled a 60 kDa protein in synaptic membranes. These antibodies were used to screen a rat hippocampal cDNA library in λZAP vector and an ~1.9 kb clone expressing the antigenic protein was identified. The open reading frame of this clone describes a unique protein of 470 amino acids (52.8 kDa). Northern blot analyses of brain RNA revealed hybridization of the cDNA probes to transcripts of ~2.0 kb. RNA levels were expressed approximately equal in hippocampus, cerebral cortex, and cerebellum. E.coli transformed with the pBS vector carrying the 1.9 kb insert expressed a fusion protein that was recognized by the anti-glycine binding protein antibodies. The fusion protein was purified from IPTG induced E.coli extracts using a 5,7-DCKA-derivatized column. The purified protein exhibited two binding sites for [^{3}H]glycine with estimated K_D's of 124 nM and 8.1 μM. The binding of glycine was displaceable by D-serine, 5,7-DCKA and HA-966. These results indicate the successful cloning of a new class of protein that may belong to a different subtype of brain NMDA receptors [Supported by grants AA 04732 and DAAL03-91-G-0167 and an unrestricted grant from Parke-Davis].

463.6

IMMUNOHISTOCHEMICAL STUDIES OF THE DISTRIBUTION IN RAT BRAIN OF A GLYCINE-BINDING PROTEIN. R. Pal*, K. T. Eggeman, K. N. Kumar, A. E. Allen, and E. K. Michaelis. Dept. Pharmacol. Toxicol. and Higuchi Biosciences Center, Univ. of Kansas, Lawrence, KS 66045

An 40-42 kDa protein that has binding sites for glycine, D-serine, HA-966, and 5,7-dichlorokynurenic acid was purified from rat brain synaptic membranes [Babcock et al., *Neuroscience Abstr., 20*, 1994]. Polyclonal antibodies were developed against proteins of 41-43 kDa size. These antibodies reacted specifically with an 60 kDa protein in synaptic membranes (Western blot analyses). Immunohistochemical studies in rat brain were performed using the antiserum to the 41-43 kDa proteins. The results of these studies indicate that in detergent-permeabilized brain sections, the pyramidal neurons of the cerebral cortex and the hippocampus exhibit strong immune reactivity with the anti-glycine-binding protein antiserum. Most heavily labeled were the pyramidal neurons, including their proximal dendrites, in the CA2 region of the hippocampus. The granule cells of the cerebellum and the dentate gyrus were also strongly labeled. A comparison between the labelling observed with the antisera to the 63-70 kDa glutamate-binding protein, the 53-58 kDa CPP-binding protein, and the glycine binding protein indicates that all three antibodies label some of the same populations of neurons with only small differences observed in the pattern of labeling. The antibodies raised against these three proteins may be useful probes for the determination of the distribution of a subpopulation of glutamate and NMDA-like receptors in brain. [Supported by grants AA 04732, DAAL03-91-G-0167 and HD 02528].

463.7

PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION OF A SYNAPTIC MEMBRANE GLYCINE-BINDING PROTEIN. K. A. Babcock, K. T. Eggeman, X. Chen, and E. K. Michaelis*. Dept. Pharmacol. Toxicol. and Higuchi Biosciences Center, Univ. of Kansas, Lawrence, KS 66045

A group of synaptic membrane proteins with sizes of 67-70, 53-58, 41-46, and 31-36 kDa has recognition sites for NMDA receptor ligands, such as glutamate, NMDA, CPP, TCP and glycine. It is possible that these proteins are breakdown products of the NMDAR1/R2 proteins or that they are distinct entities. An ~40 kDa protein that has binding sites for glycine was purified from rat brain synaptic membranes by affinity chromatography first through a matrix derivatized with 5,7-dichlorokynurenate (5,7-DCKA), then through one derivatized with 8-OH-quinoline. An 40-42 kDa protein was isolated and this protein bound [^{3}H]glycine in a strychnine-insensitive manner and with a stoichiometry of 1 mol glycine/mol protein. The glycine binding sites of the protein had a low affinity (3-8 μM) for glycine. D-Serine, 5,7-DCKA, and HA-966 inhibited glycine binding to the protein. Polyclonal antibodies developed against proteins of 41-43 kDa electroeluted from gels reacted specifically with an 60 kDa protein in synaptic membranes (Western blots). Treatment of solubilized synaptic membrane proteins with these antibodies led to the immunoextraction of approximately 60% of [^{3}H]CGP 39653-binding proteins. These observations indicate that a synaptic membrane protein of ~60 kDa size may be a strychnine-insensitive glycine receptor and that this protein is a component of a form of NMDA receptors. [Supported by grants AA 04732, DAAL03-91-G-0167, and a grant from Parke-Davis].

463.8

EXPRESSION OF HETEROMERIC SUBUNITS OF NMDA RECEPTOR IN THE CV-1 CELL LINE. A.E. Allen*, O. Hong, S. Tan, and E.K. Michaelis. Dept. of Pharmacology and Toxicology, Univ. Kansas, Lawrence, KS 66045.

A group of four smaller proteins (~66,55,42, and 33 kD) was isolated from brain synaptic membranes and shown to have ligand binding sites similar to those of NMDA receptors (Kumar et al., *Nature* **354**;70: 1991; Michaelis et al., *Ann NY Acad.* **654**:7; 1992). A ~66 kDa glutamate-binding (GBP) and the 55 kDa CPP-binding (CBP) protein were isolated from neuronal membranes and cDNA's for the GBP and CBP were recently cloned (Kumar et al., **354**;70: 1991; Johnson et al., *Molec. Pharm.* **41**:750; 1992). The present studies were designed to examine whether expression of these two proteins in mammalian cells, either singly or in combination, may lead to the formation of functional receptors. The African green monkey kidney cell line CV-1 was selected as a host for stable transfections with the two cDNA's. The cDNA for GBP was subcloned into pREP4 (pOHR4GB) and that for CBP into pREP9 (pOHR950) plasmid (Invitrogen). Each vector imparts a different antibiotic resistance in successfully transfected cells and the inserts are under the control of the RSV promoter. Following selection of cells transfected with one or both vectors by means of antibiotic resistance, the CV-1 cells were grown to confluency and subjected to Western and Northern blot analyses. An ~66 kD protein was detected in Western blots of cells transfected with pOHR4GB and an ~80 kD protein in cells transfected with pOHR950. The anti-CBP antibodies recognize a protein of ~80 kD size also in synaptic membranes. Northern blots are being used to examine the size of mRNA expressed in these cell lines and to document dual expression of the proteins in cells transfected with both vectors. Finally, all cells transfected singly or doubly will be examined for the expression of glutamate and CPP binding entities and for NMDA-sensitive ion channels. [Supported by grants GM07775 and DAAL03-91-6-0167]

463.9

AGONIST-INDUCED CURRENTS IN IDENTIFIED NEURONS OF THE RAT DORSAL HORN. H. U. Zeilhofer, J. T. Liebel, K. Brune* and D. Swandulla, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuernberg, D-91054 Erlangen, Germany.

Neurons within the dorsal horn of the spinal cord play a critical role in sensory information processing. In the present study, we have investigated ionic currents elicited by external application of the excitatory neurotransmitter L-glutamate and of the inhibitory neurotransmitters glycine and GABA.

250 µM thick transverse slices were cut from the spinal cord of 7 to 14 day old rats. Agonist-induced currents were recorded from visually identified neurons in the dorsal horn of the rat spinal cord (lamina III to V) using the tight seal whole cell configuration of the patch clamp technique.

Currents elicited by application of 100 µM L-glutamate showed a strong outward rectification in the presence of 1 mM Mg^{2+}, indicating that a large portion of the current was through NMDA receptor channels. Currents induced by 100 µM glycine displayed a transient and a maintained component. Both components reversed at the chloride equilibrium potential. Neither run down nor wash out of the current could be observed with recordings that lasted upto 90 minutes. This indicates that the current was through chloride channels that are directly gated by glycine. GABA (10 µM) at a holding potential of -80 mV elicited inward currents which showed only a steady state component. Currents elicited by GABA displayed a rapid run down within 10 minutes of whole cell recording and were blocked by the $GABA_B$ receptor antagonist CGP 35348 (200 µM) These findings indicate that GABA induced currents depended on the activation of $GABA_B$ receptors and on a readily diffusible intracellular molecule.

Supported in part by a grant from the Hildegard Doerenkamp-Gerhard Zbinden Foundation to HUZ and DS.

463.10

NEURONAL GABA AND GLYCINE RESPONSES STUDIED WITH GRAMICIDIN PERFORATED PATCH RECORDING. N. Akaike*. Dept. of Bio-Plasticity, Kyushu Univ. Fac. of Med., Fukuoka 812, Japan.

The conventional whole-cell recording of the patch-clamp technique has a great advantage over other electrical recording methods in that it could perfectly control the extra- and intracellular concentrations of ions such as Na^+, K^+, Ca^{2+} and Cl^-. However, this advantage disrupts the physiological ionic environment inside cells. Thus, various Cl^--mediated responses, γ-amino butyric acid (GABA) and glycine (Gly), have been recorded only under an artificial conditions. Subsequently, measurement of the normal concentration of intracellular Cl^- ($[Cl^-]_i$) has been much hampered. We have recently developed a "gramicidin perforated patch recording", a novel method of patch-clamp technique that enables analysis of membrane currents with intact $[Cl^-]_i$. We here report that by applying this method to acutely dissociated CNS neurons of the rat, we could analyze the GABA- and Gly-induced membrane currents in the normal $[Cl^-]_i$ environment.

463.11

CHARACTERIZATION OF A STABLE CELL-LINE EXPRESSING A RECOMBINANT $GABA_A$ RECEPTOR SUBTYPE WITH ω_1 PHARMACOLOGY. F. Besnard, P. Granger, P. Avenet, H. Depoortere, S.Z. Langer* and D. Graham, Synthélabo Recherche, 31, ave. P.V. Couturier, 92220 Bagneux, France.

The $GABA_A$ receptor is a pentameric protein composed of various combinations of subunits α_1 - α_6, β_1 - β_3, γ_1 - γ_3. At least one α, one β and one γ are necessary for a fully functional $GABA_A$ receptor exhibiting ω (benzodiazepine) pharmacology. In the present report we describe the development of a stable cell-line expressing a recombinant $\alpha_1\beta_2\gamma_2$ construct of the rat $GABA_A$ receptor. Following transfection and selection in G418 several clonal cell-lines were isolated and tested for [^{3}H]-muscimol binding and [^{3}H]-flumazenil binding activity. Among these cell-lines one exhibited 260 fmol of [^{3}H]-flumazenil binding sites per mg membrane protein and was selected for further characterization. Equilibrium saturation analysis revealed a K_d value of 0.93 nM for [^{3}H]-flumazenil in good agreement with the K_d values of [^{3}H]-flumazenil using transiently-transfected $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor constructs and rat cerebellar membrane preparations. The K_i values of diazepam, CL 218872 and zolpidem to inhibit [^{3}H]-flumazenil binding to this cell-line were 18 nM, 210 nM and 40 nM, respectively. The concentration-dependency of GABA-induced chloride current in this cell-line gave an EC_{50} value of 6.3 µM as determined by the whole cell patch-clamp technique. GABA (1 µM)-induced chloride current was potentiated by diazepam and zolpidem with EC_{50} values of 36 and 160 nM, respectively. In conclusion, these properties indicate that a functional $GABA_A$ receptor subtype with ω_1 pharmacology has been stably expressed in this transfected cell-line.

463.12

Bridging the Nicotinic Acetylcholine Receptor Channel: Prolonged Inhibition Associated with Use-dependent Binding of a Bi-functional Inhibitor. Roger L. Papke*, Wayne Gottlieb, Ben Horenstein, and Michael Francis, Depts. of: Pharm., Chem., and Neurosci., U. of F., Gainesville, FL.

The symmetrical compound bis (2,2,6,6,-tetramethyl-4-piperidinyl) sebacate (BTMPS) is a use-dependent inhibitor of nicotinic AChR. The active portions of BTMPS are the tetramethylpiperidine (TMP) rings However, inhibition of neuronal nAChR by TMP is much more rapidly reversible than inhibition by BTMPS, suggesting that prolonged inhibition of neuronal nAChR may result from binding of the conjugated compound to multiple sites within the receptor. A compound related to BTMPS, but differing by four carbons in the length of the chain joining the piperidine rings, showed a dramatic loss of inhibitory activity.

The inhibition of muscle-type nAChR by BTMPS is rapidly reversible unless a neuronal beta subunit is substituted for the muscle beta subunit. This suggests that normal muscle-type receptors may contain a single site for BTMPS inhibition and that the expression of a neuronal beta subunit provides an additional site. The sequences of the neuronal beta subunits differ from the muscle beta subunit in the putative channel forming- and extracellular vestibule forming- domains. The muscle delta subunits are similar in sequence to the neuronal beta subunits in these regions, while the muscle gamma subunit more closely resembles the muscle beta subunits. Over-expression of the delta subunit (omitting gamma subunit RNA) has the same effect on the reversibility of BTMPS inhibition as substituting a neuronal beta subunit for the muscle beta subunit. Compared with muscle-type and delta-less, receptors these gamma-less receptors also: **1)** were more sensitive to mecamylamine ($p<.01$), **2)** showed a significant increase in divalent ion permeability ($p<.05$), and **3)** had greater inward rectification (measured by chord conductance ratios) ($p<.001$). These observations suggest that the domains which control noncompetitive inhibition may also regulate ion permeation properties.

463.13

KINETICS OF ACETYLCHOLINE (ACh) RECEPTOR-CHANNEL DESENSITIZATION: RELATIONSHIP TO $\{Ca^{2+}\}_i$ IN FURA-2 LOADED CULTURED RAT MYOBALLS. J. F. Fiekers* Dept. Anatomy & Neurobiology., Univ. Vermont Coll. Med. Burl., VT 05405.

Cholinergic nicotinic receptor desensitization was studied in cultured rat myoballs using the whole cell configuration of the patch clamp technique. Whole cell currents were recorded following prolonged applications (40s-2min) of ACh. The current reached a peak in sec and declined to a steady state level during agonist application. The rate of onset of desensitization was voltage dependent and accelerated by an increase in extracellular $\{Ca\}_o$. Simultaneous measurement of $\{Ca^{2+}\}_i$ using dual excitation microspectrofluorometry with fura-2-loaded myoballs indicated a significant permeability of activated ACh receptor channels to calcium. Agonist-induced currents as well as increases in $\{Ca^{2+}\}_i$ were blocked by prior exposure of myoballs to α-bungarotoxin. Acceleration of the onset of desensitization by calcium required external calcium during receptor activation. The $\{Ca^{2+}\}_i$ reached a peak following ACh application and declined slowly to basal levels. An increase in $\{Ca^{2+}\}_i$ produced by photolysis of cytosolic DM-Nitrophen-Ca^{2+} complexes also accelerated desensitization onset in the absence of external calcium. These results suggest that Ca^{2+} entry through ACh-activated channels is an important pathway for the regulation of the ACh receptor-channel by providing Ca^{2+} required for intracellular modulatory mechanisms. The technique of photoreleased calcium will be used to determine the minimum amplitude and duration parameters of the $\{Ca^{2+}\}_i$ required for the modulation of the ACh receptor and desensitization kinetics (Supported by MDA).

463.14

ASTROGLIAL AND NEURONAL ADRENOCEPTORS LINKED TO CHANGES IN $[K^+]_i$ AND $[Ca^{2+}]_i$ EXHIBIT DIFFERENT CHARACTERISTICS. M. Nilsson[1], H. Muyderman[1], P. S. Eriksson[1], E. Hansson*[1,3] and L. Rönnbäck[1,2]. Institute of Neurobiology[1], Department of Neurology[2], University of Göteborg and Department of Cell Biology, Faculty of Health Sciences, University of Linköping[3], Sweden.

Various adrenoceptor agonists were investigated for their effects on intracellular concentrations of calcium $[Ca^{2+}]_i$ and potassium $[K^+]_i$ in type 1 astrocytes and neurons. The calcium sensitive dye fura-2 and the potassium sensitive dye PBFI were used in mixed astroglial/neuronal primary cultures from newborn rat cerebral cortex. We found considerable differencies in the adrenoceptor-evoked changes in $[Ca^{2+}]_i$ and $[K^+]_i$ between the two celltypes. Noradrenalin (NA), phenylephrine (phe; α_1-agonist), clonidine (clon; α_2-agonist) and isoproterenol (iso; β-agonist) were all able to increase calcium and decrease potassium concentrations in the astrocytes with one exception; clon could not induce potassium responses. In neurons, NA and phe evoked calcium transients while clon and iso did not. NA and clon were able to elicit potassium reductions but no responses were seen after phe and iso stimulation The astrocytes responded in general with up to 4 different types of calcium response patterns while the neurons only exhibited 2 types. After adrenoceptor stimulation, no oscillations in intracellular calcium were seen in the neurons, but could on the contrary be observed frequently in the astrocytes. In neurons, the agonist-evoked reductions in $[K^+]_i$ always slowly (after 30-50 s) returned to baseline even in the presence of the agonists. On the other hand, in the astrocytes, the agonist-induced reductions in $[K^+]_i$ always persisted at the lower level in the presence of the agonists. The results point out the astroglial cellpopulation as potentially important receivers of adrenergic input. The results also show that astroglial and neuronal changes in $[Ca^{2+}]_i$ and $[K^+]_i$ exhibit different characteristics which might be of great importance for the understanding of the basic principles involved in the homeostasis of extracellular calcium and potassium concentrations.

463.15

INOSITOL (1,4,5)-TRISPHOSPHATE RECEPTOR TYPE 1: A THIRD ALTERNATIVELY SPLICED VARIANT. F.C. Nucifora Jr., S-H. Li, S.K. Danoff, G. Pearlson*, A. Ullrich, C.A. Ross. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave., Ross 615, Baltimore, MD 21205.

The Inositol 1,4,5-trisphosphate receptor (IP_3R) is an intracellular calcium channel involved in coupling cell membrane receptors to calcium signal transduction pathways within the cell. Several isoforms of IP_3Rs have been identified, which are expressed in many tissues with differing patterns of cellular expression. Another form of diversity that exists in the type 1 IP_3R is alternative splicing. Two alternatively spliced regions have previously been identified, one in the IP_3 binding domain (S1) and a second in the coupling domain between two consensus cAMP-dependent protein kinase (PKA) sequences (S2). We now report an additional alternatively spliced region that is 9 amino acids long, located in the coupling domain. PCR primers were synthesized to flank the alternatively spliced region and products were run on 6% polyacrylamide gels. Analysis of brain and peripheral tissues from human and rat demonstrated both the short and long S3 transcripts in all tissues. The long form predominates in most brain regions (except the cerebellum) while the short form predominates in peripheral tissues. The sequence of the long form in human appears to create a consensus protein kinase C phosphorylation site. Thus, as we have suggested in the past for S2 isoforms, the S3 isoforms may differ in their regulation by phosphorylation.

463.16

A GRAPHICAL METHOD FOR USING HILL PARAMETERS TO DISTINGUISH ALTERATIONS IN THE AGONIST BINDING SITE, ALLOSTERIC COUPLING BETWEEN SITES, AND CHANNEL GATING IN MUTATED, LIGAND GATED ION CHANNELS. C.E. Spivak*. Mol. Neurobiol. Br., NIH/NIDA IRP, Box 5180, Balto., MD. 21224.

Site directed mutants of ligand gated ion channels are usually characterized empirically by parameters of the Hill equation, n_H and K_{app}, in which n_H is said to reflect "cooperativity" of the receptor, and K_{app} the affinity of an agonist for its recognition site. More realistic models of receptor occupation and transduction, however, show that these parameters are always correlated, and that this correlation can provide further insight into the effects of a mutation.

The Hill equation will best fit a log concentration response curve when the inflection of the curve corresponds to K_{app} and the slope at that point is $n_H/4$. For any model, the theoretical response function, R(A), where A is agonist concentration, can be recast to correspond to a log concentration response curve by substituting $Z \equiv \ln(A)$ for (A). The agonist concentration at the inflection point ($d^2R/dZ^2 = 0$) is K_{app}, and the slope at this point is $n_H/4$. The computer program MLAB (Civilized Software, Bethesda, MD) symbolically found the first and second derivatives for the response functions and numerically evaluated their solutions for a range of model-dependent parameters.

A linear model, such as $2A + P \rightleftharpoons A + AP \rightleftharpoons A_2P$, in which P represents the receptor, is commonly assumed. When the response is current in a voltage clamped cell, and if one allows an allosteric factor to serially enhance binding of agonist, one sees graphically that, within variations on this theme, mutations that change the channel gating equilibrium have strong effects on n_H and only minor effects on K_{app}. Conversely, mutations that change the allosteric coupling strongly affect K_{app} with much smaller effects on n_H. Similar correlations occur with other models, permitting a first mechanistic interpretation of the Hill parameters.

463.17

MOLECULAR LOCATION OF A CHARGED 1,4-DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKER IN BRAIN LIPID BILAYERS. P.E. Mason and R.P. Mason*. Neurosciences Research Center, Allegheny-Singer Research Institute, Medical College of PA, Pittsburgh, PA 15212.

Small angle X-ray diffraction is a powerful tool for ascertaining direct structure information concerning the interaction of small molecules with model and biological membranes. In this study, we examined the structure of oriented brain phospholipid/cholesterol lipid bilayers in the absence and presence (1% by mass) of the charged 1,4-dihydropyridine (DHP) calcium channel blocker, amlodipine. This compound has been shown to improve memory consolidation and retrieval in laboratory animals (*Behav. Neural Biol.* 1993;60:221). The chemistry of amlodipine includes an electron-dense halogen atom which serves as a strong scattering source for the X-rays. The results of these experiments demonstrated that amlodipine occupies a discrete, time-averaged location in the hydrocarbon core adjacent to the phospholipid headgroup region. This location would facilitate both ionic bonding with the phospholipid headgroups and hydrophobic interactions with phospholipid acyl chains in the hydrocarbon core. These results also support an intrabilayer DHP binding site on the voltage-sensitive calcium channel which is accessed from the adjacent lipid phase. The diffraction data were correlated with partition coefficient measurements for amlodipine ($K_p > 10^3$) using model membranes with a cholesterol content which reproduced neural membrane preparations from Alzheimer's disease and age-matched control cortical brain tissue. The implication of these results for the molecular pharmacology of DHP calcium channel blockers will be discussed.

463.18

GLYCINE RECEPTOR β-SUBUNIT BINDS TO THE PERIPHERAL MEMBRANE PROTEIN GEPHYRIN G.Meyer, J.Kirsch, M.Ramming, U.Grünert*, H.Betz, D.Langosch. Max-Planck-Institute for Brain Research, 60496 Frankfurt, Germany.

The inhibitory glycine receptor (GlyR) of rat spinal cord is composed of ligand binding α-subunits, β-subunits and an associated tubulin-binding protein termed gephyrin. Gephyrin contributes to the formation of GlyR clusters in the postsynaptic membrane of spinal neurons, as antisense oligonucleotides interfering with gephyrin synthesis drastically reduce clustering. Here we show that recombinant gephyrin expressed in 293 kidney cells binds to immobilized native GlyR depleted of native gephyrin. To determine the GlyR subunit(s) involved in binding, purified GlyR polypeptides were electrophoretically separated, transfered onto nitrocellulose membrane and overlayed with recombinant gephyrin. Gephyrin bound to the GlyR β-subunit, but not to the α-subunit. The binding region was localized to the large cytoplasmic loop of the β-subunit using bacterially expressed fusion proteins of β-polypeptide fragments. The GlyR β-subunit and gephyrin are widely expressed in rat brain and co-distribute in regions lacking ligand binding α-subunits. This raises the possibility that the β-subunit/gephyrin core complex participates in the formation of ligand-gated ion channels other than the GlyR.

463.19

POINT MUTANTS OF THE INHIBITORY GLYCINE RECEPTOR α1 SUBUNIT ASSOCIATED WITH HEREDITARY HYPEREKPLEXIA DISPLAY REDUCED AGONIST AFFINITY AND EFFECT SINGLE CHANNEL CONDUCTANCES

B.Laube, N.Rundström, U.Ernsberger*, H.Betz and D.Langosch, Department of Neurochemistry, Max-Planck-Institute for Brain Research, Deutschordenstraße 46, D-60528 Frankfurt, Germany.

Native adult inhibitory glycine receptor (GlyR) is a pentameric ligand-gated anion channel protein composed of two different subunits (α1 and ß) in a stoichiometry of 3:2. Hyperekplexia (STHE) is a human autosomal dominant neurologic disorder associated with point mutations in the gene encoding the α1 subunit of the GlyR (Shiang *et al.*, (1993) *Nature genetics* 3, 351-358). Accordingly, an arginine residue bordering the channel forming transmembrane region M2 at position 271 is substituted to either leucine or glutamine.

Here, we determined the functional properties of these $\alpha1^{R271L}$ and $\alpha1^{R271Q}$ mutants upon heterologous expression in *Xenopus* oocytes and kidney 293 cells. When analysing homooligomeric channels, glycine concentrations eliciting half-maximal responses (EC_{50}) were approximately 100-fold increased in comparison to wild-type α1 GlyR. Further, we observed drastic decreases in maximal whole-cell currents (up to 90%) and a reduction in single channel main conductances. In addition, the glycinergic agonists ß-alanine and taurine did not elicit detectable currents but no significant change in affinity for the competitive antagonist strychnine could be observed. Coexpression of the mutants with wild-type α1 subunit in different ratios resulted in mixed channels with intermediate glycine affinities and maximal currents depending on the ratio of the expressed subunits. Interestingly, coexpression of the mutants with both α1 and ß diminished the effects of the mutations in comparison to coexpression with α1 alone.

Our results imply that the phenotype of STHE patients is the consequence of decreased agonist affinity and efficacy of the mutant GlyRs.

463.20

ISOLATION AND CHARACTERISATION OF THE MOUSE GLYCINE RECEPTOR β SUBUNIT GENE

M.Fischer, H.Betz* and J.Kuhse, Max-Planck-Institute for Brain Research, 60496 Frankfurt a.M., Germany.

The structure of the mouse glycine receptor β subunit gene was deduced from overlapping genomic lambda clones. Subcloning and sequencing of intron/exon boundaries led to the identification of ten exons which are spread over a distance of more than 50 kb. Comparison of the β gene with the previously characterized glycine receptor α subunit genes revealed high structural homology. However, there are some notable differences. Whereas the 5´non coding region of the α genes is encoded by the first exon, the respective region of the β gene is separated by one intron. Also, β transmembrane region 2 is encoded by two exons, a finding similar to the $GABA_A$ receptor β gene structure.

The different glycine receptor subunit genes exhibit developmental and spatial specific expression in brain and spinal cord. These expression patterns should reflect complex regulation events at the level of transcription. Therefore, we are interested in a detailed characterization of the promoter and upstream regions of the glycine receptor β gene. Transcription start points were mapped 1.6 kb upstream of the start codon. To identify regulatory elements, transient expression studies with reporter gene constructs in neuroblastoma and control cell lines are being pursued.

463.21

MUTATIONS OF GLYCINE RECEPTOR SUBUNIT GENES IN THE MOUSE MUTANTS *SPASTIC* AND *SPASMODIC*.

C.-M. Becker, C. Mülhardt, B. Saul, M. Fischer^, V. Schmieden^, C. Kling, P. Gass, D. Simon-Chazottes°, J.-L. Guénet°, H. Betz^, and J. Kuhse^ (SPON: European Neuroscience Association). Neurologische Klinik and Zentrum für Molekulare Biologie, Universität Heidelberg, 69120 Heidelberg, Germany; ^Max-Planck-Institut für Hirnforschung, 60528 Frankfurt/M., Germany; °Institut Pasteur, 75724 Paris, France.

The recessive mouse mutations *spastic* (*spa*, Chr 3) and *spasmodic* (*spd*, Chr 11) result in phenotypically similar motor disorders with symptomes mimicking strychnine poisoning. The adult isoform, $GlyR_A$, of the inhibitory glycine receptor is a multi-subunit protein composed of ligand-binding α1 and structural β polypeptides. We mapped the β subunit gene *(Glyrb)* near the *spastic* locus on Chr 3. In the CNS of *spa/spa* mice, aberrant splicing reduced the level of full-length β subunit transcripts. However, exon skipping led to the formation of truncated β subunit mRNAs where translational frame shifts resulted in premature stop codons. In the *spastic* mutant, a nucleotide insertion was uncovered within intron 5 of the *Glyrb* gene which may be causal for the aberrant splicing.

In the *spasmodic* mouse, we identified a point mutation in the α1 subunit gene which predicts an amino acid exchange in the large N-terminal domain of the encoded polypeptide. Upon expression in *Xenopus* oocytes, *spasmodic* α1 subunit channels displayed drastically reduced agonist responses while ligand binding was only marginally affected. This indicates that agonistic amino acids possess lower intrinsic activities at *spasmodic* glycine receptor channels.

LIGAND-GATED ION CHANNELS: ALCOHOL MODULATION

464.1

STUDY OF ETHANOL EFFECTS ON TM II DOMAIN MUTANTS OF THE NMDA RECEPTOR-CHANNEL.

K. Masood*, C. Wu and F. F. Weight, Lab. Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892.

The N-methyl-D-aspartate (NMDA) receptor is one of the key neurotransmitte-gated ion channels, as it is involved in important physiological and pathophysiological aspects of nervous system function. NMDA receptors have been found to be affected by psychoactive drugs, such as ketamine, phencyclidine and ethanol, possibly contributing to the behavioral effects of these drugs. Earlier we reported differential ethanol sensitivity of NMDA receptor subunit combinations (Mol. Pharmacol. 45: 324, 1994). However, the mechanism by which ethanol exerts its effect on NMDA receptors is unknown. The transmembrane II (TM II) domain of NMDA receptor subunits is thought to constitute the pore of the channel. Single amino acid mutants in and around the TM II domain of NR1 were coexpressed with NR2A in *Xenopus* oocytes and the effect of ethanol on NMDA activated ion current was tested using the two-electrode voltage-clamp method. The NMDA activated-current of the native subunit combination was inhibited 41% by 100 mM ethanol. The ethanol inhibition for charged mutants was 43% for E598Q and 42% for D599N. The inhibition for polar mutants was 39% for T602G and 38% for S605G. The observations suggest that these charged and polar amino acid mutations did not significantly change ethanol inhibition of NMDA-activated current flow through the mutated channels.

464.2

ETHANOL SENSITIVITY OF RECOMBINANT GLUTAMATE RECEPTOR EXPRESSED IN XENOPUS OOCYTES IS SIMILAR TO THAT OF NEURONS

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The Ethanol(EtOH) sensitive neuronal AMPA(α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) type glutamate receptors(GluR), have multiple subunits designated as GluR1-4(*Science*,249:1033-1037,1990.), or GluRA-D(*Science*,249:556-560,1990),which exist in two distinct "splice variant" versions known as the FLIP and FLOP (*Science*,249:1580-1585,1990). We have examined the EtOH sensitivity of the recombinant GluR1-3 FLOP receptor combinations expressed in *Xenopus* oocytes , using the two electrode voltage clamp technique. EtOH concentrations of 10-500 mM consistently inhibited currents activated in oocytes by 200uM kainate. The GluR1+2+3 heteromeric combination current exhibited the highest sensitivity to EtOH, with an IC_{50} value of 176mM. The response of GluR3 homomeric receptor subunit to EtOH had an IC_{50} value of 212mM. The sensitivity of GluR3 and GluR1+2+3 subunits to inhibition by EtOH is similar to the reported sensitivity of non-NMDA glutamate-activated current in hippocampal neurons(*Ann. Med.*,22:247-252,1990). By contrast, the FLIP forms of GluR expressed in human embryonic kidney (HEK) 293 cells are reported to be more sensitive to EtOH(*Neurosci.* Letters,159:83-87,1993) than non NMDA glutamate- activated current in hippocampal neurons or the FLOP forms of GluR in our experiments.

464.3

PREGNENOLONE POTENTIATION DOES NOT ALTER ETHANOL INHIBITION OF NMDA-ACTIVATED CURRENT FOR NMDA RECEPTOR SUBUNITS ε_4/ζ_1.

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The neurosteroid pregnenolone can modulate the activity of $GABA_A$, glycine and NMDA receptors. Ethanol inhibits NMDA-activated current but whether this inhibition is affected by the modulatory action of neurosteroids is not known. We investigated the effect of pregnenolone on ethanol inhibition of NMDA-activated current using the mouse NMDA receptor subunits ε_4/ζ_1 expressed in *Xenopus* oocytes. Membrane current was recorded using the two-electrode voltage-clamp technique at a holding potential of -70 mV. Ethanol inhibited the ion current activated by 10 μM NMDA (in the presence of 10 μM glycine) in a concentration-dependent manner over the range 10 to 250 mM; the IC_{50} was 243 mM. Ethanol, 100 mM, decreased the E_{max} of the NMDA concentration-response curve but did not affect the EC_{50} or Hill coefficient. Pregnenolone sulfate potentiated NMDA-activated current in a concentration-dependent manner over the concentration range 0.3-100 μM. The EC_{50} of the concentration-response curve was 4.9 μM and the slop was 1.2; these values were not significantly changed in the presence of 100 mM ethanol. The potentiation of NMDA-activated current by pregnenolone did not affect the percentage inhibition by 100 mM ethanol of NMDA-activated current. The results suggest that the modulatory action of pregnenolone does not affect the mechanism involved in ethanol inhibition of NMDA receptor subunits ε_4/ζ_1.

464.4

POTENTIATION AND INHIBITION BY ETHANOL (ETOH) OF NMDA-EVOKED WHOLE-CELL CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS.

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The effects of EtOH on whole-cell currents elicited by U-tube application of NMDA (50 μM) plus glycine (10 μM) were studied in cultured hippocampal neurons of the rat. NMDA-elicited whole-cell currents had distinct fast and slow decay components (*JPET* **263**:859, 1992). When applied together with agonists via the U-tube, EtOH (174 μM-174 mM) caused a decrease in the peak amplitude and in the amplitudes of the fast and slow components of the NMDA response. However, the inhibition of the NMDA response was not a monotonic function of EtOH concentration, which suggests the participation of an effect opposite to the inhibitory one. This irregular pattern was evident in concentration-response curves for individual neurons and for averaged data as well, indicating that this phenomenon was not an artifact of averaging. Additionally, an amplitude enhancement (rebound) was often evident after washing out EtOH [e.g., 2 min after washing out EtOH (522 μM), 4 out of 6 neurons showed a rebound]. These results suggest a dual effect of EtOH, the potentiation being countered to different degrees by inhibitory effects, and are qualitatively similar to those reported earlier for single channels (*FEBS Lett.* **247**:61, 1989). A later onset potentiation by EtOH of the NMDA response was also observed. When EtOH was continuously present in the bath and was simultaneously applied to the cells via the U-tube together with agonists, there was a decrease followed by a progressive enhancement of the NMDA peak current. These results suggest that in addition to the inhibitory effects of EtOH on NMDA responses, two types of potentiation, one rapidly and the other of later onset, can also be observed. (Support: USPHS Grant NS25296)

464.5

CHRONIC ETHANOL TREATMENT UPREGULATES NMDA RECEPTOR FUNCTION AND BINDING IN MAMMALIAN CORTICAL NEURONS.

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In the present study, we investigated the effects of chronic ethanol exposure on NMDA receptor-mediated increase in intracellular Ca^{2+} concentration ($\Delta[Ca^{2+}]_i$), and [^{3}H]MK-801 binding in cultured cortical neurons. NMDA increased the ($\Delta[Ca^{2+}]_i$) in a dose-dependent manner with an EC_{50} ~12 μM. Chronic exposure of the cortical neurons to ethanol (50 mM, 5 days) did not produce any changes in the cell protein, morphological appearance and the resting $[Ca^{2+}]_i$; however, it significantly enhanced the NMDA-mediated increase in $[Ca^{2+}]_i$. The EC_{50} value of NMDA was not significantly altered following chronic ethanol exposure, however its E_{max} value was increased by ~40%. This enhancement of NMDA response following chronic ethanol treatment was reversed by concomitant chronic exposure of the cortical neurons to the NMDA competitive (20 μM CPP) and non-competitive (1 μM MK-801) antagonists, but not by the non-NMDA receptor antagonist CNQX (10 μM), and the L-type calcium channel blocker, nitredipine (10 μM). In addition, chronic ethanol exposure increased the specific [^{3}H]MK-801 binding in cortical neuron membrane by ~20%. Taken together, these results suggest that chronic ethanol exposure upregulated the NMDA receptor function in cortical cultured neurons, and this increased NMDA receptor function is a NMDA receptor mediated process. The altered NMDA receptor function may be responsible for the ethanol induced tolerance and withdrawal syndrome.

464.6

EFFECTS OF CHRONIC ETHANOL TREATMENT ON GLUTAMATE RECEPTOR BINDING AND mRNA LEVELS IN THE ADULT RAT BRAIN

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The amino acid L-glutamate is a major excitatory neurotransmitter that is involved in many CNS functions including memory, learning , LTP, and synaptic plasticity. To determine if chronic ethanol altered glutamate receptor binding, male Sprague Dawley rats were pair fed a liquid diet for three weeks with 36% calories coming from either sucrose or ethanol. Binding studies were performed on 6 μm brain sections using the NMDA specific ligands[^{3}H]-MK801 and [^{3}H]-CGP 39653 (CGP), as well as [^{3}H]-kainate and [^{3}H]-AMPA. Autoradiographs of the brain sections were quantitated using NIH Image software. Ethanol significantly increased MK801 binding ($p<0.05$) in motor (114%) and somato sensory (113%) cortex, stratum oriens of hippocampal CA1 (114%), and CA3 (112%). CGP binding was significantly increased ($p<0.025$) in the amygdala (110%), stratum oriens (125%) and stratum radiatum (121%) of hippocampal CA1, CA3 (124%), CA4 (123%), and dentate gyrus (117%). No significant changes were seen with either of the non-NMDA receptor specific ligands, AMPA or kainate. To determine if changes in mRNA levels corresponded to the increase in NMDA binding, total RNA was isolated from the cerebral cortex, hippocampus, cerebellum, and striatum. Northern analysis were performed using probes specific for both the NMDAR1 receptor subunit and NMDARP-71 (a glutamate binding protein). The mRNA levels for both NMDAR1 and NMDARP-71 did not change following three weeks chronic ethanol treatment. (Supported by AA06069, AA09115, and T32AA07561)

464.7

DIFFERENTIAL SENSITIVITY OF NMDA- AND GABA-ACTIVATED ION CHANNELS TO ALCOHOLS OF VARYING HYDROPHOBICITY.

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Alcohols have been shown to inhibit responses mediated by the *N*-methyl-D-aspartate (NMDA) receptor-ion channel and to enhance responses mediated by the γ-aminobutyric $acid_A$ ($GABA_A$) receptor-ion channel. Using whole-cell patch-clamp recording in mouse hippocampal neurons, we investigated the relationship between hydrophobicity of various alcohols and their potency for inhibition of NMDA-activated current and potentiation of GABA-activated current. We found that for alcohols with hydrophobicity equal to or less than heptanol, potency in inhibiting NMDA-activated current was correlated with hydrophobicity, as was potency in enhancing GABA-activated current. However, the slopes of the plots of potency vs. hydrophobicity differed, so that whereas the potencies for inhibiting NMDA and enhancing GABA were similar for more hydrophobic alcohols, they differed greatly for less hydrophobic alcohols such as ethanol. These results do not support a common mechanism of action of alcohols, such as disruption of the lipid membrane, on both NMDA- and GABA-activated ion channels.

464.8

IS PHOSPHORYLATION OF THE γ2L SUBUNIT A NECESSARY OR SUFFICIENT REQUIREMENT FOR ETHANOL SENSITIVITY OF $GABA_A$ RECEPTORS?

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Recently, it has been proposed that phosphorylation of the γ2L subunit by protein kinase C is required for ethanol sensitivity of $GABA_A$ receptors (*FEBS Lett.* **313**: 113, 1992). Neurons in rat dorsal root ganglion (DRG) have been reported to contain the γ2L subunit of the $GABA_A$ receptor (*Soc. Neurosci. Abst.* **11**: 797, 1991). We studied the effect of ethanol on GABA-activated current in neurons freshly isolated from adult rat DRG using the whole-cell patch-clamp technique and confirmed the previous observation that $GABA_A$ current in these neurons is insensitive to ethanol (*Brain Res.* **507**: 332, 1990). We also studied the ethanol sensitivity of $GABA_A$ current after procedures to phosphorylate the PKC phosphorylation site on this subunit. We found that GABA-activated current remained insensitive to ethanol despite: (i) the extracellular preapplication of 5-20 nM phorbol 12-myristate 13-acetate (PMA); (ii) the intracellular application of PKC (0.247 U/ml); (iii) altering the intracellular Ca^{2+}/EGTA ratio to raise intracellular Ca^{2+} from 6 to 100 or 600 nM; and (iv) combining the intracellular application of 1 μM okadaic acid and 30 μM peptide 3 with the extracellular application of PMA. The observations raise a question of whether phosphorylation of the γ2L subunit is a necessary or sufficient requirement for ethanol sensitivity of $GABA_A$ receptors

464.9

ALCOHOL INHIBITION OF ATP-ACTIVATED CURRENT IS NOT MEDIATED BY ACTIONS ON MEMBRANE LIPIDS OR INTRACELLULAR PROTEINS. Chaoying Li*, Robert W. Peoples and Forrest F. Weight. Lab. of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse & Alcoholism, NIH, Bethesda, MD 20892.

We have previously demonstrated, using whole-cell patch-clamp recording techniques in isolated bullfrog dorsal root ganglion neurons, that for alcohols with a molecular volume equal to or less than 42.2 ml/mol, potency for inhibiting ATP-activated current was correlated with lipid solubility (order of potency: 1-propanol = trifluoroethanol > monochloroethanol > ethanol > methanol). However, despite increased lipid solubility, alcohols with a molecular volume equal to or greater than 46.1 ml/mol (1-butanol, 1-pentanol, trichloroethanol and dichloroethanol) were without effect on the ATP-activated current. In the present study we found that the amplitude of the current activated by 2.5 μM ATP was decreased by the extracellular application of 100 mM ethanol by 45±3% (n=8 cells), and the inhibition was not significantly altered by intracellular application of 100 mM ethanol in the patch pipette (n=7; P>0.05). Inclusion of GTP-γ-S (50 μM) or GDP-β-S (100 μM) in the pipette solution also failed to reverse the inhibitory effect of ethanol. These results suggest that alcohols inhibit the function of this neurotransmitter receptor by interacting with a small hydrophobic pocket on the receptor protein, rather than with membrane lipids or intracellular proteins.

464.10

ALCOHOLS EXHIBIT A CUTOFF EFFECT FOR THE POTENTIATION OF 5-HT_3 RECEPTOR-ACTIVATED CURRENT. P. Fan* and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892

The effects of several straight chain alcohols on the inward current mediated by 5-HT_3 receptors were investigated in rat nodose ganglion neurons. The whole-cell patch-clamp technique was used to record 5-HT-activated current. When applied together with 1 μM 5-HT, propanol, butanol and pentanol potentiated the amplitude of the peak 5-HT current in all cells tested (n=19), whereas ethanol facilitated 5-HT current in 2 out of 14 cells. Potentiation of 5-HT current by propanol, butanol and pentanol was concentration-dependent and fully reversible. EC_{50} values for propanol, butanol, and pentanol were 34.2 mM, 2.7 mM and 1.4 mM respectively. Methanol (10 to 500 mM), hexanol (0.5 to 50 mM) and octanol (0.05 to 5 mM) did not enhance 5-HT current. The data suggest that the action of straight chain alcohols on 5-HT_3 receptors exhibits a cutoff effect above 5 carbon atoms. This is consistent with a direct interaction of the alcohols with a hydrophobic pocket on the receptor protein.

ACETYLCHOLINE RECEPTORS: NICOTINIC DEVELOPMENT

465.1

CONTROL OF m1 MUSCARINIC RECEPTOR GENE EXPRESSION. S. Pepitoni, M.C. Steel, C.A. Harrington*and N.J. Buckley, Wellcome Laboratory for Molecular Pharmacology, University College London, London WC1E 6BT, U.K

G-protein coupled receptors are encoded by one of the most diverse gene families in the nervous system. As a step towards understanding the factors controlling GPR expression, we have determined the ontogenic expression patterns and promoter structure of members of the muscarinic receptor gene family.

RT-PCR and *in situ* hybridisation reveal two waves of expression of the m1 gene in the embryonic rat brain. Around E12, there is a transient and widespread wave of m1 gene expression over wide areas of neuroepithelium of the developing forebrain, metencephalon and myeloencephalon - only the forebrain expresses m1 receptors in adulthood. This transient expression is followed by a restriction of expression to those areas which express the gene in adulthood. Transcripts are detectable in cortex (E16) and hippocampus (E18) concomitant with differentiation whereas in striatum expression is not detected until E18; at this stage m1 is expressed over differentiated striatal cells but not over ventricular or intermediate zone cells. Receptor gene expression appears to precede cholinergic innervation. Thus it appears that adult patterns of expression are achieved by consecutive waves of activation and repression.

In order to identify *cis* regulatory elements directing these patterns of expression we have isolated a genomic clone form a pWE 15 rat cosmid library and mapped the upstream regions of the m1 gene. Transfection of this cosmid clone into a human neuroblastoma cells NB-OK 1 - a cell line that expresses an endogenous m1 receptor - followed by RT-PCR analysis using species specific primers demonstrated transcription of the rat m1 gene in transfected NB-OK 1 cells. 5'RACE and SLIC procedures were used to gain more 5'cDNA information. Hybridisation probes derived from RACE/SLIC sequences were then used to map the non-coding exons of the m1 gene and to characterise its promoter.

465.2

REGULATION OF THE β3 NICOTINIC ACETYLCHOLINE RECEPTOR GENE IN THE DEVELOPING CHICK CENTRAL NERVOUS SYSTEM

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β3 is a member of the neuronal nAChR gene family. Its expression has been analyzed in the chick nervous system by Northern blot, in situ hybridization and immunohistochemistry during development. In contrast to other subunits such as α4 or β2 that are widely distributed, β3 expression is very restricted in the CNS. The β3 mRNA and protein have only been detected in the ganglion and amacrine cells of the retina.

To identify DNA sequences responsible for the spatio-temporal specificity of its expression, 3 kb of the 5' flanking region of the β3 gene were cloned and assayed for promoter activity by fusion to a reporter gene (CAT or lacZ) in transient transfections of primary cultures of neural cells (Matter-Sadzinski et al.,1992,EMBO J. *11*, 4529-38). A 150bp fragment located just upstream from the transcription initiation site displays specific promoter activity and drives reporter gene expression in retinal ganglion cells. This suggests that the main regulatory elements are contained in this short region. Sequence analysis reveals the presence of classical promoter elements such as TATA and CAAT boxes, as well as several putative transcription factor binding sites. Disruption of one of them, an E-box (binding site for bHLH proteins), or its displacement relative to the CAAT box lead to a tenfold reduction in promoter activity, suggesting the involvement of a bHLH factor in the control of β3 transcription.

465.3

PHARMACOLOGICAL ANALYSIS OF α-BUNGAROTOXIN BINDING SITES IN THE DEVELOPING AND ADULT RAT SOMATOSENSORY CORTEX AND HIPPOCAMPUS. R.S. Broide*, D. Acevedo and F.M. Leslie. Department of Pharmacology, University of California, Irvine, CA 92717.

Past studies of the developing rat brain have demonstrated a unique, transient expression of [^{125}I]α-bungarotoxin (α-BTX) binding sites in the sensory regions of the cortex during early postnatal development. In our studies, we have also seen a transient expression of α-BTX binding within areas of the developing hippocampus. To examine whether this transient expression is associated with a change in the binding properties of the site with age, we have used quantitative autoradiography to characterize the pharmacological profile of [^{125}I]α-BTX binding sites in somatosensory cortex (SS1) and hippocampus of rats aged embryonic day 19, postnatal day 7 and adult. Animals were sacrificed and their brains removed, frozen in isopentane at -20°C and cryostat cut onto slides. Slide-mounted tissue sections were incubated with [^{125}I]α-BTX in the presence and absence of competing ligands, washed, dried and apposed to tritium-sensitive film. The resulting autoradiograms were analyzed by computer-assisted densitometry to quantitate radioligand binding in specific brain regions. Saturation studies revealed saturable [^{125}I]α-BTX binding sites in SS1 and hippocampus, with K_D values ranging from 0.5 - 1.5 nM. At all ages and in both brain regions the following order of affinity was observed: α-BTX > α-cobrotoxin > nicotine ≥ cytisine. At E19 and P7, competition curves for agonist inhibition of [^{125}I]α-BTX binding displayed Hill slopes of ≤ 1, whereas slopes of >1 were observed in the adult. In contrast, antagonist binding curves exhibited Hill slopes of ≤ 1 at all ages. These data indicate a maturation of [^{125}I]α-BTX binding sites during development, with the appearance of positive cooperativity of agonist binding in the adult. This change in the binding profile of agonists may be associated with the downregulation of [^{125}I]α-BTX binding sites in the adult.

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465.4

ONTOGENY OF MUSCARINIC RECEPTORS IN A GENETIC ANIMAL MODEL OF DEPRESSION. L.C. Daws, D.H. Overstreet, and G.D. Schiller, School of Biological Sciences, Flinders University of South Australia, Adelaide, 5001 Australia.

The Flinders Sensitive Line (FSL) rat, selectively bred for increased responses to an anticholinesterase, is also more senitive to directly acting muscarinic agonists than the Flinders Resistant Line (FRL) rat. The muscarinic supersensitivity in the FSL rats, which mimics that observed in depressed humans, occurs very early in development and seems to be regulated by recessive genes. The present study examined muscarinic receptor binding using the nonselective ligand QNB at various ages in the FSL and FRL rat to assess whether differences in muscarinic receptors could account for differences in pharmacological sensitivity. There were no differences in receptor concentration early in development (up to part-partum Day 19)), but by Day 32 and thereafter, the FSL rats exhibited significantly greater numbers of receptors in the hippocampus and the striatum. There were never any differences in the cerebral cortex and the differences in muscarinic receptor binding in the hypothalamus only emerged in the older adult (Day 120). Thus, these global differences in muscarinic receptors, while confirming previous reports in adult rats, are not able to account for differences in the pharmacological sensitivity between the lines; the FSL rats are more sensitive to muscarinic agonists both before and after there are receptor differences. Pirenzepine, a selective M1 muscarinic antagonist, was used to displace QNB in order to estimate the percentage of M1 and M2 binding sites. The experiment indicated that the increased muscarinic receptors seen in the FSL were for both M1 and M2 subtypes.

465.5

WITHDRAWN

465.6

REGULATION OF NEURONAL NICOTINIC RECEPTORS: EFFECTS OF NICOTINE ON PRIMARY NEURONAL CELLS IN CULTURE. M.I. Dávila-García,* R.A. Houghtling, S.S. Qasba and K.J. Kellar. Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007.

The study of nicotine effects on the regulation of neuronal nicotinic acetylcholine receptors has been impeded by the lack of a suitable *in vitro* model. We have used a cell culture system from fetal rat brain to characterize the properties of neuronal nicotinic acetylcholine receptors and to begin to study the factors involved in their regulation. In the present studies we examined the effects of nicotine on [^{3}H]cytisine binding in embryonic day 18 fetal neurons from the brainstem (which included thalamus, hypothalamus, midbrain and pons), cortex, caudate and hippocampus. We found that after 15 days in culture the brainstem neurons contain the greatest number of neuronal nicotinic receptors ($\approx$ 50 fmol/mg protein) and that this receptor has high affinity for [^{3}H]cytisine (0.7 nM). Binding of [^{3}H]cytisine is competitively inhibited by nicotine ($IC_{50} \approx$ 20 nM), as well as by carbachol and dihydro-ß-erythroidine (IC_{50} values of $<$ 1uM). Nicotine induced an increase in intracellular Ca^{2+} in cortical cells. This increase in calcium was not seen in cultures previously exposed to nicotine (100 μM) for 7 days, suggesting that this response can be desensitized/inactivated by nicotine. Similar treatment of these cultures for 7 days with 100 μM nicotine increased the number of [^{3}H]cytisine binding sites. This work was supported by NIH grants DA05417 and DA06486.

ACETYLCHOLINE RECEPTORS: NICOTINIC MOLECULAR BIOLOGY

466.1

EFFECTS OF SUSTAINED NICOTINE EXPOSURE ON THE ACTIVATION OF NEURONAL NICOTINIC RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. Y.N. Hsu, J. Amin, D. Weiss and L. Wecker*. Depts. Pharmacol. and Physiol., Univ. South Florida College of Medicine, Tampa, FL 33612.

Studies suggest that nicotine induces the release of dopamine (DA) and acetylcholine (ACh) through interactions at $\alpha3\beta2$ and $\alpha4\beta2$ nicotinic receptor subtypes, respectively. The chronic administration of nicotine increases nicotine-mediated [^{3}H]DA release, but decreases nicotine-mediated [^{3}H]ACh release from brain slices, suggesting that chronic nicotine differentially affects these receptors. To test this hypothesis, cRNAs for $\alpha3$ and $\beta2$ or $\alpha4$ and $\beta2$ subunits were co-injected into *Xenopus* oocytes and nicotine-activated currents were measured before and after 24 hrs incubation with nicotine using the 2-electrode voltage-clamp technique. Currents in oocytes expressing $\alpha3\beta2$ receptors were not affected significantly by 24 hr incubation with nicotine; currents increased 6.8-fold, 5.9-fold and 5.2-fold following incubation with 0, 20 and 100 nM nicotine, respectively, due to increased receptor expression. In contrast, $\alpha4\beta2$-expressing oocytes had a reduced response to nicotine; current in control oocytes increased 5.8-fold, whereas current in oocytes incubated with 20 nM nicotine increased 2.2-fold and with 100 nM incubation, no increase was noted. This reduced response to nicotine reversed after 90 min incubation in medium lacking nicotine indicating $\alpha4\beta2$ receptors were expressed, but desensitized. Results suggest that the differential effect of chronic nicotine on the release of DA and ACh may be due, at least in part, to differences in the desensitization of the two receptor subtypes that mediate the release of these neurotransmitters. (Supported by grants #0411 from the STRC, Inc. and #AA09212 from the NIH.)

466.2

NICOTINE-INDUCED DESENSITIZATION OF RAT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT COMBINATIONS EXPRESSED IN *XENOPUS LAEVIS* OOCYTES. C.R.T. Vibat, J.A. Lasalde, M.G. McNamee, and E.L.M. Ochoa*, Section of Molecular and Cellular Biology, and Department of Pediatrics, University of California at Davis, Davis, CA 95616.

Chronic administration of nicotine up-regulates rat neuronal nicotinic acetylcholine receptors (nAChRs). A key hypothesis that explains up-regulation assumes that nicotine induces desensitization of receptor function. This is correlated to behaviorally expressed tolerance to the drug. The present experiments were conducted to obtain information on the nicotine-induced desensitization of neuronal nAChR function, a less understood phenomenon as compared to that of the muscle and *Torpedo* counterparts. *X. laevis* oocytes were injected with mRNAs encoding receptor subunits $\alpha2$, $\alpha3$, or $\alpha4$ in pairwise combination with the $\beta2$ subunit. The responses to various concentrations of ACh or nicotine were analyzed by the two electrode voltage clamp technique. $\alpha4\beta2$ is the predominant form in the rat brain that also undergoes up-regulation (Flores, *et al.*, 1992). We found that nicotine was more potent than ACh (EC50: 0.3 μM vs. 6 μM) in the $\alpha4\beta2$ combination and we observed a depression of the maximum attained response at concentrations higher than 20 μM nicotine, a clear indication of nicotine-induced desensitization. Inactivation of the response (calculated as a single exponential decay) was significantly faster for $\alpha4\beta2$ than for $\alpha3\beta2$. We constructed I/V curves at different concentrations of nicotine and the results suggest that blockade is not the mode of receptor inactivation at high nicotine concentration. By patch clamp analysis, $\alpha4\beta2$ has been shown to have three conductance states (Charnet, *et al.*, 1992). Since one or more of these states could differentially contribute to the observed desensitization, experiments are in progress to analyze desensitization kinetics at the single channel level. Taken altogether, these data suggest that the $\alpha4\beta2$ combination is desensitized by nicotine. *This research was supported by NIH grant NS22941 and in part by funds provided by the Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco-Related Disease Research Program of the University of California, Grant Number 3RT-0098.*

466.3

NICOTINE-INDUCED UPREGULATION OF NEURONAL NICOTINIC RECEPTORS RESULTS FROM A DECREASE IN THE RATE OF RECEPTOR TURNOVER

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Chronic nicotine exposure in tobacco smokers or experimental animals is known to cause an increase in brain nicotine binding sites and to cause accumulation of chronically desensitized receptors. Acetylcholine receptors of the same $(\alpha4)_2(\beta2)_3$ subunit composition as the predominant subtype of brain nicotinic receptor with high affinity for nicotine have been expressed in a permanently transfected fibroblast cell line. Chronic exposure of these cells to nicotine, other agonists, or a channel blocker is shown to result in an increase in receptor amount, indicating that nicotine induced upregulation reflects properties of the $\alpha4\beta2$ receptor protein which can be expressed in transfected fibroblasts, rather than an adaptive response unique to the neurons in which these receptors are normally expressed. The nicotine concentration-dependence, time course, and extent were similar to those reported for receptor in brain, suggesting that this intrinsic property could account for the upregulation observed in brain. The mechanism of nicotine-induced upregulation is shown to not require ion flow through the receptor and to involve a decreased rate of receptor turnover.

466.4

ANTISENSE DELETION REVEALS THAT $\alpha5$ SUBUNITS PARTICIPATE IN NATIVE CHICK NEURONAL NICOTINIC CHANNELS

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The $\alpha5$ subunit of the chick neuronal nicotinic acetylcholine receptor (nAChR) family is present in many cholinceptive neurons (Ballivet, Berg and their colleagues). Emerging evidence indicates that the $\alpha5$ subunit can participate in functional nicotinic channels by coassembly with other α and β subunits in heterologous expression systems (see Abstract by Ramirez-Latorre et al. 1993). In view of these data we have examined the role of $\alpha5$ subunits in native neuronal nAChRs.

The properties of ACh-gated macroscopic and single channel currents were assayed in embryonic chick sympathetic neurons treated with control or $\alpha5$ antisense oligonucleotides. Two different antisense oligonucleotides against different regions of the $\alpha5$ subunit gene were used and produced identical results: First, $\alpha5$ deletion alters the dose response curves to ACh, cytisine and nicotine with changes in the apparent affinity for agonist. Thus $\alpha5$-minus neurons have a higher affinity to cytisine, (EC50=24 μM) compared with control (EC50=75 μM). These results are consistent with previous findings that inclusion of $\alpha5$ subunits in heterologously expressed $\alpha4\beta2$ nAChRs decreases agonist affinity. In addition, the deletion of the $\alpha5$ subunits changes the profile of nAChR channel subtypes. Two of the three classes of channels expressed in control neurons are deleted and new channels of 14pS and 63 pS are expressed by sympathetic neurons. These results indicate that the $\alpha5$ subunit participates in native sympathetic neuronal nAChRs altering both agonist affinity and conductance. (supported by NIH29071 to LR)

466.5

MUTATIONAL ANALYSIS OF NOVEL RESIDUES IDENTIFIED WITHIN THE BINDING SITE OF *d*-TUBOCURARINE OF *TORPEDO* ACETYLCHOLINE RECEPTOR. Y. Xie, D.C. Chiara and J.B. Cohen*
Department of Neurobiology, Harvard Medical School, Boston, MA 02115

The competitive antagonist *d*-tubocurarine (dTC) binds to the two agonist binding sites of the nicotinic acetylcholine receptor (nAChR) from *Torpedo* electric organ and mouse skeletal muscle with significantly different affinities. The high-affinity and low-affinity dTC binding sites are located at the α-γ and α-δ subunit interfaces, respectively. Our photolabeling studies using [^{3}H]-dTC identified previously two non-α residues: γW55 and δW57. Here we report the identification of two additional residues photolabelled by [^{3}H]-dTC: γY111 and γY117. The tyrosine in the γ-subunit of mouse muscle nAChR corresponding to *Torpedo* γY117 has been shown to be important for the selective binding of dimethyl-TC (S. Sine, *PNAS* 90: 9346-9400, 1993). In the aligned *Torpedo* nAChR sequences, the residue which corresponds to γY111 in the δ-subunit is δR113. This difference may also contribute to the difference in dTC binding affinities between the two sites. To examine the role of these two residues in dTC binding, we made γY111R and δR113Y mutant receptors. When expressed in *Xenopus* oocytes, both mutant receptors show similar densities of ^{125}I-α-bungarotoxin (αBgt) binding and amplitudes of ACh-induced currents as wild type receptors. Competition studies of dTC against the initial rate of ^{125}I-αBgt binding to both whole oocytes and oocyte membranes reveal that γY111R mutation causes $\approx$ 3-fold decrease of dTC affinity for the high-affinity binding site, while the δR113Y mutation increases dTC affinity for the low-affinity binding site 2-3 fold. Functional consequences of these mutants in terms of changes of agonist/antagonist sensitivities will be assessed by using the two-electrode voltage-clamp technique.

466.6

STABLE RECOMBINANT EXPRESSION OF HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR $\alpha 3\beta 4$ SUBTYPE IN HEK293 CELLS. M. Akong, S. Mahaffy, L. Chavez-Noriega, A. Urrutia, K. Elliott, K. Berckhan, R. Reid, T.S. Rao, G.K. Lloyd*, E.C. Johnson, and G. Velicelebi. SIBIA, Inc., La Jolla, CA 92037.

cDNA clones encoding full-length human neuronal nicotinic acetylcholine receptor (nAChR) $\alpha 3\beta 4$ subunits were subcloned into a pCMV vector and stably transfected into HEK293 cells. Functional expression of the recombinant receptor was characterized through measurements of increases in intracellular calcium ($[Ca^{2+}]_i$) and inward currents. A binding capacity of approximately 25,000 receptors/cell was estimated from measurements of ^{3}H-nicotine binding. Nicotine, cytisine, and DMPP stimulated $[Ca^{2+}]_i$ levels in a concentration-dependent manner up to approximately six-fold above basal, with a potency rank order of DMPP > cytisine > nicotine, similar to that determined in *Xenopus* oocytes injected with hα3β4 transcripts. The competitive antagonist, d-tubocurarine, inhibited the stimulatory effects of each agonist also in concentration-dependent manner, with IC_{50} values of 0.84 $\pm$ 1.10 μM, 1.15 $\pm$ 1.06 μM, and 0.93 $\pm$ 0.33 μM against nicotine, cytisine, and DMPP, respectively. In whole-cell recordings from the stable $\alpha 3\beta 4$-transfectants, transient inward currents with magnitudes greater than 1 nA were observed at negative holding potentials following application of acetylcholine, nicotine, or DMPP. The current-voltage relationship was strongly inward rectifying, and no outward current was detected at positive potentials.

466.7

TRANSGENE MODULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR NUMBERS AND FUNCTION IN MOUSE AND HUMAN CELL LINES. Elzbieta Puchacz*, Linda Lucero and Ronald J. Lukas. Div. Neurobiol., Barrow Neuro. Inst., Phoenix, AZ 85013.

Cells of the SH-SY5Y human neuroblastoma express two types of nAChR, nicotinic α-bungarotoxin (Bgt) binding sites (nBgtS) and ganglia-type nAChR, and products of at least five human nAChR subunit genes (α3, α5, α7, β2 and β4). Here we show that transfection of SH-SY5Y cells with rat α7 constructs dramatically increases ^{125}I-labeled Bgt (I-Bgt) binding in SH-SY5Y cells without affecting ganglia-type nAChR function. Cyclosporin sensitivity of this effect, but not of I-Bgt binding to control SH-SY5Y cells, suggests that transgenic rat α7 subunits may assemble in a cyclophilin-dependent manner as homooligomers, whereas assembly of native α7-containing nBgtS does not have such a dependence. Preliminary studies indicate that transfection of SH-SY5Y cells with antisense α7 constructs produces a dramatic loss in I-Bgt binding, again without effects on ganglia-type nAChR function, suggesting that human α7 subunits contribute to native nBgtS but not ganglia-type nAChR in SH-SY5Y cells. Other studies demonstrate that adult muscle nAChR epsilon transgene expression in BC_3H-1 cells rescues nAChR from loss of function and I-Bgt binding when cells are subjected to drug treatments that mimic the motor neuronal innervation-induced loss of fetal nAChR gamma subunit expression as seen in developing muscle in vivo.

466.8

DIVERGENT EFFECTS OF CPT-cAMP ON NICOTINIC ACETYLCHOLINE RECEPTOR PROTEIN AND GENE EXPRESSION IN BC3H-1 CELLS INDUCED TO GROW OR DIFFERENTIATE. Linda Lucero, Robert S. Fisher* and Ronald J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, USA.

Changes in cyclic AMP (cAMP) levels in differentiating muscle fibers are thought to influence levels of expression of muscle nicotinic acetylcholine receptors (nAChR). We assessed effects of 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), a cyclic AMP analogue, on nAChR expression in BC3H-1 mouse muscle cells. CPT-cAMP treatment of cells maintained in a 'myoblast-like state' (where cells are grown in medium containing 10% horse and 5% fetal calf sera) inhibited the usual slow, time-dependent accumulation of nAChR. Conversely, for 'differentiated' BC3H-1 cells where a typical 2-fold increase in nAChR expression is rapidly induced by at least 2 days of growth in 1% fetal calf serum-supplemented medium, CPT-cAMP exposure produced an additional up-to-2-fold augmentation in nAChR expression. These divergent effects of CPT-cAMP treatment are reflected in nAChR function, numbers of nicotinic radioligand binding sites on the cell surface or in total particulate fractions, and levels of nAChR subunit mRNA. These findings suggest that effects of activation of cAMP-dependent signaling in muscle cells in vivo might also vary as a function of cellular differentiation state, acting to inhibit nAChR expression in myoblasts but to enhance the induction of nAChR expression after myotube formation.

466.9

SUBUNIT COMPOSITION DISTINGUISHES MULTIPLE CLASSES OF ACETYLCHOLINE RECEPTORS IN THE SAME NEURONS. W.G. Conroy* and D.K. Berg. Dept. of Biology, UC San Diego, La Jolla, CA 92093.

The rules governing subunit assembly of neuronal acetylcholine receptors (AChRs) are only beginning to be elucidated. Chick ciliary ganglion neurons express five known neuronal AChR genes. Three of the encoded gene products (α3, β4, and α5) are present in synaptic-type receptors that bind mAb 35 (mAb 35-AChRs). A fourth (α7) is present in a second class of receptors that bind α-bungarotoxin (αBgt-AChRs). Immunoprecipitations with subunit-specific mAbs now show that the fifth gene product (β2) is present in about 20% of the mAb 35-AChRs while being excluded from αBgt-AChRs. Immunoblot analysis confirms that the β2 gene product is associated with α3, β4, and α5 proteins. Immunostaining of ganglion sections demonstrates that essentially all of the neurons contain β2 gene product. Accordingly, the same neurons apparently express both classes of mAb 35-AChRs (with and without β2) as well as αBgt-AChRs. All three classes have the size expected of fully-assembled AChRs and appear on the cell surface. Other experiments indicate that the ganglion also contains a novel minor population of receptors that bind both mAb 35 and αBgt and is distinct from those described here (see abstract by Pugh et al.). The virtue of multiple AChR classes in the same neuron and the significance of β2 subunits raise new questions. (NS12601 & 25916; TRDRP)

466.10

A SUBPOPULATION OF NEURONAL ACH RECEPTORS AMONG THOSE BINDING α-BUNGAROTOXIN. P.C. Pugh*, R.A. Corriveau, and D.K. Berg. Dept. of Biology, UC San Diego, La Jolla, CA 92093.

Neuronal nicotinic acetylcholine receptors (AChRs) that bind α-bungarotoxin (αBgt-AChRs) have previously been found to contain α7, α8, or both gene products. The chick ciliary ganglion at embryonic day 18 has about 30 fmol of αBgt-AChRs containing α7 subunits. In addition it has about 4 fmol of mAb 35-AChRs that bind mAb 35 but not αBgt and, as a population, contain α3, β4, α5 and, to a lesser extent, β2 subunits. Using a sensitive solid phase assay we report a new population of putative receptors that bind both αBgt and mAb 35 with high affinity, exhibit nicotinic pharmacology similar to that of αBgt-AChRs, and constitute about 1 fmol per ganglion. The receptors sediment at 10S as expected for neuronal AChRs, are developmentally regulated, and are also found in dorsal root ganglia but not in brain or retina. Analysis with subunit-specific mAbs provides no evidence for the receptors containing any of the five neuronal AChR gene products known to be present in ciliary ganglia. The muscle AChR α1 gene product binds both αBgt and mAb 35, and RNase protection experiments surprisingly detect a small amount of α1 transcript in the ganglion. Immunoblots, however, fail to detect α1 protein in the receptors. The results raise the possibility that the receptors are composed of novel gene products yet to be described. (NS12601, NS25916, & TRDRP)

466.11

EXPRESSION OF NEURONAL ACH RECEPTOR GENES IN TRANSFECTED HEK-293 CELLS. R.C. Haselbeck, W.G. Conroy, S.J. Romano, and D.K. Berg*. Dept. of Biology, UC San Diego, La Jolla, CA 92093.

Subunit interactions and functions are poorly understood in the case of neuronal nicotinic acetylcholine receptors (AChRs). To address these issues, we have expressed three AChR genes (α3, β4, and α5) in transfected HEK-293 cells. These subunits make up the major class of synaptic-type AChRs that binds mAb 35 on chick ciliary ganglion neurons. Immunoblots of extracts from transfected cells confirm that all three genes generate proteins that co-migrate with the subunits from native AChRs. Sucrose gradient analyses of extracts demonstrate that cells transfected with α3/β4/α5, α3/β4, or α5/β4 (but not with α3/α5) contain large amounts of assembled components that bind mAb 35, immunoprecipitate with anti-α3/β4 mAbs, and sediment in the 10S region as expected for neuronal AChRs. Despite the similar amounts of such components in the three kinds of transfections, α5/β4 cells express by far the most mAb 35 binding sites on the cell surface. Surprisingly, transfections with α5 alone produce large amounts of surface sites for mAb 35 (α3 and β4 alone do not), but immunoprecipitations indicate that the α5 species cannot alone account for the large number of sites on α5/β4 cells. The results identify a new candidate neuronal AChR and suggest that α5 subunits may play a key role in targeting receptors to the surface. (NS12601, NS52516, AHA93-72)

466.12

POSTTRANSCRIPTIONAL REGULATION OF NEURONAL ACH RECEPTORS DESTINED FOR THE CELL SURFACE. B. Rothhut, S.J. Romano, S. Vijayaraghavan*, and D.K. Berg. Dept. of Biology, UC San Diego, La Jolla, CA 92093.

Mechanisms controlling receptor accumulation on neurons are largely unknown. M10 cells, stably transfected with the acetylcholine receptor (AChR) α4 and β2 genes, permit an examination of this. We find that forskolin in 24 hours stimulates a 6-fold increase in surface AChRs and a 2-fold increase in the abundant intracellular receptors. The increases are partially blocked by H89 and mimicked by 8-bromo-cAMP, implicating protein kinase A. The intracellular increase and part of the surface increase depend on protein synthesis and correlate with increases in receptor transcripts. When protein synthesis is blocked, however, forskolin still doubles selectively the number of surface AChRs, suggesting that a cAMP-dependent process either helps target receptors to the cell surface or stabilizes them there. Okadaic acid, which inhibits protein phosphatases 1 and 2A, selectively blocks the large forskolin effect on surface receptors but not the effect on intracellular receptors. Okadaic acid does not decrease total protein synthesis or AChR transcript levels in forskolin-treated cells, but does decrease the levels of AChR protein, possibly by promoting turnover. The finding that forskolin and okadaic acid have opposite but specific effects on the accumulation of surface AChRs suggests that multiple phosphorylation events may influence receptor assembly, transport, and stability. (NS12601, NS25916, & TRDRP)

466.13

DIFFERENTIAL EFFECTS ON CALCIUM LEVELS IN NEURONS CAUSED BY NICOTINIC AND MUSCARINIC RECEPTORS. M. M. Rathouz*, S. Vijayaraghavan, and D. K. Berg. Dept. Biol., UC San Diego; La Jolla, CA 92093.

Intracellular calcium levels ($[Ca]_i$) in neurons can be elevated by either nicotinic or muscarinic receptors. Chick ciliary ganglion neurons have both. The nicotinic receptors increase $[Ca]_i$ via their calcium permeability and activation of voltage-gated channels. We report M3-type muscarinic receptors on the neurons having different effects on $[Ca]_i$.

Activation of the muscarinic receptors stimulates phosphatidyl inositol (PI) turnover, and the turnover is blocked by DAMP as expected for M3 receptors. Fluorescence measurements in fluo-3-loaded neurons indicate that activation of the receptors increases $[Ca]_i$ in an oscillatory manner that is blocked by DAMP, depends on extracellular calcium, but remains after emptying caffeine-sensitive calcium stores. These features are consistent with M3 receptors acting through IP_3 to produce the oscillations.

ACh at 50 μM activates both the M3 and nicotinic receptors, generating a sustained elevation of $[Ca]_i$. Selective blockade of nicotinic receptors converts the response to an oscillatory one while selective blockade of M3 receptors causes the response to decay more abruptly. The distinctive changes in $[Ca]_i$ produced by the two receptors may allow a single neurotransmitter to modulate multiple calcium-dependent events independently in a neuron. (NS12601, NS25916, & TRDRP)

466.14

EXPRESSION OF NEURONAL ACH RECEPTOR GENES IN VERTEBRATE SKELETAL MUSCLE. R.A. Corriveau*, S.J. Romano, W.G. Conroy, L. Oliva, and D.K. Berg. Dept. of Biology, UC San Diego, La Jolla, CA 92093.

Fifteen nicotinic ACh receptor (AChR) genes have been identified; a subset of these (α2-8, β2-4) are expressed in the nervous system and are therefore considered neuronal genes. Using RNase protection assays we demonstrate that embryonic chick skeletal muscle expresses four neuronal-type AChR genes (α4, α5, α7, and β4) in developmentally regulated patterns. Of the four, α7 mRNA is relatively abundant and reaches a level about 20% that of α1 at embryonic day 8 (E8). Like α1, α7 mRNA levels increase over development, peaking at E8-E11, and fall dramatically by E17. In situ hybridizations confirm the presence of α7 transcripts in myotubes both in vivo and in culture. Immunoprecipitations and immunoblot analysis using subunit-specific monoclonal antibodies reveal α7 protein in muscle; the developmental pattern of α7 protein expression parallels that of α7 mRNA. Sucrose gradients demonstrate that the α7 protein is present as a 10S species, a size expected for an AChR. The α7-containing component binds α-bungarotoxin but does not contain α1 subunits, indicating that the two α-type gene products segregate during assembly. The finding of a putative neuronal AChR in embryonic muscle indicates that the tissue-specific division of AChR gene expression is less categorical than previously recognized and raises the possibility that neuronal AChRs participate in muscle development. (NS12601 and NS25916)

466.15

ALTERATIONS IN CYCLOSPORIN-A SENSITIVITY OF α7 HOMO-OLIGOMERIC NICOTINIC RECEPTOR EXPRESSION BY MUTATIONS WITHIN TRANSMEMBRANE DOMAINS. Santosh A. Helekar*, Hong Dang and Jim Patrick, Div. of Neuroscience, Baylor College of Medicine, Houston, TX.

Synthesis of oligomeric integral membrane receptors such as the ligand-gated ion channels may depend on the activity of folding enzymes and molecular chaperones. We showed previously that blockade of a folding enzyme, the prolyl isomerase cyclophilin (CyP), with cyclosporin A (CsA) caused a dose-dependent reduction of the expression of functional rat α7 nicotinic receptors in *Xenopus* oocytes. We now report a second effect of CsA on the small number of residual receptors that are assembled in the presence of this compound. We observed that, in addition to a decrease in receptor expression, CsA increased the extent of run down of current responses following repeated agonist applications. We wanted to investigate whether the apparent reduction in receptor expression was caused by this increase in receptor run down induced by CsA. Using a non-desensitizing rat α7 receptor mutant that had a leucine to threonine substitution at position 250 in the pore forming transmembrane domain II, we were able to dissociate the two effects of CsA. We found that CsA increased the amount of receptor run down in this mutant, as well as in the wild type. However, in contrast to the wild type receptor, CsA produced a significant enhancement of the level of expression of functional mutant receptors. These data indicate that the two effects of CsA on α7 nicotinic receptors, namely on receptor expression and on receptor run down are distinct and unrelated. The mechanisms underlying the run down effect of CsA are currently unclear. But as regards the CsA effect on receptor expression, we now have evidence that the CsA-induced enhancement of L250T mutant receptor expression can be reversed by the substitution of a glycine residue for a highly conserved proline at position 220 in the transmembrane domain I.

Supported by grants from NIH (JP) and a MDA fellowship (SAH).

466.16

ANALOGUES OF 5HT INCREASE THE RATE OF DESENSITIZATION OF NICOTINIC RECEPTORS IN OOCYTES K.M.L.CROSS, C.H.BUYKX, J.E.CHAD AND R.C.FOREMAN Department of Physiology and Pharmacology, University of Southampton, SO9 3TU, UK. (SPON: BRA)

We have expressed a variety of mammalian nicotinic receptors in *Xenopus laevis* oocytes. The $\alpha_4\beta_2$ combination gave a current of 580nA ±122nA (n=7) in response to 10μM acetylcholine (ACh), with a $t_{0.5}$ of desensitization of 51.4 ±3.6secs. In the presence of 5HT there was a dose dependent decrease (IC_{50} 100μM) of the peak current response. The rate of desensitization was increased by 5HT (100μM), the $t_{0.5}$ was reduced to 17.2 ±2.0secs, that is to 33% of the control value. The effect was readily reversed by washout. 5HT alone did not give rise to any current. 5HT also modulated the combination $\alpha\beta_2\gamma\delta$, the muscle subunits with a neuronal β subnuit substituted. The mean peak current elicited by 10μM ACh was 237 ±44nA with $t_{0.5}$ of 13.5 ±2.7secs. In the presence of 5HT the peak current was reduced with the same IC_{50} as for the $\alpha_4\beta_2$ combination. This dose of 5HT reduced the $t_{0.5}$ to 26% of control. In order to investigate the mechanism of modulation by 5HT we examined the effect of structurally similar molecules. The $5HT_2$ receptor antagonists ketanserin and cyproheptadine increased the rate of ACh receptor desensitization. Ketanserin was found to be more potent than 5HT in reducing the peak current with the IC_{50} value being 50μM. Cyproheptadine was less potent but still decreased the sensitivity of the nicotinic receptor to its natural ligand. These results suggest that 5HT acts on a specific site on the nicotinic receptor, which resembles the $5HT_2$ receptor.

Supported by the Wellcome Trust

466.17

MOLECULAR REGULATION OF HUMAN α7 nAChR GENE EXPRESSION. L.M. Monteggia*, M. Gopalakrishnan, and T. Giordano, Neuroscience Research, D47W, Abbott Labs, Abbott Park, IL 60064-3500.

Previous reports of α7 nicotinic acetylcholine receptor(nAChR) gene expression have suggested extremely complex regulatory mechanisms with differences between transcriptional activity and steady state levels of the RNA reported during chicken development. We report here the regulation of α7 in the human neuroblastoma cell line, IMR32, in response to NGF and PMA, agents which are known to alter the expression of various genes. Three transcripts of 6.0, 3.5, and 2.4 kb were observed for α7 nAChR in the IMR32 cells. An approximate 2 fold increase in the steady state levels of RNA of all transcripts was detected following 24 hr treatment with either NGF or PMA. To determine the kinetics of α7 gene induction, IMR32 cells were treated with NGF for 0.5, 1, 2, 4, 8, 16, and 24 hr, RNA was isolated and steady state levels were determined. An increase in steady state levels of RNA can result from increased transcription, stability, or both. To assess altered transcriptional activity, cells were treated with NGF or PMA for 24 hr and the nuclei have been isolated for use in nuclear runoff experiments. The stability of the α7 RNA is currently being analyzed from cells treated with actinomycin D, an inhibitor of RNA synthesis. The 3'UTR region of many mRNAs contain sequences rich in A and U nucleotides, which seem to function in stability, translation, and localization of the RNA. Using RNA fold programs to analyze the 3'UTR of the human α7 cDNA no AUUUA sequences, a motif previously shown to regulate RNA stability, were found, however a large stem loop structure was predicted. In a 9 bp loop, ATATTCTCA, bp 2-7 are identical in rat, suggesting this may be a motif for RNA binding proteins (RNP's). Studies are underway to determine whether RNPs are present which bind to the human α7 3' UTR and whether the 3' UTR from rat or other human subunits can compete for the proposed binding protein.

466.18

ACTIVITY REGULATES α7 NICOTINIC ACh RECEPTOR SUBUNIT GENE EXPRESSION IN RAT SYMPATHETIC NEURONS. P.De Koninck*+ and E.Cooper#, Depts. of Biology+ and Physiology#, McGill Univ. Montréal, Québec, Canada H3G 1Y6.

Little is known about the mechanisms by which innervation controls the expression of neurotransmitter receptors in nerve cells. Therefore, we are studying gene expression of nicotinic acetylcholine receptor (nAChR) subunits in cultured neonatal rat sympathetic neurons. These neurons express 5 nAChR transcripts: α3, α5, α7, β2 and β4. Two days after plating, mRNA levels for the α7 subunit decrease 3 fold while the mRNA levels for the α3 subunit increase and the levels of α5, β2 and β4 remain constant. These results suggest that extrinsic factors control α7 expression. One factor is neuronal activity: treating neurons with 40mM KCl (HK^+) causes a 3 fold increase in α7 mRNA levels within 24-48 hours, without causing any change in the other subunit mRNA levels except for a small (10-20%) increase in α3 mRNA levels. Treatment of cultures with α-amanitin, an mRNA synthesis inhibitor, for 24 hours, indicated that HK^+ does not affect α7 mRNA stability, providing evidence that HK^+ affects α7 gene expression. Verapamil, an L-type Ca^{++} channel blocker, inhibits the induction by HK^+, indicating that the regulation of α7 gene expression occurs via a Ca^{++} mediated pathway. Next, we examined whether serine/threonine protein kinases are involved in this induction pathway. Protein kinase C or A (PKC/PKA) activators do not increase α7 mRNA synthesis and furthermore, neither PKC nor PKA inhibitors block the induction by HK^+. KN62, an inhibitor of the Ca^{++}/Calmodulin dependent protein kinase (CaM Kinase II), completely blocks the HK^+ induction; however, we have not yet excluded the possibility that KN62 could be having its effect by blocking the L-type Ca^{++} channels. These results show that activity can influence gene expression of neurotransmitter receptors, via a PKC and PKA independent pathway. (Support: MRC Canada)

466.19

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR α4 mRNA CONSISTS OF THREE DIFFERENT TRANSCRIPTS IN RAT BRAIN: NORTHERN DETERMINATION. Z. J. Yu, D. G. Morgan* and L. Wecker. Dept. Pharmacol. and Therap., USF College of Medicine, Tampa, FL 33612.

The initial report describing the neuronal nicotinic receptor α4 RNA identified 3 different messages believed to represent alternative splicing of a single transcript (Cell 48:965, 1987). Although α4 is the most widely expressed α subunit RNA in rat brain, the 3 species of α4 transcripts have not been well described with respect to their abundance ratios in different brain regions. In this study, signals of α4 mRNA were detected by Northern hybridization with radiolabelled plasmid DNA for α4-1 or HinfI fragment of α4-1. Quantification was conducted by densitometry of X-ray film. Three transcripts homologous to α4-1 cDNA were found in all brain regions tested. The sizes of the α4 gene transcripts were approximately 2.6 kb, 4.6 kb and 6.0 kb. The ratios of the 2.6:4.6:6.0 kb transcripts were: 1.0:1.6:0.11 in basal forebrain; 1.0:1.6:0.28 in striatum; 1.0:0.41:0.14 in frontal cerebral cortex; 1.0:0.47:0.09 in midcerebral cortex; 1.0:2.6:0.10 in midbrain; 1.0:0.71:0.33 in hippocampus; and 1.0:0.65:0.34 in thalamus and hypothalamus. Further, all 3 bands diminished in parallel with increased hybridization stringency, indicating a similar degree of homology to the α4-1 probe. This study demonstrates that there are 3 alternatively processed transcripts of the neuronal nicotinic acetylcholine receptor α4 gene, and the relative abundance of these transcripts differs in different brain regions. (DGM is an Established Investigator of the American Heart Association. Supported by grant #0411 from the STRC, Inc.)

466.20

GENE EXPRESSION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN DIFFERENT AREAS OF THE HUMAN CORTEX. A. Wevers[1]*, A. Jeske[1], Ch. Birtsch[1], S. Heinemann[3], A. Maelicke[2], H. Schröder[1], [1]Inst. II für Anatomie, Univ. zu Köln, 50931 Köln, FRG, [2]Inst. für Physiologische Chemie und Pathobiochemie, Univ. Mainz, 55128 Mainz, FRG, [3]The Salk Institute, La Jolla, CA 92186, USA

In recent years the pharmacology of telencephalic nicotinic acetylcholine receptors (nAChRs) has become an important issue. To get some insights into the morphological basis of functional diversity we investigated the mRNA expression of different subunits of the pentameric nAChR in human brain on the cellular level.

In situ hybridization studies were carried out on autopsy samples of different cortex areas (A4, A10, and A17 according to Brodmann) from patients (n=7) with no history of neurological disease. Digoxigenin-labeled in vitro transcripts specific for the α3- and α4-1-subunit of the nAChR were applied and hybridization was visualized using an alkaline phosphatase conjugated digoxigenin antibody and NBT/BCIP.

We found a distinct gene expression of both nAChR subunits in neurons of different cortical areas. Whereas the α4-1 mRNA expressing neurons were larger in number and distributed throughout lamina I-VI, the α3 ones were less abundant, especially in A17, and mainly localized in the deeper layers.

The observed differential mRNA distribution pattern of the investigated nAChR subtypes in the human cortex and the possibility to perform these studies in autopsy brains will be an important prerequisite for the evaluation of changes in nAChR expression under pathological conditions.

Supported by the Deutsche Forschungsgemeinschaft, grant Schr 283/8-2.

466.21

A NOVEL *IN SITU* DOUBLE-LABELING PROCEDURE TO STUDY THE EXPRESSION OF PROTEINS AND MESSAGES. K-P. Chiu, S.A. Berman*, T. Sullivan and S. Bursztajn, Labs. of Molecular Neurosci., Harvard Medical School/McLean Hospital, Belmont, MA 02178

Nicotinic acetylcholine receptors (AchRs), the hallmark of neuromuscular junctions, express multiple receptor subunits which can be regulated by many signals. In our investigation of these phenomena, using cultured muscle cells and neurons isolated from developing embryos, it is frequently necessary to image more then one molecular response simultaneously. We devised a novel double-labeling method to detect the expression of two different signals, e.g. two different mRNA species or a protein together with its corresponding mRNA, in the same cell preparation. This method employs a separate emulsion-coated slide to detect the radioactive signal, while the other signal is detected directly in the cells on a coverslip. We have tested this method using the combination of ^{125}I-labeled α-bungarotoxin together with digoxigenin-labeled probe, or the combination of ^{35}S-labeled cDNA probe together with digoxigenin-labeled oligo probe. In either combination, the radioactive signal was detected by developing the emulsion coated on a separate slide. After exposure, the emulsion-coated slide was separated from the coverslip containing the cell sample and then developed. The cells on the coverslip were subjected to a color development procedure to detect the digoxigenin-labeled probe. After color development, the slide carrying the radioactive signal was recombined with the coverslip carrying the colored signal and the cell origin relocated. This procedure allowed us to determine the distribution of AchR protein as observed by silver grains and AchR subunit message as detected colorimetrically on the same cell. Similarly, our procedure allows for the detection of two different messages in the same cell.

466.22

IDENTIFICATION OF PHOSPHOTYROSINE CONTAINING PROTEINS FROM *T. CALIFORNICA*. S. Balasubramanian, S.L. Swope* and R.L. Huganir, Dept. of Neuroscience, Howard Hughes Med. Inst., Johns Hopkins Univ. School of Med., Baltimore, MD, 21205.

Tyrosine phosphorylation has been implicated in regulation of the synthesis, distribution and gating properties of the nicotinic acetylcholine receptor (nAChR). To better understand these processes, we would like to identify proteins which are tyrosine phosphorylated at the postsynaptic membrane. The electric organ of *T. Californica* is an abundant source of nAChR and nAChR associated proteins. We have therefore purified phosphotyrosine containing proteins from the nAChR enriched membrane fraction of homogenized electric organ by immunoaffinity chromatography using antiphosphotyrosine antibodies. Polyclonal antibodies were then generated against these tyrosine phosphorylated proteins and used to screen a λgt11 expression library made from electric organ cDNA. Both previously characterized and novel proteins have been identified by this approach. Future studies will examine the tissue distribution, subcellular localization and tyrosine phosphorylation of these proteins.

466.23

FREQUENT, LONG-LIVED SPONTANEOUS AND MONO-LIGANDED OPENINGS OF NICOTINIC ACETYLCHOLINE RECEPTORS WITH M2 MUTATIONS. W. Sigurdson and A. Auerbach*, Dept. Biophysics, SUNY, Buffalo, NY 14214.

An important functional property of muscle-cell acetylcholine receptors (AChR) is that they should remain closed in the absence of transmitter. It is well known that wt AChR open spontaneously, but such events occur at a very low frequency and have extremely brief lifetimes. When expressed in HEK293 cells and exposed to 100 nM ACh, the mutant mouse *doubly-liganded* AChR αL251C has an apparent single-channel lifetime (cell-attached patches, -80 mV, 5 kHz analysis bandwidth) of about 200 ms, ~20 times greater than that of the wt $\alpha_2\beta\delta\gamma$ receptor. At 10 nM ACh, where *mono-liganded* openings predominate, the mutant open channel lifetime is about 2.7 ms, also about 20 times longer than the wt. Without ACh added to the pipette solution, the mutant AChR exhibit a high rate of *spontaneous* activity, opening at ~1.5 s^{-1} (per patch) and having a mean open channel lifetime of ~0.5 ms. This opening rate is at least 100 times greater than that of the wt. Thus in the mutant AChR, the closing rates for vacant, mono-, and doubly-liganded receptors are slower, and the opening rate of vacant receptors is much greater than in the wt. These results suggest that in unliganded mutant receptors the energy barrier to gating is lowered, i.e. there is a destabilization of the gate. This conclusion is consistent with the notion that α251 sidechains join with homologous residues in the M2 region of other subunits to form a structure that is the major barrier to ion permeation. The high rate of spontaneous activity is consistent with the apparent poor health of cells transfected with αL251C, as the expression of this M2 mutant would result in a steady flux of Ca^{++} into the cell which could have cytotoxic effects. Thanks to M. Akabas and A. Karlin for generously providing the αL251C mutant, and to MDA and NIH for support.

466.24

MONOCLONAL ANTIBODIES PROBE STRUCTURAL TRANSITIONS OF THE ACETYLCHOLINE RECEPTOR. S. Tamamizu*, D. H. Butler, J. A. Lasalde, and M. G. McNamee. Section of Molecular and Cellular Biology, Univ. of CA, Davis, CA 95616.

We have investigated the interactions of monoclonal antibodies (mAb) with the *Torpedo californica* nicotinic acetylcholine receptor (nAChR). Using patch clamp analysis, anti-*Torpedo* monoclonal antibodies were tested to determine their effect on nAChR function. Additionally, the mAb-nAChR complexes were labeled with 3-(trifluoromethyl) 3-(m-[125I]iodophenyl diazirine) (TID) in the absence and presence of agonist. Agonist-induced desensitization of the nAChR reduces TID labeling and serves as a monitor of the resting-to-desensitized transition.

mAb 247 has previously been shown to selectively block one of the α-bungarotoxin (α-BTX) sites without altering the affinity of nAChR for acetylcholine (ACh) (Mihovilovic and Richman, J. Biol. Chem. 259: 15051-15059, 1984). Our patch clamp studies demonstrate that both mAb 247 and its respective Fab fragment completely inhibit channel opening. TID labeling determined that the mAb 247-nAChR complex undergoes the normal resting-to-desensitized transition. This supports the kinetic model which suggests that nAChR can transition from a resting state to a desensitized state without entering the active state.

mAb 387 has been previously shown to decrease α-BTX and ACh binding to nAChR (Mihovilovic and Richman, J. Biol. Chem. 262: 4978-4986, 1987). In the presence of mAb 387, we observe a 40% decrease in nAChR single-channel amplitude at -100 mV, whereas Fab 387 completely inhibits channel conductance. The labeling studies also suggest that the mAb 387-nAChR and Fab 387-nAChR complexes transition between resting and desensitized states. Interestingly, the labeling of the γ-subunit in the resting state is increased up to 2-fold in the presence of either mAb 387 or Fab 387.

466.25

MUTATIONS AT THE LIPID-PROTEIN INTERFACE OF THE ACETYLCOLINE RECEPTOR AFFECT CHANNEL GATING. J. A. Lasalde, D. H. Butler, and M. G. McNamee*. Section of Molecular and Cellular Biology, Univ. of CA, Davis, CA 95616.

Previous site-directed mutagenesis studies demonstrated that the lipid-protein interface influences channel gating of the nicotinic acetylcholine receptor (nAChR). The αC418W mutant, located in the putative M4 transmembrane domain, showed a 3-fold increase in normalized macroscopic response to ACh in voltage clamped *Xenopus laevis* oocytes (Lee Y., et al., Biophys. J. 66:646-653, 1994). We have constructed mutations at the αG421 position and extended the patch clamp analysis to further characterize the role of M4 with respect to nAChR function

At 4 μM ACh, αG421W increased the open time constant 7-10 fold without affecting the normalized macroscopic current induced by 300 μM ACh. Burst-oriented analysis showed a 5-fold increase in the open probability at 25 μM ACh. Significant ACh-dependent channel block was observed. Reconstruction of macroscopic currents from an ensemble of αG421W bursts showed that the desensitization rate was 3-fold slower than wild type. In contrast, αG421A had a 2-3 fold higher desensitization rate than wild type, but the closing rate constant/opening rate constant (α/β) values were similar (86 and 75, respectively). αG421A demonstrated a 2-fold increase in the macroscopic response to ACh. The EC_{50}s of all the αG421 mutants did not differ significantly from wild type. The hybrid nAChR, αG421WA, was produced by the co-injection of αG421W and αG421A mRNA. This mutant had kinetic properties quite different than either of the pure mutants. The α/β of 184 was 2-fold higher than αG421A and 8-fold higher than αG421W. The open probability was reduced 10-fold with no change in macroscopic response as compared to αG421W. Equivalent mutations on the β-subunit, βG450F and βG450W, showed kinetic behavior similar to wild type.

466.26

Identification of Drosophila loci whose products show structural similarity to the vertebrate nicotinic acetylcholine receptor. B.A. Chase*, Dept. of Biology, Univ. of Nebraska-Omaha, 60th & Dodge Sts., Omaha, NE 68182.

The identification of multiple α– and β–like invertebrate nAChR subunits has suggested that there may be considerable heterogeneity in neuronal nAChRs. To identify potential Drosophila nAChRs or other molecules sharing structural similarity to the vertebrate nAChR, mAb probes to the vertebrate nAChR were previously used to identify cross-reacting antigens in Drosophila (Chase et al., (1987), Neurosci. *21*, 959-976). One mAb specifically cross-reacted with neuropil having cholinergic function.

To isolate the genes that encode the antigen(s) recognized by this mAb (27.1A.16.42), a lambda-ZAP expression vector library has been screened and three cDNAs isolated. Southern analysis has indicated that these three cDNAs are unrelated. *In situ* hybridization to adult tissue sections has shown that the loci identified are expressed in a pattern consistent with the mAb's cross-reactivity in neuropil. Each cDNA probe detects transcripts in CNS cell bodies but not neuropil, consistent with these loci being transcribed in the cell bodies and their protein products being transported into axonal and synaptic regions. To infer the nature of the products of these loci, DNA sequence analysis has been initiated. Current data indicate that the cDNAs have little homology to known Drosophila nAChR genes. Chromosomal *in situ* hybridization has also indicated that these cDNAs do not derive from loci encoding known Drosophila nAChRs.

Thus, the cDNAs identified using mAb 27.1A.16.42 derive from loci that appear to be expressed in a pattern consistent with this mAb's cross-reactivity, but do not encode known nAChR subunits. As this mAb recognizes structural features of the β-subunit of the vertebrate nAChR that are presumably shared by the cross-reacting Drosophila antigens, the loci identified here may encode novel β-like subunits of a nAChR, or other proteins, perhaps members of the superfamily of structurally related neurotransmitter receptors including nACh, GABA-A and glycine receptors.

466.27

CLONING OF A SECOND ALPHA ACETYLCHOLINE RECEPTOR SUBUNIT FROM *CAENORHABDITIS ELEGANS*. C.-M. Jen[1], P. Gamez[1], T. Valdez[1], T. Miranda[1], S. An[1], J. Lye[2], T. Barnes[3], and J. Lewis[1]*. [1]Division of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249, [2]Dept. of Genetics, Washington Univ. Medical School, St. Louis, MO 63110, and [3]Dept. of Biology, McGill Univ., Montreal, PQ H3A 1B1

In a shotgun genomic sequencing effort to define the nature of *let-354*, one of us (J. Lye) found sequence fragments with high homology to nicotinic alpha acetylcholine receptor (AChR) subunits. The sequences were contained within cosmids capable of rescuing *unc-74* mutants. *unc-74* mutation is associated with loss of nicotinic acetylcholine receptor function in *C. elegans*. *unc-74* mutants are pharmacologically unresponsive to all nicotinic acetylcholine agonists effective on the wild type and are also severely deficient in binding of [^{3}H]*meta*-aminolevamisole, a nicotinic agonist of nematode AChRs. A screen of a *C. elegans* cDNA library produced two positive clones at a frequency of 1 in 100,000. Sequencing of an almost full-length cDNA confirms that the cDNA encodes a nicotinic alpha subunit as shown by its high homology to the *unc-38* alpha subunit sequence of *C. elegans* and to the sequences of other known nicotinic AChR alpha subunits. Like *unc-38*, the new alpha subunit contains about 10 additional amino acids that are not found in alpha subunits of insects between the Cys loop region and the vicinal cysteines equivalent to Cys-192 - 193. RFLP mapping studies are underway utilizing transposon and γ-ray-induced mutants of *unc-74* to determine if the new alpha subunit is indeed encoded by *unc-74*. The possibility that the new alpha subunit is *unc-74* poses a mild paradox. Mutant studies show that both *unc-74* and *unc-38* are essential to produce a pharmacologically functional levamisole nicotinic AChR. If both are alpha subunits, it appears that the nematode levamisole AChR is an obligate heterodimer of alpha subunits.

466.28

REGULATION OF ADULT-TYPE NICOTINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION BY PROTEIN TYROSINE PHOSPHATASES. M.K. Sapru* and D. Goldman. Dept. of Biol. Chem., Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48105.

Molecular mechanism(s) regulating the postnatal switch of mammalian nicotinic acetylcholine receptors (nAChR) from embryonic type ($\alpha_2\beta\gamma\delta$) to adult-type ($\alpha_2\beta\varepsilon\delta$) at the neuromuscular junction is not well understood. Previously, we observed that calcium ionophore A23187 preferentially down-regulates ε–subunit gene expression (Walke et al., submitted) in rat primary myotubes. Here we report that sodium orthovanadate, a specific protein tyrosine phosphatase inhibitor, induces ε–subunit gene expression in rat primary myotubes. RNase protection assays and gene transfer experiments further revealed that orthovanadate blocks A23187-induced down-regulation of ε–subunit gene expression. In addition, gene transfer experiments showed that overexpressing protein tyrosine phosphatases in primary myotubes selectively supresses expression from nAChR α, δ and ε–subunit gene promoters without affecting the embryonic-type specific γ–subunit gene promoter. Together, these results suggest that protein tyrosine phosphatases regulate mammalian adult-type nAChR gene expression and may mediate the local expression of these genes beneath the neuromuscular junction.

466.29

Transcription of neuronal nicotinic acetylcholine receptor subunit genes in thymic epithelial and thymic lymphoid cells. Mirta Mihovilovic[1]*, Yun Mai[1], Stephen Denning[2], Sonia Berrih-Aknin[3] and Allen D. Roses[1]. [1]Division of Neurology, Duke University, Durham, NC 27710. [2]Division of Cardiology, Duke University. [3]Departement de Recherche Medicale, Hopital Marie Lannelongue, Paris, France.

Normal thymus, hypertrophic thymus from Myasthenia gravis (MG) patients and thymomas from MG and non-MG patients express transcripts encoding the α-3, α-5 and β-4 subunits of nicotinic neuronal acetylcholine receptors (AcChRs). This pattern of transcriptional expression suggests that neuronal AcChRs similar to those expressed in ganglia are expressed in the thymus and that these receptors may be of importance in transducing signals delivered to the thymus through the autonomic nervous system.

To investigate the transcription of neuronal nicotinic AcChR subunit genes in thymus we have employed purified thymocyte preparations, primary cultures of epithelial cells and short term cultures of thymocytes. Our results indicate that thymocytes express transcripts at levels comparable to those of the thymic tissue as a whole, while epithelial cells expressed much lower transcript levels. Expression of these transcripts in primary cultures of thymic epithelial cells appears to be dependent on the presence of epidermal growth factor and occurs in cultures that have also shown binding of monoclonal antibodies that recognize neurofilaments. Taken together, these data reflect the neuroendocrine nature of thymic epithelia. In addition, establishment of thymocytes in culture appears to lead to lower levels of transcription for the α-3 AcChR subunit genes, indicating that the thymic endocrine/paracrine microenvironment is necessary to sustain this transcription in the thymic lymphoid cells.

466.30

UPSTREAM SEQUENCES REQUIRED FOR CONTROL OF *ard* EXPRESSION IN *DROSOPHILA*. L.C. Yang, Y. He, and T. Schmidt-Glenewinkel*. Department of Biological Sciences, Hunter College of CUNY, New York, NY 10021.

The *ard* genes encodes a non-α-like subunit of one of the neuronal nicotinic acetylcholine receptors from *Drosophila*. We are interested in studying the cis-acting elements and trans-acting factors required for temporal and spatial expression of the genes encoding the acetylcholine receptor subunits. 5'-flanking sequences and intron sequences were cloned into the P-element containing vectors HZ50PL and CZ20XN generating either "enhancer fusion" or "transcriptional fusion" lacZ genes respectively. P-element-mediated germ-line transformation and establishment of balanced transformed stock was carried out by standard procedure. Frozen sections of whole flies or of heads were briefly fixed and examined for expression of lacZ fusion genes using either X-gal or an antibody against β-galactosidase. "Enhancer fusion" using about 4kb of 5'-upstream sequence show spatially correct expression in *Drosophila* heads while addition of 3kb of upstream sequence showed no expression. Spatially correct expression of "transcriptional fusion" constructs required additional downstream sequences. Constructs using a series of 5'- and 3'- deletions as well as internal fragments allowed us to define several upstream elements required for *ard* expression in *Drosophila*.

466.31

CHARACTERIZATION OF TWO TIGHTLY LINKED DIVERGENT PROMOTERS AND A SILENCER ELEMENT LOCATED BETWEEN THE RAT β4 AND α3 NICOTINIC ACETYLCHOLINE RECEPTOR GENES. R.T. Boyd*. Department of Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

In order to understand the transcriptional regulation of neuronal nicotinic acetylcholine receptors by cell contact and electrical activity, it will be first necessary to identify DNA elements that control the expression of members of this family and to identify factors required for the expression of these genes. Toward this goal we have identified two promoter regions in the β4 -α3 intergenic region. One region is close to the β4 gene downstream of exon 6 and has strong promoter activity in both orientations; the other is close to the start of the α3 gene coding region. The analysis of the sense and antisense promoter constructs indicated the bidirectional promoter activity demonstrated within the β4 gene is due to two very closely linked promoters that may share common elements. The functional role for the antisense promoter is not clear. The regulated production of antisense RNA could be involved in the regulation of the β4 gene at the transcriptional or translational levels. Opposite strand RNAs could also affect the expression of the α3 gene from the upstream promoter. A region with putative silencer activity was also found near the upstream promoters. DNA-binding protein interactions within this element have been studied. The level of a protein in PC12 cells that binds to an A-T rich region in the silencer was shown to be regulated by NGF. This work supported by AHA (Ohio Affiliate), Bremer Foundation (Columbus, Ohio), and NIH Grant NS29746.

466.32

CLONING AND CHARACTERIZATION OF THE HIPPOCAMPAL α7 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FROM HUMAN POSTMORTEM BRAIN. J. Logel, C. Drebing, C. Antle, C. Breese, M. Hall, C. Adams, Y. Rollins, B. Sullivan, R. Freedman and S. Leonard. VA Medical Center and University of Colorado Health Sciences Center. Denver, CO 80262.

The full length sequence of the human α7 neuronal nicotinic receptor subunit was obtained from human hippocampus. A partial clone was obtained by screening a human hippocampal library. The amino terminus region of the cDNA was obtained with a 5' RACE clone generated from hippocampal RNA. Hippocampal α7 appears nearly identical to that cloned from the neuroblastoma cell line SH-SY5Y (X. Peng *et al.*, 1994). Sequence obtained from PCR of cDNA suggests multiple poly-adenylation sites. Northern analysis of human cingulate and hippocampal mRNA showed variation in size compared to message in the neuroblastoma cell line and rat brain. Chemical cross-linking of α-bungarotoxin to membrane preparations of human and monkey hippocampus and whole rat brain revealed two principle protein species, only one of which was competed with nicotine and methyllycaconitine. *In situ* hybridization with a probe for the cytoplasmic loop of α7 showed labeling of a small population of cells in the dentate hilus, consistent with the localization of α-bungarotoxin binding in control and schizophrenic brain tissue. Control and schizophrenic post-mortem brain was screened for polymorphisms by RFLP analysis and PCR.

466.33

EFFECT OF METHYLLYCACONITINE AND RELATED ALKALOIDS, IN VITRO AND IN VIVO, AT THE α-BUNGAROTOXIN BINDING SITE IN RODENT BRAIN. Y.D. Rollins[1], M. Hall[1], K.L. Heman[1], P. Dobelis[2], J.P. Walrond[2], G.M. Rose[1], and S. Leonard[1]*, [1]Dept. of Pharmacology, UCHSC, and Medical Research, VAMC, Denver, Colorado 80262. [2]Department of Anatomy & Neurobiology, Colorado State Univ., Fort Collins, CO.

Schizophrenia is partially characterized by an auditory gating deficit, an abnormal electrophysiological response to repeated auditory stimuli. It has recently been shown this gating deficit can be transiently reversed by nicotine. Additionally, a gating deficit can be produced in rats by the use of pharmacological agents which block a subset of the neuronal nicotinic receptors, the α-bungarotoxin binding receptor, α7. Both α-bungarotoxin and methyllycaconitine (MLA), delivered intracerebroventricularly, induce a loss of gating of auditory evoked responses as measured by evoked potential recording in the unanesthetized animal implanted with surface and depth (CA1) electrodes. We have compared the efficacy of related norditerpenoid alkaloids, isolated from the seed of *Delphinium brownii* by the Poisonous Plant Research Lab, Logan, UT, to compete with α-bungarotoxin binding in rat brain membranes and find one of these compounds, 14-deacetylnudicaline (14-DN), has a higher affinity for the α7 receptor than does either α-bungarotoxin or MLA. In whole brain homogenate, 14-DN competes [I^{125}]-α-bungarotoxin with a Ki = 50 x 10^{-12} M while MLA competes with a Ki an order of magnitude higher. Another related compound, deltaline competes with a Ki = 0.6 x 10^{-6} M. This affinity order 14-DN > MLA > deltaline found in brain membranes is similar to the potency order of these compounds in blocking transmission at the neuromuscular junction in an *in vitro* lizard muscle preparation. These compounds will also be tested for their potency in blocking auditory gating.

466.34

STUDIES OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT COMPOSITION IN THE SH-SY5Y HUMAN NEUROBLASTOMA. Renaldo C. Drisdel, Elzbieta Puchacz and Ronald J. Lukas*. Div. Neurobiol., Barrow Neurol. Inst., Phoenix AZ 85013.

At least five human nAChR subunit genes (α3, α5, α7, β2 and β4) and two types of nAChR are expressed by cells of the SH-SY5Y human neuroblastoma. One class of nAChR corresponds to nicotinic α-bungarotoxin (Bgt) binding sites (nBgtS), which bind ^{125}I-labeled Bgt (I-Bgt) with high affinity and appear to contain human α7 subunits based on sense and antisense α7 construct transfection studies. SH-SY5Y cells also express ganglia-type nAChR, which bind [^{3}H]acetylcholine with high affinity, mediate Bgt-insensitive but neosurugatoxin-sensitive monovalent cation and $^{45}Ca^{++}$ fluxes, and seem to contain human α3 and β4 subunits based on functional pharmacological profiles. To address questions remaining regarding the full subunit composition of ganglionic nBgtS and nAChR, we have undertaken a series of affinity purification and immunochemical studies of the nAChR subtypes in SH-SY5Y cells. Antibodies raised against unique peptide sequences of nAChR subunit cytoplasmic domains exhibit subunit specificity and interact with SH-SY5Y cell membrane proteins on Western blots. Bgt-based affinity purification yields material that specifically binds I-Bgt with high affinity and is recognized by anti-α7 peptide-specific antibodies, consistent with the presence of α7 subunits in nBgtS.

466.35

MAPPING NEURONAL BUNGAROTOXIN SENSITIVITY ON NEURONAL NICOTINIC RECEPTOR ALPHA SUBUNITS.

C.W. Luetje*, F. Maddox and S.C. Harvey, Molecular and Cellular Pharmacology, University of Miami, Miami FL 33101.

Neuronal nicotinic acetylcholine receptors (nAChRs) can be expressed in *Xenopus* oocytes upon injection of cRNAs encoding various combinations of α and β subunits. The α3β2 subunit combination is sensitive to neuronal bungarotoxin (NBT) blockade (98.0±1.9% block by 100nM NBT) while α2β2 is insensitive to NBT. Previous work has shown that determinants of NBT sensitivity on α3 are localized to three distinct sequence segments (84-121, 121-181, 195-215), and that gln198 of α3 (pro in α2) plays a role in determining NBT sensitivity. We have made a series of mutations within these regions of α3, changing residues from what occurs in α3 to what occurs in α2. We assay the NBT sensitivity of mutant α3 subunits in combination with β2 upon expression in *Xenopus* oocytes. Changing thr143 of α3, to lys as in α2 (T143K), results in a loss of NBT sensitivity (7.7±4.6% block by 100nM NBT). Changing amino acid residues 159-166 of α3 as a group (VLIGSSMN) to what occurs in α2 (EQMERTVD) has a modest effect on NBT sensitivity (80% block by 100nM NBT). Amino acid changes in α3 that had no effect on NBT sensitivity include K87I, Q101A, L109H, K111F, K129S and Y139Q. We have also made a mutant of the α4 subunit which forms receptors (with β2) partially sensitive to NBT (15.9±1.4 % block by 1μM NBT) and has a proline at position 198, as does α2. Changing pro198 of α4, to gln as in α3, increases NBT sensitivity (87.4±6.8% block by 1μM NBT). These results show thr143 to be a major determinant of NBT sensitivity and provide additional evidence for a role of gln/pro198 in determining NBT sensitivity.

466.36

MAPPING THE NEURONAL NICOTINIC RECEPTOR BETA SUBUNIT CONTRIBUTION TO BLOCKADE BY THE COMPETITIVE ANTAGONISTS DIHYDRO-β-ERYTHROIDINE AND NEURONAL BUNGAROTOXIN

S. C. Harvey* and C. W. Luetje Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL. 33101

Neuronal nicotinic acetylcholine receptors (nAChR) can be formed in *Xenopus* oocytes by injecting various combinations of cRNA encoding two classes of homologous subunits, α and β. Each subunit combination has distinct pharmacological properties, with both α and β subunits contributing to ligand sensitivity. We constructed β subunit chimeras to map determinants of sensitivity to two structurally distinct competitive antagonists, dihydro-β-erythroidine (DHβE) and neuronal bungarotoxin (NBT). Receptors formed by α3 and β2 are sensitive to block by 100 nM NBT (97±2.2% block), while α3β4 is insensitive. At ACh concentrations approximating the EC20, α3β2 is 50 fold more sensitive to DHβE (IC50≈400 nM) than α3β4 (IC50≈20 μM). 3 μM DHβE effectively blocks α3β2 (90±4.6% block) but has little effect on α3β4 (13.1±5.1% block). Substituting the first 135 N-terminal amino acid residues of β4 into β2, results in a subunit which forms receptors with β4-like ligand sensitivity, i.e. insensitive to both 100 nM NBT and 3 μM DHβE. Substituting the first 105 N-terminal residues had similar results. Substituting in the first 58 residues of β4 resulted in intermediate sensitivity to NBT and DHβE (80±1.7% block by 100 nM NBT; 71±6.6% block by 3 μM DHβE). These results demonstrate that at least two distinct sections of the β subunit (1-58 and 58-105) are involved in determining receptor sensitivity to competitive antagonism.

466.37

MAPPING AMINO ACIDS RESPONSIBLE FOR THE PHARMACOLOGICAL DIFFERENCES OBSERVED BETWEEN α7 AND α8 HOMOMERS.

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Homomers of α7 and α8 subunits of chick αBgt-sensitive neuronal AChRs expressed from cRNAs in *Xenopus* oocytes exhibit similar channel properties but contrasting pharmacological properties (Gerzanich et al., Mol. Pharmol. 45: 212-220, 1994). α8 homomers are more sensitive to agonists than α7 homomers (e.g. EC_{50} (μM) : ACh 2 vs 112; nicotine 1 vs 7; cytisine 1 vs 18; tetramethylammonium 10 vs 800).

To delineate the amino acid residues responsible for these pharmacological differences, we have constructed α7 and α8 subunit chimeras as well as point mutants. A chimera (α7(208)/α8) consisting of the putative extracellular domain of the α7 subunit (residues 1-208) and the rest of the residues of the α8 subunit, when expressed in oocytes, forms homomers whose pharmacological properties are indistinguishable from those of α7 homomers. A second chimera (α7(115)/α8) forms homomers whose properties are indistinguishable from those of α8 homomers. A third chimera (α7(179)/α8) forms homomers whose affinity for ACh is slightly higher (EC_{50} ~30μM) than that of α7 homomers but distinctly different from that of α8 homomers.

Mutating the α7 subunit residue F187 to the α8 subunit residue Y187 does not alter the pharmacology of the α7 mutant homomers. Thus it appears that the remaining five residues that are different between these two subunits within α179-208 contribute significantly to the pharmacological differences between α7 and α8 homomers. Further mutagenesis is in progress to verify this prediction.

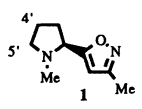
ACETYLCHOLINE RECEPTORS: NICOTINIC PHARMACOLOGY

467.1

SYNTHESIS AND RECEPTOR BINDING OF NOVEL CHOLINERGIC CHANNEL LIGANDS AS POTENTIAL COGNITION ENHANCERS.

Nan-Horng Lin, Yun He, David J. Anderson, Dave S. Garvey*, James T. Wasicak, James P. Sullivan, Stephen P. Arneric, Neuroscience Research, D-47W, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064-3500.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a global deterioration of cognitive function. (-)-Nicotine has reported beneficial effects in AD to improve cognitive performance. Preclinical testing (Arneric et. al.; Decker et. al., J. Pharmacol. Exp. Therap. in press, 1994.; Garvey et. al. J. Med. Chem., in press, 1994.) has recently demonstrated that ABT-418, **1**, [3-methyl-5-(1-methyl-2-(S)-pyrrolidinyl)-isoxazole] is a cognition enhancer that has fewer in vivo liabilities than (-)-nicotine. Like (-)-nicotine , however, ABT-418 possesses poor oral bioavailability. In order to assess what structural changes in ABT-418 are consistent with high affinity binding, an extensive SAR has been conducted on **1**. The compounds prepared were found to have binding affinity ranging from 22 -6,000 nM (displacement of [3H](-)-cytisine from whole rat brain synaptic membrane). The SAR results indicated that a bulky alkyl group or functionality at C4' or C5' position are not well tolerated for the potent binding affinity. We report here the synthesis and receptor binding of a series of pyrrolidine modified isoxazole analogs.



1

467.2

INTERACTION OF DMXBA (GTS-21), A COGNITION-ENHANCING COMPOUND, WITH CHOLINERGIC RECEPTORS.

W. R. Kem*, Vladimir M. Mahnir, and B. Lin, Dept. of Pharmacology and Therapeutics, Univ. of Florida Coll. of Med., Gainesville, FL 32610.

DMXB-Anabaseine has recently been found to enhance learning in aging rabbits (Woodruff-Pak, Li, and Kem, 1994) and rats, as well as in nucleus basalis-lesioned rats (Meyer et al., 1994). Experiments with nicotinic receptor subtypes expressed in *Xenopus* oocytes have shown that the compound acts as a very weak partial agonist upon the alpha 4-beta 2 combination, but as a strong partial agonist upon homo-oligomeric alpha 7 channels (Papke et al., 1993). It enhances LTP in the hippocampal slice (Hunter et al., 1994). We have indirectly investigated the interaction of DMXBA with several naturally expressed nicotinic receptors by measuring displacement of nicotinic radioligand binding to brain synaptosomes, modulation of PC12 rubidium efflux, and alteration of muscle contractility. DMXBA displaced 1 nM [^{3}H]CYTisine specific binding to rat brain synaptosomes with an IC_{50} of about 200 nM. Scatchard analyses of CYT binding in the presence of increasing concentrations of DMXBA showed a progressive decrease in B_{max} as well as K_d. It apparently binds to another site in addition to the ACh recognition site. Inhibition of synaptosomal alpha-bungarotoxin binding was primarily by alteration of binding affinity. DMXBA also displaced [^{3}H]QNB binding to muscarinic receptors, but with an IC_{50} about 100X higher than for displacement of CYT and BTX binding. DMXBA only inhibited nicotine-stimulated rubidium efflux from PC12 cells. Although the compound lacked nicotinic agonist action upon skeletal muscle, it did act as an antagonist at very high concentrations (>1 μM). Further studies of the actions of this compound upon different nicotinic receptor subtypes are in progress. (Supported by Taiho Pharmaceutical Co., Ltd.)

467.3

EFFECTS OF NICOTINIC ACETYLCHOLINE RECEPTOR LIGANDS ON BEHAVIORAL VIGILANCE IN RATS. S. Ruland*, J. Turchi, L.A. Holley and M. Sarter. Dept. Psychology and Neuroscience Program, Ohio State Univ., Columbus, OH 43210.

The effects of nicotinic receptor ligands on performance in a task measuring sustained attention, or vigilance, were tested. This task required the animals to discriminate between signal and non-signal events. The sequence of signal (central panel light illumination for 500, 50 or 25 msec) and non-signal presentations was randomized over 3 blocks of 54 trials each (27 signal trials, 9 per length, and 27 non-signal trials). A left lever press following a signal was counted as a hit, and a right lever press following a non-signal event was counted as a correct rejection. Hits and correct rejections were rewarded, whereas misses and false alarms (defined as incorrect right and left lever presses, respectively) were not. Baseline performance was characterized by a signal length dependent ability of the animals to discriminate between signal and non-signal events. Administration of nicotine (0.19, 0.62, 1.9 µmol) did not produce main effects on vigilance performance. The putative nicotinic receptor agonist lobeline (1.9, 6.2, 19 µmol) impaired the animals' ability to discriminate between signal and non-signal events. The antagonist mecamylamine (5, 15, 50 µmol) potently impaired vigilance performance. While the detrimental effects of lobeline may have been related to the effects of direct cholinergic stimulation in intact animals, the reasons for the differences between the effects of nicotine and lobeline remain unsettled.

467.4

EPIBATIDINE: A PROMISING LIGAND FOR NEURONAL NICOTINIC RECEPTORS R.H.Loring*, T.McHugh, X.Zhang and J.McKay. Dept. Pharm. Sci., Northeastern U., Boston, MA 02115.

Epibatidine (EB) is reported to be a potent nicotinic analgesic (Qian et al., 1993, *Eur.J.Pharm.* **250**:R14). EB agonist effects were investigated by electrophysiological recordings from perfused chick retina, and by radioligand assays for various nicotinic receptor subtypes, using anatoxin and nicotine for comparison. A 2 sec application of EB (10 μM) caused a curare-sensitive depolarization, but subsequent depolarizations due to the nicotinic agonist dimethylphenylpiperazinium (DMPP, 300 μM, 2 sec) were blocked by > 70%. Depolarizations due to glutamatergic agonists kainate or NMDA were not affected by EB. 10 μM nicotine (20 min) is the minimum dose to completely desensitize DMPP responses in retina when applied continuously, but DMPP responses recover 50% from nicotine blockade within ≈20 min. In contrast, 100 nM EB (20 min) completely blocks DMPP responses, and requires ≥1 h for 50% recovery. Preliminary data suggest that d-tubocurarine (300 μM) prevents long-term desensitization by EB. EB displaces ^{3}H-cytisine binding to $\alpha4\beta2$ receptors (K_i=3 nM) and ^{125}I-α-bungarotoxin binding to $\alpha7$-containing receptors (K_i=22 nM) immunoisolated from chick brain. The corresponding K_i's for nicotine or anatoxin were 20 nM or 8 nM for ^{3}H-cytisine and 140 nM or 22 nM for ^{125}I-α-bungarotoxin, respectively. In addition, EB blocked ^{125}I-α-bungarotoxin binding to membranes containing *Torpedo* $\alpha1$ receptors with an IC_{50} of 100 nM. These data suggest that EB is a strongly desensitizing nicotinic agonist that may be a useful ligand for several receptor subtypes. Supported by NIH NS22472.

467.5

COMPARATIVE PHARMACOLOGY OF EPIBATIDINE, A POTENT SELECTIVE AGONIST FOR NEURONAL ACHRs. V. Gerzanich, X. Peng, R. Anand, F. Wang, S. Fletcher#, J. Lindstrom*. Dept. of Neuroscience, Univ. of Pennsylvania, PA 19104-6074, #Merck Sharp & Dohme Research Laboratories, Essex CM202QR, England.

Pharmacological properties of the (+)- and (-)-isomers of the synthetic analgesic epibatidine(EB), originally isolated from the skin of the frog *Epipedobates tricolor*, were tested on different chicken and human nicotinic AChRs.

In competition binding assays EB was used on immunoaffinity isolated chicken brain($\alpha4\beta2$,$\alpha7$,$\alpha8$) and human neuronal nicotinic ($\alpha3$ and $\alpha7$)AChRs(from the SHSY-5Y cell line). Both EB isomers exhibited extremely high affinity for all neuronal AChRs tested, with IC_{50} values ranging from 1fM(human $\alpha3$AChRs) to 1µM(chicken $\alpha7$AChRs). By contrast, no EB binding was observed on human muscle type AChRs from the cell line TE671.

EB behaved as an extremely potent full agonist on chicken ($\alpha3\beta2$, $\alpha3\beta4$, $\alpha4\beta2$, $\alpha7$, and $\alpha8$) and human ($\alpha3\beta2$) neuronal AChRs expressed in *Xenopus* oocytes. Currents induced by EB were effectively blocked by the nicotinic antagonists hexamethonium and mecamylamine. Apparent affinity was 100 to 1000 times higher for EB as compared to nicotine or ACh. EC_{50} values ranged from 1nM(for homomeric chicken $\alpha8$) to 2µM (for homomeric chicken $\alpha7$). EB did not activate or block expressed *Torpedo* muscle type AChRs at concentrations up to 1mM. Currents induced by EB in oocytes expressing chicken $\alpha4\beta2$ and $\alpha3\beta2$ showed significantly slower desensitization and inactivation kinetics than did currents induced by ACh and nicotine .

467.6

FURTHER CHARACTERIZATION OF THE IN VIVO EFFECTS OF (±)EPIBATIDINE, A POTENT NICOTINIC LIGAND. A.W. Bannon, K.L. Gunther, and M.W. Decker*. Neuroscience Research, Department 47W, Pharmaceutical Discovery, Abbott Park, IL 60064-3500.

Epibatidine has been reported to be a potent nicotinic ligand that can produce analgesia (Qian et al., Eur. J. Pharmacol., 250, 1993; Badio and Daly, Mol. Pharmacol., in press). This study further characterized epibatidine-induced analgesia, and examined additional in vivo effects of epibatidine. Consistent with earlier reports, (±)epibatidine (0.1 µmol/kg; i.p.) had antinociceptive activity in mice (n = 8/group), producing an increase in jump latency in the hot-plate paradigm. The analgesic effect of epibatidine was evident at 60 min post-injection ($p < 0.05$) but not at 120 min ($p > 0.05$). Although mecamylamine (1.5, 5, and 15 µmol/kg; i.p.) pretreatment attenuated epibatidine-induced analgesia, mecamylamine (15 µmol/kg) administered after epibatidine did not attenuate the analgesic response. At the analgesic dose of epibatidine (0.1 µmol/kg) utilized in this study, significant reductions in activity and body temperature were observed. Since these effects (e.g., motor effects) possibly contributed to the apparent analgesic effect of epibatidine, additional studies were conducted with mice pretreated (i.e., 5 days prior to analgesia testing) with the nicotinic antagonist chlorisondamine (23 µmol/kg; i.p.). In mice pretreated with chlorisondamine, the analgesic effect of epibatidine was attenuated, but there were virtually no effects on the epibatidine-induced decreases in activity or temperature. These data suggest that epibatidine produces analgesia by a central (or spinal) mechanism that can be differentiated, in part, from effects on temperature and activity.

467.7

FURTHER STUDIES ON THE ROLE OF CENTRAL NICOTINIC RECEPTORS IN THE ANALGETIC EFFECTS OF EPIBATIDINE. B. Badio*, H.M. Garraffo, Dan Shi, and J.W. Daly. Laboratory of Bioorganic Chemistry, NIDDK/NIH, Bethesda, MD 20892.

Epibatidine is a potent analgetic agent, whose site of action appears to involve central nicotinic receptors. However, epibatidines elicit analgesia even in a mouse strain in which nicotine has no analgetic activity. In mice rendered tolerant to the behavioral effects of nicotine by chronic nicotine or chronic caffeine, the analgetic effects of epibatidine are reduced. Epibatidines are about 20-fold more potent than (-)nicotine at rat brain nicotinic receptors, about 200-fold more potent at ganglionic-type receptors and about 100-fold more potent at muscle-type receptors. Responses to epibatidine are blocked like those of nicotine by both competitive and noncompetitive nicotinic antagonists. Epibatidine, like nicotine, causes desensitization of nicotinic receptors. Unlike nicotine, there is little enantioselectivity in the responses of epibatidine, either *in vivo*, in binding and functional assays with brain preparations, or in cultured cells expressing ganglionic or muscle-type nicotinic receptors. Furthermore, unlike the case for nicotine/nornicotine the presence or absence of an N-methyl group has little effect on activity of epibatidines. Molecular modeling provides a rationale for the enantioselectivity and effects of N-methylation in the nicotines and the lack of such effects in the epibatidines.

467.8

ENANTIOMERS OF EPIBATIDINE AS POTENT NICOTINIC AGONISTS AT TWO IDENTIFIED SUBTYPES OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) IN RAT HIPPOCAMPAL NEURONS. M. Alkondon[1]* and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland, Sch. Medicine, Baltimore, MD 21201; [2] Lab. Mol. Pharmacol. II, IBCCF, Fed. Univ. Rio de Janeiro, RJ, Brazil, 21944.

Epibatidine, a novel azabicycloheptane alkaloid isolated from the Ecuadorean frog *Epipedobates tricolor*, is a potent analgesic in mice (Spande *et al., J. Am. Chem. Soc.* **114**: 3475, 1992). Synthetic enantiomers of epibatidine, which also have analgesic activity, bind with high affinity to the [^{3}H]nicotine-binding site in rat brain membranes and are potent nicotinic agonists in PC12 cells (Badio and Daly, *Mol. Pharmacol.* 1994, in press). We have recently demonstrated that nicotinic agonists can activate at least three subtypes of nAChRs in rat hippocampal neurons (Alkondon and Albuquerque, *JPET* **265**: 1455, 1993). Here, we investigated the agonist effects of enantiomers of epibatidine on two subtypes of hippocampal nAChRs using whole-cell patch-clamp technique and a U-tube agonist application system. Type IA, the α-BGT-sensitive current subserved by an $\alpha7$-based nAChR, was activated by epibatidine with an apparent EC_{50} of 2 μM and 4 μM for the (−) and (+) enantiomers, respectively. Type II, the dihydro-β-erythroidine-sensitive current subserved by an $\alpha4\beta2$ nAChR, was activated by epibatidine in the low nanomolar range (apparent EC_{50} between 2 and 10 nM), the natural (+) enantiomer being more potent than the (−) form. The results indicate that epibatidine enantiomers can distinguish between the subtypes of nAChRs present in the hippocampal neurons as i) they are about 1000-fold more potent in evoking the type II current than in eliciting the type IA current; and ii) the rank order of potency of the enantiomers of epibatidine was reversed for the two types of currents. The concentration of epibatidine necessary to activate type II current is compatible with the doses that evoke an analgesic effect in mice, and suggests the involvement of the $\alpha4\beta2$ nAChR subtype in such effect. Support: NINDS Grant NS25296; NIEHS Grants ES05730 and T32-ES07263.

467.9

ADENOSINE RECEPTOR LIGANDS AS ANTAGONISTS FOR ANATOXIN-A. Peter K. Chiang*, Dennis L. Butler, and Richard K. Gordon. Dept. of Applied Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

(+)-Anatoxin-a (ANTX) stimulates guinea pig ileum contraction with a potency similar to acetylcholine (ACh), and this is due to the induced release of ACh from the ileum. The postulated target of ANTX is the nicotinic receptor on ganglionic interneurons of ileum, as evidenced by the inhibition of ANTX-induced contraction by muscarinic or nicotinic antagonists. When tested on the ANTX-stimulated guinea pig ileum, it was found that adenosine receptor agonists, *N*-cyclohexyl adenosine (CHA), 5'-*N*-ethylcarboxamido-adenosine (NECA), and N6-*R*-phenylisopropyl-adenosine (PIA), all blocked the contraction with CHA being the most potent. These findings suggest that the ganglionic nicotinic receptors are under complex regulation, with possible modulation either directly or indireclty with A_1 subtype adenosine receptors.

467.10

EFFECTS OF STEROID EXPOSURE ON LIGAND BINDING AND FUNCTIONAL ACTIVITY OF NICOTINIC ACETYLCHOLINE RECEPTORS. Lei Ke* and Ronald J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, USA.

To explore potential mechanisms for the differential modulation of nicotinic acetylcholine receptor (nAChR) expression and function during development and in response to environmental challenges, we tested how exposure to steroids, such as progesterone, estradiol, testosterone, corticosterone and dexamethasone, affects function and numbers of diverse nAChR subtypes. Acute steroid exposure has no effect on [^{3}H]acetylcholine and/or ^{125}I-labeled α-bungarotoxin (Bgt) binding to human muscle-type nAChR (of the TE671/RD clonal line) or on radioligand binding to human ganglia-type nAChR containing α3 and β4 subunits or to human nicotinic Bgt binding sites containing α7 subunits (of the SH-SY5Y neuroblastoma). However, steroid exposure inhibits function (assessed using $^{86}Rb^+$ efflux) of both muscle- and ganglia-type nAChR non-competitively with IC_{50} values in the low-intermediate μM range, depending on the specific steroid studied, with progesterone displaying highest nAChR affinity. Assays done using steroids conjugated to albumin indicate that these functional effects are due to steroid interactions with extracellular domains of nAChR. These studies suggest that fluctuation in local steroid levels could influence nAChR function acutely, via apparently allosteric mechanisms.

467.11

INCREASE IN NEURONAL NICOTINIC RECEPTOR BINDING SITES FOLLOWING CHRONIC CYTISINE TREATMENT. R.D. Mellon*, R.A. Houghtling, and K.J. Kellar. Department of Pharmacology, Georgetown University, School of Medicine, Washington, D.C. 20007.

Neuronal nicotinic acetylcholine receptor (nAchR) binding sites increase following chronic administration of nicotine. Cytisine has previously been reported to increase nicotinic binding sites labeled by [^{3}H]acetylcholine in rat frontal cortex. To investigate this further, we measured nAchR binding sites in several brain areas following chronic administration of cytisine.

Male Sprague-Dawley rats received either 1.5 mg/kg cytisine, 2 mg/kg nicotine tartrate, or saline vehicle s.c. twice daily for ten days. Following decapitation, cerebral cortex, hippocampus, striatum, thalamus, and hypothalamus were dissected and nicotinic receptor binding sites were measured by [^{3}H]cytisine binding assays. Both chronic cytisine and nicotine treatment led to increased numbers of binding sites compared to saline treatment in cerebral cortex, striatum, and hypothalamus ($p<0.01$). Although similar trends were observed for the hippocampus and thalamus, statistically significant increases in these brain areas were not reached in these studies. Further, no difference in [^{3}H]cytisine binding between the nicotine and cytisine treatment was found in any brain area examined.

Recent studies in transfected oocytes found that cytisine is much less effective than acetylcholine at eliciting membrane current in receptors containing the β2 subunit, thus demonstrating properties of a partial agonist. Since chronic nicotine treatment in rats increases predominantly α4β2 nAchRs, and nicotinic antagonists such as mecamylamine and dihydro-β-erythroidine do not appear to increase nicotinic binding sites following chronic administration, our data suggest that cytisine, like nicotine, possesses enough agonist properties at α4β2 receptors to increase the number of binding sites in rat brain.

This work was supported by a grant from NIH (DA06486).

467.12

LOBELINE AND CYTISINE: EVIDENCE FOR PARTIAL AGONISM IN THE NICOTINE DRUG DISCRIMINATION PROCEDURE. M.D.B. Swedberg*, K. Rimvall and P.D. Suzdak. Novo Nordisk A/S, Pharmaceuticals Division, Novo Nordisk Park, DK-2760 Måløv, Denmark.

Lobeline (1.0 - 6.0 mg/kg, s.c., 15') and cytisine (0.1 - 0.6 mg/kg, s.c., 15') produced maximal nicotine effects of 40.2% and 48.6% at 6.0 and 0.6 mg/kg in rats trained to discriminate nicotine (0.1 mg/kg, s.c., 15'; ED_{50}: 0.02 mg/kg) from no drug. Rates of responding were reduced to 23% and 86% of vehicle controls at 6.0 mg/kg of lobeline and 0.6 mg/kg of cytisine, respectively. In ^{3}H-methylcarbachol (MCC) receptor binding to rat hippocampal membranes, cytisine (K_i: 0.9 nM) was more potent than lobeline (K_i: 3 nM) and nicotine (K_i: 5 nM). The muscarinics RS86 and oxotremorine were not effective in the drug discrimination or ^{3}H-MCC assays, respectively. The apparent discrepancy between binding affinities and effects in the nicotine drug discrimination tests is consistent with previously published data, and it has been suggested that lobeline may be a partial agonist at the nicotinic receptor (Abood et al., 1988) and that cytisine may be a partial agonist at receptors containing the α_4 and β_2 subunits (Papke and Heinemann, 1994). The effects of these compounds will be explored in other physiological systems to determine the morphological basis for the observed effects.

467.13

BUPIVACAINE BLOCKS NEURONAL NICOTINIC RECEPTORS IN THE CLOSED-CHANNEL STATE. Y-P. Yu[1], D. R. Burt[1]*, and E.X. Albuquerque[1,2]. Depts. [1]Pharmacol. & Exp. Ther., and [2]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, Brazil 21944.

The local anesthetic bupivacaine has been reported to be an open channel blocker of nicotinic receptors (nAChRs) in cultured rat hippocampal neurons (*Abs. Soc. Neurosci.* 19:1535, 1993) and in frog sartorius muscle (*Mol. Pharmacol.* 26:293, 1984). In the present study, we further investigated the interaction of bupivacaine with hippocampal nAChRs using patch-clamp technique. Whole-cell currents were recorded from fetal rat hippocampal neurons cultured for 10-30 days. Bupivacaine was applied by perfusion in the bath or in pulses together with ACh via a U-tube. The peak amplitude of the predominant, MLA-sensitive, fast-decaying currents, could be reduced by bupivacaine in a concentration-dependent manner. In addition, when the neurons were incubated with 50 μM bupivacaine prior to activation of nAChRs by ACh (3 mM), blockade increased with preincubation time (2-8 min). This time-dependent blockade is suggestive of a binding site for bupivacaine distinct from the site within the open channel that has been identified previously in neuronal and muscle nAChRs. Closed-channel blockade of nAChRs by bupivacaine was also evidenced by a decrease in peak amplitude of the fast decaying currents without a significant change in the decay time constants. The lack of voltage dependence of bupivacaine blockade of the fast-decaying currents also indicated that the binding site for bupivacaine on nAChRs was outside of the voltage-sensitive region of the channel itself. Competition experiments showed that the 18-20% blockade of fast decaying nicotinic currents by 100 μM bupivacaine remained unchanged over a wide range of concentrations of ACh (100 μM to 10 mM), indicating that the binding by bupivacaine to this type of receptor was noncompetitive and was not allosterically affected by the agonist. This action of bupivacaine on the closed channel, observed on neuronal nAChRs, was not reported for muscle nAChRs. (Support: USPHS Grant NS 25296)

467.14

NICOTINIC ACETYLCHOLINE RECEPTOR ELECTROPHYSIOLOGY AND PHARMACOLOGY OF ABT-418 IN PC12 CELLS. C.A. Briggs*, M.L. Hughes, L.M. Monteggia, T. Giordano, D. Donnelly-Roberts and S.P. Arneric. Neuroscience Research D-47W, Abbott Laboratoriess, Abbott Park, IL 60064-3500.

In view of the pharmacologic and physiologic diversity of nicotinic acetylcholine receptors (nAChRs), there are intriguing possibilities for developing novel nicotinic agonists that have reduced adverse effects while retaining the beneficial effects of (-)nicotine. One example, ABT-418, is demonstrated to activate ionotropic nAChR in PC12 cells as a model for the ganglionic subtypes.

PC12 cells were maintained in DMEM containing 10% heat-inactivated fetal calf serum and 5% heat-inactivated horse serum. After 4-7 days exposure to 100 ng/ml NGF, the whole-cell patch-clamp technique was used to record ligand-gated currents activated by substances applied at defined concentrations using the U-tube flow-reversal technique (V_h = –60 mV). The extracellular solution contained 150 mM NaCl, 2.8 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, ≥ 10 mM dextrose and 10 mM Na-HEPES buffer (7.3 pH, 325 mOsm) while the intracellular (recording pipette) solution contained 140 mM KCl, 1.0 mM $CaCl_2$, 2.0 mM $MgCl_2$, 11 mM K-EGTA, and 10 mM K-HEPES buffer (7.3 pH, 315 mOsm).

ABT-418 activated inward currents with a potency 4-fold lower than (-)nicotine (EC_{50} = 214 μM and 52 μM, respectively), while its efficacy was not significantly different from (-)nicotine (89%). Responses to 300 μM ABT-418, like those to 100 μM (-)nicotine, were reversibly inhibited 81% by 10 μM mecamylamine, 38% by 10 μM dihydro-β-erythroidine, and 82% by 100 μM dihydro-β-erythroidine. Furthermore, responses to maximal concentrations of ABT-418 (3 mM) and (-)nicotine (1 mM) were not additive. However, the Hill coefficient for ABT-418 (1.18) was smaller than that for (-)nicotine (1.77), and high concentrations of ABT-418 appeared to elicit a more rapidly decaying response than did (-)nicotine. Thus, ABT-418 activated ganglion-like nAChR channels in PC12 cells with a slightly lower potency than (-)nicotine and with subtle differences in the functional dynamics.

467.15

TOLERANCE TO NICOTINE FOLLOWING CHRONIC TREATMENT : A POTENTIAL ROLE FOR CALCIUM CHANNELS. M.I. Damaj * S.P. Welch and B.R. Martin. Department of Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298.

Recent findings support a role for the L-type calcium channels in the pharmacological effects of nicotine. The purpose of this study was to determine whether calcium channels were involved in nicotine behavioral tolerance in mice. Male ICR mice were chronically co-administered nimodipine (5 mg/kg, i.p.) and nicotine (2 mg/kg, s.c.) twice a day for 10 days. At day 11, mice were challenged with different doses of nicotine and the effect on the tail flick test was measured. In another group of mice, nicotine (2 mg/kg, s.c.) was given chronically to the animals (twice a day for 10 days). At day 11, mice were challenged with different doses of BAYK8644 and its effect on motor coordination (rotarod test) and locomotor activity was measured after i.p. administration. The effect on the tail flick test was assessed after intrathecal (i.t.) administration of BAYK8644. Chronic co-adminstration of nimodipine with nicotine, significantly reduced tolerance to the antinociceptive effects of nicotine. On the other hand, BAYK8644 effects in nicotine-treated mice decreased. Indeed, the ED_{50} for BAYK8644-induced motor impairment increased from 0.35 to 2.0 mg/kg in tolerant mice. In addition, the ED_{50} for BAYK8644-induced antinociception after i.t. injection increased from 3.7 µg/mouse to 12 µg/mouse. These results suggest that L-type calcium channels play a role in nicotine tolerance in mice. Further experiments are needed to explain the mechanisms underlying these effects. (Supported by NIDA grant #DA-05274).

467.16

CALCINEURIN AND PKC REGULATE AChR FUNCTION AT SNAKE TWITCH FIBER ENDPLATES. R. L. Parsons* and J. C. Hardwick. Department of Anatomy & Neurobiology, University of Vermont, Burlington VT 05405.

Staurosporine decreases the extent of recovery of endplate sensitivity following prolonged exposure to carbachol, presumably by inhibition of protein kinase activity (Hardwick & Parsons, Br J Pharmacol **108**: 741, 1993). Experiments have been done to determine which protein kinase is responsible for full recovery from agonist exposure. All experiments were done *in vitro* on garter snake costocutaneous muscles maintained in an isotonic potassium propionate solution. Following treatment with 50 µM sphingosine, an inhibitor of CaMK and PKC, the extent of MEPC amplitude recovery following a 10 min exposure to 540 µM carbachol was decreased by 33%. Conversely, KN-62 and lavendustin A, inhibitors of CaMK II and tyrosine kinase respectively, had no effect on MEPC amplitude recovery. Chronic treatment (18-20 hrs) with 200 nM PMA, which down regulates PKC, also reduced the extent of MEPC recovery by 27%. In contrast, the inactive analog 4αPMA had no effect. Following carbachol exposure in PKC-inhibited preparations, two populations of ACh-activated channels, one of ~50 pS and one of ~25 pS, were observed. In untreated preparations, only the large conductance channel was observed. Treatment of preparations with 0.5 µM deltamethrin, an inhibitor of protein phosphatase 2B (calcineurin) prevented the decrease in MEPC recovery associated with PKC inhibition and decreased the occurrence of small conductance channels. These observations suggest that AChRs or associated proteins are dephosphorylated by calcineurin during prolonged agonist exposure and this results in a population of ACh channels with a reduced conductance. Full recovery of these dephosphorylated AChR complexes requires rephosphorylation by PKC. Supported by NIH grant NS 23978.

467.17

ACTIVATION AND DESENSITIZATION OF NICOTINIC RECEPTORS ON ISOLATED HISTAMINERGIC NEURONS OF THE RAT TUBEROMAMMILLARY NUCLEUS. V.Uteshev, D.R.Stevens and H.L. Haas* Physiologisches Institut II, Heinrich-Heine-Universität, 40001 Düsseldorf, Germany

The actions of nicotinic agonists were examined on isolated neurons of the rat tuberomammillary nucleus utilizing rapid application and whole cell recording methods. Acetylcholine (Ach) and 1,1-dimethyl-4-phenyl-piperazinium (DMPP) activated rapidly rising inwardly rectifying currents with reversal potentials near 0 activated currents followed a dual time course while DMPP currents mV. Responses to both agonists desensitized completely. The rate of desensitization was concentration and voltage-dependent. Prolonged treatment with Ach at concentrations below the threshold for activation of inward current resulted in reduction of responses to higher concentrations of agonist. The desensitization of Ach occurred with two time constants while DMPP desensitized more rapidly with a single time course. At hyperpolarized membrane potentials there was a use-dependent reduction in responses which was long lasting and was reversed by depolarization. Projections from cholinergic regions of the basal forebrain to the tuberomammilary nucleus have been shown and rapid nicotinic neurotransmission may occur in this pathway. Supported by Human Frontier Science Program and Deutsche Forschungsgemeinschaft.

467.18

DIFFERENTIAL MODULATION OF NEURONAL NICOTINIC ACh RECEPTOR-CHANNELS BY NEUROPEPTIDES. J. Cuevas, D.J. Adams* Dept. Molec. & Cell. Pharmacology, Univ. of Miami Sch. of Med., Miami, FL 33101

Vasoactive intestinal polypeptide (VIP)- and substance P (SP)-immunoreactive neurons and nerve fibers have been found in the mammalian intracardiac ganglia (Weihe et al., 1984, Cell Tis. Res. 236, 527). The effects of VIP and SP on ACh-evoked currents were investigated in cultured rat parasympathetic cardiac neurons under voltage-clamp, using standard whole-cell, perforated-patch, and outside-out recording configurations of the patch clamp technique. Pressure ejection of VIP onto the soma increased the ACh-evoked current amplitude ~2-fold, with half-maximal potentiation occurring at 260 pM VIP (n = 3). VIP also potentiated the current evoked by nicotine (100 µM), but not those evoked by muscarine or ATP. Mecamylamine (3 µM) completely inhibited the currents elicited by ACh and nicotine in the presence of VIP. Pre-treatment of cultured neurons with pertussis toxin (200 ng/ml), intracellular application of GDP-β-S (100 µM), and bath application of L-8-K all blocked VIP-induced potentiation, suggesting that the effect of VIP is mediated by a G-protein coupled receptor. In outside-out membrane patches, bath application of ACh (4 µM) and VIP (4 nM) reduced the mean closed time between bursts of ACh receptor-channel activity by 36% (n = 3) compared to that observed with ACh alone. The mean closed time between clusters of bursts was reduced by 44%, whereas mean unitary current amplitude and apparent channel open time were unaffected by VIP. The open probability of ACh-activated channels in the patches increased ~1.6 fold, from 0.018 to 0.028, in the presence of VIP. Pressure ejection of SP onto the voltage-clamped soma reduced the peak amplitude of the ACh-evoked current, with half-maximal inhibition occurring at 46 µM and near complete block at 1 mM SP (n = 7). The decay rate of ACh-evoked currents was increased by SP, suggesting that SP may increase the rate of desensitization of the ACh receptor-channel. Since ACh is the primary neurotransmitter of extrinsic (vagal) innervation of the mammalian heart, VIP and SP may play an important role in modulating autonomic control of the heart.

467.19

SECOND PATHWAY OF NICOTINIC RECEPTOR ACTIVATION: EFFECTS OF DESENSITIZING AGENTS AND OPEN-CHANNEL BLOCKERS. T. Tano*, C.M.S. Teixeira, M.C. Heluy-Dantas, B.S. Nascimento, Y. Aracava, and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21944, Brazil; Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA.

It has been demonstrated that (-)physostigmine, certain polyamines, and 4-methylpyrazole (4-MP) can activate nicotinic receptors (nAChRs) expressed in *Torpedo* electric organ, hippocampal neurons, and muscles by binding to a nAChR site distinct from that for acetylcholine (ACh). This novel pathway of nAChR activation was shown to be insensitive to competitive nicotinic antagonists and to nAChR desensitization by high concentrations of ACh, and to be sensitive to the blockade by the nAChR-specific monoclonal antibody FK1. In this study, we evaluated whether agents known to enhance ACh-induced muscle nAChR desensitization (e.g. nortriptyline) or to produce blockade of muscle nAChR in its open conformation (e.g. atropine) also could affect the 4-MP-induced nAChR activity in *L. ocellatus* muscle fibers under cell-attached condition. Whereas nortriptyline (0.1-1 µM) reduced ACh- or anatoxin (AnTX)-induced single-channel activity, it affected neither the frequency nor the kinetic properties of 4-MP-activated single channels. In contrast, atropine (2-10 µM) blocked, in a similar manner, both ACh- and 4-MP-induced single-channel activity. Short-lived, isolated single channels and no evidence for burst activity were observed, indicating a rather slow rate of unblocking for atropine. Our results indicate that the desensitization by non-competitive blockers, similar to that induced by high concentrations of ACh, uncouples the ACh-binding site from channel gating, and does not prevent nAChR activation *via* another pathway. On the other hand, nAChR channels opened *via* either pathway are amenable to the action of an open-channel blocker. Support: Mol. Pharmacol. Train. Prog. FINEP/UMAB, FINEP and CNPq.

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RE-APPRAISING THE BUNGAROTOXIN-SENSITIVITY OF NICOTINIC RECEPTORS ON BULLFROG SYMPATHETIC NEURONS JP Horn*, W-X Shen and P Jobling Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

An earlier study reported that α-bungarotoxin (α-bgt) blocks synaptic transmission in bullfrog sympathetic ganglia (Marshall, PNAS, **78**, 1948,1981). This unusual finding suggests that nicotinic receptors on amphibian autonomic neurons may be fundamentally different from neuronal nicotinic receptors in other ganglia (see Libscombe and Rang, J. Neurosci 8, 3258, 1988).

We have studied the sensitivity of nicotinic synapses to α-bgt and neuronal-bungarotoxin (n-bgt) in the B and C cell systems of bullfrog sympathetic ganglia 9 & 10 by recording compound postganglionic action potentials from rami communicantes. High concentrations (10 µM) of α-bgt applied for up to 8 hours had no effect upon synaptic transmission in either the B or C cell system. Ganglia pretreated with collagenase were also insensitive to α-bgt. In control experiments on isolated sartorius muscle preparations, nerve-evoked twitches were fully blocked by 30-100 nM α-bgt. By contrast, 30-300 nM n-bgt blocked nicotinic transmission in the B and C cell systems. Block appeared within 25-45 min and reversed fully with a half-time of 40-80 min. This was indistinguishable from washout times after block by 100µM d-tubocurarine. Based on their bungarotoxin-sensitivity the nicotinic receptors mediating ganglionic transmission in bullfrog sympathetic B & C neurons should be classified as neuronal in type.

Supported by a Grant-in-Aid and a Postdoctoral Fellowship (PJ) from the American Heart Association, PA Affiliate and NIH grant NS01427.

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A COMPARISION OF NICOTINIC RECEPTORS ON SYMPATHETIC B AND C NEURONS: KINETICS, VOLTAGE-DEPENDENCE AND COMPETITIVE BLOCK BY D-TUBOCURARINE W-X Shen* and JP Horn Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Previous studies have shown that nicotinic receptors on sympathetic B and C neurons differ in their kinetics (Marshall, J Neurosci **6**, 590,1986) and that synaptic transmission in the C system is more sensitive to d-tubocurarine(DTC) than in the B system (Shen & Horn, Neursosci Abst 1993). We have now used two-electrode voltage clamp recordings to analyze the voltage-dependence of receptor kinetics and DTC's actions on these 2 cell types. Consistent with earlier reports, EPSC decay follows a single time constant (τ) that is shorter in B than C cells (5.1±0.6 vs 8.9±1.2 ms at -50 mV). EPSC's in both cell types have similar reversal potentials (-3.9 vs -5.6 mV) and show little rectification at negative potentials. The voltage-dependence of τ is also similar in B (-0.0033± 0.0012 mV^{-1}, n=28) and C (-0.0039± 0.0011 mV^{-1}, n=13) cells. In both cell types, 3 μM DTC reduces EPSC amplitude to the same extent, independent of membrane potential (-50 mV: 41% in B cells, n=8 and 47% in C cells, n=4, P>0.05; -100 mV: 47% in B cells, n=6, and 50% in C cells, n=3, P>0.05). DTC has no effect upon the shape of the I-V relation or τ. These results provide evidence that nicotinic receptors on B and C cells are similar in every way except for τ. In both cell types DTC acts as a competitive antagonist with similar affinity. Transmission in the C system is more sensitive to DTC because of its lower synaptic conductance (B:199nS, C:154nS). Supported by a Grant-in-Aid from the American Heart Assoc., PA Affiliate and NIH NS21065 & NS01427.

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[^{3}H]NICOTINE DOES NOT DETECT NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) IN HUMAN AND BOVINE PIAL VESSELS, CORTICAL MICROVESSELS (MVs) AND CAPILLARIES (CAPs). D.G. Linville* and E. Hamel, Montreal Neurological Institute, McGill Univ., Montréal, QC, Canada, H3A 2B4.

Previous studies have implicated nAChRs, possibly located in the basal forebrain, in the regulation of cortical cerebral blood flow in the rat (Linville et al., J. Pharmacol. Exp. Ther. 1993, 267:440). However, nAChRs have been reported in human and pig cerebral MVs (Homayoun et al. Neurosci. Abst. 1992, 337.12). In an attempt to assess the neuronal and/or vascular localization of nAChRs involved in blood flow regulation, we measured nAChRs in post-mortem human and bovine cerebral cortex, and isolated cortical MVs and CAPs. Saturation experiments were performed in human (n=2) and bovine (n=3) cerebral cortices using [^{3}H]nicotine as the radioligand and yielded the following respective binding parameters: K_D of 4.7±1.4 and 4.3±0.6 nM, and B_{max} of 14.1±7.9 and 49.7±2.4 fmol/mg protein. Specific binding in human and bovine cortices was 67±4 and 92±3 %, respectively, and Hill coefficients were 0.88±0.4 and 1.0±0.18. In contrast, cortical MVs and CAPs from both human and bovine did not yield any saturable [^{3}H]nicotine specific binding that could be analyzed with the LIGAND program. Similar results were also obtained with bovine pial vessels. In the presence of 5nM [^{3}H]nicotine, binding equilibrium was reached within 90 min in the bovine cerebral cortex (n=2) and then a time-dependent dissociation was initiated with 5μM cold nicotine which leveled off after 60 min. In contrast, pial vessels did not display any ligand-receptor complex association/dissociation characteristics. These results suggest that [^{3}H]nicotine-labelled nAChRs are not present, or are below detectable levels, in pial vessels and intracortical microvascular beds and thereby cannot significantly contribute to regulation of cortical cerebral blood flow. Supported by the Fonds de la Recherche en Santé du Québec and MRC of Canada.

467.23

MULTIPLE ACTIONS OF THE ANTICHOLINESTERASE PESTICIDE ALDICARB ON MUSCLE NICOTINIC RECEPTORS. M.F.M. Braga, L.F.F. Almeida, A.L. Almeida, L.G. França, W.S. Côrtes, W.M. Cintra, Y. Aracava*, and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21944, Brazil; Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Medicine, Baltimore, MD 21201, USA.

Previously we demonstrated that as the concentration of the cholinesterase (ChE) inhibitor Aldicarb is increased, there is a potentiation and then a depression of nerve-elicited muscle twitch, and that these effects cannot be fully accounted for by ChE inhibition (*Soc. Neurosci. Abs.* **19**:1526, 1993). In this study, we evaluated whether Aldicarb acts directly on muscle nAChRs by analyzing its effects on muscle contracture elicited by ACh or by ChE-resistant nicotinic agonists, and on ACh-evoked whole-cell and single-channel currents in preparations with negligible ChE activity. Aldicarb (10-800 μM) potentiated frog rectus abdominis contracture tension elicited by ACh (0.2-10 μM), carbamylcholine (5-60 μM), or (+)-anatoxin-a (0.1-1.0 μM). This effect of Aldicarb was inversely related to agonist concentration. In cultured rat myoballs, Aldicarb (50 μM) increased the peak amplitude of ACh (1 μM)-evoked whole-cell currents, and in single interosseal muscle fibers from the hind foot of *L. ocellatus*, Aldicarb (10-100 μM) increased the frequency of ACh (0.4 μM)-induced single-channel activity over the recording time. However, Aldicarb decreased the mean open time of ACh-activated channels and increased the number of brief closures within a burst, indicating that this carbamate-oxime can act as an open-channel blocker of muscle nAChR. Our results suggest that potentiation of nicotinic responses by Aldicarb could be due to an allosteric action of this agent on the nAChR, and that such potentiation is counteracted by its open-channel blocking activity. Support: Mol. Pharmacol. Train. Prog. FINEP/UMAB; FINEP and CNPq grants and fellowships.

467.24

INHIBITION BY LEAD OF NICOTINIC CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS. J.G. Montes[1], K. Ishihara[1], M. Alkondon[1], A.T. Eldefrawi[1*], and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, RJ 21944, Brazil.

Previous studies suggested that at least some of the neurotoxic effects of Pb^{2+} are due to inhibition of NMDA receptors (*FEBS Lett.* **261**:124, 1990; *JPET* **263**:868, 1992). However, evidence that Pb^{2+} inhibits nicotinic currents in neuroblastoma cells (*Toxicol. Appl. Pharmacol.* **103**:165, 1990) prompted us to investigate the effects of Pb^{2+} on ACh-induced currents in cultured hippocampal neurons. The neurons were obtained from fetal rats (2-3 days *antepartum*) and cultured for 13 to 34 days. Recordings were made of whole-cell currents evoked in neurons by ACh pulses (in the presence of 1 μM atropine) of 0.2 to 1 sec duration delivered via a "U" tube in the absence or presence of Pb^{2+} in the bath. Of the 4 distinct nicotinic currents that can be elicited in cultured hippocampal neurons, the most common types, IA (83 %) and IB (10 %), are fast decaying currents blocked entirely (IA) or partially (IB) by 10 nM α-Bgt and by 1 nM methyllycaconitine (*JPET* **265**:1455, 1993). In this study, Pb^{2+} (1-30 μM) reduced the peak amplitude of ACh (1 mM)-induced fast decaying currents (types IA or IB) with an IC_{50} of 3.0 μM and Hill coefficient of 1.0. In contrast, the slowly decaying (type II) currents were less sensitive to Pb^{2+}, as 30 μM Pb^{2+} inhibited the peak amplitude of this type of current by only 50 %. There was no apparent relationship between age of the neurons in culture and the sensitivity of the nicotinic currents to Pb^{2+}. Inhibition of IA currents by Pb^{2+} was noncompetitive and voltage-independent. Furthermore, Pb^{2+} did not alter the decay rate of the 2 types of currents examined. The results of our study suggest that Pb^{2+} inhibits fast decaying nicotinic currents in hippocampal neurons by binding to a single nicotinic ACh receptor (nAChR) allosteric site located outside of the electric field of the membrane. The nAChR, like the NMDA receptor (*FEBS Lett.* **261**:124, 1990; *JPET* **263**:868, 1992), may be involved in Pb^{2+}-induced neurotoxicity. Support: NINDS Grant NS25296; NIEHS Grants ES05730 and T32-ES07263.

467.25

NICOTINIC CURRENTS IN NEURONS ACUTELY DISSOCIATED FROM RAT HIPPOCAMPUS AND INHIBITION OF THE CURRENTS BY LEAD. K. Ishihara[1*], M. Alkondon[1], J.G. Montes[1], and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, RJ 21944, Brazil.

Our previous study of nicotinic currents in cultured rat hippocampal neurons revealed the presence of four distinct types of currents, of which one, type IA, was the predominant response (*JPET* **265**: 1455, 1993). To determine the most common type of nicotinic currents in neurons that have developed *in vivo*, we performed studies on neurons acutely dissociated from the CA1 region of the hippocampus of 4- to 20-day-old rats. The neurons were dissociated mechanically. Whole-cell currents induced by 1-sec pulses of ACh were recorded in the presence of 1 μM atropine. Most of the currents induced by 1 mM ACh decayed rapidly, and all of the rapidly decaying currents tested with 1 nM methyllycaconitine were blocked, indicating that, as in cultured neurons (*JPET* **265**: 1455, 1993), most of the currents were type IA. As seen in type IA currents evoked in cultured hippocampal neurons (*JPET* **265**: 1455, 1993), the peak amplitude of fast decaying currents in acutely dissociated hippocampal neurons had run down by about 60 % 15 min after the initiation of whole-cell recordings. Also as in cultured neurons (*J. Rec. Res.* **11**: 1001, 1991), the peak amplitude of currents induced by 1 mM ACh from acutely dissociated neurons increased with age of the rats (17 ± 2 pA in 4-6 days (n=7) and 71 ± 24 pA in 15-20 days (n=23)). However, the fraction of ACh-sensitive neurons (peak amplitude ≥ 10 pA) was age independent (remaining at *ca.* 60 %), while the fraction of agonist-sensitive neurons in culture varied considerably, *i.e.*, 33 - 98 %, increasing with neuron age. These results suggest that the majority of nicotinic currents that can be elicited from hippocampal CA1 neurons are type IA, and that the population density of nicotinic ACh receptors in these neurons increases with age. In addition, the sensitivity to Pb^{2+} of ACh-induced currents was similar to that seen in parallel studies that we performed on cultured rat hippocampal neurons (see J. G. Montes *et al.*, *this issue*); *e.g.*, Pb^{2+} (10 μM) inhibited ACh-induced fast-decaying currents by 70 % and 80 %, respectively, in acutely dissociated and cultured rat hippocampal neurons. Support: NINDS Grant NS25296; NIEHS Grants ES05730 and T32-ES07263.

467.26

Inhibition Of Neuronal Nicotinic Acetylcholine Receptor (nAChR) Induced Cation Efflux By Beta Amyloid Peptide Fragments- D.L. Donnelly-Roberts*, S.P. Arneric, and J.P. Sullivan Neuroscience Research (D-47W), Abbott Laboratories, Abbott Park, IL, 60064-3500

Two hallmarks of Alzheimer's disease (AD) are the 39-43 amino acid peptide beta-amyloid (βA4), the primary constituent of the plaques found in the post-mortem brains, and the loss of cholinergic markers in the neocortex and hippocampus such as nAChRs. A number of studies have shown that the βA4 peptide and various peptide fragments of βA4, i.e βA4(25-35) are neurotoxic *in vitro* through an as yet undefined mechanism. In the present study, the ability of several βA4 peptide fragments to directly modulate nAChR function was investigated. Specifically, the effects of these agents on nAChR-induced $^{86}Rb^+$ efflux in the human neuroblastoma SH-SY5Y cell line was examined. None of the βA4 peptides possessed any agonist activity at concentrations from 1 nM to 100 μM. The βA4 fragments did, however, display a differential ability to inhibit (-)- nicotine induced cation efflux. βA4 (25-35) was the most potent peptide evaluated with an IC_{50} value of 1.2 ± 0.3 μM (n=3). Substance P, which shares the same three C-terminal residues (X-Gly-Leu-Met-NH_2) as βA4 (25-35) and has been shown to modulate nAChRs *in vitro* , also inhibited (-)-nicotine-induced cation efflux (IC_{50} = 0.7 ± 0.1 μM). The triad of residues is inherent to βA4(1-40) but this peptide was 15-100 fold less potent than βA4 (25-35) depending on the aggregation state of the peptide. Thus, it may be that the conformational folding of βA4 (1-40) prevents optimal exposure of these residues especially in the aggregated state. In contrast, a control peptide, Angiotensin II, and βA4 (1-28) both of which lack these three C-terminal residues, had no effect on cation efflux at concentrations up to 100 μM. These results demonstrate that the βA4 peptide fragments can modulate the function of the nAChR in vitro and that this modulation could be linked to loss of these receptors during the pathogenesis of AD. Whether blockade of nAChR function underlies the neurotoxicity associated with the 25-35 peptide remains to be determined.

468.1

RECTIFICATION OF ALPHA-BUNGAROTOXIN-SENSITIVE, NICOTINIC WHOLE-CELL CURRENTS EVOKED IN RAT BRAIN NEURONS IS Mg^{2+}-DEPENDENT. E.X. Albuquerque[1,2]* and M. Alkondon[1]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland, Sch. Medicine, Baltimore, MD 21201; [2] Lab. Mol. Pharmacol. II, IBCCF, Fed. Univ. Rio de Janeiro, RJ, Brazil, 21944.

Recent work from our laboratory indicated the presence of multiple subtypes of nicotinic acetylcholine receptors (nAChRs) in rat hippocampal neurons (*JPET* 265: 1455, 1993). The predominant subtype, subserved by the $\alpha7$ subunit, gives rise to fast-desensitizing whole-cell currents (referred to as type IA) that rectify weakly and are blocked by α-bungarotoxin (α-BGT, 10 nM). A less predominant subtype, subserved by $\alpha4\beta2$ subunits, gives rise to slowly desensitizing whole-cell currents (referred to as type II) that rectify strongly and are blocked by dihydro-β-erythroidine (100 nM). Here, we investigated the subtype/s of nAChRs present in rat olfactory bulb (OB) neurons, and studied the mechanism of rectification of α-BGT-sensitive nicotinic currents evoked from both OB and hippocampal neurons. In OB neurons grown in primary culture, acetylcholine and other nicotinic agonists evoked fast-desensitizing whole-cell currents that were blocked by both α-BGT (10 nM) and methyllycaconitine (1 nM), suggesting that IA currents were the predominant type elicited in OB neurons. In the presence of 2 mM Mg^{2+} in the internal recording solution (F^--free), type IA currents in both types of neurons showed a marked nonlinearity in the current-voltage plots such that smaller outward and larger inward currents (inwardly rectifying) were recorded in the range of -100 mV to +80 mV. In contrast, in nominally Mg^{2+}-free internal recording solutions, a loss of rectification of type IA currents was evident as the whole-cell recording progressed in time, and a more linear current-voltage relationship was obtained under these conditions. However, type II currents recorded in hippocampal neurons revealed strong rectification even in Mg^{2+}-free internal recording solutions. The results suggest that the nicotinic currents elicited from α-BGT-sensitive nAChRs in both OB and hippocampal neurons differ from other types of nicotinic currents in their rectification property, being regulated (probably blocked from the inside) by intracellular Mg^{2+} rather than by a voltage-dependent, intrinsic channel-gating mechanism. Supp: NINDS Grant NS25296; NIEHS Grants ES05730, T32-ES07263

468.2

ELECTROPHYSIOLOGICAL EVIDENCE FOR PRE- AND POSTSYNAPTIC NICOTINIC RECEPTORS IN THE RAT INTERMEDIOLATERAL CELL NUCLEUS. **A.Bordey,M.S.Ghandour* and Feltz,P.. Lab. de Physiologie Générale, Univ.Louis Pasteur, 67084 Strasbourg Cedex, France.**

We studied the responses of cholinergic nicotinic agonists, DMPP (Dimethyl-4-phenyl piperazinium iodide) and nicotine in thin (250µM) transverse slices of the thoracic and lumbar spinal cord of neonatal rats (P0-P8) using the whole-cell recording configuration of the patch-clamp technique. Recorded neurons were identified as sympathetic preganglionic neurons (SPNs) on the basis of their location (Intermediolateral cell column (IML), morphology and electrophysiological properties (resting potential of -48.6±1.5mV (mean±sem, n=30), membrane resistance of 122.7±10.9MΩ(n=43) and presence of an A current). In SPNs, DMPP and nicotine had two effects : 1)a short term effect consisting of the induction of a postsynaptic current (n=12/21) and 2)a long term effect which is the induction of fast excitatory and/or inhibitory currents (respectively EPSCs and IPSCs) lasting 20-30min after the end of the nicotinic agonist application.At -50mV, a DMPP application either by iontophoresis or by pressure induced an inward current accompanied by an increase in conductance and characterized by two phases: a first transient peak (-181.3±100pA, n=3) showing a fast desensitization followed by a plateau phase (-77.8±14.0pA, n=7). This second phase diminished in amplitude after successive applications of DMPP (reduction between two successive applications of DMPP (5sec) spaced by 3min is 36.0±8.7%, n=3). It reversed at 0mV and was not abolished by bath perfusion of lanthanum (200µM). As regards the long lasting effect, each nicotinic agonists (10-20sec) triggered the appearance of EPSCs and/or IPSCs both in SPNs and in interneurons. These results suggest that nicotinic receptors are located postsynaptically on SPNs and presynaptically on both glycinergic interneurons that project to SPNs and on glutamate-containing terminals that project either to SPNs or to unidentified neurons of the IML.

468.3

CHARACTERISTICS OF NICOTINIC WHOLE-CELL CURRENTS IN PC12 CELLS. W. Randall, E.F.R. Pereira, K.A. Gregerson*, A. Storch, A. Maelicke, and E.X. Albuquerque. Depts. Pharmacol. Exp. Ther. & Pediatrics, Univ. MD Sch. Med., Baltimore, MD 21201, USA; Lab. Mol. Neurobiol., Inst. Physiol. Chem. & Pathobiochem., Johannes-Gutenberg University Med. Sch., Mainz D-600, Germany; Lab. Mol. Pharmacol. II, IBCCF, UFRJ, RJ 21944, Brazil.

The nicotinic receptors (nAChRs) that give rise to each of the three types (IA, II, and III) of nicotinic currents evoked in hippocampal neurons have been identified on the basis of the pharmacological and kinetic properties of the currents. An $\alpha7$-based nAChR has been assigned to type IA current, an $\alpha4\beta2$ nAChR to type II, and an $\alpha3\beta4$ nAChR to type III (*JPET*, 265:1474, 1993). Although the clonal rat pheochromocytoma (PC12) cells represent an established model for the study of sympathetic ganglionic transmission, and express various members of the nAChR gene family (*J. Neurosci*, 12:4611, 1992), the nicotinic currents evoked in these cells have not been assigned to specific nAChR subtypes. In the present study, the characteristics of nicotinic whole-cell currents activated in cultured PC12 cells were compared with those of nicotinic currents elicited in hippocampal neurons. Application of ACh (3 μM-3 mM) via a U-tube system to PC12 cells evoked whole-cell currents whose peak amplitudes increased with ACh concentration; the EC50 for ACh was $\approx$ 75 μM. Whereas nicotinic currents in PC12 cells were insensitive to dihydro-β-erythroidine (10 nM), they could be inhibited by methyllycaconitine (MLA, 1 nM), in this way resembling type IA nicotinic currents. In addition, a long-lasting blockade was observed when MLA was applied simultaneously *via* the bath perfusion and the U tube, and ACh-evoked currents in PC12 cells decayed with a slow time constant (τ_{decay} for ACh (3mM)-activated currents was $\approx$ 0.250 ms in PC12 cells). Thus, whereas the sensitivity of ACh-evoked currents in PC12 cells to competitive nicotinic blockers resembled those of the $\alpha7$ nAChR-subserved type IA currents in hippocampal neurons, the decay and the MLA-induced long-lasting blockade of nicotinic currents activated in the PC12 cells were different from those of type IA currents. It is possible that a neuronal nAChR made up of a combination of $\alpha7$ subunit(s) and other neuronal nAChR subunit(s) subserves the nicotinic currents in the PC12 cells. Support: USPHS grants NS 25296 and ES 05730.

468.4

CALCIUM PERMEABILITY OF HIPPOCAMPAL $\alpha7$ NICOTINIC ACETYLCHOLINE RECEPTOR CHANNELS. N.G. Castro[1,2], N. Brookes[1]* and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Medicine, Baltimore, MD 21201; [2]Lab. Mol. Pharmacol. II, IBCCF/UFRJ, Rio de Janeiro, RJ, Brazil 21944.

Rat hippocampal neurons express a nicotinic receptor (nAChR) channel that is sensitive to α-bungarotoxin (α-Bgt) and presumably contains the $\alpha7$ protein subunit (*J. Pharmacol. Exp. Ther.* 265:1455, 1993). The ion selectivity of this channel was investigated in cultured rat hippocampal neurons by measuring the reversal potentials (V_R) of acetylcholine-induced whole-cell currents in various ionic media. Dihydro-β-erythroidine (0.1 μM), which blocks the $\alpha4\beta2$-type nAChR, was used to isolate the α-Bgt-sensitive currents. Permeability ratios were calculated independently of the intracellular medium, using a Goldman-Hodgkin-Katz expression for V_R shifts in the presence of Ca^{2+}. The V_Rs were corrected for junction potentials, and ion activities were used instead of concentrations. When substituting extracellular Cl^- for methanesulfonate, there was a 5 mV negative shift in the V_R. This rules out a significant contribution of Cl^- to the currents. The shift was probably due to a decrease in cation activity in the presence of methanesulfonate. When switching (in the same neuron) from 1 to 10 mM Ca^{2+} in Cs^+-based extracellular solutions, the V_R became more positive by 5.6 ± 0.9 mV. Considering only Cs^+ and Ca^{2+} to be permeant, this shift yields a P_{Cs}/P_{Cs} of 6. In identical experiments, the V_R of NMDA-gated currents shifted by 8.3 ± 1.1 mV, yielding a P_{Ca}/P_{Cs} of 10. When switching from 150 mM Cs^+ to 150 mM Na^+ solutions, the nAChR current showed a small positive V_R shift. This could be accounted for by the higher activity of Na^+, with a calculated P_{Na}/P_{Cs} close to 1. Thus, the native $\alpha7$ nAChR channel in the rat hippocampus is a cation channel considerably selective for Ca^{2+}, although not as much as the NMDA channel. However, the $\alpha7$ nAChR channel shows inward rectification, conducting linearly below −20 mV. *In vivo*, these receptors may mediate a fast, transmitter-induced rise in intracellular Ca^{2+} that would be independent of membrane depolarization and maximal at near-resting membrane potentials. Support: NIH grant NS25296; CNPq fellowship, Brazil.

468.5

EFFECTS OF DENERVATION UPON ACETYLCHOLINE RECEPTOR CLUSTERS IN AUTONOMIC NEURONS AS DETERMINED BY QUANTITATIVE LASER SCANNING CONFOCAL MICROSCOPY. H.L. Wilson and P.B Sargent*. Depts. of Stomatology and Physiology and the Neurosciences Graduate Program, UCSF, San Francisco, CA 94143.

We previously showed that denervation altered the properties of acetylcholine receptor (AChR) clusters on the surface of frog cardiac ganglion cells (Neuron 1: 877-886, 1988). We have extended this analysis by using a laser scanning confocal microscope to optically section and reconstruct neurons dually labeled for AChR clusters and synaptic boutons. AChRs were labeled with rat anti-electric organ AChR mAb #22 (gift of Dr. Jon Lindstrom, UPenn), and cyanine 3.18-labeled goat anti-rat IgG. Synaptic boutons were labeled with mouse anti-SV2 mAb #10H (gift of Dr. Steve Carlson, UWash) and cyanine 5.18-labeled goat anti-mouse IgG. Cluster number per cell and relative AChR number per cluster were determined with image analysis software (Optimas). On normally innervated ganglion cells, AChR clusters are co-localized with synaptic boutons and are spatially clustered on the cell surface, since several may be associated with a single bouton, which are themselves clustered. On denervated neurons, by contrast, AChR clusters are spatially dispersed. Denervation also leads to a reduction in the relative number of AChRs per cluster, measured as the product of cluster size and average pixel value; this change is evident as soon as 3 days following surgery and is sustained for at least 6 weeks. Finally, denervation results in a transient change in the relative AChR number per cell, which is significantly reduced 18-20 days following denervation but which returns to normal by 40-42 days following denervation. These results indicate that synaptic AChR clusters are not stable in the absence of innervation. (Supported by NIH NS 24207.)

468.6

ON THE ROLE OF ACETYLCHOLINESTERASE AND ACETYLCHOLINE RECEPTORS IN DENERVATION SUPERSENSITIVITY IN THE FROG CARDIAC GANGLION. E.N. Garrett*, H.L. Wilson, S.D. Matthews, and P.B. Sargent. Depts. of Stomatology and Physiology and the Neurosciences Graduate Program, UCSF, San Francisco, CA 94143.

Chronic denervation of parasympathetic neurons in the frog cardiac ganglion leads to an increase in their sensitivity to acetylcholine but does not alter sensitivity to carbamylcholine (Streichert and Sargent, J. Physiol., 445:249-260, 1992). This suggests that denervation supersensitivity results from a reduced effectiveness of acetylcholinesterase (AChE). To learn whether denervation leads to an outright loss of AChE, we measured AChE in extracts of sham-operated and denervated ganglia. Denervation resulted in a significant *in*crease in the V_{max} for AChE. Previous work (Streichert and Sargent, J. Neurobiol. 21: 938-949, 1990) suggested that this increase is attributable to enhancement of an intracellular pool of enzyme. When extracellular AChE was measured specifically, it was found not to be altered significantly by denervation. Quantitative histochemical studies further showed no denervation-induced change in AChE activity at the surface of ganglion cells. These studies suggest that denervation supersensitivity to ACh is not caused by a loss of AChE. Nor is it apparently explained by an increase in the number of cell surface nicotinic acetylcholine receptors (AChRs), measured as the number of binding sites for ^{125}I-neuronal-bungarotoxin (κ-bungarotoxin) or for ^{125}I-mAb 22, a cross-reacting anti-electric organ AChR antibody (gift of Dr. Jon Lindstrom, UPenn). Finally, denervation does not appear to alter the distribution of AChRs relative to AChE, thus making it unlikely that supersensitivity to ACh results from the migration of AChRs to areas of the cell surface containing relatively little AChE. (Supported by NIH grant NS 24207.)

468.7

COGNITIVE EFFECTS OF NICOTINIC-CHOLINERGIC MANIPULATION IN A RAT STIMULUS DISCRIMINATION TASK A.V. Terry Jr.[1,2], J.J. Buccafusco[1,2], W.J. Jackson[1]*, M.W. Decker[3], S.P. Arneric[3]. [1]Dept. Pharmacol. Toxicol., Medical College of Georgia, [2]Dept. VA Med. Ctr. Augusta, GA 30912 and [3]Neuroscience Res., Abbott Laboratories, Abbott Park, IL 60064

The purpose of this study was to determine the nature of cognitive enhancement following central nicotinic receptor stimulation in a novel version of the delayed stimulus discrimination task. In this paradigm, an animal is presented with a stimulus, which is followed by a variable length delay (retention) interval, at the end of which the animal has the opportunity to respond, and thus earn a food reward. In our model, a tone (auditory) and a light (visual) stimulus was utilized. To diminish the rat's ability to use position and spatial strategies to solve the task, we installed computer automated, retractable doors which separate the animal from the levers during the delay interval, thus preventing positioning at the lever. After stable baselines were achieved, nicotine or lobeline was administered in a randomized dose series. Each rat received two complete series of the two drugs on different occasions. Only 2 doses per week were administered. Optimal doses of both nicotine and lobeline significantly improved overall performance in the delayed stimulus discrimination paradigm by 12.5 and 8.4% respectively. A differential pattern of improvement was observed when the data was analyzed by delay, however. The most significant enhancement with nicotine appeared at the longest delays (16.5%) whereas the most significant enhancement with lobeline occurred at the shortest delays (13.7%). In addition, mecamylamine 1.0 mg/kg (a nicotinic antagonist) administered concomitantly with nicotine completely abolished the enhancement, but did not significantly reduce the enhancement stimulated by lobeline. These data suggest that lobeline's cognitive effects may be expressed through non-nicotinic mechanisms or involve a nicotinic receptor which is not responsive to mecamylamine.

468.8

POTENTIATION OF PEPTIDERGIC TRANSMISSION IN SYMPATHETIC GANGLIA BY 1μM NICOTINE AND INCREASES IN TEMPERATURE. P Jobling*, R Thorne and JP Horn, Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Interaction between cotransmitters in sympathetic ganglia may have important implications for end-organ function. In bullfrog sympathetic ganglia co-release of LHRH with Ach causes asynchronous discharge of postganglionic action potentials (afterdischarge) after a stimulus train.

We have studied peptidergic afterdischarges (ADC) by recording extracellularly from rami communicantes and peripheral nerves that carry axons from ganglia 9&10. Changes in aortic tension associated with preganglionic C stimulation were also monitored. Repetitive stimulation of spinal nerves 7&8 (>40 stimuli at 20 Hz) evoked ADC's in rami that lasted up to 2 min and were blocked by glu-phe-trp-LHRH, an antagonist. Longer trains(ie 500 stimuli) evoked ADC's in cutaneous and pelvic nerves. The pelvic nerve responses suggest that C neurons must contribute to ADC. 0.5-1 μM nicotine markedly potentiated ADC without affecting nicotinic transmission through the ganglia. This effect of nicotine persisted for several hours and was readily reversible. 250 nM α-bungarotoxin did not block the effect of nicotine. Potentiation of ADC by nicotine was not associated with an increase in the amplitude or duration of aortic contractions. Potentiation of ADC also occurred when bath temperature was warmed from 21°C to 24° or 28°C. The duration of ADC was shorter at higher temperatures. The data reveal modulation of peptidergic transmission in sympathetic ganglia but the implications for targets remain unresolved. Supported by NS21065, NS01427 and a postdoctoral fellowship from the AHA, PA Affiliate.

468.9

INCREASED BASAL [^{3}H]DOPAMINE RELEASE EVOKED BY NANOMOLAR NICOTINE MAY PROCEED VIA A HIGH-AFFINITY RECEPTOR CONFORMATION. P.P. Rowell*, Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky 40292.

Nicotine stimulates the release of several neurotransmitters from brain tissue by acting on presynaptic receptors. We have used an *in vitro* superfusion system to measure the nicotine-evoked release of [^{3}H]dopamine (DA) from rat striatal synaptosomes. A 2-min exposure to micromolar nicotine produces a rapid increase in [^{3}H]DA release. With continued exposure the response declines, apparently due to conversion of the receptors to a high-affinity desensitized conformation. In contrast, prolonged exposure to nanomolar concentrations of nicotine, while not producing an immediate response, leads to a cumulative enhancement in basal [^{3}H]DA release. This effect is calcium-dependent and blocked by the nicotinic antagonist, dihydro-ß-erythroidine. We suggest that the gradual DA release in response to low concentrations of nicotine occurs as a result of the equilibrium between the high-affinity desensitized and active states of the receptors. Therefore, nicotine may evoke neurotransmitter release by two processes; one involving a rapid and robust increase as a result of nicotine's interaction with active receptors, and a second slower and relatively weaker process brought about by prolonged exposure to low concentrations of the drug. This dual action may have important implications with respect to nicotine delivery from cigarette smoking versus patch. (Supported by N.I.H. grant DA07479-01A1.)

468.10

THE EFFECTS OF AGING ON RAT BRAIN NICOTINIC CHOLINERGIC RECEPTOR SUBTYPES: AN AUTORADIOGRAPHIC ANALYSIS. J.R. Pauly*. Dept. of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

A reduction in the number of neuronal nicotinic receptors identified by 3H-nicotine [or 3H-cytisine] binding has been consistently demonstrated in tissue obtained from patients with Alzheimer's Disease. However, the effects of aging and Alzheimer's Disease on brain alpha-125I-bungarotoxin (BTX) receptors are not well characterized. The purpose of the present study was to compare the binding of 3H-cytisine and 125I-BTX in the brains of young and aged rodents. The brains of young (2 months) and aged (26 months) male Wistar rats were sectioned and quantitative autoradiography of 3H-cytisine and 125I-BTX binding was performed on adjacent brain sections as previously described (Pauly et al., 1991). The number of brain receptors labeled by 125I-BTX was not consistently altered by aging although region-specific increases (supraoptic nucleus, dentate gyrus) and decreases (nucleus sagulum, dorsal tegemntal nucleus) in BTX binding were determined. The effects of aging on nicotinic receptors identified by 3H-cytisine binding were more consistent and of greater magnitude than those measured for BTX binding. Age-related reductions in 3H-cytisine binding were prevalent in many brain regions including most thalamic nuclei (e.g. anteroventral, anterodorsal), the medial habenula, dorsolateral geniculate nucleus, medial geniculate nucleus, subiculum, substantia nigra, ventral tegmental area and superior colliculus. It is interesting that no age-related reduction in cytisine binding was detected in such regions as the cerebral cortex, striatum, hippocampus and interpeduncular nucleus. These data suggest that the effects of aging on the nicotinic cholinergic system in rat brain are dependent on receptor subtype. Brain regions also differ substantially in the magnitude of age-related reductions in nicotinic receptor binding. Supported by DA-08443 and a grant from the Callaway Foundation.

468.11

REGULATION OF BRAIN NICOTINIC CHOLINERGIC RECEPTORS BY CHRONIC ICV INFUSIONS OF METHYLCARBACHOL. X-H. Yang*, M.L. Hyer and J.R. Pauly. Dept. of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Methylcarbachol (MCC) has been reported to be a selective agonist for neuronal nicotinic cholinergic receptors. However, behavioral alterations elicited by ICV infusions of MCC appear to be mediated by both nicotinic (e.g. prostration syndrome) and muscarinic (e.g. polydipsia) neurotransmission. In the present study, quantitative autoradiography was used to analyze changes in cholinergic receptor binding following chronic ICV infusions of MCC. Male Wistar rats were anesthetized and a chronic ICV guide cannula was implanted into the left lateral ventricle. Animals received two daily injections (at 0900hr and 1500hr) of saline (5 μl) or MCC (30 μg in 5 μl) for 10 days. Tolerance developed for nicotinic but not muscarinic receptor mediated behaviors over the course of the chronic treatment. Following completion of behavioral testing, the brains of the animals were removed, frozen in isopentane and sectioned on a cryostat. Quantitative autoradiography was used to measure nicotinic (^{3}H-cytisine and ^{125}I-bungarotoxin) as well as muscarinic (^{3}H-QNB) receptor binding. In general, chronic MCC treatment did not alter muscarinic binding, consistent with the lack of tolerance development for MCC-induced polydipsia. However, MCC produced robust increases in nicotinic cholinergic receptor binding. Bungarotoxin binding was increased in many brain regions including the cerebral cortex, endopiriform nucleus, bed nucleus of the stria terminalis, amygdala, superior colliculus and various sites within the hippocampus. Cytisine binding was also increased following chronic MCC infusions, with the largest increases occurring in the striatum and cerebral cortex. MCC-induced changes in brain cholinergic receptor binding appear to be similar to those produced by chronic nicotine treatment and may be the result of chronic receptor desensitization. Supported by DA-08443 and a grant from the Callaway Foundation.

468.12

NEURONAL NICOTINIC RECEPTORS (nAChRs) EXPRESSED IN KERATINOCYTES CONTROL CELL ADHESION AND MOTILITY AND ARE TARGETED BY PEMPHIGUS ANTIBODIES. E.F.R. Pereira*, E.X. Albuquerque, P.G. George, R.M. Horton, B.M. Conti-Tronconi, and S.A. Grando Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21944, Brazil; Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; Depts. Dermatol. & Biochem., Univ. Minnesota, St. Paul, MN 55108.

Like cholinergic neurons, keratinocytes (KC) are capable of synthesizing, storing, releasing, and degrading ACh *in vivo* and *in vitro* (*J. Invest. Dermatol.* **101**:32, 1993). In this study, the physiological actions of ACh in human KC were studied by means of various mutually confirmatory techniques. Application of ACh (5 mM) or nicotine (1-5 mM) to suspensions of KC induced immediate cell adhesion and spreading, and establishment of intercellular contacts. In contrast, addition of nicotinic antagonists such as κ-bungarotoxin (κ-BGT, 1 nM) or mecamylamine (1 μM) to confluent KC caused cell dissociation, a phenomenon similar to acantholysis. In outside-out patches from cultured KC, ACh (10 μM) or anatoxin-a (AnTX, 1 μM), in the presence of atropine (1 μM), could activate predominantly 32-pS single channels. The frequency of channel openings induced by AnTX or ACh was reduced reversibly by mecamylamine (1 μM) and irreversibly by κ-BGT (10 nM). The pharmacological properties and the conductance of these nicotinic channels suggested that a neuronal α3β4 nAChR gives rise to the nicotinic responses in KC. Using specific antibodies and PCR probes for each of the nAChR α subunits, we confirmed that KC express neuronal nAChR α3 and β4 subunits. A high titer of antibodies that bind to the neuronal nAChR α3 subunit was found in the serum of a patient with the skin disease *pemphigus*, an immunodisease characterized by acantholysis and blistering. Thus, a reduction of nAChR activity in human KC may be related to the physiopathology of *pemphigus*. Support: USPHS grant NS 25296; Mol. Pharmacol. Train. Prog. UMAB/FINEP.

469.1

KYNURENINE-HYDROXYLASE AND KYNURENINASE INHIBITORS: FOCUS ON THE CONTROL OF KYNURENIC ACID BIOSYNTHESIS. A. Chiarugi, R. Carpenedo, M.T. Molina[#], R. Pellicciari[##] and F. Moroni.* Dept. of Pharmacology, University of Florence, Florence, Italy and Institutes of Med. Chemistry of [#]CSIC, Madrid , Spain and of the [##] University of Perugia, Italy.

Kynurenic acid (KYNA) is an endogenous antagonist of the excitatory amino acid receptors. It is synthesised from kynurenine (KYN), a tryptophan metabolite, through the action of kynurenine transaminase. The availability of KYN is the rate limiting step for KYNA production. KYN is also a substrate for kynurenine hydroxylase and kynureninase two enzymes of the metabolic pathway leading to quinolinic acid and NAD biosynthesis. The inhibition of these enzymes results in an increased KYNA synthesis and in a decreased accumulation of the excitotoxin quinolinic acid, (J. Neurochem. 57; 1630;1991). A series of kynurenine hydroxylase and kynureninase inhibitors were synthesised and tested "in vitro" on the activity of the above mentioned enzymes. Two of them were pharmacologically studied. The first one was m(nitrobenzoyl)-alanine (mNBA; J.Med. Chem 37;647; 1994) selected as an inhibitor of kynurenine hydroxylase (IC50: 1 μM) and the second was o(methoxybenzoyl)-alanine (oMBA), selected as an inhibitor of kynureninase (IC50: 5 μM). When administered to rats having a microdyalisis probe in their hippocampi, both mNBA and oMBA (20-400 mg/kg i.p.) significantly increased the concentration of KYNA in the dyalisate (up to 10 times over basal levels). mNBA was significantly more potent that oMBA suggesting that the inhibition of kynurenine hydroxylase results in a more efficient accumulation of KYNA than the inhibition of kynureninase. The simultaneous administration of both compounds had addictive effects. This increased concentration of KYNA in brain extra cellular spaces was correlated with sedation, increase of the convulsive threshold and mild analgesia. These behavioural effects are consistent with the hypothesis that the reported changes of KYNA concentration in brain extra cellular spaces cause a reduced excitatory amino acid receptor function.

469.2

NMDA RECEPTOR SUBTYPES ON RAT CHOLINERGIC NEURONS. A.C. Pawley, S. Flesher, K. Jhamandas, R.J. Boegman*, R.J. Benninger. Departments of Pharmacology-Toxicology and Psychology, Queen's University, Kingston, ON. K7L 3N6.

Differences in the agonist-antagonist potencies of a number of compounds at the NMDA receptor have led to the suggestion that NMDA receptor subtypes are present in the brain and spinal cord. Based on this hypothesis NMDA acts as an agonist at both NMDA-1 and NMDA-2 receptors, while quinolinic acid (QUIN) is an NMDA-2 selective agonist. The NMDA receptor is composed of several different subcomponents such as the agonist, glycine, channel/PCP, polyamine, magnesium, and zinc sites. The aim of this research was to examine the effect of different site selective antagonists on NMDA and QUIN toxicity to cholinergic neurons of the rat nucleus basalis (NB). Preliminary studies looked at the toxic effects of QUIN and NMDA to the cholinergic projections from the NB to the cortex and amygdala. QUIN was found to preferentially damage projections to the amygdala, while NMDA damaged both projections equally. Various antagonists were then coinjected with either QUIN or NMDA into the NB. It was found that a ten fold higher concentration of AP7 was needed to block QUIN than NMDA. With QUIN, the projections to the cortex were more easily protected than those to the amygdala by AP7. The antagonists at the remaining sites did not show any other differences between NMDA and QUIN. However, as with AP7, the cortical projections were more easily protected against QUIN, and in general, higher doses of antagonists were required to block QUIN than NMDA. This data indicates that there may be a difference between NMDA receptors on rat cholinergic neurons of the NB, and the difference might be expressed as differences in the agonist binding sites. Supported by MRC.

469.3

CHARACTERIZATION OF ^{3}H DICHLOROKYNURENIC ACID BINDING IN CLONED NMDA RECEPTORS EXPRESSED IN TRANSFECTED CELLS. N.J. Anegawa, D.R. Lynch, and D.B. Pritchett*. Departments of Pharmacology & Neurology, University of Pennsylvania; Children's Seashore House, Philadelphia PA, 19104

The NMDA subtype of receptors for excitatory amino acids contains a glycine recognition domain. Occupation of the glycine recognition site is required for receptor activation. Multiple NMDA receptor (NR) subunits have been recently cloned We have transiently expressed NR1 (a-g) and NR 2A-2C in Human Embryonic Kidney 293 cells singly or in combination. Using 293 cells expressing NMDA receptor subunits, we characterized the pharmacological properties of ^{3}H-Dichlorokynurenic Acid (^{3}H-DCK), a proposed antagonist of the glycine recognition domain. In cells transfected with NR1 a-g, saturation analysis revealed high affinity binding with similar K_Ds of 22-37 nM in all seven NR1 splice variants. This binding in all seven subunits was inhibited by glycine and MNQX with similar IC_{50}s of 0.3-3.0 μM and 3.3-6.5 μM respectively. Interestingly, similar to results previously reported by Yoneda *et al* (J. Neurochem. 60, 634 (1993)), we observed that in brain tissue, MNQX inhibited ^{3}H-DCK binding with a non-unity Hill coefficient. However, we did not observe this in NR 1 splice variants. In cells transfected with dimeric combination of NR 1a/2A or NR1a/2B or trimeric combination of NR 1a/2A/2B, saturation analysis revealed K_Ds of 24-30nM. In NR1a/2A and NR1a/2C, ^{3}H-DCK binding was inhibited by glycine with similar IC_{50}s - 1.9-2.5μM. These results demonstrate that NR1 subunit is sufficient to reconstitute ^{3}H-DCK binding. However, the observed binding displayed properties different from native NMDA receptors.

469.4

REGIONAL DISTRIBUTION AND CHARACTERIZATION OF [^{3}H]DEXTRORPHAN BINDING SITES IN RAT BRAIN DETERMINED BY QUANTITATIVE AUTORADIOGRAPHY. J.E.Roth*, T.F.Murray and P.H.Franklin. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Dextrorphan (DX), the major metabolite of the widely used antitussive dextromethorphan, is a selective noncompetitive antagonist of the NMDA subfamily of ionotropic glutamate receptors. Our previous studies in membrane homogenates (Franklin and Murray, Mol. Pharmacol. 41:131,1992) indicate that although the [^{3}H]DX binding site is associated with an "open channel" domain of the NMDA receptor-operated cation channel, it may not coincide with the channel binding sites of either [^{3}H]MK-801 or [^{3}H]TCP. Using quantitative autoradiography we have further characterized [^{3}H]DX binding sites and studied their anatomical distribution in rat brain.

[^{3}H]DX (40nM) reached equilibrium in the CA1 region of the hippocampus by 4 hr at 20°C. The inclusion of 100 μM glycine and 1 μM glutamate in the incubation droplet increased the assocation rate of [^{3}H]DX with its binding site in CA1 ($t_{0.5}$ decreased from 46.4 min to 21.0 min) without changing maximum specific binding levels. Unlike [^{3}H]MK-801 (Sakurai et al., Neurosci. 40:533,1991), [^{3}H]DX binding appears to be relatively insensitive to stimulation by glycine and glutamate in a manner that can not be explained by the presence of high endogenous levels of the same. [^{3}H]DX binding was highest in the CA1 (s. radiatum) region of the hippocampus; saturation analysis indicates that [^{3}H]DX labels a single population of binding sites (K_d=48.7 nM). High levels of binding were also seen in the outer layers of the cerebral cortex and the molecular layer of the cerebellum. (Supported by DA-07218)

469.5

OPEN CHANNEL BLOCK OF THE NMDA-ACTIVATED CHANNEL BY STRUCTURAL ANALOGUES OF PHENCYCLIDINE. J. G. Dilmore* and J. W. Johnson. Dept. of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Phencyclidine (PCP) is a dissociative anesthetic that is used as a drug of abuse. It has been shown to displace [^{3}H]MK-801 in rat brain homogenates, and to act as an open channel blocker of the ion channel activated by NMDA. In an attempt to elucidate PCP's molecular binding conformation, Kozikowski and Pang synthesized structurally constrained analogues of PCP and found that they displaced [^{3}H]MK-801 with high affinity (*Mol. Pharmacol.*, 37:352, 1990).

We have used patch clamp techniques to examine the electrophysiological behavior of the four PCP analogues with the highest binding affinities. All four have been found to antagonize the response to 5 μM NMDA and 10 μM glycine in cultured rat cortical neurons. Of these four, the drug with the lowest affinity (compound 5 in Kozikowski and Pang, 1990) was most thoroughly investigated and was determined to have an approximate IC_{50} of 358 nM (n_H = 1.4) at -60 mV. This is close to the reported binding constant of 224 nM. Compound 5 can be trapped within the closed NMDA channel. The time course of block and unblock was generally multiexponential. The time to half maximal block ($t_{1/2}$) was weakly dependent on antagonist concentration, while the $t_{1/2}$ for unblock showed no dependence on antagonist concentration. The drug with the next higher affinity in binding experiments was found in our experiments to have similarly high affinity but slower kinetics. The equilibrium blockade by these compounds is dependent on voltage with both displaying a much lower affinity at positive voltages. Preliminary experiments have indicated that the two compounds with highest binding affinities are active as NMDA antagonists at lower concentrations in our experiments. A discussion of structural and kinetic implications will be presented. We would like to thank Drs. Kozikowski and Pang for their donation of the compounds. Supported by NIMH.

469.6

RESPONSES TO EXCITATORY AMINO ACID RECEPTOR STIMULATION COUPLED TO L-ARGININE:NITRIC OXIDE PATHWAY IN RAT CEREBELLUM AND HIPPOCAMPUS. A. Balcioglu* and T.J. Maher. Mass. Coll. Pharmacy, Div. Pharm. Sci. 179 Longwood Avenue, Boston, MA 02115

Differences in the responses to stimulation of the L-arginine : nitric oxide (NO) system by N-methyl-D-aspartate (NMDA) and kainic acid (KA) in the cerebellum and hippocampus have been investigated in anesthetized rats using microdialysis and a hemoglobin-trapping technique. In the cerebellum, KA stimulated NO production more robustly than NMDA, and in a dose-dependent manner between 0.1 and 1mM. This response was potentiated by L-arginine and inhibited by N-monomethyl-L-arginine (L-NMMA) and N-nitro-L-arginine (L-NNA), with L-NNA being a more potent inhibitor. In addition, the presence of K^+ along with KA potentiated the response, which tetrodotoxin (TTX) abolished the response. In the hippocampus, hovewer, KA did not stimulate NO production while NMDA increased NO production only at the highest dose tested (1mM). This response was potentiated by L-arginine and inhibited by L-NNA. Interestingly, K^+ also potentiated the NMDA-induced response, which was diminished by TTX to levels of NO equal to those due to stimulation by NMDA alone. KA and NMDA-induced responses in the cerebellum and hippocampus were unaffected by pretreatment with L-citrulline, L-arginosuccinate and L-ornithine. These findings give further support for the coupling of NMDA and KA stimulation to NO production in the hippocampus and the cerebellum. Additionally, differences in their sensitivities to sodium channel blockade with TTX suggest relative differences in the underlying mechanisms mediating their NO responses to receptor stimulation.

469.7

EFFECTS OF NITRIC OXIDE (NO) ON AFFERENT EVOKED RESPONSES AND WIND-UP OF NOCICEPTIVE SPINAL DORSAL HORN NEURONS. D. Budai, G.L. Wilcox* and A. A. Larson. Departments of Veterinary PathoBiology and Pharmacology, University of Minnesota, Saint Paul, MN 55108.

Experiments were designed to determine the role of nitric oxide (NO) in sensory neurotransmission in the dorsal horn of the rat spinal cord. The lumbar enlargement was exposed by a laminectomy under urethane anesthesia. Extracellular recordings were made from wide dynamic range neurons responding to both innocuous and noxious mechanical or heat stimuli delivered to the cutaneous receptive field. Wind-up of the C-fiber-related responses evoked by electrical stimuli was analyzed by computing post-stimulus time histograms. Wind-up, the progressive potentiation of C-fiber responses during a train of stimuli, was increased by inhibition of NO synthase by Nω-nitro-L-arginine methyl ester (L-NAME) or L-N5-(1-iminoethyl)ornithine (L-NIO) but decreased after elevating the concentration of NO with S-nitroso-N-acetylpenicillamine (SNAP). The effects of NO on wind-up parallels that during nociceptive transmission as responses to noxious mechanical or heat stimuli were usually increased after L-NAME or L-NIO applications. Conversely, application of the NO donating compound, SNAP, led to a decrease in responses to noxious peripheral stimuli. While NMDA has been proposed as an important mediator of wind-up and of nociception, activity induced by iontophoretically applied NMDA was paradoxically inhibited by L-NAME. Responses to innocuous mechanical stimuli were mostly enhanced by SNAP and showed changes in either direction after inhibition of NO synthase. *(Supported by USPHS grant DA04090).*

469.8

COMPARISON OF DIFFERENT INHIBITORS ON THE INDUCIBLE AND CONSTITUTIVE BRAIN NITRIC OXIDE SYNTHASES. C. Demerlé-Pallardy*, A. Estival, P.E. Chabrier and P. Braquet. Institut Henri Beaufour, 1 avenue des Tropiques, 91952 LES ULIS, France.

Recently, it has been reported that aminoguanidine (AG) selectively inhibits the inducible isoform of nitric oxide (NO) synthase. The present study was designed to examine the effect of AG on the enzymatic activity of both constitutive and inducible brain NO synthases in comparison with two L-arginine analogues, N^G-nitro-L-arginine (L-NA) and 7-nitro indazole (7-NI).

Brain constitutive NO synthase inhibition was studied using neuronal cell cultures from 17-18 day old embryonic Wistar rat cortices. NO synthase was stimulated by 5 min exposure of L-glutamate (500 μM) in the presence of the inhibitors. Inducible NO synthase inhibition was assessed using C_6 glioma cells treated during 24 h with interleukin-1 (10 ng/ml). Cyclic GMP levels were used as an index of NO synthase activity and were determined by a specific radioimmunoassay.

L-NA and 7-NI inhibited with the same potency the neuronal NO synthase stimulated by L-glutamate (IC_{50}, 63 ± 23 μM and 39 ± 9 μM, respectively) while AG had no effect on the L-glutamate response. By contrast, in C_6 glioma cells, AG inhibited the inducible form of NO synthase (IC_{50}, 270 ± 5 μM) and was approximately equipotent to 7-NI (IC_{50}, 427 ± 90 μM). L-NA was 3.9 more potent than 7-NI (IC_{50}, 108 ± 17 μM).

These results indicate that in the central nervous system, AG selectively inhibits the inducible NO synthase while 7-NI and L-NA are able to block both inducible and constitutive forms of the enzyme. Moreover, it seems to be possible to selectively modulate NO production in the brain using different inhibitors.

469.9

REQUIREMENT FOR THE ACTIVATION OF NMDA RECEPTORS AND POSITIVE ALLOSTERIC INTERACTIONS OF GLUTAMATE, GLYCINE AND POLYAMINES. J.C. Marvizón* and M. Baudry. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

Activation of NMDA receptors by glutamate, glycine and spermine was studied using non-equilibrium [^{3}H]dizocilpine binding to assess increases in the association kinetic of this channel blocker. Glutamate and glycine mutually increased their efficacies and affinities to stimulate [^{3}H]dizocilpine binding, which were further increased by spermine. Glutamate and spermine appeared initially to be sufficient to enhance [^{3}H]dizocilpine binding, with no need for glycine. However, in these conditions the binding was inhibited by the glycine antagonists 7-chlorokynurenate and DNQX. Enhancement curves by glycine in the presence of increasing concentration of 7-chlorokynurenate and saturating concentrations of glutamate and spermine revealed that 1) glycine is still required in the presence of glutamate and spermine, 2) spermine markedly increases the affinity for glycine, and 3) a small glycine contamination (5 nM) was enough to stimulate [^{3}H]dizocilpine binding in these conditions, thus explaining our initial observations. The increases in glycine affinity produced by glutamate and spermine were additive.

Spermine had a biphasic effect on [^{3}H]dizocilpine binding, with a stimulatory phase followed by a inhibition at higher concentrations. The potency of spermine for both phases was increased by glutamate, but not by glycine.

These observations indicate that there are positive cooperative interactions between the glutamate, the glycine and the stimulatory and inhibitory polyamine sites of the NMDA receptor, and that glutamate and glycine, but not spermine, are required to activate the NMDA receptor.

469.10

ACTIVATION-RELATED AND ACTIVATION-INDEPENDENT EFFECTS OF POLYAMINES ON PCP RECEPTOR BINDING WITHIN THE NMDA RECEPTOR COMPLEX. S.R. Zukin*, D.C. Javitt and M.J. Frusciante. Depts. of Psychiatry and Neuroscience and Bronx Psychiatric Center, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY 10461.

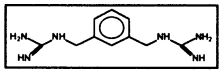
The phencyclidine (PCP) receptor is located within the *N*-methyl-D-aspartate (NMDA) receptor-gated ion channel. The functional state of the NMDA receptor complex thus influences parameters of radioligand binding to the PCP receptor, and PCP receptor ligands can serve as in vitro probes for elucidation of NMDA receptor activation mechanisms. PCP receptor binding is stimulated by NMDA receptor agonists such as L-glutamate and also by distinct classes of modulatory agents such as glycine-like amino acids and polyamines such as spermidine. The present study utilizes a kinetic approach permitting differentiation of PCP receptor binding within closed and activated conformations of the NMDA receptor complex. The results demonstrate that spermidine increases radioligand binding to the PCP receptor through two distinct mechanisms. First, spermidine, like glycine, increases the percentage of time that NMDA channels remain in the open state in the presence of L-glutamate, consistent with a role as a positive allosteric modulator of NMDA receptor activation. Second, unlike glycine, spermidine increases the affinity of the PCP receptor for its ligands. The latter effect does not appear to reflect increased NMDA receptor activation. Spermidine does not induce glycine-like alteration of the EC_{50} value for stimulation of PCP receptor binding by L-glutamate, suggesting that the effects of spermidine cannot be attributed solely to augmentation of glycine binding. These findings demonstrate first, that total specific PCP receptor binding cannot, of itself, be used as an index of NMDA receptor activation and second, that glycine and polyamines differ in the mechanisms by which they potentiate PCP receptor binding. *Supported by PHS R01 DA03383 and Dept. of Psychiatry, AECOM, T.B. Karasu, M.D., Chairman.*

469.11

EFFECTS OF RIGID ANALOGUES OF ARCAINE ON THE POLYAMINE SITE OF THE NMDA RECEPTOR COMPLEX. J. Phillips*, V. Jubian, H. Chen, A. Hamilton and I.J. Reynolds, Dept Pharmacology and Chemistry, Univ Pittsburgh, Pittsburgh PA 15261.

The limited specificity and affinity of available ligands for the NMDA receptor-associated polyamine site has limited investigation of the role of this site in modulating NMDA receptor function. With the goal of identifying more potent and specific compounds we evaluated the effects of several rigid analogues of arcaine, the most potent competitive antagonist of the polyamine site currently available, using [^{3}H] dizocilpine ([^{3}H] MK801) binding to rat brain membranes.

An examination of short chain bisguanidines suggested that a chain length of 4-5 was optimal for activity while 2- and 3-carbon arcaine analogues were less potent. Conversion of the guanidine to imidazoline diminshed activity by approximately 10-fold. Restriction of the flexibility of the chain also proved helpful. The compound shown had an affinity of 0.7μM. Replacement of the benzene ring by cyclohexane decreased the potency by about 10-fold, while substituting an acetylene into the structure of arcaine also decreased the affinity.



However, all of these compounds appeared to act at the polyamine site because they were less potent when spermidine was added to the [^{3}H] dizocilpine binding assay.

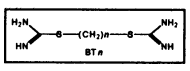
These studies have identified a novel, potent polyamine site antagonist that may represent a useful lead compound. Studies to evaluate the functional activity of this compound are currently in progress.

Supported by NIH grant DA07409 and ONR grant 413Y001.

469.12

INTERACTION OF NOVEL BISTHIOUREONES WITH THE NMDA RECEPTOR. C. Cheng*, E. Aizenman, M.L. Edwards, J.E. Matt Jr. and I.J. Reynolds Dept Pharmacology and Neurobiology, Univ Pittsburgh, Pittsburgh PA 15261, and Marion Merrell Dow Res Inst Cincinnati OH 45215.

As a part of our ongoing investigations into drug interactions at the polyamine site on the NMDA receptor complex we evaluated a number of bisthioureones of the general structure shown



using [^{3}H] dizocilpine ([^{3}H] MK801) binding to rat brain membranes and NMDA-stimulated $[Ca^{2+}]_i$ increases and whole cell currents in cultured forebrain neurons.

BT4 inhibited [^{3}H] dizocilpine binding with an IC_{50} of 2.7μM. The effect of BT4 was reduced by spermidine in an apparently competitive fashion, consistent with an action at the polyamine site, like its bisguanidine analogue arcaine. BT8, BT10 and BT12 also inhibited [^{3}H] dizocilpine binding. BT10 was most potent with an affinity of 0.19μM, some 10-fold more potent than the bisguanidine equivalent. However these longer BT compounds were insensitive to alterations in the spermidine concentration indicating a site of action other than the polyamine site. Other modifications of the BT10 structure decreased activity in this assay.

BT8, BT10 and BT12 were also potent inhibitors of NMDA-induced increases in $[Ca^{2+}]_i$, with IC_{50} values of 0.1-0.5μM, while BT4 was much less potent (0.5mM). BT10 (10μM) had no effect on responses produced by kainate (100μM), AMPA (50μM) or KCl (50mM). The action of BT10 was sensitive to membrane potential, although we found no evidence for use-dependence.

Thus, bisthioureones are a novel class of NMDA receptor ligands that exert actions on more than one site on the receptor depending on the size of the molecule.

470.1

EXTRACTS FROM *CYPERUS ARTICULATUS* (CYPERACEAE) DISPLACE [^{3}H]CGP39653 AND [^{3}H]GLYCINE BINDING FROM CORTICAL MEMBRANES AND SELECTIVELY ANTAGONIZE NMDA RECEPTOR-MEDIATED RESPONSES IN THE RAT CORTICAL WEDGE. E. Ngo Bum, C.L. Meier*, S. Urwyler, Y. Wang and P.L. Herrling. Sandoz Research Institute CH-3001 Bern and Sandoz Pharma Ltd., CH-4002 Basle, SWITZERLAND.

The marshland plant *Cyperus articulatus* (Cyperaceae) is commonly used in traditional medicine in Africa and Latin America, to treat a wide variety of human diseases ranging from headache to epilepsy. We tested the hypothesis that the reported anti-epileptic effect of this plant might be due to a functional inhibition of the N-methyl-D-aspartate (NMDA) receptor complex. One or several component(s) contained in the extracts inhibited the binding of [^{3}H]CGP 39653 to the NMDA recognition site and of [^{3}H]glycine to the strychnine-insensitive glycine site of the NMDA receptor complex from rat neocortex. Water extracts from rhizomes of *Cyperus articulatus* dose-dependently reduced spontaneous epileptiform discharges and NMDA-induced depolarizations in the rat cortical wedge preparation by a NMDA receptor-dependent mechanism. We conclude that the purported anti-epileptic effect of *Cyperus articulatus* might at least partially be due to inhibition of NMDA receptor-mediated neurotransmission.

470.2

GLUTAMATE AND GLUTATHIONE METABOLITES ON THE ACTIVATION OF N-METHYL-D-ASPARTATE RECEPTORS IN DISSOCIATED NEURONS: INHIBITION BY ETHANOL J.L.Morris and S.W.Leslie*, Div. of Pharmacol/Toxicol, Coll. of Pharmacy, Univ of Texas, Austin, Texas 78712-1074

Previous reports have shown that NMDA-stimulated increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) are inhibited by ethanol while glutathione-stimulated increases in $[Ca^{2+}]_i$ at brain NMDA receptors resist ethanol inhibition. Glutamate (G), the endogenous neurotransmitter at the NMDA receptor, is also formed as a product of glutathione (GSH) metabolism by γ-glutamyl transpeptidase (γ-GT). In addition, cysteinylglycine (CG) is formed as a consequence of GSH metabolism by γ-GT. G and CG were investigated in fura-2-loaded whole brain neonatal (<24 hr) dissociated neurons to determine if CG might act at the glycine site as a coagonist. Also, studies were conducted to determine the inhibitory effects of ethanol on G-stimulated increases in $[Ca^{2+}]_i$. Finally, the effect of CG on ethanol's inhibition of G-stimulated $[Ca^{2+}]_i$ was tested. G-stimulated increases in $[Ca^{2+}]_i$ (EC_{50}=0.7μM) were dependent on extracellular Ca^{2+}, blocked by Mg^{2+}, inhibited by 100 μM APV, and enhanced by glycine. Ethanol inhibited G-stimulated increases in $[Ca^{2+}]_i$ with an IC_{50} of approximately 48 mM. CG (0-11.2μM) did not potentiate G-stimulated increases in $[Ca^{2+}]_i$ and did not reverse ethanol inhibition of G-stimulated increases in $[Ca^{2+}]_i$. Also, glycine (0-11.2μM) was unable to reverse the ethanol inhibition. These results suggest that G-stimulated increases in $[Ca^{2+}]_i$ in dissociated neonatal whole brain neurons are highly specific for NMDA receptor-operated calcium channels under the conditions of the experiments employed. The CG product of GSH metabolism by γ-GT does not appear to act as a glycine coagonist or to change the inhibitory sensitivity of ethanol on G-stimulated increases in $[Ca^{2+}]_i$. (Supported by NIAAA grant AA09337)

470.3

ETHANOL INHIBITION OF HETEROMERIC NMDA CHANNELS IS NOT POTENTIATED BY THE PRESENCE OF Mg^{2+} OR AFTER REDUCTION/OXIDATION OF THE REDOX MODULATORY SITE B. Chu, V. Anantharam* and S. N. Treistman. Department of Pharmacology and Program in Neuroscience, University of Massachusetts Medical Center, Worcester, MA. 01655.

Previous reports indicate that the presence of Mg^{2+} enhances the magnitude of ethanol inhibition of NMDA channels in intact tissue preparations. To determine whether the presence of Mg^{2+} could also enhance ethanol inhibition in recombinant channels, we measured the degree of ethanol (50mM) inhibition of NR1b/2A, NR1b/2B, and NR1b/2C NMDA channels expressed in Xenopus oocytes in the presence of 12.5 and 3.125μM Mg^{2+}. The presence or absence of Mg^{2+} had no effect upon the degree of ethanol sensitivity (0μM Mg^{2+}: 19.5% inhibition; 12.5μM Mg^{2+}: 16.9%; 3.125μM Mg: 13.4% for NR1b/2A). Reduction or oxidation of the NMDA channel's redox modulatory site by DTT (2mM) or DTNB (1mM) was also ineffective in changing ethanol sensitivity (control: 32% inhibition; DTT: 24%; DTNB: 29% for NR1b/2A). We have previously shown that NR1a and NR1b homomeric channels were differentially sensitive to ethanol inhibition. To determine whether this difference was also present in heteromeric assemblies, we generated dose response curves for ethanol (25-100mM) to compare the sensitivity of NR1a/NR2 and NRb/NR2 assemblies. The presence of the extra 21 amino acid cassette did not result in a consistent difference in ethanol sensitivity. NR1/NR2C assemblies, with either splice variant, were significantly less sensitive to ethanol than other combinations. These results indicate that splice variants in heteromeric combinations are not differentially ethanol-sensitive, that the presence of NR2C confers less ethanol sensitivity, and that ethanol sensitivity is independent of both the redox modulatory site and Mg^{2+} binding site. Supported by NIH grant AA05542.

470.4

SUBUNIT-SPECIFIC INHIBITION OF CLONED NMDA RECEPTORS BY HALOPERIDOL V. Ilyin[§], J. Guastella[§], S. X. Cai[§]*, E. Weber[#] and R. M. Woodward[§]. [§]Acea Pharmaceuticals Inc., 1003 Health Sciences Rd. West., Irvine, CA 92715. [#]Dept. Pharmacology, U. C. Irvine, Irvine, CA 92717.

Haloperiodol, a therapeutically useful antipsychotic agent, has recently been shown to have weak inhibitory effects at neuronal NMDA receptors [Fletcher, E. J. and J. F. MacDonald, *Eu. J. Pharmacol.* 235:291 1993]. Using electrical recording techniques we assayed haloperidol on cloned rat NMDA receptors expressed in *Xenopus* oocytes, comparing effects on four heteromeric subunit combinations; NR1A co-expressed with either NR2A, 2B, 2C, or 2D. At saturating concentrations of agonists (10 μM glycine, 100 μM glutamate) only NR1A / 2B receptors were appreciably inhibited by haloperidol (IC_{50} ~3 μM), the other subunit combinations were largely or wholly unaffected (IC_{50}'s >100 μM). Inhibition of NR1A / 2B receptors by haloperidol did not show pronounced dependence on glycine or glutamate concentrations, or upon voltage (-10 to -110 mV). Furthermore, analysis of binding kinetics, though restricted by the preparation, suggested that haloperidol does not behave like a conventional use-dependent channel blocker. Whole cell patch clamp recordings showed that NMDA receptors in cultured rat cortical neurons (E17, 4-10 days *in vitro*) have similar sensitivity to haloperidol as NR1A / 2B (IC_{50} 1-3 μM) and, contrary to the earlier report [*ibid.*], that inhibition was not readily surmountable by raising glycine concentrations. Our results suggest that haloperidol selectively inhibits NMDA receptors containing NR2B subunits. Inhibition does not appear to involve simple interactions at glutamate, glycine or PCP binding sites, but may be mechanistically related to the atypical antagonist ifenprodil. Haloperidol, an old drug with a promiscuous pharmacological profile, could serve as a lead for designing novel subtype-selective NMDA receptor antagonists. (Supported in part by NIDA grant DA06726 to E. Weber.)

470.5

THE DIHYDROPYRIDINE NITRENDIPINE REDUCES N-METHYL-D-ASPARTATE - (NMDA) EVOKED SINGLE - CHANNEL ACTIVITY BY A MECHANISM CONSISTENT WITH OPEN CHANNEL BLOCK. G. A. Skeen*, R. E. Twyman†, and H. S. White. Anticonvulsant Drug Development Program; Departments of Pharmacology and Toxicology and †Neurology; University of Utah; Salt Lake City, Utah.

The 1,4-dihydropyridine nitrendipine was shown by this laboratory to reduce NMDA- (5 μM) and glycine- (1 μM) evoked whole-cell and single-channel currents of cultured rodent cortical neurons in an agonist- and a voltage-dependent manner (Skeen *et al.*, submitted to JPET, 1994). Kinetic analysis of the single-channel data (Vm = - 75 mV) for the main conductance (48 pS) showed that the nitrendipine-mediated reduction of current was explained by concentration-dependent (30 - 1000 nM) reductions in the frequency of openings and bursts, the average duration of openings and bursts, and the single open duration time constant. These results suggest that nitrendipine interacts with the open state of the NMDA receptor-associated ion channel. To investigate this possibility further, kinetic models were examined by computer simulation. The simulated and experimental data were analyzed and compared for effects on the frequency and duration of NMDA- (5 μM) evoked single-channel openings, at several concentrations of nitrendipine (30 - 1000 nM). The simulated data best approximated the experimental data when generated using a model that was similar to one which described the interaction of MK-801 with the NMDA receptor-associated ion channel (Macdonald *et al.*, J. Physiol., 410: 479-499, 1991). Consequently, the results support the hypothesis that nitrendipine interacts with NMDA receptors by an open channel mechanism similar to that described for MK-801. *Supported by a grant from Miles, Inc.*

470.6

INHIBITION BY 2,2',2''-TRIPYRIDINE OF HIPPOCAMPAL LONG-TERM POTENTIATION THROUGH BLOCKING N-METHYL-D-ASPARTATE-ACTIVATED CURRENT. K.S.Hsu[1], W.M.Fu[2]* and S.Y.Lin-Shiau[2]. [1]Dept. of Pharmacology, College of Med., National Cheng Kung Univ., Tainan, Taiwan and [2]Dept. of Pharmacology, College of Med., National Taiwan Univ., Taipei, Taiwan.

The effects of 2,2',2''-tripyridine on the long-term potentiation (LTP) and N-methyl-D-aspartate (NMDA) receptor-channel complex were studied in the rat hippocampal slices and the culture of hippocampal neurons. 2,2',2''-Tripyridine depressed the amplitude of evoked population spikes as well as LTP in a concentration-dependent manner. 2,2',2''-Tripyridine attenuated NMDA-evoked current recorded from rat cultured hippocampal neurons under whole-cell voltage-clamp, while neither kainate- nor quisqualate-activated whole-cell current was affected by 2,2',2''-tripyridine. The 2,2',2''-tripyridine block of NMDA-activated whole-cell current was non- competitive in nature and was not dependent on the extracellular glycine or spermine concentration. The block did, however, exhibit voltage- dependence. In single-channel recordings from outside-out patches, 2,2',2''-tripyridine greatly reduced the channel activity elicited by the application of NMDA but did not significantly affect the predominant unitary conductance. Consistent with an open-channel blocking mechanism, the mean open time and opening frequency were reduced by 2,2',2''-tripyridine. We conclude that the inhibitory effect of 2,2',2''-tripyridine on the NMDA-evoked currents could be used to explain the blocking action of 2,2',2''-tripyridine on the LTP (a long-lasting enhancement of synaptic efficacy) which appeared following repetitive electrical stimulation.

470.7

COMPARATIVE STUDIES ON NMDA RECEPTOR ANTAGONISM BY AMANTADINE (1-AMINOADAMANTANE) AND MEMANTINE (1-AMINO-3,5-DIMETHYL-ADAMANTANE). C.G. Parsons[1]*, O.A. Krishtal[2], U. Misgeld[3]. Dept. Pharmacol., Merz + Co., 60318 Frankfurt, Germany[1], Bogomdetz Inst. Physiol., Kiev, 252024 Ukraine[2], Inst. Physiology, Im Neuenheimer Feld 326, Univ. Heidelberg, Germany[3].

Amantadine and memantine have similar beneficial effects to classical NMDA receptor antagonists in animal models of glutamate pathology and are used in Europe for the treatment of Morbus Parkinson and dementia respectively. Whole cell patch clamp recordings from cultured superior colliculus neurones indicate that both of these aminoadamantanes selectively block NMDA receptors with rapid use- and strong voltage-dependency (IC_{50} against NMDA 200μM at -70mV, memantine = 2.3μM, amantadine = 71.0μM). Similar uncompetitive NMDA receptor blockade was observed in whole cell recordings from freshly dissociated hippocampal neurones (IC_{50} against NMDA 1mM at -100mV, memantine = 1.0μM, amantadine = 18.6μM) whereas in freshly dissociated striatal neurones, recorded under identical conditions, memantine was less potent (IC_{50}=2.9μM) and amantadine more potent (IC_{50} 12.4μM). A similar relative potency was apparent for the ability of amantadine (100μM) and memantine (30μM) to reduce the amplitude of NMDA receptor-mediated EPSPs in striatal slices. The increased *in vitro* potency of amantadine in striatum compared to other brain regions may underlie its beneficial effects in *Morbus Parkinson.* Whilst neither memantine (3-30μM) nor amantadine (10-100μM) affected current responses to AMPA (50-100μM) recorded in whole cell mode, similar concentrations of memantine, but not amantadine, weakly potentiated peak responses to AMPA 100μM recorded with the perforated patch technique (memantine 3μM, 116 ± 8% of control, n=7) implying the involvement of secondary messengers. Although this effect was weak and of variable amplitude, similar effects were seen in a separate series of experiments with memantine 30μM against AMPA 50μM (116.3 ± 4.6% of control, n=9). Chronic treatment of superior colliculus cultures for 2 weeks with memantine (5-10μM) had no discernible effect on the acute NMDA antagonistic properties of memantine but enhanced the potentiating effects of memantine 30μM on peak AMPA responses (127.9 ± 15% of control, n=8). This additional effect of memantine may underlie the reported symptomatic cognitive enhancement seen with this NMDA antagonist in clinical trials of dementia.

470.8

INCREASED POTENCY OF THE COMPETITIVE NMDA RECEPTOR ANTAGONIST D-AP5 DURING EARLY DEVELOPMENT IN HIPPOCAMPAL PYRAMIDAL CELLS. J.A. Gorter and R.J. Brady*. *Wadsworth Center for Laboratories & Research, New York State Department of Health,* Empire State Plaza, P. O. Box 509, Albany, NY 12201-0509.

NMDA receptor activation plays an important role in the mechanisms of developmental synaptic plasticity. This has prompted investigation of the pharmacological and biophysical properties of NMDA receptor mediated responses and has led to interesting findings, including a transient increase in receptor density and reduced voltage dependent block by Mg^{2+} during early life. The present study focused on the potency of the competitive NMDA receptor antagonist D-AP5 during development, using whole-cell patch clamp and extracellular recording techniques in rat hippocampal slices. NMDA responses were evoked by iontophoretic agonist application in the Schaffer-commissural synaptic fields. "Dose"-response curves for NMDA were constructed, and increasing doses of the antagonist were bath-applied to measure suppression of NMDA field- or whole-cell responses. The tissue response capability, as measured by the maximum field response upon NMDA iontophoresis, was larger in immature slices (PND10-16; 24.0±0.6mV) than in adult slices (PND>40; 18.9±1.2mV). NMDA responses in whole cell mode (clamped at -40mV) revealed no differences in NMDA evoked currents between young (maximum: 1.72±0.30nA) and mature cells (maximum: 1.90±0.15nA), suggesting that NMDA receptor densities are similar in the two groups. Using Schild analysis we found however, that D-AP5 was 2-3 fold more potent in suppressing responses to NMDA in immature pyramidal cells (Ki=3.9μM) in comparison with pyramidal cells in adult slices (Ki=10μM). Such changes in synaptic microphysiology may be explained by age-dependent alterations in the expression of specific subunit isoforms of NMDA receptors. The finding that the competitive antagonist D-AP5 has a higher potency during early development than in adult brain has to be taken into account when competitive NMDA receptor antagonists are considered for therapeutical purposes. *Supported by a HFSP grant to J.A.G. and a NIH grant (NS23071) to R.J.B.*

470.9

PHARMACOLOGICAL CHARACTERIZATION OF GLUTAMATE BINDING SITE IN RECEPTORS ASSEMBLED FROM 1a, 2A AND 2B NMDA RECEPTOR SUBUNITS S. J. Lenz, E. Greenblatt*, and D.B. Pritchett Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

Previous studies in brain suggest pharmacological heterogeneity of the NMDA sensitive glutamate binding site. We have characterized the pharmacological properties of the glutamate binding site assembled from combinations of the 1a, 2A, and 2B NMDA receptor subunits expressed in transfected cells using ^{3}H-glutamate and ^{3}H-CGP 39653 binding assays. Cells transfected with 1a and 2A, 1a and 2B, and 2A alone produced saturable, specific binding of ^{3}H-glutamate with K_D of 28 ±6 nM, 137 ±52 nM, and 123 ±20 nM, respectively. No binding was detected in cells transfected with 1a or 2B alone. NMDA inhibited ^{3}H-glutamate binding in 1a2A, 1a2B, and 2A combinations with similar IC_{50} values of 23 μM,14 μM, and 12 μM, respectively. However, the antagonist inhibition profile of ^{3}H-glutamate binding differed among the receptors assembled from 1a2A, 1a2B, and 2A with IC_{50} values of 5 ±2 μM, 25 ±13 μM, 57 ±9 μM for APV; 0.07 ±0.08 μM, 3 ±1 μM, 25 ±3 μM for CGS 19755, and 0.08 ±0.01 μM, 0.58 ±0.21 μM, 2.6 ±0.6 μM for CGP 39653, respectively. Specific, saturable binding of ^{3}H-CGP 39653 was detected in cells tranfected with both 1a and 2A but not detected in cells transfected with 1a and 2B, 1a alone, 2A alone, or 2B alone in the absence of Mg^{++}. However, in the presence of 1mM Mg^{++}, the 1a2B subunit combination displayed specific, saturable ^{3}H-CGP 39653 binding. These results suggest that the NR 2 subunits can account for heterogeneity in glutamate antagonist site.

470.10

INTERLEUKIN-1β ANTAGONISM OF NMDA-MEDIATED INCREASES IN INTRACELLULAR CALCIUM IN CULTURED CHICK CORTICAL NEURONS. J.M. Fahey*, D.G. Lindquist and L.G. Miller. Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

Interleukin-1β (IL-1β) is a proinflammatory cytokine produced in the brain which is thought to be involved in acute and chronic neurodegeneration in the CNS. Since the role of excitatory amino acids in the neurodegenerative process is well known, the present study examines the ability of this neuroactive cytokine to modulate NMDA-mediated increases in intracellular calcium of cultured chick cortical neurons using the fluorescent dye Fura2. IL-1β alone had no effect on intracellular calcium levels except at the highest concentration used (10 ng/ml). At a concentration of 1 ng/ml, IL-1β significantly attenuated the increase in intracellular calcium seen in the presence of NMDA/glycine. This inhibition was antagonized by 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA glutamate receptor antagonist. CNQX also inhibited the decrease in intracellular calcium caused by IL-1β in the presence of both NMDA/glycine and the endogenous polyamine spermine. Further experiments suggest that this modulatory effect is specific to IL-1β, since the biologically similar cytokine interleukin-6 (IL-6) produced no changes in intracellular calcium in the presence or absence of NMDA/glycine. These data indicate that the mechanism by which IL-1β antagonizes NMDA-mediated increases in intracellular calcium does not occur via the glycine or polyamine site on the NMDA receptor but may involve non-NMDA receptor subtypes of the glutamate receptor.

470.11

REGIONAL VARIATIONS IN THE PHARMACOLOGY OF NMDA RECEPTOR CHANNEL BLOCKERS. R.H.P. Porter* & J.T. Greenamyre. Univ Of Rochester, Rochester, NY, 14642

Quantitative receptor autoradiography was used to examine the regional binding characteristics of a diverse group of N-methyl-D-aspartate (NMDA) receptor channel blockers that varied in potency 10^5-fold. Full competition curves were generated in each of six brain regions for 12 different compounds. MK-801 was the most potent compound studied, with an IC_{50} of approximately 10 nM in the forebrain regions, and 24 nM in the cerebellar granule cell layer ($p < 0.05$). The binding affinities of ten and Hill slopes of five of the twelve compounds examined were significantly different in cerebellar granule cell layer than in forebrain regions. The fact that the rank order of drug potencies in cerebellum diverges from that in forebrain supports the notion that cerebellar NMDA receptor ion channels differ pharmacologically from those in forebrain. There was a general trend that drugs known to be well-tolerated in humans (remacemide and its metabolites, amantadine, budipine and memantine) had lower affinities than compounds with severe neurobehavioral or psychotomimetic effects. Also, in contrast to MK-801, TCP , PCP and ketamine, all of these compounds had a significantly higher affinity in the cerebellum than in forebrain regions. Our results suggest that we have identified a new pharmacological classification of NMDA receptor channel blockers, and that low affinity (rapid kinetics) and, possibly, subunit specificity may be important determinants of the clinical tolerability for NMDA receptor channel blockers. *(Supported by the National Parkinson Foundation Center of Excellence at the University of Rochester and Fisons Pharmaceuticals)*

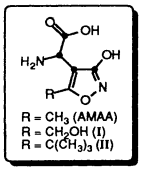
470.12

DOES ACPC DESENSITIZE THE NMDA RECEPTOR? A. Omerovic*, T. Lanthorn, and S.R. Kelso. University of Illinois at Chicago, Dept. Biological Sciences, Chicago, IL 60680.

It has been shown that chronic administration of 1-aminocyclopropanecarboxylic acid (ACPC), a specific ligand for the glycine site of NMDA receptor, results in neuroprotection from ischemic injury and reversible desensitization of behavioral responses induced by NMDA receptor activation (Skolnic et. al., Psychopharmacology, 1992, 107:489-496). The hypothesis was made that ACPC could possibly desensitize the NMDA receptor. To test this hypothesis we used Xenopus oocytes injected with rat brain total RNA and recorded NMDA-induced currents using two microelectrode voltage-clamp techniques. In the absence of glycine the measured NMDA current was very small or absent (6.8+2.8 nA vs. 62+1.8 nA in the presence of 10uM glycine). Upon addition of 1 uM and 10 uM ACPC the NMDA current increased to 57+1.8 nA (5 cells) and 93+2.3 nA (5 cells), respectively. However in all cells tested, the NMDA current remained stable over 5-15 minutes despite the continued presence of both NMDA and ACPC. Some cells, after initial exposure to ACPC for 5-10 minutes, were incubated overnight in 10uM ACPC: no significant difference was found in the size of the NMDA currents compared to previous measurements. Our results show that ACPC does not desensitize the NMDA receptors, suggesting that some other mechanisms may be involved in ACPC-induced neuroprotection.

470.13

NEW STRUCTURAL ANALOGUES OF A SELECTIVE NMDA RECEPTOR AGONIST WITH SURPRISING PHARMACOLOGICAL PROFILES. T. N. Johansen, K. Frydenvang, B. Ebert, U. Madsen and P. Krogsgaard-Larsen*. Dept. Medicinal Chemistry, Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark.



The heterocyclic acidic amino acid (*RS*)-2-amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA) is a potent and selective *N*-methyl-D-aspartic acid (NMDA) receptor agonist. AMAA is an analogue of aspartic acid in which the 3-hydroxyisoxazole moiety functions as a bioisostere of the distal carboxylate group of aspartic acid. In order to investigate the structural requirements for NMDA receptor interaction two analogues of AMAA, having different substituents in the 5-position of the isoxazole, the 5-hydroxymethyl (compound **I**) and the 5-*tert*-butyl (compound **II**) analogues, have been synthesized and tested *in vitro*.

Compound **I** is a selective NMDA receptor agonist with an electrophysiological profile in the rat cortical wedge preparation similar to that of AMAA, though slightly weaker. Surprisingly, compound **II** is a non-selective antagonist capable of inhibiting both NMDA and AMPA induced depolarizations in a dose dependant manner with IC_{50}-values of approx 100 μM. Conformational analyses of the three compounds have shown that the rotational flexibility of the 4-glycyl moiety of the antagonist (compound **II**) is markedly reduced as compared to that of the similar glycyl moieties in the two agonists (AMAA and compound **I**).

Compound **II** has been resolved using diastereomeric salt formation using (*R*)- and (*S*)-phenylethylamine and a BOC-protected derivative affording salts with a diastereomeric excess of 99.0 and 98.0 %, respectively. From the diastereomeric salts the two enantiomers have been prepared with high enantiomeric purity. The pharmacological profile of the two enantiomers is currently being investigated.

470.14

CHARACTERISTICS OF GLUTAMATE RECEPTOR IN MEDIAL VESTIBULAR NUCLEUS (MVN) NEURONS. N.Sakai, N. Murakami, N. Saito[1], H. Ujihara *[2] M.Sasa[3] and C. Tanaka[1] [1]Dept. of Pharmacology, Kobe University School of Medicine, Kobe 650, [2]Dept. of Pharmacology, Yamaguchi University School of Medicine, Ube 755 and [3]Dept of Pharmacology, Hiroshima University School of Medicine, Hiroshima 734, Japan.

A patch clamp study was performed to examine pharmacological characteristics of glutamate receptors in acutely dissociated MVN neurons. In whole cell recording, a fast application of glutamate, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) and kainic acid (KA) up to 100 μM produced dose-dependent current with reversal potentials of approximately 0 mV. These non-NMDA receptor agonists-induced currents had no desensitizing component and were completely blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) at 10 μM. An addition of 3 μM glycine potentiated glutamate-induced current. This potentiation was further augmented in the absence of magnesium. NMDA at 100 μM also induced voltage-dependent current, only in the presence of glycine in the external solution. In the presence of magnesium at 1.3 mM, NMDA-induced current was not significantly inhibited under the membrane potential of - 40 mV, although larger current was obtained in the absence of magnesium. The competitive NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (APV), also induced current with a same reversal potential of NMDA. MK-801 at 1 μM , non-competitive NMDA receptor antagonist, did not significantly block NMDA-induced current. These results suggest the existence of glutamate receptors whose characteristics are different from that observed in other neurons of central nervous system.

470.15

CHARACTERIZATION OF NMDA/GLYCINE RECEPTOR COMPLEX IN NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS. S.Y. Wu* and N.J.Dun. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614

Whole-cell patch-clamp recordings were made from sympathetic preganglionic neurons(SPNs) in transverse spinal cord slices of 10-16 day old rats. Pressure ejection of N-Methyl-D-Aspartic acid (NMDA) evoked in SPNs an inward current with a mean amplitude of 110 pA, rise time of 57±0.66 s and decay time of 9.8±2.2 s (mean±S.E, n=20). NMDA currents displayed a non-linear I-V relation with a region of negative slope conductance in the range of -90 to -60 mV in Krebs solution;the I-V relation was linear in Mg^{2+} free solution. The reversal potential,i.e. 0 mV, was the same in both solutions. Superfusion of glycine at low conc.(10-100 μM) increased and at higher conc.(0.3 - 1 mM) decreased the NMDA currents; the holding current and decay time of NMDA currents were not affected by glycine. ß-alanine increased and decreased NMDA currents in a dose-dependent manner similar to that of glycine. Methylmalonic acid, an inhibitor of glycine uptake, at low conc. (30-100 μM) enhanced and at higher conc.(0.3-1 mM) depressed the amplitude without causing a significant change of the decay time of NMDA currents or the holding current. The strychnine-insensitive glycine binding site antagonists, 7-Cl-kynurenic acid (30 μM) and HA-966 (100 μM), depressed the NMDA currents by 71% and 56%. The enhancing or depressing effects of glycine on NMDA currents were not affected by prior superfusion of strychnine (1μM) in some cells. Strychnine increased the NMDA currents evoked in several SPNs and this effect could be blocked by 7-Cl-kynurenic acid and HA-966. The inward current induced by quisqualate was not affected by glycine nor by 7-Cl-kynurenic acid and HA-966. The results suggest that in SPNs the activation of strychinine-insensitive glycine binding sites is necessary for the expression of NMDA response and that the binding site is not fully saturated, probably owing to an active glycine reuptake system present in the spinal cord slices. (Supported by NS18710).

470.16

DEVELOPMENTAL CHANGE OF THE VOLTAGE DEPENDENCY AND THE EFFECTS OF MODULATORS ON THE NMDA RESPONSE IN CENTRAL AUTONOMIC NEURONS. J. Nabekura*, H. Hamano, N. Horimoto, I. Kawamoto and T. Ogawa. Dept. of Physiology, Akita Univ. Sch. of Med., Akita, 010 Japan.

To examine the change in the characteristics of NMDA receptor mediated responses during development in the central autonomic neurons, the neurons were acutely dissociated from the nucleus tractus solitarii and ventromedial hypothalamus of the rats aged between 18 days in gestation (E18) and 21 days after birth (P21). The NMDA responses were measured by the use of a nystatin perforated patch recording configuration. Mg2+ block was less obvious in a number of fetal NTS neurons than in the neurons older than P9 and became rapidly apparent by P3. Protein kinase C (PKC) modulators such as staurosporine, H-7 and phorbol ester did not clearly affect the generation of the voltage-dependency of NMDA response in immature rats. In addition, the facilitatory effect of glycine on the NMDA response did not change in development. These evidences suggests that an appearance of the voltage-dependency of NMDA response in NTS neurons might be due to a developmental change in combinations of subunits composing the NMDA receptor and/or in the intracellular modulators of the INMDA other than PKC. In addition, Spermine enhanced the NMDA response in a dose-dependent manner in any age during development. However, the potentiation of NMDA response by spermine increased with age, without changing the NMDA concentrations for threshold, IC50, and the maximal response. The potentiation reaches the mature level by P9.

These results indicates that NMDA receptor in the central autonomic neurons rapidly changes in function and acquires the mature characteristics by the early stage after birth.

470.17

NMDA CHANNEL BLOCKADE DURING DEVELOPMENT PRODUCES AGE-SPECIFIC ALTERATIONS IN NMDA RECEPTOR DISTRIBUTION PATTERN. J. He, R. Sircar*, Laboratory for Developmental Neuroscience, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, New York.

The NMDA receptor has been shown to play an important role in developmental plasticity. Phencyclidine (PCP) and PCP-like drugs (MK-801, ketamine) act as non-competitive antagonists at the *N*-methyl-D-aspartate (NMDA) receptor. We have earlier shown that postnatal PCP treatment in rats produced long-term changes in seizure susceptibility. Here we report the effects of chronic postnatal PCP treatment on the NMDA receptor distribution pattern. Pups were treated with PCP (5 mg/kg/day) for 11 days from postnatal days 5 to 15, intraperitoneally. Control pups received saline (1 ml/100 g). All rat pups were weaned on postnatal day 21. On postnatal days 21, 40, 60, and 180, separate groups of saline- and PCP-treated rats were sacrificed, their brains removed and immediately frozen in crushed dry ice. Brain sections were incubated with [^{3}H]MK-801 in the absence and presence of excess nonradiolabeled MK-801, washed, dried and juxtaposed against tritium-sensitive film. Quantitative densitometric analyses of the autoradiographic films showed age-specific changes in [^{3}H]MK-801 distribution patterns following postnatal PCP exposure.

Supported by: National Institute on Drug Abuse

470.18

DECREASED MAGNESIUM (Mg^{2+}) BLOCK OF NMDA MEDIATED EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN AGED FISCHER 344 RAT HIPPOCAMPUS. M.C. Jasek* and W.H. Griffith. Dept. of Medical Pharmacol. & Toxicol., Texas A&M Univ Hlth Sci Center, College Station, TX 77843.

The purpose of the present study was to examine age-related changes in the Mg^{2+} sensitivity of N-methyl-D-aspartate (NMDA) synaptic potentials *in vitro* in F344 rat hippocampus. We utilized hippocampal CA1 field EPSPs in young (1-2 months) and aged (24-25 months) rats. Synaptic NMDA potentials were studied in isolation in the presence of 10 μM DNQX. EPSP area was measured and all values were normalized to initial EPSPs prior to any drug application. In the presence of 10 μM DNQX and no Mg^{2+}, the normalized EPSP area was 1.45±0.17 in young (n=6) and 1.86±0.24 in aged (n=6). When 0.1 mM Mg^{2+} was added EPSP area in young decreased to 0.78±0.18 while in aged, the area remained almost unchanged at 1.88±0.19. In young 0.3 mM Mg^{2+} decreased the normalized area further to 0.39±0.12 while in aged 0.3 mM Mg^{2+} decreased the response to 0.88±0.09. There was a significant difference ($p<0.01$, independent *t*-test) between the two age groups at concentrations of 0.1 mM and 0.3 mM Mg^{2+} indicating an age-related decrease in the sensitivity to the Mg^{2+} block. Similar experiments testing the non-NMDA synaptic potential (using 50 μM APV instead of DNQX) resulted in no significant difference in Mg^{2+} sensitivity between age groups. These latter results suggest that the age-related changes in NMDA synaptic potentials were due to alterations in postsynaptic sensitivity and not due to changes in neurotransmitter release.

(Supported by NIH grant AG07805)

471.1

SPECIFIC DETECTION OF NEUROTENSIN METABOLITES IN THE RAT BRAIN BY *IN VIVO* MICRODIALYSIS/ELECTROSPRAY MASS SPECTROMETRY. P.E. Andrén*, M.R. Emmett and R.M. Caprioli. Analytical Chemistry Ctr., Dept. of Biochemistry & Molecular Biology, Univ. of Texas Medical School, Houston TX 77030

We have developed a highly sensitive microelectrospray mass spectrometry (uES MS)-technique in order to detect attomole levels of neuropeptides from brain microdialysis (Md) perfusates. Md samples were desalted on a nano-LC (50 um i.d.) C18-column at a flow rate of 800 nL/min which was then eluted directly into the mass spectrometer for ES MS analysis. In the present experiments we have studied *in vivo* neurotensin (NT) metabolism in rat striatum. NT is a putative neurotransmitter or neuromodulator in brain and several of the NT-fragments retain biological activity. The detection limit of the nano-LC/uES MS system was less than a total of 100 attomol when synthetic NT was injected.

A Md probe was stereotaxically implanted into the striatum of an anesthetized rat and an infusion of 1 uM NT through the microdialyis probe was performed at 0.3 uL/min. NT was metabolized *in vivo* and NT-fragments were obtained in the Md perfusate and analyzed by nano-LC/uES MS. MS spectra showed that NT was metabolized to fragments 1-12, 1-11, 1-10, 1-8, 3-8 and 7-11. We will also show results of experiments which are designed to monitor endogenous NT and its metabolic fragments.

471.2

STRUCTURE SPECIFIC DETECTION OF ENDOGENOUSLY RELEASED NEUROPEPTIDES BY MASS SPECTROMETRY AND IN VIVO MICRODIALYSIS. Mark R. Emmett*, Per Andren and Richard M. Caprioli. The Analytical Chemistry Center, Dept. of Biochemistry & Molecular Biology, University of Texas Medical School, Houston, Texas 77030.

In vivo microdialysis (Md) has been used to monitor a variety of endogenous neurotransmitters. Md has not been widely used to study endogenous neuropeptides due to low extracellular concentrations coupled with poor recoveries and salt contamination of the samples. Because of the dilute nature of the microdialysates (attomole/μL), previous experiments have been performed with radioimmunoassays of wide specificity which interact with all proteins/peptides containing short homologous sequences.

Micro-electrospray, an ultra-high sensitivity mass spectrometric ionization technique, has been developed that permits the detection of endogenous neuropeptides from in vivo microdialysates with structural (amino acid sequence) specificity. We have coupled nano-LC (C-18 packed, 50 micron i.d., 40 nL bed vol. columns) for the separation of neuropeptides. This system has been used to measure the specific release of endogenous methionine enkephalin (M-enk) from the globus pallidus/ventral pallidum area of rat brain in response to local depolarization by 100 mM KCl and the specific release of endogenous neurotensin from rat hypothalamus under similar conditions. In addition, we have demonstrated the pharmacologically stimulated release of M-enk after the systemic administration of morphine (10 mg/Kg, I.P.).

471.3

DOPAMINE D-2 REGULATION OF NEUROTENSIN RELEASE FROM STRIATUM AND NUCLEUS ACCUMBENS AS MEASURED BY MICRODIALYSIS. J.D. Wagstaff*, J.W. Gibb, and G.R. Hanson. Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Neurotensin (NT) is closely associated with extrapyramidal and limbic dopamine (DA) systems. It is speculated that this peptide neurotransmitter plays an important role in the regulation of these DA pathways and, in turn, NT release is controlled in part by alterations in DA activity. This has not been satisfactorily tested *in vivo* as the low levels of extracellular NT are difficult to detect consistently. Through a modification of the methods described by Maidment et al. (*Neurosci.* 45:1, 1991), we have developed a highly reliable method of measuring changes in extracellular NT in the striatum and nucleus accumbens in awake rats by *in vivo* microdialysis. Using this technique, release of NT is evoked by infusing a high concentration of potassium (100 mM) through the microdialysis probe; this potassium-evoked release is calcium-dependent. Further, we have examined the effect of stimulating the DA D-2 receptor subtype on NT release. Systemic administration of the D-2 agonist, quinpirole (5 mg/kg), induced a rapid increase of approximately 200% in extracellular NT in the striatum and 40-60% in the nucleus accumbens. This study shows that DA systems are important factors in controlling NT release in extrapyramidal and limbic DA terminal fields, and that stimulation of D2 receptors activates associated NT pathways in awake animals. (This work was supported by USPHS grants DA 04222 and DA 00869.)

471.4

MOLECULAR HETEROGENEITY OF NEUROPEPTIDE Y IN CSF. K. Maeda*, M. Yasuda, H. Kaneda, K. Sakai and S. Maeda. Hyogo Inst. for Aging Brain Cognitive Dis., Himeji 670, Kobe Univ. Sch. of Med. Kobe 650 Japan.

We have examined the molecular heterogeneity of neuropeptide Y-like immunoreactivity (NPY-LI) and somatostatin-like immunoreactivity (SLI) in human cerebrospinal fluid (CSF). Gel chromatography, not high performance liquid chromatography (HPLC), suggested two NPY immunoreactive materials in CSF. Both gel chromatography and HPLC revealed three SLI components in CSF: somatostatin 14, somatostatin 28 and a higher molecular weight precursor. We have also measured peptide-LI in control subjects and in patients with various neurologic disorders. We observed a significant reduction in CSF SLI in control subjects over 60 years of age, compared with the younger controls. CSF SLI was significantly decreased in multiple sclerosis (MS), or Guillain-Barre syndrome, compared with that of age-matched control subjects. A reduced concentration of NPY-LI was found in CSF of patients with MS. Our results suggest that (1) the possible heterogeneity of NPY molecules occurs in human CSF, and (2) somatostatin neurons might be more susceptible than NPY neurons in various pathological conditions and aging.

471.5

ACIDIC SULFUR-CONTAINING AMINO ACIDS, γ-GLUTAMYL AND β-APARTYL PEPTIDES IN HUMAN CEREBROSPINAL FLUID

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A method for the determination of acidic amine containing substances in human cerebrospinal fluid (CSF) is presented. The automated technique is based on precolumn derivatisation of primary amines with o-phthaldialdehyde/2-mercaptoethanol, separation by reversed phase HPLC and fluorescence detection. Three different techniques with complementary selectivities were employed in order to minimize false identification. Addition of 2-mercaptoethanol to the CSF was necessary to avoid oxidation of cysteine to cysteine sulfinate. The dominant peptides were γ-glutamylglutamine and glutathione followed by β-aspartyltaurine, β-aspartylglycine and γ-glutamylcysteine. The sulfur-containing amino acids cysteine sulfinate, homocysteate, homocysteine sulfinate and sulfo-S-serine could not be detected.

471.6

SPINAL CORD DYNORPHIN NEURONS: DO DIFFERENT NOCICEPTIVE STATES ACTIVATE DIFFERENT BIOCHEMICAL ADAPTIVE MECHANISMS TO COPE WITH INCREASED DEMAND? **V.M. Medina* and A.R. Gintzler, Dept. of Biochemistry, SUNY Hlth. Sci. Ctr. at Brooklyn, N.Y. 11203**

Late gestation and parturition are associated with an opioid analgesia that is mediated, in part, by a maternal lumbar spinal cord dynorphin/κ analgesic system. Recently, this laboratory demonstrated that the activation of spinal dynorphin neurons during the gestational period is accompanied by a marked decrease in dynorphin precursor content (50%). This suggests that increased rates of processing of dynorphin intermediates is a major biochemical adaptation by which spinal dynorphin neurons adjust to the increased demands of pregnancy. In order to assess the generality of this coping mechanism, spinal cord dynorphin precursor content was assessed in a rat model of arthritis, a condition in which lumbar dynorphin neurons are also activated. In these experiments, peripheral inflammatory lesions were produced by injecting Freund's adjuvant into the hind ankle. The magnitude of tryptic generation of leucine-enkephalin-arginine was used as an indicator of dynorphin precursor content. In contrast to the pregnant vs nonpregnant state, there was no significant difference in dynorphin precursor content in cervical, thoracic or lumbar spinal cord obtained from Freund's Adjuvant-treated vs vehicle-treated controls. These results suggest that different nociceptive states can activate different biochemical adaptive mechanisms that enable spinal cord dynorphin neurons to cope with increased demand.

471.7

PARATHYROID HORMONE-RELATED PEPTIDE (PTHRP) IS A CEREBELLAR GRANULE CELL NEUROPEPTIDE PRODUCED IN RESPONSE TO VOLTAGE-SENSITIVE CALCIUM CHANNEL ACTIVATION. E. H. Holt, B. E. Dreyer, A. E. Broadus, and M. L. Brines*, Departments of Cellular and Molecular Physiology and Internal Medicine, Yale University, New Haven, CT 06510.

Parathyroid hormone-related protein (PTHrP) is a secretory protein widely expressed in mammalian tissues. In the adult rat brain it is expressed in the cerebellum and hippocampus, as well as in isolated neurons in the cerebral cortex. PTHrP is encoded by a complex gene distinct from that of parathyroid hormone (PTH). Although PTHrP bears strong homology to PTH in its N-terminus, the rest of the protein is unique.

Primary cultures of neonatal (P8) rat cerebella are ideal for studying the regulation and function of PTHrP because the highly enriched granule cells possess high levels of the peptide and its receptor. On immunocytochemistry, PTHrP is seen in the cell bodies of neurons; preliminary studies on PTHrP receptor mRNA in neuron- or glia-enriched cerebellar cultures suggest the receptor is present on glia as well as neurons.

Cerebellar cultures require chronic depolarization with 25 mM potassium (K^+) for survival. Under these conditions, they produce and release high levels of PTHrP. Changing these cultures to media with physiologic (5 mM) K^+ is associated with a rapid decay of PTHrP mRNA. When these cells are again depolarized in 25 mM K^+ media, PTHrP mRNA returns to its original level within 4-6 h. Release of the peptide into culture media follows similar kinetics. PTHrP mRNA expression in response to depolarization is blocked by the L-type voltage-sensitive calcium channel (L-VSCC) antagonist nitrendipine, or by omitting extracellular calcium (Ca^{2+}). The L-VSCC agonist Bay K 8644 mimicks the effects of 25 mM K^+ on PTHrP mRNA expression. In contrast, the calcium ionophore A23187 does not stimulate PTHrP mRNA expression, even at high concentrations (20 uM). These results demonstrate the fundamental importance of Ca^{2+} influx through L-VSCC's in controlling PTHrP expression.

In summary, we have described the localization and regulation of a newly identified cerebellar neuropeptide, PTHrP, whose expression requires L-VSCC activity. The presence of the peptide and its receptor in the same primary cultures makes these cultures ideal for further studies of the function of this protein.

471.8

EFFECTS OF NEURONAL DIFFERENTIATION ON LUTEINIZING HORMONE-RELEASING HORMONE IN SK-N-SH HUMAN NEUROBLASTOMA CELLS. Anna Ratka*, Scott E. Mambourg, Bruce E. Torian, Becky Flores, Deptartment of Pharmaceutical Sciences, College of Pharmacy, Idaho State University, Pocatello, ID 83209.

In our attempts to elucidate the nature of functional interactions between Luteinizing Hormone-Releasing Hormone (LHRH) and opioid receptors at the neuronal level we have employed an *in vitro* model - opioid receptors-containing SK-N-SH human neuroblastoma cells. The SK-N-SH cells differentiate into a neuronal phenotype after exposure to retinoic acid (RA). Recently we have found that the SK-N-SH cells possess the gene to express LHRH. To further characterize the LHRH system in SK-N-SH cells, the concentrations of LHRH and the LHRH mRNA levels were measured during neuronal differentiation. Cells were treated with RA (10 μM) for 1 hr, 2, 4, and 6 days. The amount of LHRH was measured by the ELISA procedure. The longer the treatment with RA the more pronounced decrease in LHRH concentration. LHRH concentrations (μg/mg protein) changed as follows (no RA vs RA): 3.2 ± 0.1 vs 2.5 ± 0.2 after 1 hr, 5.1 ± 0.3 vs 1.9 ± 0.1 after 2 days, 5.2 ± 0.1 vs 3.0 ± 0.2 after 4 days, and 7.1 ± 0.4 vs 1.8 ± 0.1 after 6 days. Treatment of SK-N-SH cells with RA for 6 days caused more than two-fold reduction of the LHRH mRNA level; 20.3 vs 8.7 OD x mm^2. We conclude that in SK-N-SH human neuroblastoma cells the LHRH mRNA as well as LHRH concentrations decrease during the process of differentiation into a neuronal phenotype.

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471.9

INTRACELLULAR ROUTING AND METABOLISM OF ANGIOTENSIN II IN BOVINE ADRENAL MEDULLARY CELLS IN PRIMARY CULTURE. J.M. Wang[1], W. Annaert[1], F. Goossens[2], C. Van Broeckhoven[3]* and W.P. De Potter[1]. [1]Lab. Neuropharmacol., Dept. Medicine, [2]Lab. Med. Biochem., Dept. Pharmacy, and [3]Dept. Biochem., Univ. Antwerp (UIA), 2610 Wilrijk, Belgium

Previous work has demonstrated that angiotensin II (AII) is internalized in bovine adrenal medullary (BAM) cells via a process of receptor mediated endocytosis. Here we report new data on the fate of the internalized ligand in cultured BAM cells. After long term incubation with [^{126}I]AII or pulse-chase experiments followed by sucrose density gradient centrifugation of a post-nuclear fraction, the distribution of the internalized ligand parallelled the distribution of horseradish peroxidase (HRP, 4-5 min incubation as a marker for early endosomes). The determination of subcellular organelle markers indicated that the internalized AII was not present in lysosomes. When the cells were pulsed with [^{126}I]AII for 5 min, followed by 0, 10, and 30 min chase, increasing amounts of radioactivity were released to the extracellular medium. At 0 min no significant radioactivity could be detected in the medium, after 10 min, 33% of the initial radioactivity was recovered and this value increased to 57% after 30 min chase. In order to investigate the nature of the released AII-related radioactivity, samples were applied to gel filtration followed by HPLC on a Vydac C^4 column. The elution profile revealed 3 peaks, of which the two main ones could be identified as AIII and A(1-7), two breakdown products of AII. Our results demonstrate that, after internalization, the majority of the metabolites is recycled to the extracellular medium. On the basis of these results we assume that the degradation takes place in the endosome compartment.From the physiological point of view, it is possible that the metabolized AIII and A(1-7) exert either autocrine, paracrine or both effects on the surrounding chromaffin or cortex cells.

471.10

IMPULSE FLOW DEPENDENCY OF GALANIN RELEASE IN VIVO IN THE RAT VENTRAL HIPPOCAMPUS. S. Consolo*, G. Baldi, G. Russi, T. Bartfai°, A. Vezzani. Istituto "Mario Negri", 20157 Milan, Italy; °Dept. Neurochemistry and Neurotoxicology, Stockholm University, S-10691 Stockholm, Sweden.

Using microdialysis and a sensitive radioimmunoassay we have studied the in vivo release of the neuropeptide galanin (GAL) from the ventral hippocampus of freely moving rats. The spontaneous outflow of GAL-like immunoreactivity (GAL-LI) (1.8 fmol/ml/20 min) was dependent on the presence of extracellular Ca^{2+} and was inhibited by tetrodotoxin (TTX). Evoked release induced by infusion of KCl (60 mM) or veratridine (148 μM) was also Ca^{2+}-dependent and sensitive to TTX. Electrical stimulation of the ventral limb of the diagonal band nuclei induced a frequency-dependent (50-200 Hz) and TTX-sensitive overflow of GAL-LI in the hippocampus. In vitro GAL-LI release, studied in slices of rat ventral hippocampus, was also Ca^{2+} dependent and increased in a concentration-dependent manner by KCl depolarization. This study provides the first demonstration of in vivo GAL release in the ventral hippocampus. This release is related to the activity of the cholinergic GAL-LI containing cells in the septal diagonal band nuclei.

We thank CNR, Rome, Italy, Convenzione Psicofarmacologia.

471.11

IN VITRO AND IN VIVO EVIDENCE FOR A ROLE OF AMINOPEPTIDASE A (APA) IN CCK8 DEGRADATION. M. Migaud, E. N. Chauvel, M.-C. Fournié-Zaluski, B. P. Roques, and C. Durieux* Laboratoire de Pharmacochimie Moléculaire et Structurale, INSERM U266, CNRS URA D 1500, 4, Av. de l'Observatoire 75006 PARIS.

Cholecystokinin octapeptide (CCK8) is widely distributed in the mammalian brain where it may act as a neurotransmitter Although previous studies have shown that many peptidases can cleave CCK8 *in vitro*, the enzymatic processes responsible for its inactivation *in vivo* are not clearly established. Using the brain slices superfusion technique, a selective aminopeptidase A (APA) inhibitor, Homo-Glu-thiol, was shown to increase significantly the endogenous CCK-like immunoreactivity ($p<0.01$) from rat hippocampal slices. Under the same conditions the serine peptidase inhibitor Ala-Ala-Pro-Val-$COCH_2Cl$ produced a small but non significant increase and Leu-thiol, an aminopeptidase N inhibitor was ineffective. The affinity of CCK8 alone or in association with peptidase inhibitors was determined using *in vivo* binding experiments in mice and compared with the fully enzymatic-resistant and potent agonist pBC 264. CCK8 was 200-fold less potent than BC 264 in inhibiting the specific binding of [^{3}H]pBC 264. Among the peptidase inhibitors tested, Homo-Glu-thiol was the most potent in protecting CCK8 from degradation, leading to a 100-fold increase in its affinity. Taken together, these results show the critical role played by aminopeptidase A in the interruption of CCK8 conveyed messages.

471.12

PYROGLUTAMYL PEPTIDASE II ACTIVITY IS NOT IN THE PROCESSES OF RAT BULBOSPINAL TRH-ERGIC NEURONS. P. Joseph-Bravo*, M. E. Fresán, M. Cisneros, M. A. Vargas and J.-L. Charli. Instituto de Biotecnología, Universidad Nacional Autónoma de México, A.P.510-3, Cuernavaca, Mor., México 62271.

Pyroglutamyl peptidase II (PPII) [E.C. 3.4.19.6] is a neuronal ectoenzyme enriched in synaptosomes. Various of its properties, including its narrow specificity, suggest it is involved in the inactivation of TRH released into the synaptic cleft. Because synaptosomes contain pre and postsynaptic elements, we do not know whether the enzyme is pre or postsynaptic. No specific antibodies, irreversible inhibitors or mRNA sequence information have so far been obtained. Therefore, in an attempt to define if it is present in the pre or postsynaptic membrane we induced neuronal degeneration of serotonin-TRHergic cells that project from raphe nuclei to the spinal cord. TRH levels were measured by RIA and PPII activity by a specific radiochemical assay. Two to four weeks after intracisternal injection of 200 ug 5,7-dihydroxytryptamine to adult male Wistar rats, TRH levels decreased over 70% in the cervical, thoracic or lumbar regions of spinal cord. In contrast, PPII activity was unaffected in each region. However, 6-8 weeks after injection, a 59-66 % increase in activity was detected in the lumbar region. Previous studies had demonstrated that destruction of bulbospinal TRH neurons lead to an increase in spinal cord TRH receptors. Our data suggest that PPII is not localized in the processes of bulbospinal TRHergic neurons but probably in the target cells. (Supported in part by grant IN-204791 from DGAPA-UNAM).

471.13

PURIFICATION AND CHARACTERIZATION OF RAT BRAIN TRANSGLUTAMINASE. H. Ohashi , Y. Itoh, P.J. Birckbichler and Y. Takeuchi.* Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba 300-33, JAPAN and Noble Center for Biomedical Research/OMRF, 825 N.E. 13th Street Oklahoma City, OK 73104

Transglutaminase (TGase) in neuronal system has been suggested to be involved in neurotransmitter release, long term potentiation, Alzheimer's disease, and so forth. In order to have molecular basis on the enzyme in the central nervous system, the enzyme was purified to apparent homogeneity from the centrifugal supernatant of male SD rat brain homogenate, using DEAE ion exchange, heparin affinity, and casein agarose affinity column chromatographies. The purified enzyme has a molecular weight of 75kDa on SDS-polyacrylamide gel electrophoresis analysis. The TGase activity was Ca^{2+} dependent (EC_{50} = 0.38mM), and was maximal in a pH range of 8.0 - 9.5. Km values for putrescine and N-dimethylated casein were approximately 0.3mM and 0.05mM, respectively. GTP inhibited the enzyme activity 100-fold more potently than ATP did. Iodoacetate completely inhibited the enzyme activity, suggesting the involvement of cysteine residue in brain TGase. Zn^{2+} potently inhibited the enzyme activity, while Mg^{2+} was not inhibitory at all. The rat brain TGase did not give immunoreactivity to antibodies against known types of TGase such as tissue type TGase, epidermal TGase and coagulation factor XIIIa. The results suggest a novel type of TGase in mammalian brain.

471.14

METABOLIC CONVERSION AND *IN VITRO* BLOOD-BRAIN BARRIER PERMEABILITY OF AN ENKEPHALIN ANALOG PRO-DRUG. D.L. Greene*, V.S. Hau, S.J. Weber, T.J. Abbruscato, V.J. Hruby and T.P. Davis, Dept. of Pharmacology and Chemistry, Univ. of Arizona, Tucson, AZ 85724.

To improve blood-brain barrier (BBB) penetration of the delta opioid receptor ligand [D-Pen^2, D-Pen^5] Enkephalin (DPDPE), a pro-drug Phe^o-DPDPE was synthesized to increase lipophilicity. BBB penetration studies used an *in vitro* bovine brain microvessel endothelial (BMEC) model. *In vitro* conversion was studied using mouse serum and brain resuspended at 15% protein. Phe^o-DPDPE (100 μM) was added to samples time-course incubated at 37°C. Samples were analyzed by HPLC. To study the rate of conversion to DPDPE, specific aminopeptidase inhibitors were added. To study BBB penetration, BMECs were grown to a confluent monolayer, suspended and seeded onto membrane filters. Permeability coefficients (P.C.) were determined to give the rate of penetration in cm/min. The resultant conversion half-time for Phe^o-DPDPE to DPDPE was 6.8 min in serum and 3.9 min in brain. After adding 6 μM amastatin (aminopeptidase M inhibitor) to the brain homogenate and 50 μM bestatin (leucine aminopeptidase inhibitor) to the serum, the half life of Phe^o-DPDPE conversion increased to >500 min. The P.C. (cm/min) for Phe^o-DPDPE ($62.0 \pm 4.1 \times 10^{-4}$) was 21% higher than that of its parent compound DPDPE ($49.2 \pm 2.8 \times 10^{-4}$). These results show that the pro-drug Phe^o-DPDPE has a longer conversion time in serum than in brain. The results also show that two aminopeptidases are responsible for cleaving Phe^o-DPDPE to DPDPE and the addition of phenylalanine to the amino terminus of DPDPE increases the BBB penetration to the brain using the BMEC model of the BBB. (Supported by N.I.D.A. #DA06284 and N.I.H. - HLB7479-09)

471.15

ONO-1603 INHIBITS BRAIN PROLYL OLIGOPEPTIDASE *IN VITRO, IN VIVO* AND *IN SITU*. N. Katsube, H. Maegawa, Y. Kagamiishi, M. Yamamoto* and H. Aishita. CNS Division, Discovery Research Laboratories, Minase Research Institute, Ono Pharmaceutical Co., Ltd., Osaka 618, Japan.

ONO-1603 , (S) - 1 - [N - (4 chlorobenzyl) succinamoyl] pyrrolidine - 2 -carbaldehyde), inhibited prolyl oligopeptidase (POP) purified from bovine brain *in vitro* with competitive manner (Ki value, 12 nM). Oral administration of ONO-1603 also inhibited POP of rat brain in several regions *in vivo* for 0.25 - 4 hrs. To examine the inhibitory effects of ONO-1603 on brain POP *in situ*, we established a novel method for the evaluation of continuous POP activity in the brain of freely moving rats using the microdialysis technique. 7-(N -succinyl-Gly-Pro)-4-methylcoumarinamide, a specific substrate for POP, was continuously perfused into the hippocampus of the rat brain through the dialysis probe, and the 7-amino-4-methylcoumarin (AMC) level in the dialysate (the product by enzymatic reaction with POP) was monitored. In dialysate, the AMC was continuously and stably appeared, and was decreased by the administration of carbobenzoxy-proline-prolinal, a prototype inhibitor of POP, indicating that the AMC level in dialysate reflects the brain POP *in situ*. Following the oral administration of ONO-1603, the AMC levels in dialysate from the hippocampus markedly decreased in a time - and dose-related manner, and the AMC was gradually recovered to the basal level. From these observations, it is demonstrated that ONO-1603 is an orally active inhibitor of the brain POP.

471.16

THE ISOLATION OF FMRFamide-LIKE PEPTIDES FROM THE NEMATODE *Haemonchus contortus*. C. Keating, M. C. Thorndyke*[1], L. Holden-Dye, R. G. Williams & R. J. Walker. Dept Physiology and Pharmacology, University of Southampton, Southampton. UK, SO9 3TU and [1] Dept Biology, Royal Holloway and Bedford New College, Egham, Surrey, UK.

Two FMRFamide (Phe-Met-Arg-Phe-NH_2) like peptides (FLPs) have been isolated from the nematode *Ascaris suum*. These are Lys-Asn-Glu-Phe-Ile-Arg-Phe-NH_2 which can be abbreviated to KNEFIRFamide or AF1 and Lys-His-Glu-Tyr-Leu-Arg-Phe-NH_2, KHEYLRFamide or AF2.

We are presently attempting to purify novel FLPs from the nervous system of the parasitic nematode *Haemonchus contortus* using reverse phase high pressure liquid chromatography (rpHPLC). We are monitoring the purification steps with a radio-immunoassay, using an anti-KYSALMFamide antibody (Elphick *et al*, 1991). We have already demonstrated SALMFamide like immunoreactivity in the nervous system of this parasite (Keating *et al*, 1993). In this study we have purified a FLP from this organism and identified it as AF2. We have also purified a second immunoreactive peptide which we are presently characterizing. We have shown that AF2 potentiates the effect of acetylcholine in *Ascaris* dorsal muscle strips and that AF2 induces spontaneous activity in *Ascaris* dorsal muscle.

These findings show that AF2 has now been shown to exist in a second parasitic nematode and it maybe that this peptide is ubiquitous throughout this Phylum.

Elphick, M.E., Reeve, J.R., Burke, R.D., Thorndyke, M.C. (1991), *Peptides*. **12**, 455-459.

Keating, C., Thorndyke, M.C., Holden-Dye, L., Franks, C.J., Williams, R.G., & Walker, R.J. (1993). *Br.J. Pharm*. **111**, 307P.

471.17

CHARACTERIZATION AND PHYSIOLOGICAL FUNCTION OF LUQIN IN THE CENTRAL NERVOUS SYSTEM OF *APLYSIA CALIFORNICA*. R. S. Aloyz, A. Angers and L. DesGroseillers'. Department of Biochemistry, University of Montreal, Montreal, Canada, H3C 3J7.

We are investigating the role of neuropeptides in renal physiology. Left Upper Quadrant (LUQ) neurons and neuron L10 in the central nervous system of *Aplysia* have been shown to extensively innervate the kidney and regulate renal functions. Three neuropeptide precursor genes (LUQ-1, L5-67 and L10-M), which are differentially expressed in these cells, have previously been cloned. However, the nature of the physiologically relevant peptides which they express is still unknown. In this study, metabolic labeling of the LUQ cells and RP-HPLC separation of their peptide content allowed us to identify the L5-67 precursor and the processed peptides. Cleavage of the signal peptide occurred between amino acids 23 and 24 of the prepropeptide and generated a propeptide of 89 amino acids. Further processing by endopeptidases at the twin basic residues Lys^{12}-Arg^{13} of the precursor generated a peptide of 76 amino acids, as well as an amidated decapeptide, LUQIN. Immunoprecipitation of labeled LUQ peptides and RT-PCR on mRNA isolated from neurons L2-6 allowed us to identify a 4 kDa LUQ peptide which could arise by differential splicing. The sequence of LUQIN was determined by amino acid sequencing and by its comigration with the synthetic peptide Ala-Pro-Ser-Trp-Arg-Pro-Gln-Gly-Arg-Phe-amide in three different RP-HPLC systems. The amidation of LUQIN was further demonstrated by its resistance to carboxypeptidase A digestion. When applied to neuron L10, LUQIN induced a long-lasting inhibition of L10 activity. No other neurons in the abdominal ganglion, when tested at random, responded to LUQIN. Finally, LUQIN has also been purified from the kidney.

472.1

CHARACTERIZATION OF cDNAs ENCODING *APLYSIA CALIFORNICA* FURIN AND PC2. G.T. NAGLE*, A.T. GARCIA, S.L. KNOCK, E. GORHAM AND A. KUROSKY. Marine Biomedical Institute and Departments of Anatomy & Neurosciences, Humanities & Basic Sciences, and Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX 77555.

The neuroendocrine bag cells and exocrine atrial gland of *Aplysia californica* express genes belonging to the egg-laying hormone (ELH) family and process the resulting precursor at mono-, di-, tri-, and tetrabasic sequences. Some of these cleavages may result from proteolysis by a furin-like or PC2-like enzyme; these enzymes have been identified in organisms as diverse as yeast and humans. The atrial gland is particularly interesting because the organ produces large (milligram) amounts of ELH-related peptides and may be a relatively rich source of prohormone processing enzymes. An atrial gland cDNA library was constructed and screened using a furin-related PCR probe and a clone encoding a furin-like protein was isolated. The insert is identical to a furin-related PCR product from the bag cells. The library was also screened using a *Lymnaea* PC2 probe (provided by A.B. Smit and W.P.M. Geraerts, Holland) and a clone encoding a PC2-like protein was isolated. Both clones have relatively long 3′ untranslated regions (UTR's). The 3′-UTR's contain multiple polyadenylation sites which could be used to generate multiple mRNAs. Furthermore, the 3′-UTR's also contain relatively long microsatellite repeat sequences $(CA)_n$ and $(TG)_n$. The eventual goal of these studies is to elucidate the potential role of these enzymes in prohormone processing in *Aplysia*. Supported by NIH Grant NS 29261.

472.2

Yeast Aspartic Protease 3 Cleaves Prohormones at Selective Paired and Mono-Basic Residues with Preference for a Lys/Arg Residue Upstream from the Cleavage site. N. X. Cawley[1,3], H-C. Chen[2], M. C. Beinfeld[4] and Y. Peng Loh[1,3]*. From the [1]Lab. of Develop. Neurobio. and [2]Endocrin. and Reprod. Research Branch, NICHD, NIH, Bethesda, MD 20892 and the [3]Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and the [4]Dept. Pharmacological and Physiological Sciences, St. Louis University Medical Center, St. Louis, MO 63104.

The novel yeast aspartic protease 3 (YAP3) was overexpressed by induction of yeast strain BJ3501 that was transformed with a plasmid containing the YAP3 gene under the control of the galactose inducible promoter. YAP3 was secreted into the growth media and was partially purified by concanavalin A affinity chromatography. The specificity of YAP3 was studied using a number of polypeptide substrates: porcine cholecystokinin 33 (CCK33), porcine dynorphin A(1-11), porcine dynorphin B/rimorphin(1-13) and bovine proinsulin. Analysis of the products generated from each substrate demonstrated that YAP3 cleaved at selective paired and mono-basic residue sites generating CCK8 and CCK22 from CCK33; Leu-enkaphalin and Leu-enkaphalin-Arg from dynorphin A and dynorphin B respectively and an extended form of insulin from proinsulin. Cleavage at mono-basic residues occurred only when a Lys/Arg was present in the -4, -5 or -6 position relative to the cleavage site.

472.3

PROHORMONE CONVERTASE 2 IS EXPRESSED IN PROENKEPHALIN CONTAINING NEURONS IN DEVELOPING AND ADULT RAT CEREBELLUM. Y. P. Loh, A.M. Bamberger*, W. P. Hayes, and L. P. Pu. Lab. of Devel. Neurobiol, NICHD NIH, Bethesda, MD 20892.

The enzymatic processing of peptide precursors at basic amino acids is a key step in neuropeptide biosynthesis. Prohormone convertase 2 (PC2) may participate in such processing. We have examined the distribution of PC2 mRNA in rat cerebellum during postnatal development and in adult and compared it with that of proenkephalin mRNA and enkephalin precursor. Using in situ hybridization, we showed that PC2 mRNA was expressed in a distinct layer pattern in adult rat cerebellum. The Purkinje cell layer expressed very high levels of PC2, while the granular cell layer showed low levels of expression. High expression of PC2 mRNA was also found in Golgi cells. Similarly, proenkephalin mRNA was detected in Purkinje cells and Golgi cells. During cerebellar development, PC2 and proenkephalin mRNAs were present as early as postnatal day 1 (P1). With age, there was a gradual increase in the expression of PC2 mRNA in Purkinje and Golgi cells through adult, which correlated well with that of proenkephalin mRNA. Interestingly, a transient expression of PC2 mRNA was observed in granule cells, which appeared as early as P1 and increased to a maximum at P28, an age corresponding to cell division and maturation. Further, by in situ hybridization combined with immunocytochemistry, a cellular coexistence of PC2 mRNA and proenkephalin was found in enkephalin expressing Purkinje cells and Golgi cells. These findings implicate the role of PC2 in neuronal proenkephalin processing in both developing and adult cerebellum.

472.4

EFFECT OF NICOTINE ON PROENKEPHALIN mRNA LEVEL, SECRETION OF [MET^5]-ENKEPHALIN, AND AP-1 DNA BINDING ACTIVITY IN BOVINE ADRENAL MEDULLARY CELLS. [1]H. W. Suh*, [2]P. M. Husdson, [2]M. K. MaMillian, [2]K. P. Das, [2]B. C. Wilson, [2]G. C. Wu, and [2]J. S. Hong. [1]Dept. Pharmacol. Coll. Med., Hallym Univ., Chunchon, Korea, and [2]LMIN/NIEHS/NIH, RTP, NC U.S.A.

The effects of nicotine on the secretion of [Met^5]-enkephalin (ME) in addition to proenkephalin A (proENK) mRNA levels, AP-1 DNA binding activity, and on the transcriptional activity of the proENK gene were studied in bovine adrenal medullary chromaffin (BAMC) cells. Nicotine (10 μM) caused a rapid secretion of ME followed by a long-term secretion into the medium. Post-treatment with hexamethonium plus atropine, nimodipine, calmidazolium or KN-62 up to 6 hr after the nicotine treatment significantly inhibited increases of proENK mRNA level, the secretion of ME, and AP-1 DNA binding activity induced by nicotine. However, nicotine-induced responses were not affected when these agents were added 9 or 12 hr after the nicotine treatment. The results of the nuclear run-on assay showed that nicotine increases the transcriptional rate for the proENK gene after 30 min and the response continues for up to 9 hr with a maximal increase of 2- to 2.5-fold. Our results suggest that the long-term stimulation (for at least 6 hr) of nicotinic receptors is required for the increases in proENK mRNA levels and the long-term secretion of ME in BAMC cells. The nicotine-induced long-term secretion of ME followed an increased biosynthesis of ME during the first 6 hr which appeared to result from increased transcription of the proENK gene followed by increased syntheis of proENK mRNA.

472.5

IDENTIFICATION OF AT_4 LIGANDS IN THE RAT BRAIN AND DEMONSTRATION OF *in vivo* SYNTHESIS INDEPENDENT OF ANGIOTENSIN CONVERTING ENZYME ACTIVITY. B.A. Mungall*, A.E. Ball, J.W. Harding and J.W. Wright. Program in Neuroscience and Departments of Veterinary and Comparative Anatomy, Pharmacology, Physiology and Psychology, Washington State University, Pullman, WA 99163.

We have recently discovered a unique angiotensin AT_4 receptor site, assumed to bind endogenous angiotensin IV. We have provided definitive evidence regarding the identity of the naturally occurring ligand acting at this site. Utilizing acetic acid peptide extraction, High Performance Liquid Chromatography separation and Radioimmunoassay detection, we have identified and quantified angiotensin IV-like N-terminal peptides in rat cerebellum, cortex, striatum, hippocampus, thalamus and hypothalamus with high binding affinity for this AT_4 receptor. In addition, *in vivo* metabolism studies utilizing radiolabelled peptide infusion via push pull cannulation, have revealed cleavage of angiotensin I to angiotensin I(2-10) and then subsequently to angiotensin IV-like peptides with high affinity for the AT_4 receptor both in control (untreated) and captopril treated rats. *In vitro* metabolism studies of cerebellum homogenates have confirmed these findings. These studies indicate the presence of high affinity endogenous AT_4 ligands, and formation of these peptides independent of the classical renin-angiotensin pathway involving angiotensin converting enzyme (ACE). ACE inhibitors are widely used in cardiovascular therapy as antihypertensive agents for treatment of congestive heart failure, myocardial infarction and stroke. The possibility of AT_4 activation playing a role in the observed effects of captopril therapy, especially on mood and cognition may provide an insight into the mechanism of ACE inhibitors.

472.6

ANATOMICAL RELATIONSHIP BETWEEN GENES ENCODING PROHORMONE CONVERTASES AND PRO-TRH IN ADULT RAT BRAIN: IMPLICATION IN DIFFERENTIAL PROCESSING OF PRO-TRH. L. P. Pu*, W. Ma, I.L. Barker and Y. P. Loh. Labs. of LDN, NICHD and LNP, NINDS, NIH, Bethesda, MD 20892.

Pro-TRH in the CNS is differentially processed at paired-basic residues to generate several copies of TRH along with other neuropeptides. The mechanism for such differential processing is not yet known. Recently, prohormone convertases (PC1 and PC2), responsible for paired-basic residue cleavage, are found to be expressed in neuroendocrine system. We have used *in situ* hybridization histochemistry to analyse the anatomical relationship between genes encoding PC1, PC2, and pro-TRH in rat brain. PC1 and PC2 mRNA expressing cells were widely distributed throughout the brain. Expression levels of PC1 and PC2 mRNAs differed in various brain regions with PC2 showing a broader distribution. Regions with high concentrations of PC1 and PC2 cells demonstrated a good correspondence with that of pro-TRH cells. They included olfactory bulb, forebrain areas, hippocampus, hypothalamic nuclei, and several nuclei in brainstem. However, not all brain areas with high levels of prepro-TRH mRNA had high levels of PC1 and PC2 mRNAs. For example, in thalamic reticular nucleus prepro-TRH mRNA was expressed, but neither PC2 mRNA nor PC1 mRNA was detectable. By performing double-labeling *in situ* hybridization histochemistry, a cellular coexistence of PC1 or PC2 mRNAs with prepro-TRH mRNA was found in a differential manner in several brain regions. These results suggest that the different expression patterns of PC1 and PC2 in pro-TRH neurons may be responsible for the differential processing of pro-TRH in various rat brain regions.

472.7

TISSUE DISTRIBUTION AND DEVELOPMENTAL STUDY OF APLYSIA PRO-HORMONE CONVERTASE PC2. T. Ouimet* and and V.F. Castellucci. Lab. of Neurobiology and Behavior, IRCM, Univ. de Montréal, Montréal, Québec, H2W 1R7.

The nervous system of the marine mollusc *Aplysia californica* contains many neuropeptides which must undergo proteolytic cleavage at multibasic sites to release their bioactive moiety. We have recently characterized the cDNA structure of a subtilisin-like serine protease of the nervous system of aplysia (aPC2) which appears to be a homolog of the vertebrate PC2, of the pro-hormone convertase family. *In-situ* hybridization shows a large distribution of aPC2 mRNA in the nervous system. Study of the tissue distribution of the aPC2 transcript by Northern blot analysis also revealed its presence in the atrial gland as well as in muscle. This last result was also confirmed by *in-situ* hybridization. Northern blot analysis of early stages of Aplysia development reveals the presence of a shorter transcript. Thus, aPC2 seems to have a larger distribution in Aplysia than in vertebrates where it seems to be restricted to neuroendocrine tissues. Furthermore, different aPC2 transcripts seem present during development. Funded by MRC of Canada (MT-12099).

472.8

REGULATION OF CHOLECYSTOKININ mRNA BY RETINOIC ACID AND ß-ADRENERGIC AGENTS IN CELL LINES. **B. L. Mania-Farnell*, L.W. Botros, and T.P. Davis. Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.**

Regulation of cholecystokinin (CCK) mRNA expression was studied in two CCK expressing cell lines, the human neuroepithelioma cell line SK-N-MCIXC and a rat medullary thyroid carcinoma cell line WE 4/2. SK-N-MCIXC cells express the CCK gene at high levels and perform post-translational processing of CCK (Konings et al., Neuropeptides 25, 1993; Verbeeck and Burbach, FEBS 268, 1990). WE 4/2 cells contain high levels of CCK and process pro-CCK to biologically active forms (Beinfeld, Peptides 13, 1992). To examine the effect of ß-adrenergic agents on CCK mRNA we used the ß-adrenergic agonist isoproterenol. We also treated cells with a combination of isoproterenol and the ß-adrenergic antagonist propranolol. The affect of retinoic acid on the modulation of CCK gene expression was also examined. Messenger RNA levels were quantitated using Northern blot analysis with a cRNA CCK probe. Isoproterenol significantly increased CCK mRNA levels in SK-N-MCIXC cells after 6, 12 and 24 hr treatments, the increase was blocked when isoproterenol was combined with propranolol. In WE 4/2 cells isoproterenol increased CCK mRNA levels after 6 hrs, although not significantly, the increase was no longer present after 12 hrs. Retinoic acid had no affect on CCK mRNA levels in WE 4/2 cells at 6 or 12 hr treatments; however, there were significant decreases in CCK mRNA levels in SK-N-MCIXC cells after 6, 12 and 24 hrs. Our results indicate that CCK mRNA levels may be differentially regulated in these two cell lines in response to ß-adrenergic agents and retinoic acid. (Supported by N.I.H. Grant DK 36289 and MH42600 and MRC Grants MT11268 and PG2.)

472.9

GLUTAMATERGIC REGULATION OF STRIATAL PEPTIDE GENE EXPRESSION IN RATS. J. Jolkkonen*, P. Jenner and C.D. Marsden. Dept. of Neurology, Univ. of Kuopio, Kuopio, Finland, Pharmacology Group, King's College, London, U.K. and Univ. Dept. of Clinical Neurology, The National Hospital, London, U.K.

Nigrostriatal dopaminergic neurons are known to regulate enkephalin and substance P gene expression in the striatum. We studied possible modulation of striatal peptide mRNA levels by corticostriatal glutamate input following disruption of glutamate neurons, glutamate release and receptor binding.

Glutamate afferents were bilaterally lesioned by partial frontoparietal ablation of the cerebral cortex, glutamate release was inhibited by lamotrigine treatment (5 and 20 mg/kg, i.p., once a day) and NMDA receptors were blocked by intrastriatal infusion of CPP (0.12 and 1.2 µg/day). Striatal peptide mRNA levels were measured one week following the start of the experiment by using *in situ* hybridization histochemistry according to Young *et al.* (1986). Coronal brain sections (12 µm) cut at three different rostrocaudal levels of the striatum were incubated with ^{35}S-labelled oligonucleotide probes, apposed to X-ray film and average optical densities were analyzed by computerized densitometry.

Following decortication both enkephalin and substance P mRNA levels were decreased particularly in dorsolateral part of the rostral striatum (17.0-25.8%). The overall hybridization signal for striatal peptide mRNA was not affected by subchronic administration of lamotrigine. NMDA receptor blockade by CPP infusion decreased enkephalin (14.4-34.6%) and substance P mRNA levels (13.9-38.2%) in a dose-dependent manner in the striatum.

It seems that enkephalin and substance P gene expression in the rat striatum is also regulated by glutamate in addition to the well-established dopaminergic regulation.

472.10

ISOLATION OF THE GC-RICH, TATA-LESS PROMOTER FOR THE GENE ENCODING THE BIFUNCTIONAL ENZYME PAM. Hand, T.A. Mains, R.E., Eipper, B.A. * Dept.of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

PAM (peptidylglycine α-amidating monooxygenase) is a bifunctional enzyme catalyzing the 2-step conversion of glycine extended COOH-terminal neuroendocrine peptides to α-amidated bioactive products. The 27 protein coding exons of the single copy PAM gene were isolated from rat genomic libraries and span more than 160 kb of DNA. Since the expression of PAM is tightly regulated in a tissue and developmentally specific manner, isolation of the promoter was undertaken. Exon 2 of the PAM gene contains 301 nts of 5'-UTR and the start methionine; primer extension data indicated that the longest cDNA clone isolated lacked the most 5'-end of PAM. Using RACE, products isolated from atrium and neurointermediate lobe (NIL) contained 98 nts of upstream coding sequence preceding Exon 2. Screening a genomic library yielded a clone containing 69 nts of the sequence in the RACE product (Exon 1) flanked by consensus intron/exon junction sequences. Subsequent screening of a cDNA library (made from the NILs of haloperidol-treated rats) with a 5'-specific probe revealed a clone having an additional 21 nts of upstream coding sequence. Exon 0 was identified by screening the genomic library with an oligomer complimentary to the most 5'-sequence. The clone identified contained the 50 nts of Exon 0 followed at the 3' end by a consensus exon/intron junction sequence. A highly GC-rich sequence having several consensus SP1 binding sites but lacking TATA or CAAT sequences preceeded Exon 0. Two kb of the upstream sequence (PAM-US) was subcloned into a reporter vector (pGL2, Promega) in the sense or antisense orientation and transiently transfected into GH3 cells, which endogenously express PAM. The PAM-US sense construct yielded levels of luciferase expression at least 150 times those of the antisense construct. Support DK 32949.

472.11

ONTOGENY OF PROENKEPHALIN IN RAT: ITS CORRELATION WITH PROTEOLYTIC PROCESSING ENZYME GENE EXPRESSION. M. Zheng* and J. E. Pintar. Dept. of Anat. and Cell Biol., Columbia Univ. P&S, New York, NY 10032 and Dept. of Neurosci. and Cell Biol., UMDNJ Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Proenkephalin (PE) is a proprotein which, through selective endoproteolytic cleavage, gives rise to opioid peptide enkephalins and many larger enkephalin-containing peptides (ECP). Gene transfer experiments have shown that the endoprotease PC2 is capable of performing extensive cleavage of PE, whereas PC1 and furin cleave PE only to a limited extent. It is not known which of these enzymes are coexpressed with PE and therefore may actually be involved in PE processing in vivo. Using in situ hybridization we have examined the ontogeny of PE mRNA expression and compared it with proteolytic processing enzyme gene expression. PE expression is first detected at a low level in the wall of truncus arteriosus at e10. By e12 its expression has expanded to cover the aortic sac and common ventricular chamber, which overlaps with furin expression. In the nervous system, PE expression is detected in the restricted regions of the thalamus, striatum, pons, medulla, and the spinal cord from e14 onward. At these stages, PC2 is expressed widely in the CNS and overlaps extensively with PE expression, whereas PC1 expression overlaps with PE expression only in the medulla. In mid- and late gestational stages PE expression is also detected at a high level in a variety of mesenchymal tissues, which overlaps with furin expression. Together these data suggest that PE expressed in development may be processed in a tissue-specific manner by different sets of endoproteases. In the nervous system PE may be processed extensively by PC2 and to a small extent by PC1, generating enkephalin pentapeptides or smaller ECPs. PE in the peripheral tissues, in contrast, may be processed to only a limited extent by furin, which is consistent with reported lack of detectable free enkephalins in tissues outside of nervous system. Supported by DA-08622 (JEP).

472.12

DEVELOPMENTAL EXPRESSION OF PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE IN THE RAT. J. Zhang*, M. Zheng, B. A. Eipper, and J. E. Pintar. Dept. Neurosci. & Cell Biol., UMDNJ Robert Wood Johnson Med. Sch., Piscataway, NJ 08854 and Dept. Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Posttranslational processing of peptide precursors frequently includes amidation of C-terminal glycine residues following proteolytic cleavage, which is performed by peptidylglycine α-amidating monooxygenase (PAM). To evaluate the contribution of this peptide modification process in development, we have begun to examine the ontogeny of PAM gene expression in the Sprague-Dawley rat by in situ hybridization. Using a probe that recognizes all PAM isoforms, we thus far have detected a high level of PAM expression in the heart (in both atrium and ventricle) as early as embryonic day 10 (e10). In the developing nervous system PAM mRNA is present at least as early as e14 in regions outside the ventricular layer and is particularly high in the cortical plate, hypothalamus, and spinal cord. By midgestational stages (e16-e18), PAM transcripts are also widespread in tissues outside of the nervous system and are particularly abundant in mesenchyme surrounding developing limb cartilage and are also notable in the lung, muscle, adrenal, and testis. We also have used a variety of probes that distinguish alternatively-spliced forms of PAM to determine the relative abundance of different PAM forms and whether there is cell-type specific accumulation of different PAM transcripts. Results from these studies suggest that exon-B containing PAM transcripts are the predominant PAM mRNAs at all ages examined and are found in most, if not all, tissues. In addition, rPAM-4 transcripts, which encode only the peptidylglycine α-hydroxylating monooxygenase portion of PAM, are relatively enriched in the dorsal horn of the spinal cord but are essentially absent from the ependymal region of the intermediate zone, an area rich in exon-B containing PAM transcripts. Taken together, these results provide evidence for widespread expression and alternative splicing of PAM during development. Supported by DA-08622 (JEP) and DA-00266 (BAE).

472.13

AN *IN VITRO* MODEL FOR THE STUDY OF NEUROTRANSMITTER REGULATION OF HYPOTHALAMIC GENE EXPRESSION D.M. Witt* and H. Gainer, Laboratory of Neurochemistry, NINDS, NIH Bethesda, MD 20892.

Determination of the specific neural mechanisms involved in stimulus-coupled neuropeptide gene expression is limited by the complexities of the *in vivo* environment which include a multitude of neural networks and feedback circuits. Conventional primary tissue cultures, devoid of these complexities, are usually maintained for long periods of time *in vitro* before they can be used for experiments, and often alter their normal properties during the culture period. We have developed an *in vitro* model for examining neurotransmitter / neurosteroid effects in brain slices that are acutely maintained in a controlled environment.

By combining electrophysiological preparations, organotypic slice explant technology, and immunocytochemistry we have been able to study immediate early gene (IEG) expression in acute slice explants of the paraventricular nucleus of the hypothalamus (PVN) from postnatal rats. Following *in vitro* stimulation (1.5 hrs), using either K^+ (40 mm) depolarization or glutamate (10-100 μM) stimulation, IEG expression was enhanced in oxytocin / vasopressin neurons, primarily in magnocellular regions of the PVN. This new approach enables the assessment of the efficacy of specific stimuli to regulate neuropeptide gene expression in the mammalian hypothalamus.

472.14

EXPRESSION OF THE PRE-PROSOMATOSTATIN GENE AND SOMATOSTATIN IMMUNOREACTIVE FORMS IN THE HUMAN NEUROBLASTOMA LA-N-2 CELL LINE.

Ronnie Folkesson*, Lena Bergström, Lars Nilsson and Bengt Winblad, Karolinska Institute Clinical Neuroscience, Geriatric Medicine, Novum KFC Huddinge, Sweden.

Initial screening of different human neuroblastoma cell lines for expression of Somatostatin immunoreactivity (SS-ir) and prepro-SS mRNA revealed only one candidate, the neuroblastoma LA-N-2 cell line. Extracted SS-ir material from LA-N-2 cells was analyzed and compared with SS-ir extracted material from rat hypothalamus after gel filtration. The SS-ir in each fraction was analyzed with RIA revealing three major peaks of SS-ir forms in the rat hypothalamus extract.The predominate peak coeluted with SS-14 the second peak coeluted with SS-28 and the third peak was found close to the void volume and corresponded to an estimated molecular weight of 15 kDa. In contrast the SS-ir extracted from the LA-N-2 cell line showed, a quite different elution pattern.Three peaks of SS-ir were measurable, one which corresponded in elution position to synthetic SS-28 and two others which corresponded to approximately 8 kDa and 15 kDa. No immunoreactivity was observed in the fractions coeluting with synthetic SS-14. Total cellular RNA from LA-N-2 cells was prepared and examined for the expression of prepro-SS mRNA by Northern blot analysis revealing the presence of a single prepro-SS mRNA of about 850 nucleotides in LA-N-2 cells.In addition this cell line is cholinergic which providing opportunities to investigate how the somatostainergic and cholinergic systems interact. Such interactions are of interest considering the marked reduction of these two transmittors in cognitive disorders including Alzheimer's disease.

472.15

NEURONAL SPECIFIC PROCESSING OF VGF PROTEIN LEADS TO THE PRODUCTION AND REGULATED SECRETION OF PEPTIDES DERIVED FROM ITS CARBOXY-TERMINAL. R. Possenti°*, A.M. Rinaldi°, N. Canu, A. Levi, G.L.Ferri§, M.T. Ciotti, E. Trani. Institute of Neurobiology CNR, Via C. Marx 15, Rome, Italy; §Dept. Citomorphology, University of Cagliari; ° Dept. Experimental Medicine, University of Tor Vergata, Rome.

VGF, a protein of 617 aminoacids, has a restricted expression in vivo in sub-populations of neuronal and endocrine cells. By the use of polyclonal antibodies directed against different domains of VGF we demonstrated that in vivo as well as in vitro neuronal cells are able to cleave this protein in smaller peptides. This processing is not observed in adrenal medulla chromaffin cells. PC12 cells, a line established from a rat pheochromocytoma, are unable to process VGF, but acquire the ability to mature this protein upon neuronal differentiation in response to NGF. In primary cultures of cerebellum granule cells the in vitro maturation correlates with increased processing of VGF.The major products of the VGF cleavage are a polypeptide of apparent molecular weight 20 KDa and a doublet in the 14-10 KDa range which are derived from the COOH-terminal of the protein. These products are enriched in preparation of dense core vesicles from neuronal cells and are preferentially secreted upon depolarisation in neuronal primary colture and cell lines. We suggest that these VGF-derived peptides are the biologically relevant species which may play a role in neuronal communication.

Supported by a grant from P.F. Invecchiamento C.N.R.

472.16

ALTERED PRODUCTION OF PROHORMONE CONVERTASE 2 (PC2) mRNA IN THE ARCUATE (ARC) OF MIDDLE AGED FEMALE C57BL/6J MICE. **D. Joshi,* M.M. Miller, N. G. Seidah and R. Day, Departments of Experimental Medicine, Obstetrics and Gynecology, Anatomy, and Center for Studies on Aging, McGill University; Clinical Research Institute of Montreal, Montreal, Quebec, Canada**

We have previously reported a significant increase in the proportion of β-endorphin (β-endo)(1-27) and (1-26) forms in ARC of middle aged irregularly cycling female C57BL/6J mice (Joshi et al. 1993). PC2, an endoproteinase of subtilisin/kexin family, mediates cleavage of β-endo(1-31) to β-endo(1-27). The objective of this study was to determine if an increase in PC2 expression is associated with the observed change in β-endo processing. PC2 mRNA was measured using an antisense cRNA probe to mouse PC2. ARC of young (4-5mo) normally cycling (n=5) and middle aged (12-13mo) irregularly cycling (n=5) female mice in diestrus of the estrous cycle were pooled for Northern blot analysis. A significant ($p<0.01$) increase in the PC2 mRNA was observed in ARC of middle aged animals as compared to young mice. For *in situ* hybridization, two sections each from five ARC subregions were examined in both young (n=3) and middle aged (n=3) mice. Film autoradiograms of *in situ* hybridization revealed a significant proportional increase in mid ARC PC2 mRNA ($p<0.05$ ANOVA) of middle aged mice vs. young females. These data suggest that there is an increase in the level of PC2 mRNA in ARC in middle aged female mice, which may account, at least in part, for altered processing of β-endo in middle aged irregularly cycling female mice.

Funded by NIH AG07795 (MMM) and MRC MT11268 (RD).

472.17

DIFFERENTIAL EFFECTS ON NEOSTRIATAL NEUROPEPTIDE mRNA ABUNDANCE BY EXCITATORY AMINO ACID RECEPTORS M. Villegas, L. Lucas‡, B. McEwen‡ and J. Angulo*, ‡Lab of Neuroendocrinology, Rockefeller University and Dept. of Biological Sciences, Hunter College CUNY, New York, N.Y. 10021.

Cortical, thalamic and amygdaloid inputs to the neostriatum employ excitatory amino acids as transmitters. These inputs play a central role in excitation of neostriatal neurons most of which utilize neuropeptides as transmitters and/or modulators. The effects of NMDA and AMPA/kainate receptor blockade on the expression of proenkephalin (PE), protachykinin (PT) and prodynorphin (PD) mRNAs in the caudate-putamen (CPU) and nucleus accumbens (NAc) were assessed with MK801 and CNQX (NMDA and AMPA/kainate antagonists, respectively). Daily systemic injections with MK801 for seven consecutive days increased the abundance of all three neuropeptide mRNAs in the caudate-putamen and accumbens. (1) PE mRNA abundance was increased in the anterior CPU (26%), dorsal and ventral CPU (46% and 39%, respectively) but was unaffected in the NAc. (2) PT mRNA was increased in the NAc (33%) as well as anterior CPU (27%), dorsal CPU (43%) and ventral CPU (67%). (3) PD mRNA was elevated in dorsal and ventral regions of the CPU (49% and 24%, respectively) and in anterior CPU (50%). In the NAc PD mRNA was increased only at the higher dose (0.1 mg/kg) of MK801. In contrast, systemic injections of CNQX for seven consecutive days decreased PE and PT mRNA abundance approximately 30% below controls in dorsal and ventral aspects of the CPU and accumbens. These observations demonstrate that NMDA and AMPA/kainate receptors differentially modulate neuropeptide expression in the neostriatum of the rat brain.

472.18

TRANSCRIPTIONAL REGULATION OF THE NPY GENE IN RESPONSE TO NGF.

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Nerve Growth Factor has been reported to regulate the expression of the NPY gene in PC12 cells. The study of the intracellular mechanisms involved in mediating such responses may provide important clues about how neuronal cells integrate a variety of stimuli in order to produce a coordinated response. Using a CAT reporter system, we have shown that an AP-1 consensus sequence is responsible for most of the NGF-induced transcriptional activity of the NPY gene. However, a residual response to NGF was observed after the deletion of this AP-1 site, suggesting that other sequences are also involved. This residual response is mediated through both PKC and PKA dependent mechanisms. Specific inhibitors such as calphostin C (PKC) and H-89 (PKA) were shown to block only partially this residual response to NGF when applied alone, whereas combined application of both inhibitors completely blocked it. Two consensus sites for AP-2 transcription factors which are also present in the NPY promoter region, appear to be responsible for the residual activity of NGF. The deletion of these three sequences completely abolished the response to NGF. Basal levels of expression of the NPY gene in PC12 cells were also found to be mainly dependent on the two adjacent AP-2 sites.

Funded by the Wellcome Trust.

472.19

FRAMESHIFT MUTATIONS AT HOTSPOTS IN VASOPRESSIN TRANSCRIPTS IN RAT AND HUMAN POST-MITOTIC NEURONS. F.W. van Leeuwen*, D.A.P. Evans and J.P.H. Burbach†. Neth. Inst. Brain Res., 1105 AZ Amsterdam, †Dept. Med. Pharmacol., Rudolf Magnus Inst., 3584 CG Utrecht, The Netherlands.

Mutations in DNA underlie carcinogenesis, inherited pathology and aging and are generally thought to be introduced during meiosis and mitosis. Here we report that in post-mitotic neurons specific frameshift mutations occur at high frequency. These mutations were identified in vasopressin transcripts in magnocellular neurons of the vasopressin-deficient homozygous Brattleboro rat and predominantly consist of a GA deletion at GAGAG-motifs. In homozygous Brattleboro rats substituted with vasopressin for 40 weeks and displaying a normalized water balance a 25% reduction in the number of vasopressin cells displaying a GA deletion was found. This indicates that the diseased state of the Brattleboro rat, resulting in a permanent activation of vasopressin neurons, enhanced the mutational rate. Using antibodies against peptides predicted from the +1 reading frame of vasopressin mRNA, immunocytochemical evidence was obtained for similar events in the hypothalamus of wild-type rats and human. These data have revealed hitherto unrecognized somatic mutations in non-dividing neurons. Such mutations are not restricted to the Brattleboro rat and may occur more widely in neuronal systems affecting other neuronal genes. Evidence for frameshift mutations in other genes will be presented.

CATECHOLAMINES: MEASUREMENTS, SPECT, LESIONS—IMMEDIATE EARLY GENES

473.1

VOLTAMMETRIC CHARACTERIZATION OF ELECTRICALLY-STIMULATED SOMATO-DENDRITIC DOPAMINE RELEASE IN MESENCEPHALIC SLICES. S.J. Cragg*, M.E. Rice and S.A. Greenfield. Univ. Dept. Pharmacology, Oxford OX1 3QT, UK; Dept. Physiology & Biophysics, NYU Medical Center, New York, NY 10016.

Somato-dendritic release of dopamine (DA) from the substantia nigra (SN) and ventral tegmental area (VTA) may represent a non-classical form of signaling in those regions. We have used fast-scan cyclic voltammetry (FCV) with 8 μm carbon fiber microelectrodes to detect endogenous dopamine efflux *in situ* from tyrosine-hydroxylase (TH)- positive regions of superfused slices from guinea pig mid-brain. Release was monitored during local electrical stimulation, using a train of 100 pulses delivered at 10 Hz. Signals attributable to DA were identified on the basis of anatomical, electrochemical and pharmacological criteria. The response exhibited site-specific variation that correlated well with TH staining: release was significantly higher in VTA (1.04 ± 0.19 μM) than in SN pars compacta (0.52 ± 0.05 μM), which was in turn significantly higher than in SN pars reticulata (0.34 ± 0.03 μM).

The voltammogram of the released substance had oxidation and reduction peak potentials that corresponded to those of DA and the signal evoked in the DA-rich striatum, and were distinguishable from those of 5-HT. The response had apparently no contribution from DOPAC, since it was unaffected by 20 μM pargyline. Electrically-stimulated DA release was Ca^{2+}-dependent, but TTX-independent. The selective DA uptake blocker, GBR 12909 significantly increased the response (260% of control), while desiprimine, a selective inhibitor of NE uptake had no significant effect. We conclude that the direct monitoring of stimulated DA release *in situ* with carbon fiber electrodes can provide new insights in to the mechanism and function of dendritic release.

Supported by Bristol-Meyers Squibb and NIH grant NS-28480.

473.2

REDUCTION/OXIDATION RATIOS AND NEUROTRANSMITTER IDENTITY: EFFECT OF NAFION COATING THICKNESS. R. T. Marrocco*, C. R. Harris-Collazo & M. C. Davidson. Dept. of Psychiatry, UC San Diego School of Medicine, La Jolla, CA 92093 and Institute for Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Studies utilizing in vivo voltammetry to investigate the evoked release of catecholamines commonly use Nafion coated carbon fiber electrodes. Electrochemical signals from cations may be detected chronoamperometrically by applying a positive potential step (vs. an Ag/AgCl reference electrode) to the working electrode to oxidize electroactive molecules. The reverse current flow generated by the return of the potential to its previous value reduces the oxidized electroactive species. The ratio of the reduction to oxidation current at the maximum oxidation current is termed the reduction/oxidation (RO) ratio. It has been suggested that the RO value gives some indication of the identity of the major species measured (Gratton *et al., Neurosci.* **29**: 57-64, 1989).

We have recently found that the RO ratio may be related to the thickness of the electrode's Nafion coating. Nafion was applied by simple immersion or with electric current. Our results showed that as the amount of Nafion coating increased, the RO ratio also increased when the only substance added to the calibration medium was noradrenaline, dopamine, or serotonin. Moreover, increases in the duration of applied voltage also produced systematic changes in RO ratio. A three fold-increase in plating time caused a comparable increase in RO ratio for noradrenaline. While dopamine may usually exhibit a larger RO ratio than noradrenaline or serotonin, that ratio may be different for each electrode. Also, once the electrode is in the brain, the Nafion coating may begin to thin and RO ratios will change. Thus, RO ratios depend on the neurotransmitter present and the properties of the detecting electrode.

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473.3

Multicomponent Analyses of Monoamines and Related Compounds in Cerebrospinal Fluid by High Performance Liquid Chromatography (HPLC) Coupled With Electrochemical Array Detectors. C.J. Hope*, P. Huettl, F. Issa[1], D. Kirch[2] and G.A. Gerhardt. Dept. of Psychiatry, Univ. of Colorado Health Sciences Center, Denver, CO, [1]NIMH, Washington D.C., and [2]Medical College of Georgia, Augusta, GA.

Recent studies in our laboratory have involved the detailed analyses of monoamine neurotransmitters and related species in CSF using HPLC coupled with multichannel coulometric electrochemical array detectors. Such approaches allow for the analysis of up to 30 neurochemicals in a single sample. Frozen CSF samples (0.5 ml) are thawed, diluted to 1 ml with mobile phase, filtered and divided into two 0.5 ml fractions. An isocratic HPLC system consisting of a single pump, C18 reverse phase column (4.6 mm X 100 mm, 3 μm particles, Hypersil; Keystone) and a dual-channel electrochemical array detector (ESA 5100A, E_1 oxidation and E_2 reduction using a 5011 cell) is used to measure low levels of dopamine, norepinephrine, DOPAC and other species using a pH 4.0 citrate-acetate mobile phase with 4-7% MeOH and 0.32-0.37 mM OS. A gradient HPLC with sixteen coulometric electrochemical detectors set at increasing potentials ranging from 0 mV to 1200 mV (CEAS; ESA Inc.) is used for the measurement of tyrosine, tryptophan, homovanillic acid and up to 20 other neurotransmitter related compounds. Separations performed with the CEAS are carried out with a combination of both phenyl (4.6 mm x 150 mm, 5 μm particles, BDS Hypersil Phenyl; Keystone) and sulfonic acid (4.6 mm x 30 mm, 5 μm particles, 100 SCX; Keystone) columns using a gradient elution with a pH 3.0 phosphate buffer containing 0-17.5% ethanol. The use of the electrochemical array chromatography systems for studies of monkey and human CSF samples will be presented.

473.4

A STABLE LOW MAINTENANCE HPLC FOR THE SIMULTANEOUS ANALYSIS OF CATECHOLAMINES, INDOLES AND THEIR METABOLITES. J.D. Harvey-White, and I.J. Kopin*, Clinical Neuroscience Branch, National Institute of Neurological Disorders and Stroke, 9000 Rockville Pike, Bethesda, MD 20892.

Procedures for obtaining a consistently stabile, sensitive, and low maintenance HPLC with electrochemical detection for the simultaneous analysis of norepinephrine, epinephrine, dopamine, dihydroxyphenylalanine(L-DOPA), dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylalanine (3OMDOPA), 4-hydroxy-3-methoxyphenylethylamine, homovanillic acid(HVA), 5-hydroxyindoleacetic acid(5HIAA), and serotonin(5HT) are described. Conditions to meet different experimental needs for optimal selectivity, and sensitivity have been determined. 1 - Heptanesulfonic acid(HSA) is used as the ion-pairing agent. The retention times(RT) for HVA, 5HIAA, and DOPAC are inversely related to the concentration of HSA (all other compounds are proportionately related). The RT of L-DOPA and 3OMDOPA (relative to other compounds) are greatly increased as pH is decreased. Resistance to complete drying of the mobile phase(MP) facilitates pump maintenance, the MP can be recycled for months, and the columns last 1 to 2 years. Addition of 1% EDTA/0.02% ethanol to dialysates stabilized all compounds of interest: with 0.1M perchloric acid tissue homogenates this addition stabilized DOPAC and 5HT, and increased the stability of 5HIAA.

473.5

COMPARISON OF TWO I-123 LABELED SPECT PROBES, FOR THE DOPAMINE TRANSPORTER IN NONHUMAN PRIMATE BRAIN. M. S. Al-Tikriti*, M. S. Gandelman, B. E. Scanley, Y. Zea-Ponce, R. M. Baldwin, S. S. Zoghbi, M. Laruelle, P. B. Hoffer, D. S. Charney, S. Wang, J. L. Neumeyer, R. B. Innis. Yale University and VA Med Ctr, West Haven, CT 06516 and Research Biochemicals Int., Natick, MA 01760.

A comparative SPECT evaluation of the regional uptake of 2ß-carboisopropoxy-3ß-(4-iodophenyl)tropane (IPCIT) and 2ß-carbomethoxy-3ß-(4-iodophenyl)tropane (ß-CIT) was performed to assess the improved specificity of IPCIT over ß-CIT for the dopamine (DA) transporter. For IPCIT and ß-CIT five single bolus injection studies (n=10) ranging from 7-10 h with 6.9-15 mCi injected dose, were completed in 3 baboons *(Papio anubis,* 10 kg). Peripheral metabolism of the two ligands were similar as demonstrated by their respective clearances, 175±35 l/h (mean ± SD) for IPCIT and 134±39 l/h for ß-CIT.

The SPECT images utilized ROIs over striatum (which reflect DA transporters), midbrain (previously shown for ß-CIT to reflect primarily serotonin transporters), and the cerebellar lobe (a region of nonspecific uptake). The time to peak specific striatal uptake (striatal minus cerebellar activity) was similar for IPCIT and ß-CIT (373±51 and 360±55 min, respectively). At time of peak specific striatal activity, striatal to cerebellar ratios were 2.9±0.3 for IPCIT and 7.5±0.8 for ß-CIT. However, the normalized striatal uptake values (µCi/cc per µCi injected dose per g body mass) for IPCIT (5.14±0.8) were similar to those of ß-CIT (5.8±1.4), whereas for the midbrain the normalized value were (3.8±0.7) and (5.6±1,1) for IPCIT and ß-CIT, respectively. In contrast, the normalized nonspecific uptake measured in cerebellum was higher for IPCIT (1.8±0.4) than for ß-CIT (0.8±0.3). In conclusion, [^{123}I]IPCIT demonstrated a higher DA transporter specificty and higher level of nonspecific uptake.

473.6

CLINICAL APPLICATION OF A KINETIC ANALYSIS OF I-123 IBZM SPECT IN NEUROPSYCHIATRIC PATIENTS. R. Coppola *, M. B. Knable, S. S. Wolf, D. W. Jones, T. M. Hyde, D.R. Weinberger, NIMH Neuroscience Center, NIH, St. Elizabeths, Washington, D.C., 20032

I-123 IBZM, a dopamine D2 receptor antagonist, has been used for in vivo SPECT imaging to obtain measurements of dopamine receptor density. We have developed a simple kinetic analysis for time dependent IBZM uptake as an approximation of synaptic dopamine activity that emphasizes radioligand association and presumably endogenous dopamine release. We tested this approach in normals and in two neurological conditions in which alterations of striatal dopamine activity are thought to contribute to the pathophysiology. In patients with asymmetric clinical signs of Parkinson's disease (PD) (n=13) there was a nonsignificant increase in mean basal ganglia/occipital ratio of IBZM activity at 2 hrs contralateral to maximal clinical signs. Likewise, in MZ twins with Tourrette's Syndrome (TS) discordant for ratings of severity (n=6 twin pairs), there was no difference between twins in bg/occ ratio. The rate of accumulation of IBZM (a parameter proportional to association and dissociation rates, receptor density and concentration of endogenous dopamine) in the basal ganglia over the 4 hr scan time revealed in the PD patients a mean slope that was significantly higher in the contralateral striatum (p=0.02). The more severely affected TS twins had a significantly decreased slope when compared to the less affected twins (p=0.03) and to normal controls (p=0.03). We conclude that the rate of accumulation of striatal IBZM activity reflects synaptic dopamine activity and possibly dopamine release since it is increased in the setting of decreased striatal dopamine concentration (PD) and is decreased in a disease purported to have increased striatal dopamine activity (TS). Furthermore, the rate of accumulation of IBZM activity is a more sensitive indicator of synaptic dopamine activity than single time point measurements of striatal/occipital ratio.

473.7

NIMODIPINE REVERTS THE DEPLETION OF EXTRACELLULAR DOPAMINE DURING ETHANOL WITHDRAWAL. Z.L. Rossetti, S. Carboni, R. Isola and J. DeVry[a], Department of Neuroscience, University of Cagliari, Italy and [a]Tropon AG, Germany.

Withdrawal of chronic ethanol (EtOH) treatment in rats is associated with a profound inhibition of the mesolimbic dopamine (DA) system. DA depletion has been hypothesized to constitute a neurochemical correlate of the dysphoric and depressive state associated with wthdrawal. However the cellular mechanisms underlying this phenomenon are poorly understood. Chronic EtOH treatment is known to be associated with an overactivity of L-type calcium channels. To assess whether L-type calcium channels have a role in the withdrawal-associated inhibition of the DA system, we studied the effect the dihydro-pyridine calcium antagonist nimodipine on the extracellular DA in the ventral striatum of EtOH-withdrawn rats. In EtOH-dependent rats, twelve hrs following the last treatment (5g/kg every 6 h for 6 days), dialysate DA levels were about 30% of control, sucrose-treated rats. Nimodipine (2.5-10 mg/kg s.c.) dose dependently reverted the fall in DA output toward control values. At the dose of 10 mg/kg nimodipine raised DA output to about 160% of control. In contrast, the drug had no effect in control rats. Thus, overactivation of L-type calcium channels can mediate the inhibition of the mesolimbic dopaminergic system during EtOH withdrawal, presumably through inhibitory afferences to dopamine cells. In addition, our results suggest a potential use of nimodipine in the dysphoric and depressive state associated with EtOH abstinence syndrome.

473.8

THE SIGMA LIGAND RIMCAZOLE ACTIVATES CATECHOLAMINERGIC NEURONS IN THE RAT HYPOTHALAMUS. M.J. Eaton*, K.J. Lookingland and K.E. Moore. Dept. Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

The purpose of the present study was to investigate sigma receptor-mediated regulation of catecholaminergic neurons in the hypothalamus by examining the acute effects of rimcazole on concentrations of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in various hypothalamic nuclei containing cell bodies or terminals of dopaminergic (DA) neurons and terminals of noradrenergic neurons. Rimcazole produced dose- and time-related increases in DOPAC concentrations in terminals of tuberoinfundibular DA neurons in the median eminence which were accompanied by decreases in plasma concentrations of prolactin. Within 15 min., rimcazole increased DOPAC concentrations without affecting concentrations of dopamine in periventricular, paraventricular, medial preoptic and dorsomedial nuclei of the hypothalamus; a neurochemical profile reflecting activation of DA neurons within these brain regions. By 30 min., concentrations of both DOPAC and dopamine within periventricular, paraventricular, medial preoptic and dorsomedial nuclei of the hypothalamus were elevated. This neurochemical profile is consistent with activation of noradrenergic neurons within these brain regions. These data suggest that rimcazole activates both DA and noradrenergic neurons within the hypothalamus. The stimulatory effects of rimcazole on tuberoinfundibular DA and noradrenergic neurons in the hypothalamus are similar to those produced by selective DA agonists such as quinelorane, therefore the ability of the D2 receptor antagonist raclopride to block the effects of rimcazole in these brain regions was assessed. Raclopride did not antagonize the effects of rimcazole indicating that its actions on catecholaminergic neurons within the hypothalamus are not mediated by D2 DA receptors. (Supported by NIH grant NS 15911)

473.9

FURTHER STUDIES ON THE RELATIONSHIP BETWEEN DOPAMINE CELL NUMBER AND NEUROLEPTIC RESPONSE. B. Hitzemann, K. Dains and R. Hitzemann*. Dept. of Psychiatry SUNY at Stony Brook, NY 11794 and Research Service, VAMC Northport, NY 11768.

Previous studies have shown that the genetic variability in the sensitivity to haloperidol-induced catalepsy is associated with the number of dopamine neurons in the substantia nigra zona compacta (SNZc) (JPET 266:431, 1993). To further investigate this relationship, we have used three different genetic strategies. Neuroleptic responsive (NR) and neuroleptic non-responsive lines were selected from the new heterogenous stock/Northport (HS/Np). At S_4, the lines differed 6-fold in their sensitivity to haloperidol-induced catalepsy. Confirming previous results, tyrosine hydroxylase (TH) cell number was significantly higher in the NNR line; the difference was most pronounced in the rostral SNZC where TH cell number was increased 23%. Fifty-two C57BL/6:DBA/2 (B6D2) F_2 hybrids were phenotyped for haloperidol response prior to determining TH cell number. Paralleling the results in the selected lines, TH cell number was significantly higher (range 10-28%) in the most non-responsive animals. TH cell number was determined in 10 inbred mouse strains that were previously phenotyped for haloperidol response. Among the inbred strains there was no significant correlation between response and cell number. The reasons for the differences between the first two and third genetic strategy are not clear but may suggest that epistatic interactions are present in the HS and F_2 animals but not the inbreds. These data bring into question the validity of using genetic correlations obtained from inbred strains.

473.10

D_2 DOPAMINE RECEPTOR DENSITY IN THE NEUROLEPTIC RESPONSIVE (NR) AND NEUROLEPTIC NON-RESPONSIVE (NNR) LINES SELECTED FROM THE HETEROGENEOUS STOCK/NORTHPORT (HS/NP). S. Kanes*, L.Cipp, K.Dains, B. Hitzemann and R.Hitzemann. Depts. Psychiatry, and Pharmacology, SUNY at Stony Brook, NY 11794 and Research Service, VAMC, Northport, NY 11768.

We have previously demonstrated (JPET 61:341, 1992 and Psychopharmacol. 103:244, 1991) that NR and NNR lines selected from HS/Ibg stock differ significantly in D_2 receptor density; the NNR line has a significantly higher somatodendritic receptor density throughout the midbrain but a lower receptor density in the lateral striatum. Recently, we confirmed that among 8 inbred mouse strains (C57BL/6, DBA/2, C3H, BALB/c, LP, A, AKR and CBA), the strains least sensitive to haloperidol-induced catalepsy had the highest somatodendritic receptor density (JPET 267: 538, 1993). However, differently than the selected lines, there was a trend to higher receptor density among the non-responsive strains in the lateral striatum. These eight inbred strains were crossed to form a new HS stock. After 8 generations of random breeding, a new selection of NR and NNR lines was begun. At S_4 the lines differed 6 fold in their ED_{50} for haloperidol-induced catalepsy. Receptor binding to the D_2 family of receptors was determined using ^{125}I-epidepride and quantitative receptor autoradiography. The data obtained parallel the results obtained from the inbred strains - receptor density was higher both in the substantia nigra and striatum. Similar results have also been obtained in C57Bl/6:DBA/2 F_2 animals phenotyped for haloperidol response and D_2 receptor density.

473.11

MAPPING THE GENES CONTRIBUTING TO CHOLINERGIC AND DOPAMINERGIC NEURON DENSITY USING THE BXD/TY RI STRAINS. K. Dains,* B. Hitzemann and R. Hitzemann. Departments of Neurobiology and Behavior, and Psychiatry, State University of New York at Stony Brook, Stony Brook, NY 11794 and Psychiatry Service, VAMC, Northport, NY.

Two traits associated with neuroleptic-induced catalepsey are the density of cholinergic cells in the caudate-putamen and the density of dopaminergic cells in the substantia nigra (Hitzemann, et al., 1993). By analyzing the BXD/Ty series of mice we were able to obtain significant quantitative trait loci (QTL) associated with haloperidol-induced catalepsey (Kanes, et. al., 1994) and the above traits. These QTL include the microstatellites D9MIT22, D9MIT4 and D9MIT21 which map near the dopamine D_2 receptor gene (DRD2). F_2 hybrids generated from C57BL/6J and DBA/2J strains were used to confirm the associations between significant QTL by typing for haloperidol sensitivity and cholinergic and dopaminergic cell density, then genotyping for the significant QTL. This analysis has also revealed a significant QTL for cholinergic cell number at microsatellite D12MIT17, which is within proximity of the serotonin transporter gene. These markers are suitable candidates for positional cloning.

473.12

LESIONING THE MEDIAL PREFRONTAL CORTEX DECREASES MIDBRAIN DOPAMINE NEURON ACTIVITY. S.S. Shim*, W.-X. Shi and B.S. Bunney. Depts of Psychiatry & Pharmacology, Yale Univ Sch of Med, New Haven, CT 06510

Lesions in the prefrontal cortex (PFC) have been shown to profoundly affect subcortical dopamine (DA) transmission in animals, and this effect is believed to be mediated by cortical projections to DA terminal areas (see reviews by Grace, *J Neural Transm* **91**:111, 1993 and Deutch, *J Neural Transm* **91**:197, 1993). However, since the PFC is also anatomically connected to DA neurons located in the midbrain, lesions in the PFC may directly affect the electrical activity of DA neurons which should in turn influence DA release in terminal areas. To examine this possibility, we used extracellular single unit recording techniques to record DA neurons from both A10 and A9 in control and PFC lesioned rats. To lesion the medial PFC, we either made a glass knife cut immediately caudal to the mPFC or locally injected ibotenic acid into the mPFC. One hour after mechanical lesioning the number of spontaneously active DA neurons in both the A9 and A10 areas was decreased (A9: from 1.15±0.10 to 0.57±0.07 cells/track, A10: from 1.41±0.09 to 0.77±0.09). Furthermore, the burst activity of A10 DA neurons was also reduced from 26.70±1.80% to 17.50±2.46%. However, unlike the mechanical lesion, local injection of ibotenic acid only decreased the activity of A10 neurons. Thus, four to 20 days after the injection, the number of spontaneously active DA neurons in the A10 area was significantly reduced from 1.50±0.13 to 0.56±0.11 cells/track, whereas that in A9 area were not significantly affected. Surprisingly, the basal activity of DA neurons in the A9 area was increased (control: 3.65±0.28 spikes/sec, lesioned: 4.89±0.21 spikes/sec) in these chemically lesioned rats. These results suggest that lesions in the mPFC can significantly affect DA neuron activity located in the midbrain and this effect may contribute to the change in DA transmission in subcortical areas seen after PFC lesions. Supported by PHS award MH28849, the NPF, the NARSAD, and the State of Connecticut.

473.13

APOMORPHINE INCREASES EXTRACELLULAR GABA LEVELS IN THE PREFRONTAL CORTEX OF THE FREELY MOVING, CONSCIOUS RAT. A.C. Grobin* and A.Y. Deutch. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT 06510.

It has been hypothesized that dopamine (DA) in the prefrontal cortex (PFC) regulates GABA function. DA axons form synapses with GABA interneurons in the PFC. Furthermore, *in vitro* studies have reported that DA agonists will stimulate GABA release in the PFC. To test this hypothesis in the intact animal, the D_2/D_1 agonist apomorphine (APO) was administered to male Sprague-Dawley rats and GABA levels determined in the PFC using *in vivo* microdialysis. APO was delivered directly to the PFC through the dialysis probe, or systemically through an indwelling subcutaneous catheter. GABA levels and relative glycine amounts in dialysates were determined using pre-column derivatization with o-phthaldialdehyde followed by HPLC-EC. Local administration of APO (0.01 and 1.0 μM) produced a large increase in extracellular GABA. Systemic administration (0.5mg/kg) produced a profound increase in extracellular GABA of short duration; no increase in glycine was observed. Thus, both local and systemic administration of a DA receptor agonist increased extracellular GABA levels in the PFC. This report provides the first *in vivo* data to show that DA stimulates GABA release in the PFC. Thus, DA may inhibit pyramidal cell firing through stimulation of inhibitory GABA interneurons, as well as by directly synapsing on pyramidal cells. This arrangement has important implications for our understanding of the functional consequences of the loss of interneurons in the cortex of schizophrenics.

These studies were supported by MH-45124 and GM-07324.

473.14

CHRONIC RESERPINE ADMINISTRATION DECREASES ^{3}H-MAZINDOL BINDING: EVIDENCE FOR RESERPINE-INDUCED NEUROTOXICITY. J. L. Neisewander* and A. N. Sussman. Dept. Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

Chronic administration of reserpine produces spontaneous oral dyskinesia that persists for at least 60 days following termination of treatment. This response is also associated with persistent neurochemical changes, including a 64% depletion of dopamine and a 10-15% increase in D2 receptor density in the caudate-putamen (CPu). The increase in D2 receptor density is region-specific since it is not observed in the nucleus accumbens (NAc). In light of this long-lasting dopamine depletion and receptor up-regulation, we hypothesize that reserpine may be neurotoxic to dopamine neurons in the CPu. To test this hypothesis, we examined whether reserpine-treated animals would exhibit a decrease in dopamine transporter binding sites using ^{3}H-mazindol. Rats were treated every other day with either vehicle (N=6) or reserpine (1.0 mg/kg, SC; N=8) for 21 days. The animals were sacrificed 24 hours after their last injection. Sections of rat brain containing the CPu and NAc were labeled with 15 nM ^{3}H-mazindol in the presence or absence of 1μM GBR-12909 in buffer containing 300 nM desmethylimipramine. Quantitative autoradiographic analysis revealed a significant decrease in ^{3}H-mazindol binding in the CPu of reserpine-treated animals relative to vehicle-treated animals. In contrast, there was no significant difference in ^{3}H-mazindol binding in the NAc. These findings are consistent with our hypothesis that reserpine may be neurotoxic to dopamine neurons in the CPu, and may have implications for tardive dyskinesia and L-DOPA-induced dyskinesia.

473.15

3-METHOXYTYRAMINE-ACCUMULATION FOLLOWING PARGYLINE ADMINISTRATION REFLECTS THE EXTRACELLULAR DOPAMINE CONCENTRATION IN DIALYSATE FROM THE STRIATUM BUT NOT FROM THE SUBSTANTIA NIGRA. H. Nissbrandt*, A. Elverfors, J. Jonason, F. Bergquist Department of Pharmacology, University of Göteborg, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Indirect biochemical methods, as accumulation of the dopamine (DA) metabolite 3-methoxytyramine (3-MT) after monoamine oxidase inhibition has been used as an indirect measurement of DA release. Results obtained with this indirect method are in accordance with results obtained with more direct methods, as microdialysis technique, when DA release is measured in terminal regions, as the striatum, but not when measured in somatodendritic regions, as the substantia nigra (SN). This study was undertaken to further investigate the discrepancy in the SN between the different methods to measure DA release. The DA and 3-MT concentrations were measured by microdialysis in freely moving rats in the SN and in the striatum following pargyline (75 mg/kg) treatment and d-amphetamine (3 mg/kg) plus pargyline treatment. Pargyline treatment only increased the DA concentrations to 160% of basal level in the striatum and to 300% of basal level in the SN. Combined treatment with pargyline and d-amphetamine increased the DA concentrations to 650% of basal level in the striatum and to 1600% of basal level in the SN. In the striatum, the 3-MT concentrations in dialysate increased when the animals were treated with d-amphetamine plus pargyline as compared to when treated with pargyline only. However, in the SN the 3-MT concentrations following pargyline plus d-amphetamine was equal with the concentrations following pargyline only. This suggest that pargyline-induced 3-MT accumulation in the SN does not reflect the extracellular concentrations of DA. A possible explanation for this discrepancy between the striatum and the SN could be that the metabolic enzyme COMT is located in dopaminergic neurons in the SN but not in the striatum.

473.16

PRENATAL HALDOL EXPOSURE REDUCES THE NUMBER OF SPONTANEOUSLY ACTIVE MIDBRAIN DOPAMINE NEURONS IN NEONATAL OFFSPRING. **L. Wang*, J. Zhang and D.K. Pitts. Dept. of Pharmaceutical Sci., Coll. of Pharmacy & A.H.P., Wayne State Univ., Detroit, MI 48202.**

The dopamine (DA) receptor antagonist, haloperidol (HAL, 5 mg/kg) or the vehicle, dimethlyl sulfoxide (DMSO), was administered to pregnant Sprague-Dawley rats by s.c. injection (200 μl/kg). Injections began on gestational day (GD) 8 and ended on GD 20. The control dams (n=2) were pair-fed and appeared to gain weight at the same rate as HAL treated dams (n=2). The average dam weights (g) were as follows: (HAL, DMSO): 234, 268 on GD 8; 296, 330 on GD 20. The body weight (g) of 2-week-old male offspring from HAL-treated dams (26.8 +/-0.4, n=12) were significantly ($P < 0.001$) lower than those from DMSO-treated dams (31.2 +/-0.8, n=8). The two-week-old male offspring were anesthetized with chloral hydrate and the number of spontaneously active midbrain DA neurons were determined in a stereotaxically defined block of tissue using standard extracellular electrophysiological recording techniques. The number of spontaneously active DA neurons per electrode track in HAL-treated offspring was found to be significantly decreased in both the A9 (0.85 +/- 0.09) and A10 (0.65 +/-0.11) regions (treatment effect, $P < 0.001$; cell type by treatment interaction, $P > 0.50$) relative to DMSO controls (A9: 1.27 +/- 0.25; A10: 1.10 +/- 0.13). There was a trend towards A10 DA neurons from HAL-treated offspring having a higher discharge rate than controls ($P < 0.07$; cell type by treatment interaction). Experiments examining non-injected controls, the effects of lower HAL doses and cross-fostering are underway. These preliminary results suggest that DA receptors may influence the development of both A9 and A10 midbrain DA neurons during gestation. Supported in part by MH47857 (DKP).

473.17

THE RESPONSE OF VENTRAL PALLIDAL NEURONS TO DOPAMINE AGONISTS IS NOT ALTERED BY 6-HYDROXY-DOPAMINE-INDUCED LESIONS. B.A. Heidenreich*, I. Mitrovic, F. Rehman & T.C. Napier. Dept. Pharmacol., Stritch Sch. Med., Loyola Univ. Chicago, Maywood, IL 60153.

The ventral pallidum (VP) receives a dopamine (DA) input from the substantia nigra and ventral tegmental area. Iontophoretic application of DA or the D_1 agonist SKF38393 alters the firing rate of many VP neurons (Napier and Maslowski-Cobuzzi, Synapse, in press). Also, systemically administered D_1 agonists increase the activity of many VP neurons (Maslowski and Napier, EJP, 200:103). The present study investigated the effects of chronic DA depletion on the responses of VP neurons to DA and SKF38393. Rats received infusions of 6-hydroxy-DA (6-OHDA, 8 μg/4 μl) into the medial forebrain bundle after treatment with pargyline (50 mg/kg i.p.). Desipramine (30 mg/kg i.p.) was given to protect other monoamine neurons. Seven to ten days later, rats were anesthetized with chloral hydrate and single neuron recording techniques were used to assess changes in sensitivity to microiontophoretic DA and SKF38393, and i.v. SKF38393. As observed previously, increases and decreases in activity were produced by iontophoretic DA and SKF38393. Treatment with 6-OHDA did not alter the E_{max} of DA to increase or decrease firing rate. Similarly, there was no change in the E_{max} of i.v. SKF38393 to increase neuronal activity. Because chronic removal of DA input to many brain regions produces supersensitivity to DA agonists, the present results highlight differences between the effects of DA in the VP and other DA-receptive regions. Supported by USPHS MH45180 (TCN).

473.18

WHY CAN'T HEMIPARKINSON RATS WALK NORMALLY?. I. Q. Whishaw* and E. I. Miklyaeva, Department of Psychology, University of Lethbridge, Lethbridge, AB, Canada, T1K3M4

The normal walking sequence of most tetrapods involves patterns of diagonal support in which one set of diagonal limbs supports weight while weight is unloaded from the others. We have examined walking in hemiparkinson rats (6-OHDA in the nigrostriatal bundle) using high speed video, kinematic, and weight support analysis. They use abnormal gaits or turn ipsilateral in tests of spontaneous locomotion rather than displaying the normal diagonal pattern of walking. An investigation of the underlying basis for this change showed that they are not able to use their "bad" limbs in to shift posture and so contribute to the normal diagonal walking pattern. To compensate for this impairment they alter their movement patterns to place more reliance on their good limbs. Thus, it is suggested that hemiparkinson rats do not walk normally because because they are relying upon two limbs rather than four to shift their weight.

473.19

WHY DO HEMIPARKINSON RATS TURN?. E. I. Miklyaeva* and I. Q. Whishaw, Department of Psychology, University of Lethbridge, Lethbridge, AB, Canada, T1K3M4

Hemiparkinson rats (6-OHDA in the nigrostriatal bundle) turn ipsilateral to their lesion spontaneously and under amphetamine and contralateral after apomorphine. They display sensorimotor abnormalities when eating, rearing, reaching and competing for food. Control rats move from a "diagonal" postural supporting pattern, unloading the limb opposite to the lifted one and loading the other pair of limbs, when performing most behaviors. Hemiparkinson rats are not able to use their "bad" limbs in this way. Consequently they modify their posture and movement to rely on the ipsilateral "good" limbs to actively adjust support. This change biases their behavior such that they display a variety of anomalous movements that include ipsiversive rotation spontaneously and under amphetamine, backward dodging when they protect their food from robbers, atypical sitting to eat food, and atypical standing to reach for food. Apomorphine restores more normal movements. The results show that following DA-depletions the new supporting patterns are related to the many changes in behavior typical of hemiparkinson rats. Thus, it is suggested that they turn because they have to.

473.20

ADENOSINE ANTAGONISTS AND A D2 DOPAMINE AGONIST COOPERATIVELY REGULATE C-FOS INDUCTION IN THE RAT STRIATUM. A.E. Pollack* and J.S. Fink. Molecular Neurobiology Laboratory, Massachusetts General Hospital and Department of Neurology, Harvard Medical School, Boston, MA 02114.

Adenosine (AD) antagonists potentiate dopamine (DA)-mediated behaviors and receptors for these neurotransmitters are abundantly expressed in striatum. The immediate early gene c-Fos is induced in the anterior dorsolateral striatum 3 hours following a single injection of reserpine (RES) (10mg/kg). Pretreatment with the D2 DA agonist quinpirole (QUIN) (0.5mg/kg) blocked c-Fos induction, indicating that this induction of c-Fos is mediated by D2 DA receptors. We tested whether co-administration of AD antagonists with a low dose of QUIN (0.05mg/kg) could block the induction of striatal c-Fos, detected by immunohistochemistry, following acute RES. Pretreatment with QUIN, theophylline (25mg/kg), or DMPX (25mg/kg) slightly attenuated c-Fos induction, while QUIN+theophylline or QUIN+DMPX completely blocked the induction of striatal c-Fos following acute RES. The A2a AD antagonist CSC (5mg/kg) did not block c-Fos induction, while QUIN+CSC attenuated c-Fos induction following RES. These results suggest that D2 DA agonists and AD antagonists cooperatively regulate c-Fos in the striatum. Since A2a AD receptors and D2 DA receptors are co-localized on the same striatal neurons these results support an functional interaction between A2a AD receptors and D2 DA receptors in a subset of striatal neurons.

473.21

PRIOR EXPOSURE TO INTRA-VTA AMPHETAMINE ENHANCES THE INDUCTION OF IMMEDIATE-EARLY GENE EXPRESSION IN THE RAT FOREBRAIN BY SYSTEMIC AMPHETAMINE. P. Vezina* and G.S. Robertson. Department of Psychiatry, University of Chicago, Chicago, IL 60637 and Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

Injections of amphetamine into the ventral tegmental area (VTA) have been shown to enhance the locomotion and the increase in extracellular nucleus accumbens (N.Acc.) dopamine (DA) produced by a subsequent systemic amphetamine challenge. The present experiment investigated whether prior exposure to such injections would also affect the subsequent induction by systemic amphetamine of immediate-early gene activation in the N.Acc., the antero-medial striatum and the medial prefrontal cortex, forebrain projection fields of A10 DA perikarya in the VTA. Rats were administered three injections of either d-amphetamine (2.5 μg/0.5 μl/side) or saline into the VTA, one injection every third day. One week following the last injection, animals were challenged with amphetamine (1.0 mg/kg, i.p.) and, 90 minutes later, brains were prepared for assessment of immediate-early gene induction by immunohistochemistry using antibodies that recognize FOS, FOS-B, JUN-B and NGFI-A. Animals preexposed to VTA amphetamine showed increased (generally two fold) expression of FOS, FOS-B and JUN-B in all A10 projection fields when compared to VTA saline preexposed animals. The two groups did not differ in amphetamine induced expression of NGFI-A in any site. This enhanced induction by amphetamine of immediate-early genes of the *fos/jun* (leucine-zipper) family in VTA amphetamine preexposed animals is consistent with the sensitized DAergic response to this drug reported in these animals in one of the DA terminal fields tested (N.Acc.). While NGFI-A (of the zinc finger immediate-early gene family) is induced by amphetamine, it does not appear to be involved in the differential expression of the behavioral and DAergic responses to amphetamine by these two groups.

473.22

Changes in cochlear tyrosine hydroxylase immunoreactivity after repeated exposure to cocaine. B.G. Shivapuja, S.Y. Liu, D.Z. Pitovski, E.P. Schoener*. Dept. of Otolaryngology, Henry Ford Hospital, Detroit, MI- 48202.

The site of action of cocaine in the auditory system may include structures peripheral to the ventral cochlear nucleus, including the cochlea. Previous studies in our laboratory examined the acute and chronic effects of cocaine on cochlear function. Experiments designed to study acute effects of cocaine on the cochlea demonstrate a significant decrease in both the amplitude of the compound action potential of the auditory nerve (N_1) and cochlear blood flow. On the other hand, experiments designed to study chronic effects of cocaine on the cochlea show an enhancement of the N_1 amplitude of the auditory nerve.

In order to understand the underlying mechanisms involved in chronic cocaine action in the cochlea, this experiment was designed to examine the effects of repeated administration of cocaine on the cochlear catecholaminergic systems in chinchilla using immunohistochemical techniques. Twelve chinchillas were randomly assigned to either control or experimental groups. Animals in control group were administered saline while animals in experimental group were administered 15 mg/kg cocaine IP daily for 42 days. Animals were sacrificed 24 h after the last treatment. The catecholamine system was evaluated using a polyclonal antibody to tyrosine hydroxylase (TH).

Microscopic examination of whole-mounted tissue revealed TH immunoreactive fibers that have been described previously as sympathetic. Changes in fiber density, arborization and varicosity were observed in the cocaine-treated animals. Fiber density was quantified along the organ of Corti from the apical to the basal region by counting the number of fibers below the plane of the inner sulcus cells. Statistical comparison of the total number of nerve fibers per cochlea between the two groups demonstrated a significant decrease in cocaine-treated animals. (Supported in part by NIDA/NIH grant DA07524)

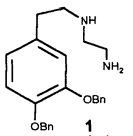
473.23

INHIBITORY EFFECTS OF DOPAMINE ON SINGLE-UNIT ACTIVITY OF DORSOMEDIAL ARCUATE NEURONS ARE POTENTIATED BY COCAINE. J.T. Pan*, S.H. Yen and C.J. Lai. Inst. Physiol., Natl. Yang-Ming Med. Coll., Taiwan R.O.C.

Whether tuberoinfundibular dopaminergic (TIDA) neurons contain dopamine (DA) autoreceptor has been a controversial issue. Earlier study from this lab using single-unit recording of dorsomedial/ventrolateral arcuate (ARC) neurons in rat brain slices found that DA only inhibited 37% of ARC neurons recorded. Recent immunohistochemical studies, however, reveal that DA neurons are localized in the dorsomedial part of the ARC only. By focusing our recording in that region, we found that DA in 50-250 nmol ranges now inhibited a significant number of ARC neurons tested (74.2% of 182 units). Apomorphine, a DA agonist, was less effective (38.1% of 21 units). Cocaine, an abusive drug whose effect is believed to increase DA concentration in synaptic clefts by inhibiting DA reuptake, also inhibited a significant number of ARC neurons by itself (51.5% of 97 units), but with a lesser efficacy. Cocaine co-administered with DA, however, potentiated the inhibitory effect of DA in 82% of DA-responsive units (n=39). The results demonstrate clearly that DA indeed had a predominantly inhibitory effect on presumed DA neurons in the dorsomedial ARC. The effects of cocaine, either by itself or with DA, further support this notion.

473.24

ACUTE AND DELAYED EFFECTS OF THE DOPAMINE DERIVATIVE BBADE ON COCAINE-INDUCED HYPERACTIVITY. H.M. Deutsch* and M.M. Schweri#. School Chemistry and Biochemistry, Georgia Tech, Atlanta GA 30332 and #Mercer U. School of Medicine, Macon GA 31207.



1

The dopamine derivative BBADE (1), a pseudoirreversible inhibitor of [^{3}H]methylphenidate binding (unpublished data), was examined as a potential antagonist against the behavioral effects of cocaine (COC). After overnight acclimatization in separate activity monitor cages, male Sprague-Dawley rats were injected with BBADE·HCl (20 mg/kg, i.v.) or water (controls). Total (T), ambulatory (A), and nonambulatory (N) activities were recorded at 5 min intervals for 30 min thereafter. BBADE alone caused a very slight, but statistically significant increase in all activities over controls at 15-30 min after injection [two-way (time x treatment) ANOVA with repeated measures of the log-transformed activity counts ($p \leq .03$), followed by post hoc t-tests ($p \leq .03$)]. In the acute study, rats were then immediately challenged with COC (40 mg/kg, i.p.) and their activity recorded at 5 min intervals for 60 min. The BBADE treated rats continued to show significantly greater T and N activity in the first 20 min after acute COC injection, but not in the subsequent 40 min; a similar, but nonsignificant, trend was found for A activity. When the COC challenge was delayed for 24 h, opposite results were obtained: T and A activity in BBADE treated rats were decreased by approximately 30% from controls ($p \leq .01$); N activity also decreased, but nonsignificantly. The overnight activity counts of the treated rats were significantly less than controls ($p \leq .001$), suggesting that a nonspecific depressant effect may supplant the initial mild activating effect of BBADE. Supported by NIDA (DA06305).

SEROTONIN RECEPTORS: 5-HT3

474.1

DIFFERENTIAL EXPRESSION REGULATION OF THE TWO SPLICE VARIANTS OF 5-HT$_3$ RECEPTORS BY DIFFERENCIATION IN NG108-15 CELLS. M.B. Emerit*, E. Doucet, M.C. Miquel, M. Riad, M.P. Martres, S. El Mestikawy, and M. Hamon. INSERM U288, CHU Pitié -Salpêtrière, 91 Bd de l'Hopital, 75634 Paris Cedex 13, France.

The 5-HT3 receptor mRNA has recently been shown to be expressed as two forms (5-HT3aL and 5-HT3aS) varying by 18 base pairs in the third intracellular loop domain (Hope et al., Eur. J. Pharmacol. 245:187, 1993). As the short form of the 5-HT3 receptor lacks a casein kinase II phosphorylation site in the region corresponding to the alternative splicing, the question of the functional roles of the two variants has been raised. We have studied the regulation of the expression of mRNAs coding for the two variants in NG108-15 clonal cells under various culture conditions. Relative abundance of the two mRNAs was determined by quantitative PCR over the linear range of the assay by using primers flanking the site of alternative splicing. Quantitation of mRNAs for both forms (L+S) was performed by competitive PCR using as an internal standard a deleted RNA corresponding to a 460 base pair fragment starting at transmembrane domain-2 and ending at TMD-4. Differenciation significantly reduced the abundance of the overall mRNA coding for 5-HT3 receptors in different proportions depending on the inducing agent (dibutyryl cyclic AMP, with or without TPA, theophyllin + PGE1...). In addition, the relative proportions of the two splice variants changed significantly when compared with control cells, since the ratio S/L increased with exposure time to the differenciating agents (Control: 2.75, DBcAMP 1 mM, 1 day: 3.76, 2 days: 4.29, 3 days: 4.43, 5 days: 5.36, 7 days: 6.42). As functional changes have already been described for 5-HT3 receptors upon differenciation in these cells (Shao et al., J. Neurophysiol. 65: 630, 1991), our results suggest different functional roles for 5-HT3aL and 5-HT3aS. We are currently testing this hypothesis by quantifying S/L ratios during development in various central and peripheral tissues.

474.2

DEVELOPMENTAL EXPRESSION OF 5-HT$_3$ RECEPTOR mRNA IN RAT SYMPATHETIC AND SENSORY NEURONS. M. Rosenberg#, P. Séguéla*, and E. Cooper#. Dept. of Physiology#, and the Montreal Neurological Institute*, McGill University, Montreal, Quebec, Canada, H3G 1Y6.

Serotonin, acting on 5-HT$_3$ receptors (5-HT$_3$Rs) on autonomic and sensory neurons, affects a variety of physiological systems, including circulation, respiration, digestion and pain. Yet little is known about the factors that influence the expression of these receptors on peripheral neurons. To learn more, we are investigating the developmental expression of 5-HT$_3$Rs in neonatal rat vagal sensory neurons from nodose ganglia, and in sympathetic neurons from the superior cervical ganglia (SCG). We have quantified the levels of 5-HT$_3$R mRNA expression in these neurons at various stages of development using RNase protection assays. Our results show that, at P_1, the level of 5-HT$_3$R mRNA expression is 10-15 fold higher in nodose ganglia than in SCG. We find no significant change in the level of 5-HT$_3$R mRNA expression in nodose ganglia during the first three weeks of postnatal development. However, in SCG, the level of 5-HT$_3$R mRNA increases 3-5 fold during the first postnatal week and an additional 2-3 fold during the next two weeks of development, such that, by P_{21}, the 5-HT$_3$R mRNA level in nodose is only 2 fold higher than in SCG. These results indicate that: (1) for the vagal sensory neurons, much of the 5-HT$_3$R gene expression occurs before birth; (2) for sympathetic neurons, there is a significant increase in 5-HT$_3$R gene expression during the first three postnatal weeks, suggesting that 5-HT$_3$R expression is controlled by epigenetic influences after birth. (Supported by the Canadian Heart and Stroke Foundation and the Medical Research Council of Canada.)

474.3

DEVELOPMENT AND CHARACTERIZATION OF ANTIBODIES AGAINST THE 5HT$_3$ RECEPTOR. M. Morales*, E. Battenberg, L. de Lecea, P. Sanna and E. E. Bloom. The Scripps Research Institute, Neuropharmacology. 10666 N. Torrey Pines Rd., La Jolla CA 92037.

Based on the predicted amino acid sequence of the cloned rat 5-HT$_3$ receptor cDNA (Johnson and Heinemann. Soc. Neurosci. Abstr. 18: 249), seven different peptides of 15-19 residues that were expected to be antigenic were conjugated to bovine serum albumin and used as immunogens to develop anti-5-HT$_3$ receptor antibodies in rabbits. Three peptides were derived from the amino terminal, extracellular region, and four from the large intracellular loop, between transmembrane regions 3 (M3) and 4 (M4) of the receptor protein. Antibody production was evaluated by solid-phase ELISA and immunodot blot analyses. Peptides corresponding to the extracellular region of the protein gave antibodies of very low titers. Two of the peptides (P_6 and P_7) corresponding to the large intracellular loop produced antiserum of high titer. These antisera when tested on a dot blot analyses were able to detect 0.2 ng of synthetic peptide at dilution of $1x10^3$, and 2 ng at dilution of $1x10^4$.

Membranes from brain and NG 108-15 cells were solubilized with Triton X-100. By western blots, anti-5-HT$_3$-R antibodies recognized a single band (molecular weight close to 35 KDa) in the detergent soluble fractions. Western blot and tissue immunolabeling was abolished when antibodies were preadsorbed with the corresponding peptide. These antibodies labeled cells of different sizes and shapes on rat brain tissue: small round cells in the striatum; medium bipolar and multipolar cells in cortical areas and hippocampus. Immunolabeled neurons were also found in the olfactory bulb, olfactory tubercle and amygdala. The pattern of distribution of the immunolabeled cells is in agreement with that reported for the localization of rat 5-HT$_3$-R transcripts (Johnson and Heinemann. Soc. Neurosci. Abstr. 19: 632). Our data indicate that 5-HT$_3$R immunolabeled neurons are prominent in areas associated with the DA mesolimbic system. (Supported by AA 06420).

474.4

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF NEURONS EXPRESSING THE 5-HT$_3$ RECEPTOR. E. Battenberg*, M. Morales, L. de Lecea, D.S. Johnson and E. E. Bloom. The Scripps Research Institute, Neuro-pharmacology. 10666 N. Torrey Pines Rd., La Jolla CA 92037.

The type 3 serotonin receptor (5-HT$_3$ R) is a ligand-gated ion channel present in peripheral and central neurons and in some neuronally derived cell lines (i.e. NG 108-15 cell line). Valuable information concerning the distribution of this receptor had been obtained with the use of specific radioligands and riboprobes. To better understand the role of 5-HT$_3$R in the CNS, we have begun to characterize the biochemical features of the rat neurons endowed with this receptor.

By immunocytochemical and *in situ* hybridization techniques, we found that some hippocampal neurons express both the 5-HT$_3$R and GABA. Brain sections hybridized with a radioactive antisense RNA probe for detection of 5-HT$_3$R transcripts and immunolabeled with antibodies against GABA, revealed double labeled neurons in several strata of the hippocampus, s. oriens, s. pyramidale, s. lacunosum moleculare and s. lucidum. The coexistence of 5-HT$_3$R mRNA and GABA immunoreactivity in hippocampal interneurons supports electrophysiological data indicating that 5-HT$_3$R-mediated depolarization occurs in non-pyramidal GABAergic neurons (Ropert & Guy. J. Physiol. 441:21, 1991). Additionally, brain sections hybridized with the same RNA probe and immunolabeled with antibodies against somatostatin (SS) demonstrated that the hippocampal neurons containing the 5-HT$_3$R and GABA are a distinct subset of cells that do not belong to the previously described subclass of GABA/SS cells in the hippocampus.

Some neocortical neurons expressing the 5-HT$_3$R also contained GABA. Furthermore, 5-HT$_3$R hybridizing neurons appear to receive afferent fibers immunoreactive for tyrosine hydroxylase. These data suggest that a subset of cortical neurons containing the 5-HT$_3$R might be regulated by both catecholamines and serotonin. (Supported by AA 06420).

474.5

FURTHER CHARACTERIZATION OF 5-HT_3-LIKE RECEPTORS IN THE RAT MEDIAL PREFRONTAL CORTEX. J.Y. Zhang*, E. Edwards and R.Y. Wang. Dept. Psychiatry and Behav. Sci. (J.Y.Z. and R.Y.W.), SUNY at Stony Brook, Stony Brook, NY 11794-8790 and Dept. Pharmacol. (E.E.), Univ. Maryland at Baltimore, Baltimore, MD 21201-1180.

In contrast to studies indicating that applications of 5-HT_3 receptor agonists produce a fast depolarizing action, which is rapidly desensitized, on peripheral or cultured cells, we have found that iontophoresis of 2-methyl-5-HT elicits a slow depressant action with no desensitization on cells in the medial prefrontal cortex (mPFC). In addition, 5-HT_3-like receptors in the mPFC might be coupled to the phosphoinositide (PI) hydrolysis. To further characterize the pharmacological properties of 5-HT_3-like receptors in the mPFC, using the technique of single cell recording and microiontophoresis, we have compared the depressant action of various 5-HT_3 receptor agonists on the firing of mPFC cells. The rank order of effectiveness was: 5-HT > SR 57227A = 2-methyl-5-HT = mCPBG (1-(m-chlorophenyl)-biguanide) = PBG = pCPBG = oCPBG. Furthermore, in the mPFC, d-tubocurarine not only blocked the depressant action of 5-HT_3 receptor agonists but also antagonized that of dopamine and GABA. In fact, d-tubocurarine alone often accelerated the firing of mPFC cells. In another research paradigm, the effect of various 5-HT_3 receptor agonists on PI turnover were investigated and compared using the tissue of the mPFC. The rank order of potency was: 5-HT > SR 57227A = 2-methyl-5-HT > mCPBG = PBG = pCPBG = oCPBG. In summary, the following two new findings further indicate that 5-HT_3-like receptors in the mPFC are pharmacologically different from those in the periphery: 1) in the mPFC, mCPBG is not the most potent 5-HT_3 receptor agonist, and 2) d-tubocurarine is not a specific antagonist for 5-HT_3-like receptors.

474.6

THE PIGLET: AN ANIMAL MODEL FOR STUDYING CISPLATIN-INDUCED DELAYED EMESIS. S. Milano*, L. Grélot, P. Blower[1] & D. Romain[2], URA CNRS 1832, St Jérôme, 13397 Marseille Cedex 20, France; SmithKline Beecham Pharmaceuticals, UK (1) and France (2).

The pathogenesis of cisplatin-induced delayed emesis being obscure, we investigated the suitability of the piglet as a model for studying delayed emesis. We implanted surgically in 13 weaned piglets a cannula in the jugular vein and electrodes for recording ECG and abdominal EMG. After a 4-5 days recovery, piglets were hydrated (iv, saline+furosemide, 100ml/hr for 10hr) and then given cisplatin (5.5mg/kg, iv). Recordings were made continuously for the following 60 hr. Animals were not fed but received continuous iv infusions of glucose and polyionic solutions. All piglets responded with both acute and delayed emesis. In the acute phase of emesis, the first vomiting occurred with a latency of 131±43 min after cisplatin administration. Emetic intensity reached a peak (5 vomits/hr) within 120 min and then decreased rapidly, so that no vomiting were observed between the 15th and 16th hr. The mean number of vomits during the first 16 hr was 18.6±6. Delayed emesis started at the 17th hr and was observed to last until the 58th hr. Emetic intensity of the delayed phase reached a peak (0.8 vomit/hr) between the 19th and the 21th hr and the mean number of vomits during the whole of the delayed phase was 9.2±8.6. Cisplatin inducing delayed vomiting in the piglet without any lethality and with a weak toxicity, this animal is a suitable model in which to study the pathogenesis of delayed emesis.

474.7

EFFECT OF GRANISETRON ON ACUTE AND DELAYED CISPLATIN-INDUCED EMESIS IN THE PIGLET. L. Grélot*, S. Milano, P. Blower[1] & D. Romain[2], URA CNRS 1832, St Jérôme, 13397 Marseille Cedex 20, France; SmithKline Beecham Pharmaceuticals, UK (1) and France (2).

Emesis in cancer chemotherapy are classified as acute or delayed when occuring during or after the 24 hr following cytotoxic treatment, respectively. It remains unclear whether serotonin 5-HT3 receptors are involved in delayed emesis. In 19 weaned piglets, granisetron was tested on both acute and delayed emesis. Vomiting was induced by an iv infusion of cisplatin (5.5mg/kg) and animals were observed for 60 hr. In group 1, 12 piglets received an initial (15 min prior to cisplatin) single dose (0.25mg/kg, n=2; 0.5mg/kg, n=3; 2mg/kg, n=3 or 7mg/kg, n=4) of granisetron. In group 2, 7 piglets received in addition to the initial dose (0.25mg/kg, n=2; 1mg/kg, n=5), a supplementary injection (same dose as first one) each 5 hr during 30 hr. In group 1, single doses of granisetron prevented vomiting during the first 5-18 hr in a dose dependent manner. All animals exhibited a delayed phase of vomiting, the number (13.5±8.8) of which was higher to those of animals not treated with ganisetron (9.2±8.6). In group 2, repetitive administration of granisetron was beneficial in reducing both the acute and delayed phases of vomiting. Indeed, 6/7 piglets (0.25mg/kg, n=1; 1mg/kg, n=5) during the acute phase and 4/7 (1mg/kg, n=4) during the delayed phase did not vomit. These results suggest that serotonin 5-HT3 receptors might be involved in the development of delayed emesis in this species.

474.8

5HT_3 RECEPTOR MODULATION OF FROG SPINAL CORD REFLEXES. A.M. Holohean*, J.C. Hackman and R.A. Davidoff. Neurophysiology Laboratory, Veterans Affairs Medical Center and Dept. Neurology, Univ. Miami School of Medicine, Miami, FL 33101.

Functional 5HT_3 receptors are present in spinal gray matter and are presumably involved in modulating nociception. Recently, the 5HT_3 agonist, 2-Me-5HT (2-methyl-serotonin maleate), has been shown to activate ionotropic channels in dorsal root ganglion cells. To understand the role of 5HT_3 receptors in spinal cord function, sucrose gap recordings from isolated, hemisected *Rana pipiens* spinal cords superfused in HCO_3^- Ringer's were used. Dorsal (DRPs) and ventral root (VRPs) potentials were evoked by stimulating an adjacent dorsal root.

Application of 2-Me-5HT (100 μM) did not affect either motoneuron or primary afferent terminal resting membrane potential, but the amount of spontaneous motoneuron and afferent terminal activity was substantially decreased. No significant effect on the height of evoked VRPs was produced by prolonged exposure (20-30 min) to 2-Me-5HT. However, a substantial decrease in the area of VRPs (66 + 4%) and DRPs (71 + 7% of control) was noted. We have previously shown that this area is sensitive to NMDA antagonists, and believe that activation of interneuronal excitatory amino acid receptors is responsible for the width of root potentials. In this regard, 2-Me-5HT decreased the depolarization (VR, 81 + 7%; DR, 69 + 4%) evoked by applications of NMDA (100 μM, 10 sec), but not AMPA (30 μM), KA (30 μM), or K^+ (10 mM). This decrease was prevented by exposing the cord to TTX but was not affected by naloxone.

These results argue for a role of 5HT_3 in interneuronal firing patterns in the frog spinal cord. Supported by VAMC MRIS #1769 and 3369 and USPHS #NS17577).

SEROTONIN RECEPTORS: EFFECTOR MECHANISMS

475.1

AGONIST POTENCY AND AFFINITY AT 5-HT_{1A} RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY (CHO) CELLS. J.G. Hensler*, K.D. Burris, L.S. Cervera, H.A. Miller and P.B. Molinoff. Dept. of Pharmacololgy, Univ. of Texas Health Sci. Center, San Antonio, TX. 78284; Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine,Philadelphia, PA. 19104.

The relationship between agonist potency and affinity was investigated in CHO cells stably expressing the 5-HT_{1A} receptor (CHO-5HT_{1A} cells). In membranes from CHO-5HT_{1A} cells, the specific binding of the radiolabeled 5-HT_{1A} receptor agonist ^{3}H-8-OH-DPAT (1-1.2 nM) was inhibited essentially completely (97±1.5%) by GppNHp (EC_{50} = 2.8 ± 0.3 μM). Saturation experiments using membranes from CHO-5HT_{1A} cells indicated that the binding of ^{3}H-8-OH-DPAT (0.05 - 50 nM), in the absence of guanine nucleotides, was to a single site (Kd = 1.1 nM, Bmax = 1 pmol/mg protein). Taken together, these data suggest that in CHO-5HT_{1A} cells all of the 5-HT_{1A} receptors are coupled to G proteins and exist in a high affinity state as determined by radiolabeled agonist binding. To determine whether agonist potency can be related to the high affinity state of the receptor, EC_{50} values of agonists to inhibit forskolin-stimulated ^{3}H-cAMP accumulation in CHO-5HT_{1A} cells were compared with their Ki values for the inhibition of ^{3}H-DPAT binding. The ratio of Ki values to the EC_{50} values were near unity: 8-OH-DPAT, 1.4/0.9 nM; 5-HT, 2/2.4 nM; gepirone, 55/37 nM; ipsapirone, 26/32 nM. Thus in CHO-5HT_{1A} cells, the potency of agonists appears to be related to their affinity at the high affinity state of the receptor. Supported by USPHS grants MH48125 and MH52369.

474.2

INTERACTION BETWEEN HUMAN 5-HT_{1A} AND 5-HT_{1C} RECEPTOR IN VITRO. J. Akiyoshi*, I. Fujii. Dept. of Neuropsychiatry, Oita Medical University, Hasama-Machi, Oita 879-55, Japan.

The 5-HT_{1A} and 5-HT_{1C} receptors were important role for depression, anxiety and schizophrenia. In vivo there was some reports of interaction between 5-HT_{1A} and 5-HT_{1C} receptor. 5-HT_{1C} receptor selective agonists induced penile erection. In contrast, selective agonists of 5-HT_{1A} receptor such as 8-OH-DPAT produced an opposite effect on penile erection. But, in vivo there is no adequate cells for studying the interaction between 5-HT_{1A} and 5-HT_{1C} receptor.

Recently, we found the cell line which had both of human 5-HT_{1A} and 5-HT_{1C} receptor. Total RNA was isolated from these cells and cDNA was synthesized. We made a primers for human 5-HT_{1A} and 5-HT_{1C} receptor. PCR amplification allowed the identification of these receptors.

We studied the interaction between human 5-HT_{1A} and 5-HT_{1C} receptor, using the Ca^{++} sensitive dye fura-2/AM. The fluorescence of fura-2/AM was measured in a fluorescence spectrophotometer, with excitation at 340 and 360 nm. Pretreatment of 5-HT_{1A} receptor agonist 8-OH-DPAT decreased Ca^{++} mobilization induced by DOI (5-HT_{1C} receptor agonist) in this cells.

These results show that there is interaction between human 5-HT_{1A} and 5-HT_{1C} receptor in vitro.

475.3

5-HT_{1D} AND 5-HT_{1A} RECEPTORS ARE RESPONSIBLE FOR THE MITOGENIC EFFECT OF SEROTONIN IN A HUMAN TUMOR OF NEUROENDOCRINE ORIGIN

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Small cell lung carcinoma (SCLC), an aggressive human tumor often associated with tobacco smoking, possesses neuroendocrine features. These include the presence of neurosecretory granules containing hormones and neuropeptides and the expression of neuronal nicotinic receptors and voltage-dependent Ca^{2+} channels. We have found that serotonin (5-HT) is contained, is released and is mitogenic in the two SCLC cell lines GLC8 and NCI-N-592. The mitogenic effect of 5-HT is not counteracted by ketanserin, ICS 205-930 and GR 113-808 which are antagonists of the 5-HT_2, 5-HT_3 and 5-HT_4 receptors, respectively. We found that the 5-HT_{1D} agonist sumatriptan and the 5-HT_{1A} agonist 8-OH-DPAT are capable of increasing DNA synthesis in the SCLC cell lines, with lower efficacy but higher potency than 5-HT (EC_{50} = 25 nM and 90 nM respectively). Their effects on DNA synthesis were completely additive and togheter they reached the maximal effect elicited by 5-HT alone. Spiperone (1 μM) and the 5-HT_{1A} antagonist SDZ 216-525 (1 μM) inhibited the 5-HT mitogenic response by about 40%.
When cells were cultivated at higher density, the two 5-HT antagonists were able to inhibit basal cell proliferation.
Taken togheter, our results suggest that 5-HT should be added to the list of autocrine growth factors for SCLC cells. The mitogenic effect of 5-HT is due to stimulation of both 5-HT_{1D} and 5-HT_{1A} receptor types. The design of specific antagonists might be useful for the growth control of human small cell lung carcinoma.

475.4

CONSTITUTIVELY ACTIVE SEROTONIN 2C (5HT_{2C}) RECEPTORS STIMULATE DNA REPLICATION IN A TRANSFECTED CELL LINE.

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Agonist activation of the serotonin 2C (5HT_{2C}) receptor expressed in NIH 3T3 fibroblasts has been shown to result in the development of a transformed phenotype. In light of recent evidence demonstrating 5HT_{2C} receptor constitutive activity, the contribution of agonist independent receptor activation to transformation of NIH 3T3 fibroblasts transfected with 5HT_{2C} receptor cDNA was examined. [^{3}H]Thymidine incorporation was used as a measure of cell growth in serum starved NIH 3T3 fibroblasts transfected with 5HT_{2C} receptor cDNA. Three classes of 5HT_{2C} receptor ligands were distinguished. In transfected, but not nontransfected, fibroblasts basal levels of [^{3}H]thymidine incorporation were increased by agonists, decreased by inverse agonists and minimally affected by neutral antagonists. The effects of agonists and inverse agonists were blocked by a neutral antagonist. Pharmacological characterization of agonist-mediated increases in [^{3}H]thymidine incorporation was consistent with properties of the 5HT_{2C} receptor. In addition, the rank order of potency of inverse agonists to decrease basal [^{3}H]thymidine incorporation was consistent with the rank order to decrease basal 5HT_{2C} receptor-mediated phosphoinositide hydrolysis. Thus, in NIH 3T3 fibroblasts transfected with 5HT_{2C} receptor cDNA, the basal level of [^{3}H]thymidine incorporation is reciprocally modulated by 5HT_{2C} receptor agonists and inverse agonists. These data suggest that constitutively active 5HT_{2C} receptors stimulate cell division and may contribute to the development of a transformed phenotype.(Supported by the National Institute of Mental Health, MH 34007).

475.5

5-HT_{2C} RECEPTOR ACTIVATION INCREASES ARACHIDONIC ACID RELEASE.

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The 5-HT_{2C} (formerly 5-HT_{1C}) receptor is a member of the 5-HT_2 receptor family which have been shown to couple to phospholipase C (PLC)-mediated phosphatidyl inositol (PI) lipid hydrolysis. Recently it has been shown that some PLC-coupled receptors can also activate phospholipase A_2 (PLA_2) to liberate arachidonic acid (AA). We have investigated the coupling of 5-HT_{2C} receptors to the PLA_2-AA pathway. CHO cells were transfected with human 5-HT_{2C} cDNA and a line stably expressing 200 fmol/mg protein was selected for study. Cells were incubated for 4 h at 37° C with 0.1 μCi/ml of [^{14}C]-AA. After washing, 5-HT_{2C} agonist-induced release of [^{14}C]-AA was measured at various time points (5 - 30 min). 5-HT (EC_{50} = 28 nM), DOI (EC_{50} = 100 nM) and quipazine (EC_{50} = 1.2 μM) each increased AA release. DOI and quipazine were partial agonists with intrinsic activities of 0.43 and 0.32, respectively. The EC_{50} values for each of these agonists for PI hydrolysis were similar to those for AA release (28 nM, 133 nM and 1.5 μM for 5-HT, DOI and quipazine, respectively) however, the intrinsic activities for DOI and quipazine were reversed (0.6 and 0.8 for DOI and quipazine, respectively). 5-HT-mediated AA release desensitized by 40% after 15 min treatment with 10 μM 5-HT, whereas ATP-mediated AA release was unaltered. 5-HT-mediated AA release was insensitive to treatment with pertussis toxin. Both 5-HT- and melittin-induced AA release was reduced (50%) in the absence of extracellular Ca^{2+}. Inhibitors of PLC (U-73122 and ET-18-OCH_3), at concentrations that reduced PI hydrolysis by greater than 90%, had little or no effect on 5-HT_{2C} agonist-mediated AA release. However the PLA_2 inhibitor mepacrine completely blocked 5-HT_{2C}-mediated AA release but did not affect 5-HT-mediated PI hydrolysis. These results suggest that 5-HT_{2C} receptors couple to the PLA_2-AA pathway in CHO cells. (Supported by UPHS grants GM 34825, DA06620, MH48125, HD26437).

475.6

5-HT_{2C} RECEPTORS INHIBIT 5-$HT_{1B\text{-like}}$ RECEPTOR FUNCTION VIA ARACHIDONIC ACID METABOLISM.

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5-$HT_{1B\text{-like}}$ receptor-mediated inhibition of forskolin-stimulated cAMP accumulation (FScA) is inhibited by activation of transfected human 5-HT_{2C}, but not 5-HT_{2A}, receptors in CHO cells (Berg *et al.*, Mol. Pharmacol., in press). We have investigated the role of phospholipase A_2 (PLA_2)-mediated arachidonic acid (AA) metabolism as a mediator of the effect of 5-HT_{2C} receptor activation.

In CHO cells stably expressing 5-HT_{2C} receptors (200 fmol/mg protein), 5-HT_{2C} agonists (such as DOI) increase AA release via a PLA_2-dependent mechanism (see Clarke *et al.*, this meeting). As reported previously, activation of 5-HT_{2C} receptors with DOI (1 μM) abolished 5-carboxamidotryptamine (5-CT, 5 nM)-induced inhibition of FScA. This effect of DOI was blocked following incubation with the PLA_2 inhibitor mepacrine (100 μM). The inhibition of FScA (mean % ± SE, n=5) was as follows: 55 ± 5 for 5-CT alone vs. 9 ± 7 for 5-CT + DOI; in the presence of mepacrine, 59 ± 8 for 5-CT alone vs. 61 ± 8 for 5-CT + DOI. Furthermore, the cyclooxygenase inhibitor indomethacin (2 μM) also blocked the DOI-mediated reduction of 5-CT inhibition of FScA (73% ± 5% vs 84% ± 4% for 5-CT and 5-CT + DOI, respectively, n=5). Activation of PLA_2 with melittin (2.5 μg/ml) as well as activation of naturally expressed ATP receptors abolished 5-CT-mediated inhibition of FScA and mepacrine blocked these effects. These data suggest that PLA_2-AA release mediates the 5-HT_{2C} receptor regulation of the 5-$HT_{1B\text{-like}}$ receptor pathway in CHO cells. (Supported by UPHS grants GM 34825, DA06620, MH48125, HD26437).

475.7

2-AMINO-5-PHOSPHONOVALERATE (APV) POTENTIATES SEROTONIN-2 RECEPTOR-MEDIATED SIGNALLING IN C6BU-1 CELL AND ATT-20 CELL

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We investigated the effects of various N-methyl-D-aspartate (NMDA) receptor antagonists on serotonin-2 (5-HT2) receptor-mediated Ca^{2+} mobilization and inositol 1,4,5-trisphosphate (IP_3) formation. In C6BU-1 rat glioma cell, D(-)-2-amino-5-phosphonovalerate (D-APV) potentiated 5-HT-induced Ca^{2+} mobilization and IP_3 formation, while other NMDA antagonists such as MK-801, phencyclidine and HA-966 did not. D-APV enhanced the fluoride-activated Ca^{2+} mobilization. 5-HT2 receptor-mediated Ca^{2+} mobilization and D-APV-induced enhancement were not affected by the pretreatment with pertussis toxin. D-APV-induced potentiation of 5-HT2 receptor-mediated signalling was also observed in AtT-20 mice pituitary tumor cell. These results suggest that enhanced GTP binding protein (G protein) function is one of the mechanisms responsible for the enhancement of 5-HT response induced by D-APV. In addition, both in C6BU-1 cell and AtT-20 cell thrombin-induced Ca^{2+} mobilization was also potentiated by D-APV suggesting that D-APV may activate G protein coupled to phosphoinositide metabolism.

475.8

INTERACTION BETWEEN INTERLEUKIN-1β AND 5-HT-2 RECEPTOR-MEDIATED SIGNALING SYSTEMS.

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Cytokines play an important role in the central nervous system as well as in the immune systems. In this study, we have studied the effect of several cytokines on serotonin (5-HT) -mediated signaling systems in C6 rat glioma cells. Interleukin-1β (IL-1β) inhibited 5-HT-induced Ca^{2+} mobilization (which is mediated by 5-HT-2 receptor) in a dose and time dependent manner after the cells were treated with it. However, heat-inactivated IL-1β or IFNγ did not alter the response to 5-HT. 5-HT induced cGMP generation following Ca^{2+} mobilization and this generation was inhibited by ketanserin and L-NMMA (nitric oxide synthase inhibitor), suggesting 5-HT-2 receptor and nitric oxide synthase (NOS) are responsible for the cGMP generation. IL-1β, which enhanced cGMP production mediated by NOS, attenuated 5-HT-induced cGMP response. These results suggest an interaction between IL-1β and 5-HT-2 receptor-mediated signaling systems.

475.9

PROTEIN KINASE C (PKC) INHIBITORS ENHANCE 5-HT ACTIVATION OF INTERNEURONS IN RAT PIRIFORM CORTEX MEDIATED BY THE 5-HT-2A RECEPTOR.

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The excitation by 5-HT of interneurons in rat piriform cortex is known to be mediated via stimulation of 5-HT_{2A} receptors. Since activation of the phosphoinositide (PI) second messenger by 5-HTacting at 5-HT_{2A} receptors leads to activation of protein kinase C (PKC), we explored the role of PKC in the 5-HT_{2A} signal transduction mechanism by testing structurally distinct PKC inhibitors. Extracellular studies were performed on interneurons excited by 5-HT near the border of layers II and III in piriform cortex slices. A specific PKC inhibitor, the benzophenanthridine alkaloid chelerythrine (25-50 μM), increased the excitation of interneurons by 5-HT (10-100 μM). The isoquinolinesulfonamide kinase inhibitors H7, H8 and HA 1004 (100 μM) were also tested. H7 application robustly enhanced the excitation of interneurons by 5-HT. In contrast, H8 application only weakly enhanced the excitation of interneurons by 5-HT (10-100μM) and HA 1004 had no effect. None of these PKC inhibitors altered the excitation of interneurons by NE (100 μM). The rank order potency of the isoquinolinesulfonamides is in agreement with the rank order potency of these compounds for inhibition of PKC, but not for PKA, PKG, or MLC kinase. Based on these results we propose that: 1) The activation of PKC by 5-HT rapidly desensitizes the 5-HT_{2A}-mediated excitation; 2) PKC inhibitors prevent this rapid desensitization, unmasking the full 5-HT_{2A} receptor response. Thus, activation of PKC, rather than mediating the excitatory action of 5-HT at the 5-HT_{2A} receptor, appears to have a negative feedback role.

475.10

INTERACTION OF PROTEIN KINASE C (PKC) AND PROTEIN KINASE A (PKA) IN THE MEDIATION OF 5-HT_{1P}-INDUCED SLOW DEPOLARIZATION OF TYPE 2/AH NEURONS OF THE GUINEA PIG MYENTERIC PLEXUS. H. Pan and M D. Gershon*, Columbia Univ. P&S New York, NY 10032

5-HT evokes a slow depolarization of myenteric type 2/AH neurons by decreasing a Ca^{2+}-activated K^+ conductance (gK_{Ca}). This effect is seen in the presence of 5-HT_{1A}, 5-HT_3, and 5-HT_4 antagonists and is mediatd by a novel an operationally-defined receptor (agonist = 5-hydroxyindalpine [5-OHIP]; antagonist = a dipeptide, 5-HTP-DP), called 5-HT_{1P}. Since 5-HT and 5-OHIP specifically cause immunoprecipitated $G_{o\alpha}$ to become labeled by $GTP\gamma^{35}S$ and this labeling is blocked by 5-HTP-DP, it has been proposed that 5-HT_{1P} receptors are G_o-coupled. Experiments were done to test this hypothesis and to identify further steps in the signal transduction pathway. Responses to 5-HT were potentiated and prolonged by intracellular injection of GTPγS and inhibited by GDPβS. Pertussis toxin (1 μg/ml, 1.5 hrs) blocked responses to 5-HT. Responses to 5-HT were also antagonized by an inhibitor of phospholipase C (D609; 0.1mM), inhibitors of PKC (chelerythrine, 3-6 μM; a pseudosubstrate PKC inhibitor [$PKCI_{(19-31)}$; injected intracellularly], and down-regulation of PKC (by incubation with phorbol dibutyate [PDBU; 10 μM] for > 5 hrs). Activators of PKC (PDBU, (-)-7-octylindolactam V, oleoyl-acetyl-glycerol) mimicked responses to 5-HT. Responses to 5-HT, however, can also be mimicked by activators of PKA and antagonized by the PKA inhibitor, Rp-cAMPS (injected intracellularly) and by 2',5'-dideoxyadenosine (0.4-0.6 mM), an inhibitor of adenyl cyclase. Okadaic acid (2 μM), an inhibitor of protein phosphatases 1 and 2A, potentiated and prolonged the response to 5-HT. We propose that PKC and PKA are both activated by 5-HT and mediate the decrease in gK_{Ca}. We suggest the following pathways: 5-HT → 5-HT_{1P} → $G_{o\alpha}$ → ↑PLC → ↑1,2 diacylglycerol → ↑PKC → ↑adenyl cyclase → ↑cAMP → ↑PKA → ↓gK_{Ca}. PKC may also directly ↓gK_{Ca}. (Supported by NIH grant NS12969).

475.11

5-HT_4 RECEPTORS MEDIATE EXCITATORY RESPONSES IN HIPPOCAMPUS THROUGH cAMP AND THE ACTIVATION OF PROTEIN KINASE A. G. Torres*, I. L. Holt and R. Andrade. Pharmacological and Physiological Sciences, St. Louis Univ. School of Medicine, St. Louis MO 63104.

The aim of the present study was to examine the effect of serotonin (5-HT) on the calcium-activated afterhyperpolarization (AHP) in hippocampal neurons. Intracellular recordings from the CA1 region of hippocampal slices show that administration of serotonin in presence of the 5-HT_{1A} antagonist BMY 7378 produces a slow depolarization and a reduction in the AHP amplitude leading to an increase in cell excitability.

We have previously identified tentatively the serotonin receptor involved in the reduction of the AHP as belonging to the 5-HT_4 subtype. This identification was confirmed in the present study by using the selective 5-HT_4 receptor antagonists DAU 6285, SDZ 205-557 and GR 113808. All these compounds were found to act as competitive antagonists, with GR 113808 showing the highest affinity.

We also examined the signaling mechanisms involved in the 5-HT_4 receptor mediated reduction in the AHP. This effect of serotonin was mimicked by 8-Br cAMP and blocked by the protein kinase inhibitors staurosporin and Rp-cAMPS. However, it was not enhanced by IBMX at concentrations that could clearly enhance β-adrenergic responses in the same cells. The reason for this paradox is currently under investigation. In conclusion, the results of these study show that the excitatory response associated with serotonin in the hippocampus is mediated by cAMP/PKA and involves the 5-HT_4 receptor subtype. Supported by MH 43985 and the Alfred P. Sloan Foundation.

SEROTONIN RECEPTORS: MOLECULAR BIOLOGY

476.1

SITE-DIRECTED MUTAGENESIS OF THE 5-HT_{2C} RECEPTOR: IDENTIFICATION OF RESIDUES INVOLVED IN LIGAND BINDING AND RECEPTOR ACTIVATION. N.J. Stam*, P. Vanderheyden, J. Kelder, Th. de Boer, C. van Alebeek and D. Leysen, Scientific Development Group. N.V. Organon. P.O. Box 20, 5340 BH Oss, The Netherlands.

Throughout the central and peripheral nervous system multiple 5-HT receptor subtypes are expressed that belong to the superfamily of G protein-coupled receptors. The molecular processes by which agonist and antagonists bind to 5-HT receptors are currently unknown.

In order to investigate the molecular basis for ligand binding to the 5-HT_{2C} receptor we have constructed a three-dimensional model for this receptor using the atomic coordinates of the structurally related bacteriorhodopsin as a template. Based on our model and data from other studies we have chosen six residues, proposed to be involved in 5-HT binding, for site-directed mutagenesis studies. First, we investigated the role of two aromatic residues (Phe328 and Phe329) in transmembrane domain VI, that are conserved among all G protein-coupled 5-HT receptors. To investigate the role of these aromatic residues in ligand binding and receptor activation we have substituted these residues for alanines.

Additional site-directed mutations were performed for two serine residues in transmembrane domain IV and one serine in transmembrane domain V. Both serine residues in transmembrane domain IV are conserved among all members of the 5-HT receptor family, with the exception of the 5-HT_{1A} receptor in which Ser 187 is replaced by a Gly, and in the recently cloned 5-HT_{2B} receptor in which this residue is replaced by an Ala. The Ser in transmembrane domain V is present in all members of the 5-HT_2-like (5-HT_{2A}, -2B, -2C) receptors but not in 5-HT_1-like receptors. In our model, these three Serine residues are in a position that they may interact with 5-HT. Finally, we investigated the role of an aspartic acid in transmembrane domain III, which is conserved only in cationic amine receptors and which is postulated to interact with the positively charched amine group of 5-HT. Mutant and wild-type receptors have stably been transfected into NIH3T3 cells and are currently pharmacologically characterized. Initial experiments indicate that some of the mutations affect differentially agonist binding as compared to antagonist binding.

476.2

Mutagenesis studies of the human 5HT_{2A} and 5-HT_{2C} receptors. N. Almaula, L. Chi, B. Ebersole, S. Maayani, J.A. Ballesteros, H. Weinstein, and S.C. Sealfon*. Mount Sinai School of Medicine, New York, N.Y 10029.

The 5-HT_2 receptor subtypes are thought to mediate the action of hallucinogenic drugs of abuse. Mutagenesis studies of human 5-HT_2 subtype receptors have been initiated in an attempt to clarify the receptor structures and the mechanism of ligand binding and activation by partial and full agonists. The targets for study have been selected based on differences between the two subtypes, interactions of transmembrane helices (TMHs) proposed in other receptors, molecular modeling, and published mutations. 9 mutations have been studied in the 5-HT_{2A} receptor at postions 120, 134, 242, 372, and 376. Corresponding mutations at 5-HT_{2C} positions 112 and 222 have also been generated. Mutation of 5-HT_{2A} S242-A causes an increase in mesulergine affinity, as reported (Kao et al. FEBS 307:324,1992) and a decrease in the affinity for the hallucinogens LSD and psilocin. Mutation or exchange of residues at two conserved loci, TMH2:D120 and TMH7:N376, residues predicted to be in proximity in the GnRH receptor (Zhou et al. Mol. Pharm. 45:165,1994) did not affect ketanserin affinity. Mutations corresponding to published mutations in the rat 5-HT_{2A} receptor yield results that differ from those reported for certain drugs. Several mutations are being evaluated to probe and refine a model of the mechanism of activation of the 5-HT_{2A} receptor (Zhang and Weinstein J.Med. Chem. 36:934,1993). (Supported by NIH-NIDA DA06620 and DA00060 and USPHS GM 34852).

476.3

AN AROMATIC RESIDUE AT POSITION 340 IS ESSENTIAL FOR ERGOT BUT NOT ERGOPEPTINE BINDING TO THE 5-HT2A RECEPTOR. MS Choudhary*^, A Uluer^, RB Westkaemper+, RA Glennon+ and BL Roth^, ^Department of Psychiatry, Case Western Reserve University Medical School, Cleveland, OH 44106 and +Department of Medicinal Chemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond,VA.

We have investigated the molecular requirements for drug binding to serotonin$_{2A}$ (5-hydroxytryptamine$_{2A}$; 5-HT$_{2A}$) receptors and previously discovered a single, highly conserved, aromatic residue, Phe340, which is essential for ergot binding to the 5-HT$_{2A}$ receptor. To clarify the details of ergot-receptor interactions, an additional 8 point mutations were constructed and expressed in COS-7 cells. The effects of these point mutations on the binding of 14 different ergot and ergopeptine derivatives were then determined. Mutations at position 340 in which an aliphatic residue was substituted for an aromatic residue (e.g. F340L, F340A) greatly diminished ergot (100-1000 -fold decrease in Ki) but not ergopeptine (<10 fold decrease in Ki) affinities. By contrast, mutations at position 340 in which an aromatic residue was conserved (e.g. F340Y) did not appreciably affect ergot or ergopeptine affinities. Mutations at position 339, in general, had either no effect (F339A, F339L) or modest effects (F339Y) on ergot and ergopeptine Ki's. The F125 mutations had little effect on ergot and ergopeptine binding. Two molecular models (one in which ergots and ergopeptines bind to different residues, another in which they utilize similar residues) were evaluated using three-dimensional computer-graphic models of 5-HT2A receptor-ligand interactions. The results suggest that an *aromatic* residue at position 340, but not 339, is essential for ergot but not ergopeptine binding to the 5-HT2A receptor.

476.4

A SMALL REGION IN THE SIXTH AND SEVENTH TRANSMEMBRANE DOMAINS OF THE HUMAN 5-HT$_{1D\beta}$ AND 5-HT$_{1E}$ RECEPTORS DETERMINES 5-CARBOXAMIDOTRYPTAMINE AFFINITY

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Two 5-hydroxytryptamine (5-HT) receptor subtypes, the 5-HT$_{1D\beta}$ and 5-HT$_{1E}$ receptors, have primary structures that are approximately 50% identical. However, the 5-HT$_{1D\beta}$ receptor has high affinity for 5-carboxamidotryptamine (5-CT) whereas the 5-HT$_{1E}$ receptor has low affinity for this compound. In order to ascertain the structural basis for this difference, a series of chimeric 5-HT$_{1D\beta}$/5-HT$_{1E}$ receptors was constructed. Ligand binding experiments with the chimeric receptors showed that a stretch of 57 amino acids extending from the beginning of the sixth to the middle of the seventh transmembrane domain of these two 5-HT receptors was largely responsible for determining 5-CT affinity. All the chimeric receptors had high affinity for 5-HT and lysergol, suggesting that the gross three dimensional structure of the chimeric receptors was not altered. A mutant 5-HT$_{1D\beta}$ receptor in which two residues in the sixth transmembrane domain were changed to the corresponding residues in the 5-HT$_{1E}$ receptor ($I^{333}S^{334}$ converted to $K^{333}E^{334}$) showed markedly lower affinity for 5-CT than did the wild type 5-HT$_{1D\beta}$ receptor but retained high affinity for 5-HT and lysergol. Neither single mutation alone (i.e. I^{333} converted to K^{333} or S^{334} converted to E^{334}) was sufficient to alter 5-CT affinity. These data suggest that two amino acids in the sixth transmembrane domain are largely responsible for determing the affinity of the 5-HT$_{1D\beta}$ and 5-HT$_{1E}$ receptors for 5-CT.

476.5

MOLECULAR CLONING AND CHARACTERIZATION OF SEROTONIN AND OCTOPAMINE RECEPTORS OF *APLYSIA*. X.-C. Li*, J.-F. Giot, René Hen, K.R. Weiss‡, X. Lo‡, and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032; ‡Dept. Physiol. & Biophys., Mt. Sinai School of Medicine, NY, NY 10029

Serotonin, a modulatory neurotransmitter, acts on different receptors to activate a variety of postsynaptic responses. In particular, serotonin can elicit short- and long-term facilitation of the monosynaptic connections between the sensory and motor neurons of the *Aplysia* gill-withdrawal reflex. The short-term actions of 5-HT likely involve activation of at least two types of 5-HT receptors, those coupled to cAMP and PKA and those coupled to PKC. To analyze this transduction mechanism on the molecular level, we isolated two 5-HT receptor genes from *Aplysia*, Ap5HT2A and Ap5HT2B. and one gene encoding an octopamine receptor, ApOctR. Both 5-HT receptors contain characteristic seven hydrophobic regions. The amino acid sequence in this putative transmembrane region is homologous to those of other serotonin receptors. The protein homology between the two receptors is 80%. Homology with mammalian 5-HT receptors 5HT1A, 5HT1C, 5HT2, *Drosophila* 5-HT receptors Dro1, Dro2b, *Lymeanus* 5-HT receptor Lym17, are 37%, 39%, 41%, 38%, 35%, and 32% respectively. When expressed in mammalian cells both *Ap*5HT2A and Ap5HT2B stimulate phosphoinositide turnover by phospholipase C activation and mobilize intracellular Ca^{2+}. Both are expressed in the *Aplysia* CNS. To further analyze the role of cAMP we also isolated an octopamine receptor that stimulates adenyl cyclase to increase cAMP over 500-fold in mammalian cultured cells. Since the sensory neurons of *Aplysia* lack octopamine receptors, transfection of this receptor into the sensory neurons should allow study of the short- and long-term consequences of the selective activation of the cAMP pathway.

476.6

STRUCTURE AND CHARACTERIZATION OF THE RAT 5-HT1C RECEPTOR GENE, Junko Aimi*, Hung-Teh Kao, and Roland D. Ciaranello, Nancy Pritzker Laboratory of Developmental and Molecular Neurobiology, Dept. Psychiatry, Stanford University, Stanford, CA 94305

The structure of the rat 5-HT1c receptor gene has been determined by two overlapping genomic clones which span approximately 30 kb. Exon/intron junctions were defined by polymerase chain reaction (PCR) and confirmed directly by sequence analysis. Cloning the 3' region of the mRNA enabled us to determine the sequence of the entire cDNA. The 5' end of the transcript was mapped by primer extension analysis and determined to be 691 nucleotides (nt) upstream of the initiator ATG. The full length transcript is 4732 (nt). This consists of a coding sequence of 1380 nt, flanked by a long 3'UTR of 2666 nt, and 691 nt of 5'UTR.

Sequence analysis of the 5' flanking region suggests that the 5-HT1c receptor gene lacks a classical TATAA box in the proximity of the transcription initiation site. However, a number of consensus binding sequences for cis-acting elements including AP1 and AP2 have been identified in this region. To characterize the regulatory sequences of the 5-HT1c gene promoter, a series of promoter deletions have been constucted and engineered into a luciferase reporter system. By utilizing a transient expression assay in two neuronal cell lines, RS1 (a PC12 derivative) and Neuro 2a, regions of the 5' flanking sequences have been identified which confer positive (between nt+1 and -165) and negative (between nt-1193 and -309) gene expression in a cell-type specific manner. To examine the regulation of 5-HT1c gene expression in a more physiologically relevant context, these studies are also being employed in primary rat choroid plexus cultures as well as in other rat epithelial cell lines. This will enable us to identify cis- and trans-acting element which play a functional role in 5-HT1c gene expression.

476.7

HUMAN AND MOUSE 5-HT$_{5A}$ AND 5-HT$_{5B}$ RECEPTORS: CLONING AND FUNCTIONAL EXPRESSION. [1]R. Grailhe, [3]N. Amlaiky, [1]A. Ghavami, [1]S. Ramboz, [2]F. Yocca*, [2]C. Mahle, [4]C. Margouris, [4]F. Perrot, and [1]R. Hen. [1]Center for Neurobiology and Behavior, Columbia University, 10032 New York, NY. [2]Bristol-Myers Squibb, 06492 Wallingford, CT. [3]L.G.M.E CNRS, 67085 Strasbourg, France. [4]Rhône-Poulenc Rorer, Vitry-sur-Seine, France.

We isolated from a mouse brain cDNA library and a human genomic library the genes encoding the 5-HT5A and 5-HT5B serotonin receptors. Amino acid sequence comparisons revealed that the human 5-HT5A receptor is 85% homologous to the mouse 5-HT5A receptor. We only found one human 5-HT5B pseudogene raising the possibility that there is no functional human 5-HT5B receptor. When expressed in Cos-7 cells, the mouse 5-HT5A receptor displayed high (Kd=0.84nM) and low affinity (Kd=13nM) binding sites for the radiolabelled ligand [3H]5-CT. Both sites were insensitive to GppNHp suggesting that the 5-HT5A receptor is not coupled to G proteins in Cos-7 cells. The pharmacological profile of the mouse 5-HT5A receptor resembled the profile of 5-CT sensitive sites that were found in the mouse brain and that were insensitive to GppNHp. In Cos-7 and NIH 3T3 cells expressing the mouse 5-HT5A receptor we did not detect any change in adenylate cyclase or phospholipase activity in response to serotonin. We are currently analyzing the possible coupling of 5-HT5 receptors to ion channels in a variety of neuronal cell lines. In order to study the function of the 5-HT5A receptor we are using gene targeting techniques to generate mutant mice lacking this receptor.

476.8

CLONING AND CHROMOSOMAL LOCALIZATION OF A HUMAN 5-HT$_6$ SEROTONIN RECEPTOR. R.Kohen[1], M.A.Metcalf[1], T.Druck[2], K.Huebner[2], D.R.Sibley[3], M.W.Hamblin[1]*. [1]GRECC, Seattle VAMC and the University of Washington, Seattle, WA 98108, [2]Jefferson Cancer Institute, Jefferson Medical College, Philadelphia, PA 19107; [3]NINDS/NIH, Bethesda, MD 20892.

The 5-HT$_6$ serotonin receptor, one of three known subtypes of mammalian Gs-coupled serotonin receptor, has received interest for its high affinity for the atypical neuroleptic clozapine. We previously reported the cloning of the rat 5-HT$_6$ receptor and now report the isolation of the corresponding human gene and its chromosomal localization.

The human 5-HT$_6$ receptor is a 440 amino acid polypeptide that is 96% identical to its rat homologue within the transmembrane regions. The nucleic acid sequences of the open reading frames from the two species are approximately 84% identical. Comparison of genomic and cDNA sequences reveals the presence of two introns -- a 3 kb intron occurs after base 714 and a 190 bp intron occurs after base 873.

Using hybridization to a somatic cell hybrid panel, the 5-HT$_6$ receptor gene was localized to 1p35-pter. This localization indicates that the 5-HT$_6$ and the 5-HT1Dα loci may be closely linked. Such clustering has not been previously found within the serotonin receptor family. We have tentatively identified an Rsa I polymorphism within the 5-HT$_6$ coding region, which may be informative in linkage studies examining the role of this receptor in various human diseases.

477.1

Increased Platelet $5HT_2$ Binding in Bipolar Patients Requiring Anticonvulsants. E. Risby, W. N. Morgan, M. Stipetic*, M. J. Owens, C.B. Nemeroff, Emory University School of Medicine, Atlanta, GA 30322.

The pathophysiologic abnormality responsible for bipolar mood disorders is unclear. Abnormalities in the serotonin system have been proposed based on preclinical data suggesting that lithium augments serotonin functioning. However, it is unclear why some patients respond well to lithium monotherapy, when others require the addition of an anticonvulsant to achieve optimal control of their bipolar illness. We compared serotonin receptor binding ($5HT_2$) in the platelets of 12 bipolar subjects whose illness was controlled on lithium monotherapy with 8 patients who required an anticonvulsant (6 on divalproex, 2 on carbamazepine) and 7 normal controls. Patients who required anticonvulsants had significantly higher mean ^{3}H-LSD binding (mean = 123 ± 21 fmol/mg protein) than pure lithium responders (mean = 81 ± 27 fmol/mg protein) and normal controls (mean = 70 ± 21 fmol/mg protein). This preliminary data suggests there are no abnormalities in $5HT_2$ receptor binding in lithium responders. However, patients requiring an anticonvulsant (either in combination with lithium or as monotherapy) tend to have higher $5HT_2$ platelet receptor binding sites. It is unclear if the anticonvulsants are responsible for this elevation in binding sites or if high ^{3}H-LSD binding predicts clinical response to anticonvulsants.

477.2

SEROTONIN IA RECEPTOR BINDING IN THE ENTORHINAL CORTEX IN SCHIZOPHRENIA AND SUICIDE. D.C. Ohuoha, T.M. Hyde, M.M. Herman, C.F. Spurney, D.J. Sabourin*, and J.E. Kleinman. Clinical Brain Disorders Branch, NIMH, Neurosciences Center at St. Elizabeths Hospital, 2700 M.L.King Ave S.E., Washington, DC 20032.

Over the years, there has been a growing interest in the role of the entorhinal cortex in various neuropsychiatric disorders, such as Alzheimer's disease and schizophrenia. In this study we examined 5HT1a receptor density in post-mortem entorhinal cortex, using quantitative autoradiography comparing patients with schizophrenia to normal controls, nonpsychotic suicides and non-schizophrenic neuroleptic-treated psychiatric patients. Blocked sections of fresh frozen brain containing the entorhinal region were obtained for study. Quantitative autoradiography of 5HT1a receptors was performed using 2nM [3H]8-OHDPAT. Nonspecific binding was determined by incubating consecutive sections in the presence of 10uM 8-OHDPAT. Preliminary analysis of the autoradiograms using computerized image analysis did not reveal any statistically significant differences between patients with schizophrenia and other groups, confirming a prior study of schizophrenics and suicides of this brain region.

477.3

EFFECT OF DESIPRAMINE, LITHIUM AND ADINAZOLAM ON SEROTONIN RECEPTOR SUBTYPES IN RAT BRAIN. Y. Dwivedi, S.C. Pandey, X.Ren, P. Tueting*, and G.N. Pandey. Department of Psychiatry, College of Medicine, University of Illinois, Chicago, Illinois 60612.

We have examined the effect desipramine, lithium and adinazolam on 5HT receptor subtypes ($5HT_{1C}$, $5HT_2$, $5HT_3$ receptors) in rat brain. Male Sprague-Dawley rats were treated with desipramine (10 mg/Kg), lithium (2 meq/Kg twice) and adinazolam (10 mg/Kg) for 15 days and sacrificed after 24 hrs of last injection. The brain regions were used for measurement of $5HT_2$, $5HT_{1C}$, and $5HT_3$ receptors. It was observed that treatment with desipramine decreased B_{max} of $5HT_2$ receptors whereas lithium or adinazolam had no effect on $5HT_2$ receptors in rat cortex. We also observed that lithium or adinazolam treatment has no effect on $5HT_{1C}$ and $5HT_3$ receptors in rat cortex and hippocampus. The function of $5HT_{1C}$ receptor in rat choroid plexus was studied by measuring 5HT-stimulated inositol-1-phosphate (IP_1) formation. It was observed that treatment with adinazolam increased, whereas, desipramine treatment had no effect on 5HT-stimulated IP_1 formation in rat choroid plexus. These results suggest that modulation of 5HT-neurotransmission by lithium does not involve $5HT_{1C}$, $5HT_2$, $5HT_3$ receptors in rat brain. Furthermore, adinazolam treatment has no effect on $5HT_{1C}$, $5HT_2$, and $5HT_3$ receptor in rat brain but significantly increases the 5HT-stimulated inositol phosphate formation in rat choroid plexus.

477.4

THE RELATIONSHIP BETWEEN 5-HT_{1A} RECEPTOR DENSITY AND mRNA LEVEL IN RAT BRAIN IS AGE AND REGION-DEPENDENT. **L. Lu*, R.K. Raghupathi, M. Munir, M.-P. Kung, H. Kung and P. McGonigle Depts. of Pharmacology and Radiology Univ. of Pennsylvania, Philadelphia, PA 19104.**

The relationship between the expression of 5-HT_{1A} receptors and the level of receptor mRNA was examined in coronal sections of brain from 3, 7, 10, 14, 21 and 60 day-old Sprague-Dawley rats. Sections were labelled with [^{125}I]-8-OH-PIPAT to visualize 5-HT_{1A} receptors. Adjacent sections were labelled with a 183 base ^{35}S-labelled riboprobe complementary to rat 5-HT_{1A} receptor mRNA. Preliminary experiments demonstrated that [^{125}I]-8-OH-PIPAT selectively labels 5-HT_{1A} receptors. In the hippocampus and cortex, the density of receptors increased significantly between days 3 and 21. After day 21, receptor density increased in CA1, plateaued in CA3 and dentate gyrus, and decreased in cortex. The pattern of mRNA expression paralleled the expression of receptors in each region between days 3 and 21. However, mRNA and receptor expression diverged in all of these regions between days 21 and 60. In contrast, the density of receptors in the thalamus increased between days 3 and 14 and then declined. The density of receptors in the cerebellar cortex declined steadily to almost undetectable levels between days 3 and 60. The pattern of mRNA expression paralleled receptor expression in both of these regions at every age. These results suggest that 5-HT_{1A} receptor expression is directly related to mRNA levels during development. However, it is likely that additional mechanisms regulate receptor expression in the mature brain. (Supported by USPHS MH 43821 and MH-48125)

477.5

Transcriptional Regulation of Hippocampal 5-HT_{1a} Receptors by Glucocorticoid Hormones. P. Zhong*, R.D. Ciaranello, Dept. of Psychiatry and Beh. Sci., Stanford Univ Med Ctr, Stanford, CA 94305.

5-HT_{1a} receptors in the hippocampus play a critical role in modulating limbic system output. The activity and level of 5-HT_{1a} receptors are modulated by glucocorticoid levels. The present study was undertaken to test the hypothesis that glucocorticoids attenuate the transcriptional activity of the 5-HT_{1a} receptor gene. Using *in situ* hybridization and RNase protection assays, we observed a substantial increase in 5-HT_{1a} mRNA expression after adrenalectomy in the same hippocampal regions in which 5-HT_{1a} binding sites are increased. This increase in 5-HT1a mRNA expression occurs as early as one hour after adrenalectomy and precedes the increase in receptor binding sites. Further *in situ* hybridization analysis showed that 5-HT_{1a} mRNA is increased within individual hippocampal cells after adrenalectomy, whereas no evidence suggested recruitment of cells that do not express 5-HT_{1a} receptors normally. Nuclear run-on assays showed that the rate of transcription of 5-HT_{1a} mRNA after adrenalectomy increased from 60% to 75% above the rate from control preparations and could be reduced to basal levels by the administration of dexamethasone. These results suggest that transcription of hippocampal 5-HT_{1a} receptor mRNA is under negative regulation by corticosteroid hormones.

477.6

THE REGULATORY ROLE OF CORTICOSTERONE ON THE 5-HT1A RECEPTOR DURING ONTOGENY. H.J.J. van Oers, P. Zhong, R.D. Ciaranello and S. Levine*, Dept. of Psychiatry and Beh. Sci., Stanford Univ. Sch. of Med., Stanford, CA 94305.

Corticosterone (CORT) appears to have a role in regulating the 5-HT system. Stress, resulting in increased circulating CORT, acutely facilitates 5-HT turnover. Studies in adult rats have shown that following acute adrenalectomy (ADX) 5-HT1a receptor density is elevated. CORT replacement at the time of ADX maintains the normal levels of receptor density and hippocampal 5-HT1a receptor mRNA expression.

In the neonatal rat between post-natal-day (pnd) 4-14 CORT levels are normally low and non-circadian. The adrenal is hyporesponsive to endogenous and exogenous ACTH. This period is designated as the stress-hyporesponsive period (SHRP). In these experiments we studied the role of CORT on the 5-HT1a receptor during development in order to determine the regulatory role of CORT on the 5-HT system. The 5-HT1a receptor density in the rat hippocampus and brain stem was examined at pnd 6 (in the SHRP) and 21 by *in vitro* binding studies using the selective 5-HT1a agonist [^{3}H]8-OH-DPAT. The 6-day-old pups showed an increase in 5-HT1a receptor density in the brain stem 24 hr post-ADX compared to SHAM animals. No discernable changes ocurred in the hippocampus at this age. In the 21-day-old animals the reverse was found: no changes were observed in the brain stem and binding was increased in the hippocampus following ADX. The level of glucocorticoid receptors (GR) in the hippocampus at the early age (pnd 6) is about half of that seen at pnd 21. The suppressive effect of CORT on the 5-HT1a receptors might be due to the low availability of GRs. These data suggest clear distinctions in the regulatory role of CORT on the 5-HT system during development.

477.7

EFFECTS OF ADRENALECTOMY AND CHRONIC GLUCOCORTICOID TREATMENT ON BRAIN 5-HT_{2C} RECEPTOR BINDING IN WISTAR RATS. Bridget A. Hulihan-Giblin*, Charanjit S. Aulakh, and Dennis L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

In a previous report from this laboratory, chronic glucocorticoid treatment was shown to attenuate m-chlorophenylpiperazine (m-CPP, a 5-HT agonist)-induced increases in plasma prolactin, decreases in food intake and locomotor activity (Bagdy et al., 1989). m-CPP decreases food intake and locomotor activity (Kennett and Curzon, 1988; 1991) and increases plasma prolactin (Aulakh et al., 1992) by selective stimulation of 5-HT_{2C} receptors. In the present study, we investigated the effects of chronic glucocorticoid treatment and adrenalectomy on [^{3}H]-mesulergine-labeled 5-HT_{2C} receptors in the frontal cortex, hippocampus, striatum and hypothalamus in male Wistar rats. The animals were divided into four different groups; sham-operated, sham-operated + corticosterone treatment, adrenalectomized (Adx) and Adx+ corticosterone treatment.

Adrenalectomy produced a significant decrease in Bmax values of 5-HT_{2C} receptors only in the striatum relative to sham-operated controls which was restored to control values by chronic corticosterone treatment. On the other hand, chronic corticosterone treatment produced a significant increase in Bmax values of 5-HT_{2C} receptors only in the hippocampus relative to sham-operated controls. There were no parallel changes in K_d values. These findings demonstrate modulation of 5-HT_{2C} receptor binding by the glucocorticoids.

477.8

CHRONIC ESTROGEN TREATMENT OF FEMALE RATS ENHANCES 5-HT_{1A}-MEDIATED cAMP ACCUMULATION IN HIPPOCAMPAL SLICES. Y. Chen*[1], K.A. Berg[2] and W.P. Clarke[1]. Departments of Pharmacology[1] and Anesthesiology[2], Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Female sex homones and 5-HT_{1A} receptor systems have been implicated in the etiology of affective disorders such as anxiety and depression. Recently, we showed that chronic treatment of ovariectomized (OVX) female rats with estrogen (E) enhances 5-HT_{1A}-mediated electrophysiological responses in rat hippocampal slices. E-treatment also enhances the 5-HT_{1A}-mediated inhibition of adenylyl cyclase activity in hippocampal membranes. but to a much smaller degree. To study effects of E on both responses coupled to the 5-HT_{1A} receptor in hippocampus we have developed a method to assess adenylyl cyclase activity in rat hippocampal slices that are maintained under similar conditions as those for electrophysiological studies.

OVX female rats received sc implants of either cholesterol (control) or 10% 17ß-estradiol in cholesterol. Treatment of rats with E for 5 days enhanced 5-HT_{1A}-mediated inhibition of forskolin-stimulated cAMP accumulation (FScA) in hippocampal slices. For 5-carboxamidotryptamine, values for E_{max} (% inhibition), pEC_{50} and slope factor were 33.8±1.4, 8.27±0.06 and 1.05±0.17 for controls and 44.0*±0.76, 8.44*±0.04 and 0.87 ±0.06 for E-treated rats, n=12). For the 5-HT_{1A} partial agonist buspirone, values were 24.9 ±1.25, 6.34±0.06 and 1.01±0.11 for controls and 34.8±1.0*, 6.48±0.08 and 1.2 ± 0.17, n=8). FScA (pmol/mg protein) was higher in slices from E-treated rats (191 ± 1.2) than in controls (167±1.2). 5-day E treatment did not alter $GABA_B$-mediated inhibition of FScA. 5-HT_{1A}-mediated inhibition of FScA was not altered after 1 day E treatment *in vivo* nor after 30 min or 2 hr treatment with 10 nM E *in vitro*. In preliminary experiments we have begun to test the hypothesis that the effect of E on 5-HT_{1A}-mediated signal transduction may be mediated by inhibition of protein kinase C (PKC), which can desensitize the 5-HT_{1A} receptor system. 5-day E treatment increased maximal PKC activity measured in vitro in membrane and cytosol fractions of hippocampus. Western blot analysis revealed small increases in the quantities of PKC's α and δ in hippocampal cytosol and membrane fractions and a slight decrease in PKCγ in hippocampal membranes in response to E treatment. Treatment of hippocampal slices from OVX rats with the PKC inhibitor staurosporine for 90 min did not mimic E's effect on 5-CT CRCs. More work is needed to evaluate a role for PKC as a mediator of the E effect on 5-HT_{1A}-mediated signal transduction. (Supported by HD26437 and MH48125).

477.9

CHRONIC BUSPIRONE TREATMENT DIFFERENTIALLY REGULATES 5-HT_{1A} AND 5-HT_2 RECEPTOR mRNA IN VARIOUS REGIONS OF THE RAT HIPPOCAMPUS. H. Chen*, L. Zhang, D. R. Rubinow and D.-M. Chuang. Section on Molecular Neurobiology and Section on Behavioral Endocrinology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892

Busiprone, a 5-HT_{1A} receptor agonist with antidepressant and anxiolytic efficacy, has been studied for its chronic effects on the levels of 5-HT_{1A} and 5-HT_2 receptor mRNA in the rat hippocampus. Groups of rats were treated with buspirone (1mg/kg/day) or vehicle for 21 days using osmotic minipumps implanted subcutaneously under anesthesia. Levels of 5-HT_{1A} and 5-HT_2 receptor mRNA were analyzed by in situ hybridization in various subhippocampus regions. In untreated hippocampus, the rank order of the abundance of 5-HT_{1A} receptor mRNA was: dentate gyrus (DG) > CA_1 > CA_2 > CA_3 > CA_4. After buspirone treatment, 5-HT_{1A} receptor mRNA levels were significantly decreased in the CA_1 and CA_2 but were markedly increased in the CA_3, CA_4 and DG. The rank order of the hippocampal 5-HT_2 receptor mRNA abundance was: DG > CA_3 > CA_4 > CA_1> CA_2. Buspirone treatment markedly increased levels of 5-HT_2 receptor mRNA in the CA_3, CA_4, DG and CA_2, without affecting the mRNA level in the CA_1. These results demonstrate that chronic buspirone treatment differentially regulates 5-HT_{1A} and 5-HT_2 receptor mRNA in various regions of the hippocampus. Moreover, buspirone, through activation of 5-HT_{1A} receptors, appears to " cross-talk" with the 5-HT_2 receptors. Experiments are in progress to examine whether these changes in receptor mRNA result in corresponding changes in the 5-HT receptor binding sites.

477.10

MICROTUBULES ARE INVOLVED IN THE AGONIST-INDUCED UP-REGULATION OF 5-HT_2 RECEPTOR BINDING SITES IN CEREBELLAR GRANULE CELLS. D.-M. Chuang*, H. Chen, H. Li and C. Hough. Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892

We have reported that microtubules (MT) mediate the regulation of muscarinic and β-adrenergic receptor expression in cultured cells (Mol Cell Neurosci 2: 315, 1991; J. Neurochem 62: 430, 1994). Here, we examined the role of MT in the paradoxical up-regulation of 5-HT_2 receptors induced by persistent agonist stimulation (Mol Pharmcol 43: 349, 1993). Pretreatment of cerebellar granule cells with colchicine (10 μM), a MT disruptor, did not affect basal level of 5-HT_2 receptor binding sites but largely attenuated the up-regulation of 5-HT_2 receptor sites induced by a 16-hr stimulation with DOI, a 5-HT_2 receptor agonist. This effect of colchicine was dose-dependent and completely blocked by taxol, a MT stabilizer, which by itself was inactive. Nocodazole (10 μM), another MT disruptor, also attenuated DOI-induced 5-HT_2 receptor up-regulation; the nocodazole's effect was, however, not readily reversed by taxol. β-Lumicolchicine, an inactive derivative of colchicine, and cytochalasine B or D, a disrupter of microfilaments, did not affect levels of basal or DOI-stimulated 5-HT_2 binding sites. These results suggest that the structural intergrity of MT plays an essential role in the agonist-induced up-regulation of 5-HT_2 receptor binding sites in cerebellar granule cells.

477.11

EFFECTS OF LESIONS ON THE 5-HT_{1A} RECEPTOR AND 5-HT TRANSPORTER AND THEIR mRNAs IN THE RAT DORSAL RAPHE. R. Cortés*, L. Puiols, G. Mengod, and F. Artigas. Dept. Neurochemistry, Centre d'Investigació i Desenvolupament, CSIC, Barcelona E-08034, Spain.

In the present study we used receptor autoradiography and *in situ* hybridization to examine the response of dorsal raphe neurons to drug-induced lesions of the 5-HT system. We analyzed the effect of 5,7-DHT (5,7-dihydroxytryptamine) and PCPA (*p*-chlorophenylalanine) on the 5-HT transporter and the 5-HT_{1A} autoreceptor, as labeled with ^{3}H-citalopram and ^{3}H-8-OH-DPAT, respectively. Their corresponding mRNAs were visualized using ^{35}S-labeled oligonucleotides. Both mRNA species were present at high densities in dorsal raphe cells. Fifteen days after *i.c.v.* administration of 5,7-DHT (200 μg/20 μl) no hybridization signal for the mRNAs encoding the 5-HT transporter and the 5-HT_{1A} receptor could be detected in the dorsal raphe. This depletion of messengers ran in parallel with a marked reduction in the density of the encoded proteins in this nucleus. The two proteins were, however, differently affected. Thus, ^{3}H-citalopram binding was reduced by about 40% in the ventral third of the dorsal raphe, whereas no binding could be observed in the dorsal parts of the nucleus, nor in surrounding structures of the midbrain and hippocampus. In contrast, 5-HT_{1A} receptor concentrations decreased homogeneously throughout the dorsal raphe (85% depletion), but were apparently unaffected in other brain structures. After administration of a single dose of PCPA (300 mg/kg i.p.) a transient but marked reduction in both 5-HT transporter and 5-HT_{1A} receptor mRNA was observed in the dorsal raphe. A similar time course profile was followed by 5-HT_{1A} receptor binding, although its recovery was slower. In the same animals ^{3}H-citalopram binding was not significantly affected by PCPA. The results show that damage to 5-HT neurons differentially alters presynaptic 5-HT markers.

477.12

[^{3}H]8-OH-DPAT BINDING SITES IN RAT CEREBRAL CORTEX DURING TREMOR INDUCED BY KAINIC ACID INJECTION F. Radja* and T.A. Reader. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Faculté de médecine, Université de Montréal (Québec) CANADA

Recent investigations, carried out using membrane homogenates from rat cerebral cortex and hippocampus, have shown that [^{3}H]8-OH-DPAT binds to two differents components. The 5-HT1A^{HIGH} site corresponds to the known 5-HT1A receptor while the novel 5-HT1A^{LOW} site requires a high range of tritiated ligand to be saturated[1]. The excitatory amino acid agonist kainic acid (KA), a conformationally restricted analog of *L*-glutamic acid, is selective for the kainate receptor subtype and is known to cause convulsions. Here we examined cortical [^{3}H]8-OH-DPAT binding early after an i.p. injection of KA (10 mg\kg). One to two hours after treatment the first seizures take place, and at that time the animals were decapited; their brains were rapidly removed and frozen. Saturation curves were carried out with a wide range (0.02 to 160 nM) of [^{3}H]8-OH-DPAT concentrations. The Scatchard transformations of the data showed concave curves, and their analyses using SCAFIT[2] revealed two different sites. Preliminary results indicate that binding to the HT1A^{HIGH} receptor is unaltered by KA treatment; in contrast, the binding capacity of 5-HT1A^{LOW} sites is highly (70%) increased with no major changes in the affinity. Since there were no shifts of high- to low-affinity sites, the results argue against the existence of affinity states to explain the two [^{3}H]8-OH-DPAT binding components. These findings lend further support to the proposal that a novel 5-HT1A receptor could be present in rat brain. *[Supported by the Savoy Foundation for Epilepsy].* References: [1]Nénonéné et al *J Neurochem* (1994) **62** in press. [2]De Léan et al *Am J Physiol* **235**:E97-E102.

477.13

5-HT1A RECEPTOR MODIFICATION IN HIPPOCAMPUS AND CORTEX FOLLOWING 5,7-DHT LESIONS IN THE FIMBRIA-FORNIX AND CINGULUM BUNDLE. T. D. Patel* and F. C. Zhou. Program in Medical Neurobiology and Department of Anatomy. Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

Microinjection of the serotonin (5-HT) neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the cingulum bundle (CB) and fimbria-fornix (FF) denervates the hippocampus and cortex of 5-HT fiber projections and evokes denervation supersensitivity of the 5-HT1A receptor on target neurons by 14 days postlesion (Patel and Zhou, 1994). This study seeks to examine early changes in the cellular distribution and expression of the 5-HT1A receptor subsequent to 5-HT denervation.

Adult female Sprague-Dawley rats were pretreated with desipramine (1mg/100 g.b.w.) and microinjected with 5,7-DHT (5ug/0.5ul) into the CB and FF. At postlesion day 5, animals were perfused and brain sections were immunocytochemically stained for 5-HT and 5-HT1A receptor using the antibody against peptide 170-186 of the rat 5-HT1A receptor (Azmitia et al., 1992). 5-HT immunoreactivity (IM) was virtually absent in lesioned hippocampus. No qualitative difference in 5-HT1A-IM staining was detectable between lesioned and nonlesioned brains on pyramidal cells of the CA fields which exhibited 5-HT1A localization to the axon hillock. However, in the molecular layer of the dentate gyrus, brains with lesion exhibit a greater number of 5-HT1A-IM cells as compared to non-lesion controls. Some of these appear to have a neuronal somatodendritic morphology, while others appear to be astrocyte-like. Similarly, in layer I of cortex, brains with lesion exhibit polymorphic 5-HT1A-IM elements which are not detected in nonlesion controls.

These findings suggest that (1) the loss of 5-HT innervation might be involved in signaling changes in the cellular distribution and expression of the 5-HT1A receptor, (2) cells which do not normally express the 5-HT1A receptor can be induced to express the receptor following 5-HT denervation, and (3) these receptor changes occur as early as 5 days postlesion.

477.14

QUANTITATIVE AUTORADIOGRAPHY OF 5-HYDROXYTRYPTAMINE$_{1B}$ BINDING SITES IN RATS WITH NEONATAL 5,7-DIHYDROXYTRYPTAMINE LESIONS. M. R. Pranzatelli*, M. M. Durkin, M. Farmer. Dept. of Neurology, Pediatrics, and Pharmacology, The George Washington University, and Center for Neurosciences, Children's Research Institute, Washington, DC 20010.

We previously found different effects on behavior, 5-HT levels, and 5-HT$_{1A}$ sites of neonatal 5,7-dihydroxytryptamine (5,7-DHT) lesions depending on the route of 5,7-DHT injection. To study the impact of early lesions on 5-HT$_{1B}$ sites, the terminal autoreceptor and species homolog of the human 5-HT$_{1D\beta}$ receptor, we labelled 5-HT$_{1B}$ sites autoradiographically with [^{3}H]5-HT four months after intraperitoneal (ip) or intracisternal (ic) 5,7-DHT injection during the first postnatal week. Specific binding at 5-HT$_{1B}$ sites was greatest in substantia nigra and subiculum, but 21 other areas were quantitated as well. Both routes of 5,7-DHT injection were associated with increases in specific binding in subiculum (+37% and 18%, respectively) and posterior-subiculum (+24% and +47%). In contrast, there was a 32% increase in specific binding in substantia nigra in rats with lesions made ic but not ip. No significant differences were found in nucleus accumbens, caudate-putamen, or other brain areas. In saturation homogenate binding studies using [^{125}I]iodocyanopindolol one month after ip injections, neonatal (5,7-DHT) lesions did not significantly alter 5-HT$_{1B}$ maximal binding site density in cortex, striatum, diencephalon, or brainstem. These data suggest differential effects of route of neonatal 5,7-DHT injections on plasticity of 5-HT$_{1B}$ receptor recognition sites.

477.15

5,7-DIHYDROXYTRPYTAMINE ALTERS 5-HT$_{1B}$ AND 5-HT$_{1D}$ SEROTONIN RECEPTOR mRNA EXPRESSION IN THE RAT BRAIN. J. F. Neumaier*, P. Szot, D. M. Dorsa, and M. W. Hamblin. GRECC, SVAMC, and the Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98108.

The 5-HT$_{1B}$ and 5-HT$_{1D}$ serotonin receptors were initially described as presynaptic autoreceptors which regulate the release of serotonin. However, these receptors have also been found to regulate the release of several other neurotransmitters in various regions of the rat brain. In this study we investigated the effects of chemical lesioning of serotonergic neurons on the levels of mRNA expression of these receptors.

5,7-Dihydroxytryptamine (5,7-DHT) or vehicle was infused sequentially into the fourth and lateral ventricles using standard protocols. We used previously reported *in situ* hybridization protocols. In the olfactory bulb and subregions of the hippocampus, 5-HT$_{1D}$ receptor mRNA levels did not appear to be altered by the 5,7-DHT lesions. In the striatum and CA1 region of the hippocampus, 5-HT$_{1B}$ mRNA levels also appeared unchanged. However, there was a greater than two-fold increase in hybridization signal density detected for both receptor mRNAs in the dorsal raphe, a major source of serotonin projections to the forebrain. The increases in mRNA levels detected in the dorsal raphe may reflect sprouting of new axonal processes and increased synthesis of axonal proteins.

These results may suggest that these receptors are differentially regulated when expressed in presynaptic vs. postsynaptic neurons, and that the postsynaptic receptor mRNA levels may be relatively insensitive to decreases in endogenous ligand concentration.

477.16

SELECTIVE PROTECTION AGAINST EEDQ OF RAT CORTICAL 5-HT2A RECEPTORS BY 5-HT2 LIGANDS. K.F.Martin, S.C. Cheetham, C.J. Kettle & D.J.Heal. (SPON:Brain Research Association) Boots Pharmaceuticals Research Dept., Nottingham, NG2 3AA, U.K.

N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) irreversibly inactivates 5-HT2A receptors (Battaglia et al., 1986, J. Neurochem., 46, 589). We have now determined the ability of selective and non-selective 5-HT receptor agonists and antagonists to protect rat brain 5-HT2A receptors in vivo. 5-HT2A receptor binding parameters were quantified using [3H]ketanserin with the functional status determined using 5-HT-stimulated phosphoinositide (PI) metabolism. EEDQ reduced the number of [3H]ketanserin binding sites in frontal cortex by 57-73% without altering affinity. Single injections of the 5-HT2 agonist (DOI) or the antagonists (ritanserin, ketanserin, metergoline) significantly decreased the number of 5-HT2A receptors by 15-44%. However, these drugs also protected 5-HT2A receptors from EEDQ-induced inactivation by 49-77%. Drugs with no 5-HT2A affinity (8-OH-DPAT, RU 24969, MDL 72222) were without effect. Consistent with the binding data, DOI and ketanserin decreased the PI response to 5-HT (10 μM) by 50% and also prevented the 75% EEDQ-induced decrease. These data indicate, firstly, that 5-HT2A ligands down-regulate 5-HT2A receptors regardless of whether they are agonists or antagonists, and secondly, that they protect against the effects of EEDQ on receptor number and function. In addition, the data show that EEDQ does not differentiate between the high and low affinity state of the 5-HT2A receptor.

477.17

FUNCTIONAL SUBSENSITIVITY OF 5-HT$_{2A}$ AND 5-HT$_{2C}$ RECEPTORS MEDIATING HYPERTHERMIA FOLLOWING LONG-TERM TREATMENT WITH 5-HT$_{2A}$/5-HT$_{2C}$ RECEPTOR ANTAGONISTS IN RATS. Pascale Mazzola-Pomietto, Charanjit S. Aulakh, James Tolliver*, and Dennis L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We have recently demonstrated that hyperthermia induced by (±) 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and m-chlorophenyl-piperazine (m-CPP) is mediated by selective stimulation of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively (Mazzola-Pomietto et al., 1993; Murphy et al., 1993). In the present study, we investigated whether long-term administration of 5-HT$_{2A}$/5-HT$_{2C}$ receptor antagonists would produce either functional supersensitivity or subsensitivity of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors mediating hyperthermia. Therefore, we studied the effects of daily administration of low (1.0 mg/kg/day) and high (5.0 mg/kg/day) doses of mianserin, ritanserin, metergoline or mesulergine (5-HT$_{2A}$/5-HT$_{2C}$ receptor antagonists) on DOI and m-CPP-induced hyperthermia in Wistar rats.

Daily administration of high but not low doses of mianserin for 6-7 days significantly attenuated DOI-induced but not m-CPP-induced hyperthermia. Daily administration of both low and high doses of ritanserin for 13-14 days significantly attenuated m-CPP-induced hyperthermia while DOI-induced hyperthermia was attenuated only by the high dose. These findings demonstrate functional subsensitivity of both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors mediating hyperthermia following long-term administration of 5-HT$_{2A}$/5-HT$_{2C}$ receptor antagonists. We will also present data on the effects of long-term administration of metergoline and mesulergine on DOI and m-CPP-induced hyperthermia.

477.18

REGULATION OF SEROTONIN$_{2A}$ RECEPTORS IN HETEROLOGOUS EXPRESSION SYSTEMS. M. Grotewiel, H. Canton* and E. Sanders-Bush. Department of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600

The serotonin$_{2A}$ and serotonin$_{2C}$ receptors are unique among receptors coupled to guanine nucleotide-binding proteins in that chronic treatment *in vivo* with agonists as well as antagonists decreases receptor density. To uncover molecular events involved in down-regulation of the serotonin$_{2A}$ receptor, the ability of agonists and antagonists to alter receptor density was examined in three heterologous expression systems: transfected NIH 3T3, transfected Madin-Darby Canine Kidney and transfected AtT-20 cells. All three transfected cell lines exhibited pharmacological properties consistent with that predicted for cells expressing the serotonin$_{2A}$ receptor. However, the three cell lines displayed different receptor regulation properties after treatment with drugs acting at the serotonin$_{2A}$ receptor. In transfected NIH 3T3 cells, neither agonist nor antagonist treatment altered receptor density. Treatment with agonist as well as antagonist led to up-regulation of the serotonin$_{2A}$ receptor in transfected Madin-Darby Canine Kidney cells. In transfected AtT-20 cells, treatment with agonist led to receptor down-regulation, whereas antagonist treatment increased receptor density. Thus, the cellular background in which the serotonin$_{2A}$ receptor is expressed appears to determine the regulation properties of the receptor. (This work was supported by a Pharmaceutical Manufacturer's Association Foundation Fellowship and NIH grant MH34007.)

477.19

MOLECULAR MECHANISM OF AGONIST-INDUCED REGULATION OF THE RAT 5-HT$_2$ RECEPTOR. A.C. Chin* and R.D. Ciaranello, Nancy Pritzker Lab, Dept. of Psychiatry, Stanford University, Stanford, CA 94305

The rat serotonin 2 (5-HT$_2$) receptor is expressed in a wide variety of neuronal and non-neuronal tissues. Interestingly, the regulation of the 5-HT$_2$ receptor in response to agonist exposure differs in the various cell types. In primary cultures of rat uterine myocytes, the 5-HT$_2$ receptor is involved in the regulation of collagenase expression; exposure of the cultures to 5-HT$_2$ agonists results in the up-regulation of both the 5-HT$_2$ receptor and collagenase. We present data demonstrating that the agonist-induced regulation of the 5-HT$_2$ receptor is mediated by protein kinase C (PKC) and results in an increase in 5-HT$_2$ mRNA. Furthermore, this up-regulation by agonists (and PMA) is blocked in the presence of PKC inhibitors such as staurosporine and chelerythrine. Experiments addressing whether this regulation is at the transcriptional or post-transcriptional level are currently underway and the results will be presented. In addition, we have recently described the structural organization of the gene including a characterization of promoter elements required for expression of the receptor gene in different cell types. Promoter deletion plasmids will be utilized to define the *cis*-sequences required for affecting the agonist-induced increases in 5-HT$_2$ mRNA levels.

477.20

DIFFERENTIAL REGULATION OF SEROTONIN 5-HT$_{2A}$ AND 5-HT$_{2C}$ RECEPTORS AFTER CHRONIC TREATMENT WITH CLOZAPINE, CHLORPROMAZINE AND SOME NOVEL PUTATIVE ATYPICAL ANTIPSYCHOTIC DRUGS. M. Kuoppamäki, E.-P. Pälvimäki, E. Syvälahti and J. Hietala*, Dept. of Pharmacology, University of Turku, FIN-20520 Turku, Finland.

Previous studies have suggested that 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors may be important for the atypical effects of clozapine. We have used quantitative autoradiography to study the number of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor binding sites after chronic treatment (14 days, s.c. injections once a day) with saline (SAL, 1 ml/kg), clozapine (CLOZ, 25 mg/kg), chlorpromazine (CPZ, 15 mg/kg), ORG 5222 (ORG, 0.1 mg/kg), risperidone (RIS, 0.3 mg/kg), and amperozide (AMP, 5 mg/kg). Receptor binding was measured 68h after the last injections.

In the doses used, CLOZ, CPZ, and ORG decreased the frontal cortical 5-HT$_{2A}$ receptor binding of [^{3}H]ketanserin and [^{125}I]DOI by 40-60 %. AMP also significantly decreased 5-HT$_{2A}$ receptor [^{3}H]ketanserin binding by 30%, whereas RIS did not affect 5-HT$_{2A}$ receptor binding. In contrast to 5-HT$_{2A}$ receptors, only CLOZ decreased significantly (by about 50%) the 5-HT$_{2C}$ receptor [^{3}H]mesulergine and [^{125}I]DOI binding in the choroid plexus. For comparison, we also determined the number of striatal D_1 and D_2 receptor binding sites with [^{3}H]SCH 23390 and [^{3}H]spiperone, respectively. CPZ was the only drug to significantly upregulate D_2 receptor binding sites. None of the drugs affected D_1 receptor binding to any extent. In conclusion, this study suggests that chronic treatment with CLOZ, CPZ, ORG, RIS and AMP differentially regulate 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

477.21

DOWN-REGULATED CORTICAL [^{3}H]KETANSERIN BINDING IN ANOREXIC MUTANT MICE EXPRESSING SEROTONERGIC HYPERINNERVATION. E.S. Corp*, N. Min, T.H. Joh and J.H Son, Burke Medical Research Institute and Bourne Laboratory, Cornell University Medical College, White Plains, NY 10605, and Neuropsychology Program, Queens College , CUNY, Flushing, NY.11367

The murine recessive autosomal mutation *anorexia (anx)* is characterized by pre-weaning weight loss resulting in death at about 21 days. Weight differences can be seen as early as post-natal day (PD) 6, and by PD 16 mutants (*anx/anx*) can weigh as little as 30% of normal littermates. *Anx/anx* mice display dysfunctions in ingestive and motor behaviors that are consistent with defects in the central serotonergic system. Previously, it was demonstrated that the *anx* mutation is associated with profound serotonergic hyperinnervation in frontal cortex and olfactory areas. We now report that in 20-day-old *anx/anx* mice, specific [^{3}H]Ketanserin binding in the frontal cortex is reduced in comparison with normal littermates. *In vitro* autoradiographic binding methods and computerized image analysis were employed. Binding parameters were determined using nonlinear regression assuming a single-site model. Differences were assessed using t-tests (n=4 per group, * p<0.02).

	Kd (nM)	Bmax (fmol/mg)
Normal	12 ± 3.7	145 ± 15.0
Mutant	7 ± 1.5	71 ± 9.3 *

Although Kds were slightly higher than would be predicted, the location of [^{3}H]Ketanserin binding sites in the frontal cortex strongly suggests that binding is to 5HT$_2$ receptors. There were 51% fewer receptors in the frontal cortex of the mutant mice compared to their normal littermates. Since serotonin hyperinnervation is a key feature of the *anx* mutation, the reduced receptor number most likely represents down-regulation of post-synaptic 5HT$_2$ receptors in response to high synaptic levels of serotonin.

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477.22

REGULATION OF THE 5-HT$_6$ SEROTONIN RECEPTOR IN STABLY TRANSFECTED 293 CELLS. Steven J. Max*, Frederick J. Monsma, Jr.† and David R. Sibley, Molecular Neuropharmacology Section, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892 and †F. Hoffman-LaRoche, AG, Basel, Switzerland.

We investigated the regulatory properties of the cloned rat 5-HT$_6$ serotonin receptor in stably transfected HEK-293 cells. The cell line was characterized using the radioligand ^{3}H-lysergic acid diethylamide (^{3}H-LSD) and exhibited K_d and B_{max} values of approximately 5 nM and 1 pmol/mg protein, respectively. Exposure to 5-hydroxytryptamine (5-HT) resulted in a dose-dependent stimulation of adenylyl cyclase activity in both intact cell and membrane preparations. Stimulation of cAMP accumulation by 5-HT was maximal at 100 µM with an EC_{50} of 1.5 µM. Pretreatment of the cells with 100 µM 5-HT for 20 hours resulted in a sixty percent decrease in the maximal response for cAMP accumulation with no appreciable change in the EC_{50}. This effect was time-dependent, reaching maximal levels at 8 hours with a $t_{1/2} \leq 1$ hour. The 5-HT pretreatment appeared to have no effect on the maximum binding capacity of ^{3}H-LSD nor the K_d value. Treatment of the cells with various intracellular activators of protein kinases had mixed effects on 5-HT$_6$ receptor function. Exposure to 1 µM phorbol-12-myristate 13-acetate (PMA), an activator of protein kinase C, for 20 hours appeared to have no effect on receptor function or binding capacity. On the other hand, treatment with 1 mM 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), an activator of cAMP-dependent protein kinase, resulted in a 55% reduction in the maximal 5-HT cAMP response with no apparent change in potency. In contrast, there was no effect on the B_{max} nor the K_d as measured by ^{3}H-LSD binding. Overall, the data suggest that agonist-induced desensitization of the 5-HT$_6$ serotonin receptor occurs via stimulation of cAMP-dependent protein kinase as measured by a decrease in maximal cAMP accumulation. Furthermore, there does not appear to be a concomitant agonist-induced down-regulation of the 5-HT$_6$ receptor as detected with ^{3}H-LSD.

477.23

EXPERIMENTAL HEPATIC ENCEPHALOPATHY ASSOCIATED WITH CHANGES IN 5-HT BINDING SITES IN THE RAT BRAIN. P.B.F. Bergqvist*, D. Bratanis, G. Apelqvist, M. Bugge, and F. Bengtsson, Dept. of Clinical Pharmacology, Lund University Hospital, S-221 85 Lund, Sweden.

Previous studies on rats suffering from hepatic encephalopathy (HE) have shown a profound increase in the 5-HT turnover in the brain. A recent preliminary microdialysis study by us failed, however, to show any change in the neocortical 5-HT release associated with chronic experimental HE. Further, the effects on different 5-HT receptors in the brain in HE has not been adequately investigated. We studied the brain regional 5-HT$_{1A, 1B,}$ and $_2$ subreceptor types in rats with chronic experimental HE. The regional densities of these 5-HT subreceptor types were determined in 16-25 (depending on 5-HT subreceptor type studied) brain regions by the use of autoradiography. The results showed that the 5-HT$_{1A}$ binding sites were decreased in the HE rats compared to controls only in the dorsal part of the lateral septal nucleus ($p<0.05$) and in the CA1 field of Ammon's horn in the hippocampus ($p<0.05$). The 5-HT$_{1B}$ bindning sites were unaltered in all brain regions investigated in the HE rats, but an increase ($p<0.05$) in the density of the 5-HT$_2$ binding sites in the substantia nigra was observed. We conclude that chronic experimental HE may be associated with some alterations in the density of at least two separate brain 5-HT subreceptor types. Whether or not this observation is related to an indoleamine pertubation of relevance to the pathogenesis of HE should be further addressed in future work.

478.1

DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE AXONS IN RELATION TO MELANOTROPES AND GLIAL-LIKE CELLS OF THE RAT PITUITARY INTERMEDIATE LOBE. B.M. Chronwall[1] and K.A. Gary[2]. [1]School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64108. [2]Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

The majority cell population in rat pituitary intermediate lobe (IL) is the melanotropes which release β-endorphin and α-MSH. Melanotrope secretion is regulated by tonic inhibition via dopaminergic axon terminals. A smaller population of glial-like cells is present in the IL, and synaptic contacts have been described between these cells and IL axon boutons in amphibian and rat. This study utilizes image analysis methods to evaluate the anatomical relationship of tyrosine hydroxylase (TH) immunoreactive axons to cellular elements in the rat IL. Pituitaries were fixed and Vibratome sectioned at 75 μm. Sections were incubated in anti-TH serum, processed for immunohistochemistry, and embedded in Epon. One micron serial sections were toluidine blue stained. Individual melanotropes associated with TH immunoreactive axons were identified, digitally imaged, and followed through successive sections. This process was repeated with individual glial-like cells associated with immunopositive axons. Melanotropes among other melanotropes exhibited lower numbers of TH immunopositive boutons than those sharing cell membrane surface with glial-like cells. Morphometric measurement of cell membrane surface area in single sections revealed higher densities of immunopositive boutons associated glial-like cell structures than with individual melanotropes. Using Image-1 image analysis software, three dimensional reconstruction of individual melanotropes and glial-like cells indicate that TH immunopositive axons were closely apposed with glial-like cell processes and followed these processes over several microns. These observations suggest interactions between dopaminergic axons and glial-like cells of the rat IL.

478.2

GLUTAMIC ACID DECARBOXYLASE mRNA IS EXPRESSED IN BOTH EXCITATORY AND INHIBITORY NEURONS IN CULTURED HIPPOCAMPAL NEURONS. [1,2]M.A. Dichter*, [2]Y. Cao, [1]K.S. Wilcox, [2,3]J. Eberwine, Depts. of [1]Neurology, [2]Pharmacology, and [3]Psychiatry, Univ. of Penn. School of Medicine and the Graduate Hospital, Phila, PA 19104

The recently developed procedure of single cell mRNA amplification, when coupled with the whole cell patch clamp technique, can be used to investigate the expression and regulation of mRNA of a variety of proteins in identified cell types. The goal of the present set of experiments was to develop specific molecular "profiles" of excitatory and inhibitory neurons. It was hypothesized that the mRNA encoding glutamic acid decarboxylase (GAD), the enzyme which catalyzes the conversion of glutamic acid to GABA, would be present in detectable quantities only in neurons which utilized GABA as a neurotransmitter. Experiments were performed in low density hippocampal cultures. The whole cell patch clamp technique was used to record from monosynaptically connected pairs of neurons. The presynaptic neurons were positively identified as either excitatory or inhibitory based on the reversal potential and waveform of the evoked postsynaptic current. Excitatory neurons release neurotransmitter which activates postsynaptic glutamate receptors, whereas inhibitory neurons use GABA as a neurotransmitter. Ten neurons were identified as inhibitory and six neurons were identified as excitatory. Inclusion of dNTPs and reverse transcriptase in the recording pipettes insured that amplification of cellular mRNA would be initiated during the recording. Messenger RNA for GAD65 was found in all sixteen neurons at a relatively high level when compared on slot blots to brain derived neurotrophic factor. However, immunocytochemisty for GABA and GAD in the cultures demonstrated that the protein was only being expressed in approximately 40% of the neurons. These results suggest that neurotransmitter phenotype may be posttranscriptionally regulated. Supported by AG9900 (JE).

478.3

COMPARISON OF GLUTAMIC ACID DECARBOXYLASE (GAD) IMMUNOREACTIVITY IN DORSOLATERAL SEPTAL (DLSN) NEURONS RESPONSIVE AND UNRESPONSIVE TO NICOTINIC AGONISTS E.M. Sorenson*, W.C. McDaniel and J.P. Gallagher. Dept. of Pharm. & Tox., Univ. of Texas Med. Br., 301 University Blvd. Galveston, TX 77555-1031

Using intracellular recording, we have previously reported that nicotine and 1,1-methyl-4-phenylpiperazinium (DMPP) hyperpolarize the majority of DLSN neurons by a postsynaptic mechanism. We have now compared the morphology, electrophysiology and GAD immunoreactivity of DMPP responsive (RES) and unresponsive (UNR) neurons.

Neurobiotin (1%, Vector) was injected into neurons at the end of recording sessions. After cutting the fixed tissue into 75 μM sections, injected neurons were visualized using the ABC method (Vector) with DAB as the chromogen. Sections containing stained neurons were processed for GAD immunohistochemistry using primary antibody at a 1:500 (Johansen et al., 1989) or 1:2000 (Chemicon) dilution. Vector® SG was the second chromogen.

Of the 26 neurons visualized, 18 were DMPP RES (69%). Neither membrane potential nor input resistance were significantly different between DMPP RES and UNR neurons. Furthermore, no other electrophysiological differences were observed, such as, the occurrence of low threshold calcium action potentials, AHPs following single action potentials or slow ADPs following a train of action potentials. DMPP RES cells were either multi- or bipolar whereas the DMPP UNR neurons were all multipolar. While no morphological differences were apparent between multipolar DMPP RES and UNR cells, all of the DMPP UNR cells tested (6/6) were GAD-negative whereas only 60% (6/10) of the DMPP RES cells were GAD-negative. In summary, DMPP UNR cells appear to be GAD-negative, multipolar neurons. Supported by DHHS 1F32 DA 05447 to EMS and The Council for Tobacco Research, USA, Inc. to JPG.

478.4

LOCALIZATION OF MONOAMINE OXIDASE A (MAO-A) IN RAT BRAIN AND IN VITRO REGULATION. J.W. Jahng[1], T.C. Wessel[1]*, K. Chen[2], K.S. Kim[1], and J.C. Shih[2]. Burke Med. Res. Inst., Cornell Univ.[1], White Plains, NY 10605 and Dept. of Molec. Pharmacol. and Toxicol., USC[2], Los Angeles, CA 90033.

The distribution of MAO-A gene products in the adult rat brain and adrenal medulla (AM) was examined by *in situ* hybridization and by immunocytochemistry with a polyclonal antiserum. The highest levels of MAO-A mRNA and protein expression were found in the locus ceruleus; moderate levels were detected in the serotonergic dorsal raphe nucleus (DRN), habenula, and the adrenergic neurons of the medulla oblongata; low levels were found in the substantia nigra compacta (SNc), hypothalamus, hippocampus, and the cortex. Northern blot analysis of microdissected brain regions confirmed this distribution. Compared to young adult rats, aged rats showed a clear increase in MAO-A mRNA in the SNc, DRN, and AM by *in situ* hybridization. In vitro experiments using the rat PC12 cell line revealed no significant upregulation of the MAO-A gene in response to the protein kinase A (PKA) activator, forskolin. This is in contrast to the rapid increases observed in tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) mRNAs. PKA-deficient pheochromocytoma cell lines showed normal MAO-A mRNA levels but decreased TH and DBH levels, suggesting that the basal transcription of the MAO-A gene is not dependent on cyclic AMP. Promoter analysis of the MAO-A gene will further delineate the responding cis-acting elements in this important gene. Supported by NIMH grants 48866, 39085, 37020, 00796 and the Welin Professorship to J.C.S.

478.5

CLONING OF A NOVEL MONOAMINE OXIDASE (MAO) cDNA FROM TROUT LIVER K. Chen, H.-F. Wu, J. Grimsby and J.C. Shih*. Dept. Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033.

A trout liver monoamine oxidase (MAO) cDNA was cloned by screening a cDNA library with a human MAOA cDNA probe. The trout MAO cDNA encodes 499 amino acids with a molecular weight of 56.6 KDa. The deduced amino acid sequences between trout MAO and human MAOA or MAOB show 70% and 71% identity respectively. Trout MAO contains the pentapeptide sequence Ser-Gly-Gly-Cys-Tyr motif in which the cofactor FAD is covalently bound. Transient expression of the cDNA in COS7 cells shows that trout MAO oxidizes both serotonin (5-HT) and ß-phenylethylamine (PEA), unlike human MAOA or MAOB which oxidizes only 5-HT or PEA, respectively. The Km for 5-HT is similar for trout MAO (188.0 μM) and human MAOA (119.3 μM). The Km for PEA is similar for trout MAO (4.8 μM) and human MAOB (4.6 μM). When 5HT was used as a substrate, similar to human MAOA, trout MAO is more sensitive to clorgyline (IC_{50} 2.0 x 10^{-8} M) than deprenyl (1.1 x 10^{-6} M). However, trout MAO is less sensitive to clorgyline than human MAOA (3.7 x 10^{-10} M). Trout MAO is less sensitive to deprenyl (IC_{50} 5.0 x 10^{-7} M) than human MAOB (IC_{50} 2.7 x 10^{-9} M) when using PEA as the substrate. These results indicate that trout MAO displays substrate and inhibitor selectivities that are not identical to either MAOA or B and therefore represents a novel type of MAO. The structure of trout MAO will provide insights into the substrate and inhibitor selectivity of the MAOs. (Supported by NIMH grants R37 MH39085 (MERIT Award), R01 MH37020 (Research Scientist Award), K05 MH00796 and Welin Professorship).

478.6

DYNAMICS OF SEROTONIN-DEGRADATIVE PATHWAYS IN THE BRAIN OF SOME FROGS. Naokuni Takeda*, Department of Biotechnology, COSMO Research Institute, Satte, Saitama, 340-01, Japan

Bufotenine(BUTN) has been found in the brain of *Bufo bufo japonicus* (Soc. Neurosci.19,1171; Comp.Biochem.Physiol.107C,275,'94). The main pathways are as follows; Serotonin - *N*-methyl serotonin(*N*-MET) - BUTN and Serotonin - *N,N*-di methyltryptamine (DMT). In the brain of *Rana japonics*, serotonin was degraded to N-MET, but not to BUTN. The pathway to DMT was not found. By the injection of NIALAMIDE into the body cavity, amounts of each monoamine were highly increased. Furthermore, N-MET was gradually degraded to BUTN, and the pathways to DMT were also detected. In *Bufo*, NIALAMIDE was directly injected into the brain by micro-dialysis. The amounts of N-MET, BUTN and DMT were highly increased. The monoamine oxidase inhibitor was shown to evoke the appearance of intrinsic metabolic pathways from Serotonin to BUTN and to DMT.

478.7

LOCALIZATION OF TRANSMITTER RECEPTORS IN THE CHICK BRAIN IN RELATION TO AUDITORY IMPRINTING. R. Schnabel and K. Braun.*Federal Institute for Neurobiology, 39118 Magdeburg, FRG

The learning process of auditory imprinting in the domestic chick is associated with a significant, regionally restricted increase in the incorporation of radioactive deoxyglucose in the rostro-medial neostriatum/hyperstriatum (MNH), the lobus parolfactorius (LPO) and a dorsal part of the caudal neostriatum (Ndc). After successful imprinting the MNH shows a marked decrease in spine density and the remaining synapses have prolonged postsynaptic densities. We assume that these synaptic changes are controlled by transmitter/receptor systems and that changes at the receptor level may occur during the learning process.

We investigated the distribution and density of the glutamatergic NMDA and kainate (KA) receptors, the dopaminergic D1 and D2 receptors, and the GABA A receptor associated benzodiazepine binding sites by ligand autoradiography .

The MNH expressed a high density of NMDA and benzodiazepine receptors homogeneously. D1 and KA receptors showed a relative low density in the neostriatal part of MNH compared to the hyperstriatal part where a significant higher density could be observed. In Ndc a high density of NMDA, benzodiazepine and D1 receptors was detected. KA showed a low binding level. The LPO expressed a high density of D1, a moderate density of KA receptors, and a low density of NMDA and benzodiazepine receptors. D2 receptors were not detectable in the MNH and Ndc but clearly visible in the LPO. Generally, an almost complementary localization of NMDA and KA receptors was found throughout the forebrain.

We have started to compare the receptor densities in imprinted, unimprinted (trained but not imprinted) and naive, isolated chicks at several time points after imprinting.

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478.8

NMDA RECEPTOR IMMUNOSTAINING IN MOUSE DORSAL COCHLEAR NUCLEUS AND CEREBELLUM. M. Bilak, S. Bilak, and K. Morest*, Anatomy Dept., Univ. CT Health Ctr., Farmington 06030.

With mAb to NMDAR1 (Pharmingen) light microscopic labeling (Vectastain) of cell surfaces was the same in 3 ICR adults. In cerebellum Purkinje cell body staining varied from intense to negative; dendritic staining was only on 1°/2° shafts. No labeling of 3° dendritic, synaptic, or glial structures was evident in the molecular layer. Golgi II cell bodies were only moderately stained, but more intense labeling was associated with granule cell bodies, dendrites, and rosettes. In dorsal cochlear nucleus staining of fusiform cell bodies varied; their dendritic shafts were negative. In the molecular layer there was an intense pattern resembling that of the 3° dendrites reflecting the synaptic form of parallel fibers, as seen by synaptophysin immunostaining. Granule cells were negative. There was also staining of cartwheel, corn, and giant cell bodies and dendrites. We conclude that a significant component of the NMDA receptors differs in the granule cells, inhibitory interneurons, and projection neurons of cerebellum and cochlear nucleus. This may reflect differing roles for NMDAR1 in the processing carried out in these different circuits.

Supported by NIH grants R01DC12716, T32DC00025.

478.9

IMMUNOPURIFICATION AND CULTURE OF SEPTAL CHOLINERGIC NEURONS FROM RAT EMBRYOS. P. Taupin*, D.A. Peterson, J. Ray and F.H. Gage, Dept. Neurosciences, UCSD, La Jolla, CA 92093-0627, USA

The ability to investigate primary cholinergic neurons of the central nervous system *in vitro* is limited due to the presence of a low number of cholinergic neurons. Attempts to enrich this population *in vitro* has met with some limited success. We used antibodies to choline acetyltransferase (ChAT) to purify the cholinergic neurons of the septal area from E 17 Fischer rat embryos, by immunomagnetophoresis. At this developmental stage, these neurons may express a membrane associated form of ChAT. This form may be important for neuron-neuron and neuron-glia interaction during the neurogenesis of the cholinergic neurons. The enrichment in cholinergic neurons was assassed by ChAT enzymatic assay and by immunocytofluorescence with double and triple staining using antibodies against ChAT, neurofilament and GFAP. A highly enriched population of viable cholinergic neurons was isolated. After purification, the cells were plated at a low density, in the presence of bFGF (20 ng/ml) and cell culture conditioned medium. The cholinergic neurons grew and extended processes. These results suggest that growth factors and environmental signals influence the growth of the purified cholinergic neurons in culture. This method will allow for the purification of other types of cells (e.g. dopaminergic neurons) expressing membrane associated form of neurotransmitter synthesizing enzymes. We are presently examining antibodies against the low and high affinity NGF receptors as reagents for immunopurification of cholinergic neurons. The diversity of populations of septal cholinergic neurons and the potential of these cells to proliferate and differentiate *in vitro*, in presence of trophic factor(s), are currently under investigation. Supported by France Parkinson and NIH.

478.10

IMMUNOHISTOCHEMICAL LOCALIZATION OF D-SERINE IN RAT BRAIN. M.J. Schell * and S.H. Snyder. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent biochemical evidence indicates that D-serine comprises fully 30% of the total pool of free serine in many areas of the telencephalon. Since D-serine--but not L-serine--is a potent agonist at the glycine modulatory site of the NMDA receptor, its presence in areas known to contain abundant NMDA receptors makes it a candidate neurotransmitter. To address this possibility, we have generated a highly specific antibody against glutaraldehyde conjugates of D-serine. In dot blot screens against various amino acids coupled to dialyzed rat brain protein, the antiserum diluted 1:5000 detected 2 pmoles of D-serine with a 1000-fold selectivity over L-serine, L-glutamate, glycine, and D-aspartate conjugates. The signal was blocked by glutaraldehyde\D-serine conjugates. Immunohistochemistry revealed a heterogeneous distribution of D-serine in rat brain that matched the regional distributions reported with biochemical techniques. Intense staining was observed in the gray matter of the cerebral cortex, striatum, and hippocampus. Less intense staining was seen in the olfactory bulb, and staining in the cerebellum and brainstem was undetected. Liquid phase glutaraldehyde conjugates of D-serine but not L-serine abolished staining. Stained cells were scattered throughout the striatum and hippocampus and had a morphology resembling small interneurons. Stained cell bodies were also seen isolated in certain white matter tracts, suggesting that D-serine is also present in some glia. These results show a distribution of D-serine consistent with its possible role as an endogenous ligand for the NMDA receptor.

478.11

LOCALIZATION OF TAURINE AND GLUTAMATE IN PRIMARY OLFACTORY NEURONS IN THE RAT. A. Didier*(1), O.P. Ottersen(2) and J. Storm-Mathisen(2), Lab Physiologie Neurosensorielle, URA 180, UCBL, F-69622 Villeurbanne, France(1) and Anatomical Institute, University of Oslo, PO Box 1105 Blindern, N-0317, Oslo, Norway(2).

Glutamate and taurine are widely distributed aminoacids in the CNS. In the olfactory system, information on their precise cellular distribution, critical with regard to their function in this system is still lacking. EM semi-quantitative immunocytochemistry of taurine and glutamate was performed, using previously fully characterized antibodies raised in rabbits against glutareldehyde conjugates of glutamate or taurine. Ultrathin sections of plastic-embedded olfactory bulb were incubated with the primary antibody and subsequently treated with a second antibody coupled to colloidal gold. We report here analysis of labelling in the peripheral layers of the OB (olfactory nerve layer and glomerular layer). Taurine and glutamate are co-localized in the primary olfactory terminals within glomeruli. The density of gold particles was about 50% higher in primary olfactory terminals profiles compared to dendritic profiles for glutamate ($p < 0.0001$) and taurine ($p < 0.05$). In addition, quantification of glutamate labelling of the olfactory axons running in the olfactory nerve layer revealed that the particle density in primary terminals is 40 % higher than in the axons ($p < 0.0001$). The opposite gradient was found for taurine ($p<0.005$).

The enrichment in glutamate of the olfactory primary terminals compared to their axons is a strong argument favoring the hypothesis of a neurotransmitter role for glutamate in olfactory receptors. Further functional studies are required to resolve the role of taurine in these receptors.

478.12

AUTORADIOGRAPHIC DETECTION OF REDUCED SEROTONIN UPTAKE SITES IN RAT BRAIN FOLLOWING REPEATED ADMINISTRATION OF (+) 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) R. Lew*, K.E. Sabol G. Vosmer and L.S. Seiden, Dept. Pharmacology and Physiology, University of Chicago, Chicago, IL 60637.

In the present study, [^{125}I]RTI-55 autoradiography to serotonin uptake sites showe repeated MDMA administration to cause a long lasting reduction in serotonin uptake sites in rat brain that persisted up to 52 weeks. Age-matched Holtzman Sprague-Dawley rats (275 - 300 g) were injected i.p with either saline (0.9% NaCl) or MDMA (20 mg/kg free base) twice daily for 4 days and allowed to recover for 2, 8, 16, 32 an 52 weeks. Following recovery, brains from saline and MDMA treated animals were histologically processed for autoradiography. Coronal brain sections (10 µm) were incubated for 120 min with [^{125}I]RTI-55 (40 pM) in the absence and presence of the HT uptake blocker citalopram (300 nM). Afterwards sections were washed in PBS a air dried. Autoradiograms were generated using Kodak X-ray film and quantitated using Image 1.51. Preliminary autoradiography studies showed inclusion of RTI-121 (100 nM) and propranolol (10 µM) could inhibit [^{125}I]-RTI-55 binding to dopamine uptake sites and lipid sites without affecting binding to serotonin uptake sites. In the experiment proper, [^{125}I]-RTI-55 autoradiography showed an almost complete loss o serotonin uptake sites in prefrontal, frontal and somatosensory cortex, thalamus, hypothalamus and hippocampus in brain sections from MDMA treated animals that had recovered for only 2 weeks (1.8 - 4.8 % of control). By 16 and 52 weeks after treatment, the density of serotonin uptake sites in the above regions had recovered to 52 - 65 and 65 - 79 % respectively of control, suggesting regeneration of serotonerg terminal fields. In contrast, cell body regions such as substantia nigra, ventral tegmental area and dorsal raphe were not affected by MDMA treatment. In conclusic repeated treatment with high doses of MDMA causes a reduction of serotonin uptake sites that persists up to 52 weeks following administration and that this effect is confined to terminal fields. (This work was supported by research grant DA 00085; L.S.S is supported by RSA MH-105 62)

478.13

COMPARATIVE IMMUNOCYTOCHEMISTRY OF GLYT1 and GLYT2 GLYCINE TRANSPORTERS IN MOUSE AND RAT BRAIN. F.Jursky, S.Tamura and N. Nelson*. Roche Institute of Molecular Biology, Roche Research Center, Nutley NJ 07110)

Using immunocytochemistry with polyclonal antibodies raised against recombinant fusion proteins, as well as conjugated oligopeptides, we compared the distribution of the GLYT1 and GLYT2 glycine transporters in mouse and rat brain. GLYT1 consists of two almost identical subtypes GLYT1a and GLYT1b differing only in a stretch of 10 aa in the N-terminus. We raised antibodies against the common C-terminal part as well as the N-terminal region specific for the GLYT1b subtype . Both antibodies gave the same patern in both Western blot analysis and on brain sections examined with light microscopy. This suggests that the major pattern of immunoreactivity arises in both cases from the GLYT1b subtype. GLYT2 was localized in the rat brain in the same pattern as in the mouse : it was most abundant in the gray matter of the brainstem, less in the cerebellum, midbrain, thalamus, with only traces in pallidum, cerebral cortex, hippocampus and was completely absent in the olfactory bulb. In contrast to localized presence of the strychnine matching GLYT2, GLYT1 immunoreacivity was distributed throughout the whole brain. It was most abundant in the gray and white matter of the brainstem, cerebellum, midbrain, thalamus, olfactory bulb and less present in the cerebral cortex and hippocampus. While GLYT2 immunoreactivity was localized exclusively in the varicosities and processes, GLYT1 antibody stained processes and round shaped cells distinguishable mainly in the cerebral cortex and hippocampus where the GLYT1 immunoreactivity is more scarce.

478.14

PHARMACOLOGICAL PROPERTIES OF [^{3}H]3-OH-PCP BINDING SITES IN RAT BRAIN BY AUTORADIOGRAPHIC STUDIES T.Suzuki[1]*, T.Yamamoto[2], S.Abe[1], T.Hori[1], T.Moroji[2], H.Shiraishi[1], T.Ito[3], I.K.Ho.[3] 1)Dept. of Psychiatry, Univ. of Tsukuba, 2)Dept. of Psychopharmacology, Tokyo Institute of Psychiatry, 3)Dept. of Pharmcology & Toxicology, Univ. Mississippi Medical Center.

[^{3}H]3-OH-PCP binding in rat brain was examined using an autoradiographic study. [^{3}H]3-OH-PCP binding reached equilibrium by 90 min at 22°C. The B_{max} and $(Kd)_{app}$ values were determined in 11 discrete regions. [^{3}H]3-OH-PCP binding appeared to label a single high affinity site with $(Kd)_{app}$ of 3.5 to 5.5 nM. The highest level of density was observed in the stratum oriens of the CA1. Relatively high amounts of binding were present in the dentate gyrus, superficial layer of cerebral cortices. In contrast, little binding was found in the globus pallidum, cerebellum and brain stem. Both MK801 and 3-OH-PCP were more potent than SKF10047 in displacing [^{3}H]3-OH-PCP binding sites in a monophasic manner in the different regions. D-AP5(NMDA antagonist), 7-chlorokynurenic acid(glycine antagonist), arcaine and diethylenetriamine inhibited [^{3}H]3-OH-PCP binding with 1.0 of n_H. These results suggest that [^{3}H]3-OH-PCP binds to NMDA/PCP ionchannel complex in preference to sigma site.

478.15

SPATIO-TEMPORAL HETEROGENEITY OF THE GENE EXPRESSION FOR THE *N*-METHYL-D-ASPARTATE RECEPTOR CHANNEL SUBUNITS IN THE MOUSE NERVOUS SYSTEM. M. Watanabe* and Y. Inoue, Dept. of Anatomy, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan

A number of electrophysiological, pathophysiological, and pharmacological studies have shown the functional heterogeneity of the *N*-methyl-D-aspartate (NMDA) receptor channel in the nervous system. In order to clarify relationships between the molecular anatomical organization of the channel subunits and the functional heterogeneity, *in situ* hybridization histochemistry has applied to reveal spatio-temporal expressions in the mouse nervous system, of five NMDA receptor channel subunit mRNAs designated as the ε1-4 and the ζ1 subunit mRNAs. The ε1 subunit mRNA is expressed postnatally and distributed widely in the central nervous system at mature stages. On the other hand, the ε2 subunit mRNA is found throughout the fetal central nervous system, but its expression becomes restricted to the forebrain and several subregions of the brainstem and spinal cord in the postnatal maturation. The ε3 subunit mRNA appears after birth and is found predominantly in the cerebellar granule cells. Although high transcriptic signals are detected widely in the central nervous system at fetal stages, signal levels of the ε4 subunit mRNA decrease to the background level in the postnatal maturation. As a consequence of the drastic developmental changes, heterogeneous organization of the ε subunit mRNAs, whose distributions are consistent with the known pharmacological and electrophysiological heterogeneity of the channel, is accomplished in the central nervous system by the postnatal third week. In contrast, the ζ1 subunit mRNA is expressed ubiquitously throughout the central and peripheral nervous system at various developmental stages. These findings suggest that the NMDA receptor channel undergoes a developmental reorganization of the ε subunits, through which the central nervous system would acquire the channel with functional diversity and spatial heterogeneity.

478.16

SIGMA RECEPTOR LOCALIZATION IN RAT STRIATUM AND HIPPOCAMPUS. **G.M. Gonzalez-Alvear*, D. Thompson-Montgomery and L.L.Werling. Dept. Pharmacology, The George Washington University Medical Ctr., Washington, D.C. 20037.**

We have previously reported that the sigma agonists (+)pentazocine and BD737 inhibited NMDA-stimulated [^{3}H]DA and [^{3}H]NE release from rat striatal and hippocampal slices, respectively, in a concentration-dependent manner. We have now investigated the effects of these sigma ligands on NMDA-stimulated catecholamine release in the presence of tetrodotoxin (TTX).

Striata or hippocampi were dissected, chopped, and washed in Mg$^+$-free modified Krebs-HEPES buffer, then incubated with 15 nM [^{3}H]DA or 50 nM [^{3}H]NE, respectively, for 30 min. Slices were loaded into a superfusion apparatus and superfused with buffer containing 1 μM TTX. Tissue was stimulated to release [^{3}H]DA or [^{3}H]NE by a 2 min exposure to 100 μM NMDA. After return to the non-stimulating buffer, tissue was stimulated a second time for 2 min in the presence of a sigma agonist.

Tetrodotoxin blocks the propagation of action potentials and thereby eliminates the contribution of interneuron-mediated effects. The addition of TTX to the superfusion medium had no effect on the inhibition of [^{3}H]DA release by (+)pentazocine (1 μM) and BD737 (1 μM). Both sigma agonists inhibited approximately 40% of NMDA-stimulated DA release from rat striatal slices in the presence of TTX. In addition, TTX (1 μM) had no effect on NMDA-stimulated [^{3}H]DA release in the absence of a sigma agonist. In contrast, (+)pentazocine (500 nM) and BD737 (100 nM) failed to inhibit NMDA-stimulated [^{3}H]NE release from rat hippocampal slices in the presence of TTX. Furthermore, TTX alone had no effect on NMDA-stimulated [^{3}H]NE release in the absence of (+)pentazocine or BD737. Therefore, whereas it appears that sigma receptors regulating dopamine release in the striatum are located on dopaminergic nerve terminals, sigma receptor-mediated regulation of NE release in the hippocampus appears to require functional interneurons. (Supported by a grant from NIDA to LLW and by NIGMS predoctoral fellowship to GMG.)

478.17

COMPARISON OF SIGMA RECEPTOR BINDING IN MOUSE BRAIN AND SPINAL CORD. K. J. Kovács, D. Budai* and A. A. Larson. Department of Veterinary Pathobiology, University of Minnesota, Saint Paul, MN 55108.

Sigma binding sites in mouse brain areas and spinal cord were characterized using labeled (+)pentazocine (PENT) and 1,3-di(2-tolyl)guanidine (DTG). [^{3}H](+)PENT, a selective ligand for sigma$_1$ sites, labeled a single population of binding sites (K_d=1.19-1.69 nM) with an increasing density along the neuraxis yielding B_{max} values of (in fmol/mg protein) 543 in the cortex, 725 in the cerebellum and 934 in the spinal cord. [^{3}H]DTG is a nondiscriminant ligand with high affinity for both sigma$_1$ and sigma$_2$ sites and with moderate affinity for a recently described low affinity site. [^{3}H]DTG labeled two sites in mouse cortex, cerebellum and spinal cord membranes. The K_d (8.1-9.6 nM) and B_{max} (617-687 fmol/mg protein) values calculated for the high affinity [^{3}H]DTG-labeled sites did not differ in these tissues. [^{3}H]DTG was displaced by (+)PENT and (+) N-allylnormetazocine (SKF 10047) in a clearly biphasic manner. These ligands inhibited only approximately 30% of [^{3}H]DTG high affinity binding in all tissues indicating the presence of [^{3}H]DTG-labeled high affinity sigma$_2$ sites throughout the CNS. Besides its high affinity sites, [^{3}H]DTG labeled an other, bigger receptor population with lower affinity whose K_d and B_{max} values were significantly higher in the spinal cord than in the cortex. Experiments with boiled membranes showed that a portion of those low affinity [^{3}H]DTG binding sites represent ligand binding to heat-resistant nonspecific sites. *(Supported by USPHS DA04090).*

478.18

QUANTIFICATION OF THE SEROTONIN HYPERINNERVATION IN ADULT RAT NEOSTRIATUM AFTER NEONATAL LESION OF ITS DOPAMINE INNERVATION BY INTRAVENTRICULAR 6-OHDA. A. Mrini*, J.P. Soucy, F. Lafaille, P. Lemoine and L. Descarries. Centre de recherche en sciences neurologiques and Département de pathologie, Université de Montréal, Montréal, Québec, Canada.

A previously described technique (Doucet et al., *Brain Res.* 441:233, 1988) was used to quantitate serotonin (5-HT) innervation density after the specific labeling of these axon terminals (varicosities) by uptake and storage of [^{3}H]5-HT, in transverse rat brain slices radioautographed as 4 μm-thick sections of fixed and resin-embedded tissue. With the aid of an image analysis system, counts of the labeled varicosities were obtained from 3 sectors, dorsal, mediolateral and ventral, at each of 3 transverse levels, rostral (r), intermediate (i) and dorsal (d), across the neostriatum (NS). Eight 3-month-old rats, cerebroventricularly injected with 6-OHDA at 3 days (50 μg f.b. on each side, after DMI pretreatment) and seven littermate, normal controls were sampled. After correction for incomplete radioautographic exposure and for section thickness, and based on previous electron microscopic measurements of the average diameter of these terminals, the results could be expressed in millions of axon varicosities per mm3 of tissue. Control values ranged from 4.8 in rNS to 6.3 in cNS (5.8 in iNS), for an average of 5.6, somewhat higher than previously reported (Soghomonian et al., *Brain Res.* 425:85, 1987). The corresponding values for hyperinnervated NS ranged from 9.7 rostrally to 7.7 caudally (8.8 in iNS), for an average of 8.7 and increases of 22%, 52% and 102% above control in the cNS, iNS and rNS, respectively (average increase of 55%). These results confirm the predilection of the 5-HT hyperinnervation for the rNS, but also demonstrate its presence in the cNS, as well as an inversion of the normal gradient of neostriatal 5-HT innervation density under these conditions. (MRC grant MT-3544).

478.19

AUTORADIOGRAPHIC DISTRIBUTION OF NICOTINIC RECEPTOR SITES LABELED WITH [^{3}H]CYTISINE IN THE HUMAN BRAIN: COMPARISON WITH MUSCARINIC RECEPTORS. I.Aubert*, D.Cécyre, S.Gauthier and R.Quirion. Douglas Hospital Research Center, Departments of Psychiatry, Neurology & Neurosurgery McGill University, Montreal, Quebec, Canada H4H 1R3.

The localization of nicotinic (nAChR) and muscarinic receptor sites might provide insights on the organization of the cholinergic synapse in various brain areas. Using *in vitro* receptor autoradiography, the distribution of nAChR ([^{3}H]cytisine, 20 nM), muscarinic M_1 ([^{3}H]pirenzepine, 15 nM) and M_2 ([^{3}H]AF-DX 384, 2 nM) binding sites was investigated in postmortem human brains from Alzheimer's, parkinsonian and control patients. [^{3}H]Cytisine generated high qualities autoradiograms in comparison to previously used nAChR radioligands. The lateral geniculate body and the thalamus contained the highest amounts of nAChR sites. In the frontal, temporal and entorhinal cortices, the middle layers contained high amounts of nAChR sites. In the hippocampal formation, [^{3}H]cytisine labeling is enriched in the subiculum and dentate gyrus. The molecular layer of the cerebellum is also enriched in nAChR sites. In the frontal and temporal cortices, the distributions of M_1 (superficial laminae) and M_2 (deep laminae) sites are distinct from that of nAChR, while in the entorhinal cortex, hippocampal formation, lateral geniculate body and thalamus, the localization of M_2 sites is similar to that of nAChR. However, in the cerebellum, M_2 sites are localized mostly in the granule cell layer. In addition to nAChR sites, M_1 receptors are detected in the substantia nigra. The striatum is enriched with nAChR, M_1 and M_2 sites. A detailed quantitative analysis could clarify some of the current discrepancies regarding to the status of cholinergic receptors in neurodegenerative disorders. Supported by the Alzheimer Society of Canada and MRCC.

478.20

A volkensin lesion of striatonigral neurons produces a marked decrease in mRNA expression for the m4 subtype of the muscarinic acetylcholine receptor. M.B.Harrison*, M.Tissot and R.G.Wiley+, UVA, Charlottesville, VA 22908 and +DVAMC and Vanderbilt Univ., Nashville, TN 37212

By *in situ* hybridization, 3 subtypes of the muscarinic acetylcholine receptor are expressed in rat striatum, the m1 subtype in the majority of striatal neurons and the m2 subtype, a probable autoreceptor, in cholinergic interneurons which also express m1 and m4. The m4 subtype is consistently expressed on striatal neurons which express PPT mRNA but only in a subgroup of neurons expressing PPE mRNA. This colocalization data suggests that m4 mRNA may be preferentially expressed by striatonigral neurons. To further define the localization of the m1 and m4 receptor, striatonigral neurons were lesioned by injection of the suicide transport agent volkensin into the left substantia nigra of adult male rats (n=5). After 10 days, alternate sections through the striatum were hybridized with ^{35}S-labelled oligonucleotide probes for m1 or m4, exposed to film and optical density (OD) determined. Expression of m4 mRNA decreased by 63% (OD 0.134 ± 0.007 vs 0.05 ± 0.006; mean ± se, $p<0.01$), with an 18% decrease in m1 mRNA (OD 0.311 ± 0.03 vs 0.256 ± 0.03, $p<0.05$). These results are consistent with the preferential localization of m4 receptors to striatonigral neurons and suggest that acetylcholine release may differentially affect the two striatal efferent projections.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS III

479.1

DIFFERENTIAL EXPRESSION OF SOMATOSTATIN RECEPTOR SUBTYPES QUANTIFIED BY POLYMERASE CHAIN REACTION (q-PCR) IN MOUSE EMBRYONIC HYPOTHALAMIC CELL CULTURE. C. Viollet, A. Faivre-Bauman, R. Gardette*, C. Kordon,C. Llorens-Cortes, C. Loudes and J. Epelbaum U.159 and U. 36 INSERM, Paris, 75014 and 75005, France.

By whole cell patch clamp, somatostatin (SRIF) (0.1 µM) significantly decreased glutamate sensitivity by a pertussis toxin-dependent mechanism in neuronal cultures from foetal (E16) mouse hypothalamus after the onset of neuronal maturation [14 days in vitro (div)]. Since five subtypes of G-protein linked SRIF receptors have recently been cloned, we developed a q-PCR in order to detect which subtypes are expressed in the developing hypothalamus. At 14 div, SSTR1 mRNA levels were twice higher than those of SSTR2 and SSTR4 while SSTR3 and SSTR5 mRNAs were not detected. By ^{125}I-SRIF receptor radioautography, a small population of hypothalamic neurons (<10 %) was endowed with SRIF binding sites localized on both cell bodies and processes. Octreotide, a SSTR2/SSTR5 prefering agonist displaced 70 % of total ^{125}I-SRIF specific binding displaceable by SRIF14 while BIM 23056, a SSTR3 selective agonist, was totally inactive. These results indicate that the great majority of neuronal SRIF binding sites in primary hypothalamic cell cultures corresponds to the SSTR2 subtype and that no direct correlation occurs between the amount of mRNA expressed and the SRIF receptor protein moieties.

479.2

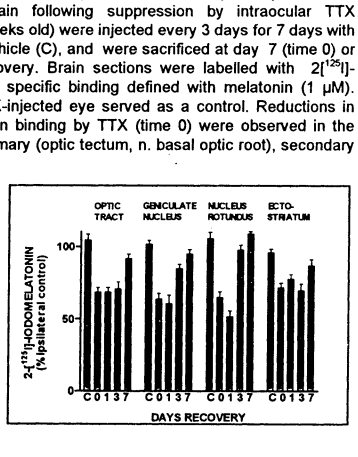
LOCALIZATION AND CHARACTERIZATION OF ML-2 BINDING SITES WITH 2-[^{125}I]-MCA-NAT TO CNS AND PERIPHERAL TISSUES OF VARIOUS SPECIES. E.J. Molinari[1], P.C. North[2], and M.L. Dubocovich[1]*. [1]Dept. Mol. Pharmacol. and Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 606ll, and [2]Dept. Medicinal Chemistry, Glaxo, Ware, UK.

2-[^{125}I]-MCA-NAT (2-[^{125}I]-5-methoxy-carbonylamino-N-acetyltryptamine) selectively labels ML-2 binding sites in hamster brain membranes (Dubocovich, et al., IUPHAR Abstract, 1994, in press). Here we report the pharmacological characteristics of the ML-2 binding site in hamster kidney and testis as well as the presence of specific ML-2 binding to brain membranes of various species. The binding of 2-[^{125}I]-MCA-NAT to whole washed hamster brain, testis and kidney membranes shows high affinity, saturable binding (Kd = 328 ± 104 pM; B_{max}= 20 ± 1.9 fmol/mg protein, n=3) and rapid kinetics of association/dissociation as demonstrated for binding of 2-[^{125}I]-iodomelatonin to the ML-2 receptor. In testis and kidney, melatonin analogues competed for 2-[^{125}I]-MCA-NAT binding with an order of pharmacological affinities [2-iodomelatonin > 6-chloro-2-methyl-melatonin > 6-chloromelatonin > prazosin > 5-MCA-NAT > N-acetylserotonin > luzindole >> serotonin] identical to that reported in hamster brain (r = 0.989, slope: 0.80, n=10, brain vs. testis; r = 0.989, slope: 1.1, n=4, brain vs. kidney). The specific binding of 2-[^{125}I]-MCA-NAT (55-65 pM) sites is similar (fmol/mg protein) in brain (4.6 ± 0.4, n=26), testis (6.3 ± 0.2, n=16) and kidney (5.1 ± 0.4, n=12). High affinity specific 2-[^{125}I]-MCA-NAT binding was also demonstrated in C3H/HeN mouse, but not in rabbit, rat, guinea pig and chick brain membranes. In conclusion, 2-[^{125}I]-MCA-NAT allowed detection of specific ML-2 binding sites in CNS and peripheral tissues of hamster and mouse brain. These results suggest that the distribution of ML-2 sites is widespread. Supported by a Glaxo grant and a USPHS MH-42922 grant to MLD.

479.3

PLASTICITY OF ML-1 MELATONIN RECEPTORS IN CHICK BRAIN VISUAL AREAS FOLLOWING INTRAOCULAR TETRODOTOXIN (TTX) M.L. Dubocovich[1], S. Iacob[1] and D. N. Krause[2]*. [1]Dept. Molec. Pharmac. Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 606ll, & [2]Dept. Pharmac., Univ. CA, Irvine, CA.

This study demonstrates the recovery of melatonin receptor expression in visual areas of chick brain following suppression by intraocular TTX administration. Chicks (3 weeks old) were injected every 3 days for 7 days with either 6 µg TTX or saline/vehicle (C), and were sacrificed at day 7 (time 0) or after 1, 3 and 7 days recovery. Brain sections were labelled with 2[^{125}I]-iodomelatonin (50 pM) and specific binding defined with melatonin (1 µM). Areas ipsilateral to the TTX-injected eye served as a control. Reductions in specific 2-[^{125}I]-iodomelatonin binding by TTX (time 0) were observed in the contralateral optic tract, primary (optic tectum, n. basal optic root), secondary (n. triangularis) and tertiary (ectostriatum) visual areas (also see Fig.). Binding remained depressed for 2-3 days but was completely recovered by 7 days following TTX. The extent of recovery correlated with the degree of pupillary reflex suppression. We conclude that expression of ML-1 melatonin receptors in chick brain visual areas is selectively modulated by the visual input. MH-42922.



479.4

MELATONIN RECEPTORS IN THE CHICK OPTIC TECTUM: PRESYNAPTIC EFFECTS AND ACTIVITY-DEPENDENT REGULATION. D.N. Krause[1], J.C. Dye*[2], S. Iacob[3], H.J. Karten[2] and M.L. Dubocovich[3]. [1]Dept. Pharmacol., College of Med., U.C. Irvine, CA 92717; [2]Dept. Neurosci., U.C. San Diego, La Jolla, CA 92093; and [3]Dept. Molec. Pharmacol. & Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We previously reported a high density of 2-[^{125}I]-iodomelatonin binding sites in the retinorecipient layers of the chick optic tectum (*stratum griseum et fibrosum superficiale*, SGFS) and lower levels in the optic nerve (ON). Binding in both regions is decreased by ON transection (*Brain Res.* 590:325, 1992), which suggests transport of melatonin receptors in the ON from the retina to terminals in the SGFS. We have examined the electrophysiological effects of melatonin on retinotectal transmission in a brain slice preparation of the hatchling chick. Bath application of 10-20 nM melatonin suppressed tectal field potentials evoked by stimulation of retinal afferents at the level of the ON or *stratum opticum*. Alterations in the presynaptic components of the field potential isolated in 0 calcium saline support the involvement of a presynaptic site of action for melatonin. We have also found that *in vivo* intraocular injections of tetrodotoxin (TTX, 6 µg), which block the electrical activity of the retinal ganglion cells, decreased 2-[^{125}I]-iodomelatonin binding in the ON and optic tectum to a similar extent as that seen following ON transection. TTX (in one eye of 3 week-old chicks, every 3 days for 7 days) decreased binding in the contralateral ON (31%), SGFS (outer retinorecipient layers: 58%; inner layers: 33%) and *stratum griseum centrale* (34%). Binding in these areas recovered to control levels by 3-7 days following cessation of the TTX treatment. This suggests that expression of melatonin receptors in the retinotectal pathway, including presynaptic receptors which modulate visual input, is regulated by the activity in the pathway. (Supported by NS 24560 and EY 06890 to HJK and MH-42922 to MLD.)

479.5

DOPAMINE D3 RECEPTORS ARE ELEVATED IN SCHIZOPHRENIA AND ALZHEIMER'S (AD) CASES

E. Gurevich*[1], M.-P. Kung[2], H. Kung [2]and J.N. Joyce[1]. Dept. Psychiatry[1], and Radiology[2], Univ. Penn. Sch. Medicine, Philadelphia, PA

The binding of [^{125}I]-7-OH-DPAT (PIPAT; 0.2 nM, or 1/2 K_d), the ligand that binds selectively to D_3 dopamine receptors, was analyzed in sections of human brain containing caudal striatal region. The binding varied from 4 to 6 fmol/mg protein in different striatal areas, which is approximately 5-7 times lower that of D_2 receptors. The highest concentration of D_3 receptors was observed in the ventral putamen, the caudate nucleus, and the internal part of the globus pallidus (GPi) with slightly lower binding in the external globus pallidus (GPe) and ventral pallidum (VP). The nucleus basalis of Meynert exhibited relatevely low PIPAT binding. The distribution of D_3 receptors in the striatal region differs from that of D_2 receptors visualized with [^{125}I]epidepride in the presence of 50 nM of 7-OH-DPAT. D_2 receptor density was 4-5 times higher in the dorsal caudate-putamen than in the GP and VP. The only brain regions outside the striatum that displayed D_3 receptor density comparable to that of the striatum were the SN and amygdala. We compared PIPAT binding in caudal striatum in schisophrenia cases (n=12, 76.0±1.2 yrs) with neurologically normal control (n=12; 67.3±4.7 yrs) and AD (n = 11; 79 ± 10 yrs). The binding to D_3 receptors was significantly elevated in schizophrenics in all striatal areas except for the VP. The highest increase was observed in the GPe (81%) followed by the nucleus basalis (53% average increase). There were even higher elevations in all regions for AD. The highest increase was observed in the GPe (101%) and ventral putamen (73% average increase) followed by the GPi (63%). The results suggest that alterations in the mesolimbic DA systems leads to selective elevation in D_3 dopamine receptors. **Funded by MH 43880, AG 09215.**

479.6

EFFECT OF THE ANESTHETIC PROPOFOL ON 5-HT and FOS IN THE RAT BRAIN. T. Diab, A. W. Gelb and D.F. Cechetto*. Robarts Research Inst., Univ. of Western Ontario London, Ont., Canada N6A 5K8

The effects of anesthetics on neurotransmitters at specific sites in the brain are largely unknown. In this investigation, we studied immunohistochemically the effect of propofol on 5-HT and fos in the rat brain. Paired control and experimental male Wistar rats were chronically cannulated for blood pressure monitoring and anesthetic or vehicle infusion 7 days prior to the experiment. Following a 6 hour infusion of propofol (20-25 mg/kg/hr) or the intralipid vehicle, the immunoreactivity for serotonin (5-HT) and fos were studied. Changes in immunostaining levels between pairs were quantified using computerized imaging analysis. Fos activity was determined by counting the positively stained nuclei. The 5-HT signal was significantly increased by 30% in the dorsal raphe and significantly decreased by 15% in the area postrema compared to control. Numerous positively stained fos nuclei were observed in the inferior olive in the experimental animals which was not seen in the controls. The lateral parabrachial nucleus had double the number of labelled nuclei for fos compared to the control. These results suggest that an anesthetic such as propofol can initiate site specific changes in gene activity and selective changes in neurotransmitter levels which may have functional implications for the autonomic and behaviourial effects of anesthetics. (Supported by the Heart and Stroke Foundation of Ontario)

479.7

ß-ADRENOCEPTOR AUTORADIOGRAPHIC LABELLING IN HUMAN BRAIN: SUBTYPE DISTRIBUTION, EFFECTS OF AGE AND POST MORTEM DELAY AND MODIFICATIONS IN ALZHEIMER'S DISEASE. A. Pazos[*1], B. Grijalba[1], J. González-Gil[1] and J. Pascual[1,2]. [1]Dept. of Physiol. & Pharmacol., [2]Dept. of Medicine, Univ. of Cantabria, Santander, Spain.

The density and distribution of ß-adrenergic receptor subtypes was studied by means of quantitative autoradiography in post mortem human brain, in both control and Alzheimer's disease (AD) cases. Tissue sections were incubated with ^{125}Iodocyano-pindolol (ICYP). ICI-89406 and ICI-118551 were used as displacers. Our results showed a clear predominance of the $ß_1$-subtype in areas such as the basal ganglia and the neocortex. $ß_2$-adrenoceptors were mainly distributed in cerebellum and hippocampus. There was no effect of post mortem time (4-72h) on the density of ICYP binding. In contrast, a general general tendency of ß-receptors to decrease with age was found. However, this decrease was statistically significant in telencephalic areas, such as basal ganglia, frontal and visual cortex and thalamus, but not in other areas studied such as the mesencephalon and the cerebellum. This decrease was far noticeable in the $ß_1$-subtype. Regarding ß-receptors in AD, a general tendency to decrease (20%-60%) in total receptor binding was observed in neocortex, basal ganglia, hippocampus and cerebellum. While in the neostriatum the receptor loss was due to a decrease of $ß_1$-receptor binding (42%), in the cerebellum and hippocampus the receptor loss was secondary to a decrease of $ß_2$-receptors (39%-60%). The decrease of ß-adrenoceptor density during normal aging and in AD can be explained by the neuronal loss besides the degeneration of the locus coeruleus, known to occur in these processes. (Supported by DGICYT SAF93-265)

479.8

CELLULAR CO-LOCALIZATION OF α_2A AND α_2B ADRENERGIC RECEPTOR SUBTYPES IN PRIMARY CULTURES OF RAT SPINAL CORD NEURONS. *Y. Huang, M.V. Langston, H.E. Laird II*, P. A. St.John and J.W. Regan.* Departments of Pharmacology & Toxicology, Physiology, and Anatomy, University of Arizona, Tucson, AZ 85724.

The results of molecular cloning have revealed three subtypes of the α_2-adrenergic receptor. These subtypes have been defined as the α_2-C10 (α_2A), α_2-C2 (α_2B) and α_2-C4 (α_2C). α_2-Adrenergic receptors have been characterized in the central nervous system and spinal cord where they are involved in a number of physiological activities, including the control of blood pressure and nociception; however, the specific cellular localization and the subtypes which mediate these effects are largely unknown. For each of the α_2-adrenergic receptor subtypes, polyclonal antibodies were raised in chickens using recombinant fusion proteins containing the third intracellular loops of these receptors. The antibodies were characterized in transfected COS cells expressing each of the subtypes individually and were found to be selective and did not cross-react. Primary cultures of rat spinal cord were prepared from 14 day embryos. After 9 days in culture, they were examined by immunofluorescent microscopy. Positive immunoreactivity was detected with antibodies to the α_2A and α_2B, but not with antibodies to the α_2C. The labeling was on neuronal, but not on glial cells. It was blocked by preincubation of the antibodies with the appropriate fusion protein. The labeling in both subtypes was distributed diffusely across the cell body and neurites, and about 80% of the neurons in heterogeneous spinal cord cultures showed the labeling in both cases. Using dual-labeling techniques, positive immunofluorescence was co-localized to neurons with both anti-α_2A and anti-α_2B antibodies. The rat spinal cord appears to contain α_2A and α_2B adrenergic receptors which are present on neurons and may be co-expressed in the same cell. (Supported by the ADCRC)

479.9

α2C10-ADRENERGIC RECEPTOR mRNA IN THE MONKEY CEREBRAL CORTEX: NON-RADIOACTIVE *IN SITU* HYBRIDIZATION COMBINED WITH IMMUNOCYTOCHEMICAL LABELING OF CELL TYPE-SPECIFIC MARKERS. E. Wang* and M.S. Lidow. *Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.*

The noradrenergic system is involved in regulation of high order cortical functions such as learning and memory. It has also been shown that α2C10 subtype of adrenergic receptors is among the most abundant in the cerebral cortex. The present study investigates the distribution and type of cells synthesizing this receptor subtype in the prefrontal, motor, somatosensory and visual cortical regions of the adult rhesus monkey. For this purpose, animals were perfused with 4% paraformaldehyde in 0.1M PBS buffer. Brains were dissected out, postfixed in the same fixative, briefly immersed in isopentane at -30°C, and then, stored at -70°C. *In situ* hybridization was conducted on the glass slide-mounted cryostat brain sections using digoxigenin-labeled riboprobes complimentary to *AccI-PstI* fragment of human α2C10 receptor cDNA which is within the sequence encoding the third cytoplasmic loop of the receptor. Some *in situ*-labeled sections were simultaneously processed for fluorescent immunocytochemical visualization of GABA, parvalbumin, SMI-32 and other cell markers. We found that α2C10-receptor mRNA is expressed by cells in all cortical areas and layers observed. Nevertheless, there were significant areal differences in the laminar distribution of this mRNA. For example, in the visual cortex, the labeling was particularly prominent in layers IVa and VI. In the somatosensory cortex, the strongest signal was observed in layers III and V, and in the motor cortex, the message concentrated in layer V. Finally, the prefrontal cortex had fairly homogenous laminar distribution of the message with only slight increase in layer V. The double labeling studies showed that mRNA encoding α2C10 receptors is expressed by a variety of cells including GABA and parvalbumin-containing interneurons and SMI-32 immunoreactive pyramidal cells. Such wide distribution of α2C10-adrenergic receptors suggests that they may play an important role in cortical functions.

479.10

AGONIST BINDING TO α_2-ADRENOCEPTORS IN ROSTRAL LOCUS COERULEUS FROM DEPRESSED SUICIDE VICTIMS AND CONTROLS. V. Klimek, C.A. Stockmeier, H.Y. Meltzer, J. Overholser, A.E. Halaris*, I.A. Paul, G.A. Ordway. Dpt. Psychiatry & Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS 39216; Dpts. Psychiatry & Psychology, Case Western Reserve U., Cleveland, OH 44106

Alterations in brain norepinephrine have been implicated in depression, schizophrenia, and anxiety. The locus coeruleus (LC) is the principal source of brain norepinephrine, and release of norepinephrine is modulated by α_2-adrenoceptors on LC neurons. Alterations in α_2-adrenoceptors in suicide and depression have been reported previously. In this study, the binding of the α_2-adrenoceptor agonist, *p*-[^{125}I]-clonidine, was measured at several levels of rostral LC (rostral 4.8 mm) from 8 victims of suicide and 8 age-matched control subjects. Suicide victims were retrospectively diagnosed with major depression (n=7) or bipolar depression (n=1) using DSMIII-R criteria. In all subjects, agonist binding significantly increased in a rostral to caudal gradient within the rostral LC, parallelled by a significant elevation in the number of neuromelanin-containing cells. When agonist binding was corrected for cell number at each level, no gradients were observed. Two regions of LC could be distinguished anatomically and the density of agonist binding was significantly different between these regions. There were no significant differences in agonist binding in the rostral LC between control subjects and suicide victims. [Supported by USPHS MH46692 and American Suicide Foundation.]

479.11

Functional Localization of Alpha-2 Receptors in vivo Using Idazoxan and PET. W.W. Hong, M. Schmidt, R. Risinger, G.E. Alexander, N. Azari, J.M. Maisog, D. Mangot, C.L. Grady, M.J. Mentis, M.B. Schapiro, S.I. Rapoport, J.T. Little*, U. Freo, M. Kurkjian, W.Z. Potter, J.W. VanMeter. Laboratory of Neuroscience, National Institute on Aging, Bethesda, MD 20892.

As a prelude to studies of alpha-2 receptor function in dementia of the Alzheimer's type and normal aging, regional cerebral blood flow (rCBF) was measured using H_2O^{15} PET (Scanditronix PC-2048-15B, 6 mm FWHM, 15 slices) before and during infusion of the specific alpha-2 receptor antagonist idazoxan. Five screened healthy controls (aged 20-30, four male, one female, all right handed) underwent 5 scans at rest (eyes and ears occluded) and 5 during visual stimulation with isodipole textures in an alternating fashion. The first two scans (off drug) were followed by a 200 mcg/kg 30 minute infusion of idazoxan, with a maintenance drip for the remaining eight scans. All ten scans for each subject were interpolated from 15 to 43 slices, roll-yaw corrected, registered, normalized to Talairach space, and smoothed using a gaussian filter of 20 mm x 20 mm x 12 mm. Each scan for each subject was normalized to its own global mean, then multiplied by the mean of the two non drug scans for that subject. Pixel-by-pixel analysis of rCBF was performed for each subject and for the group to identify regions affected by idazoxan. In addition, changes in rCBF were measured in each subject across time in the regions with the largest drug effect. In spite of a significant decrease in global blood flow across time ($p<0.0001$), blood flow to visual cortex was significantly increased by idazoxan in the group in both the resting and visually stimulated conditions ($p<0.05$). Moreover, each subject showed an rCBF increase in visual cortex after idazoxan in the same areas identified by the group analysis. This metabolic increase is consistent with the high density of alpha-2 receptors in the human visual cortex and enhancement of neurotransmitter release. Intravenous idazoxan and PET may be a useful way to assess central alpha-2 receptor function in neuropsychiatric illnesses.

479.12

OCTOPAMINE,DOPAMINE AND NORADRENALINE IMMUNOCYTOCHEMISTRY IN THE RAT BRAIN: THREE DISTINCT PATTERNS OF DISTRIBUTION. S. Burchett, T.P. Hicks*, J. Rapus and M. Eckert. Depts. of Biology and Psychology, UNCG, Greensboro, NC, 27412-5001.

The distributions of three amines normally present in the mammalian nervous system: octopamine (OA), dopamine (DA) and noradrenaline (NA), were compared in rat brain following immunocytochemical treatment with polyclonal antibodies. The distributions of NA and DA conformed well with previously published accounts, in that somatic NA staining was confined to the locus coeruleus (A6, A5, A2 and A1 zones) while that for somatic DA was present in the ventral tegmental area and substantia nigra. The development of a novel antibody to OA allowed us to perform the first screening study for this compound in mammalian CNS. We found somatic label to be distributed increasingly along the rostro-caudal axis; staining in more caudal regions being relatively more intense and widely distributed than rostrally. OA immunoreactivity was demonstrated best in rats perfused with fixative solutions having relatively high glutaraldehyde levels (6%), causing a high background level of stain, and using low serum dilutions (1:250). Caudally-situated structures showing the most intense reactivity included: cranial nerve nuclei (especially V and VII); throughout the central core of the long axis of the reticular formation and ventrolateral to the cerebral aqueduct; and the deep cerebellar nuclei. The distribution of label throughout CNS was heterogenous even though immunoreactive product was dispersed very widely within somata and axons, with few areas not showing labelled cells. Background label was also quite uneven, suggesting the possibility of unequal patterns of terminal immunoreactivity. These results are not inconsistent with the view of OA as a modulator of synaptic transmission within mammalian brain.

479.13

DISTRIBUTION OF NITRIC OXIDE PRODUCING NEURONS IN THE CNS OF THE AXOLOTL, *AMBYSTOMA mexicanum*. M. León-Olea, M. Sánchez-Alvarez, F. Pellicer *, E. Talavera, E. Sánchez-Islas, G. Martínez-Lorenzana Lab. Histología, Div. Neurociencias, Instituto Mexicano de Psiquiatría. Av. México-Xochimilco 101, C.P. 14370, México, D. F., México.

Nitric oxide (NO) is a highly reactive and diffusible molecule which participates in a wide variety of functions such as in the citotoxic reaction of leucocytes, as mediator of neurotransmitters in the relaxation of vascular endothelium, and as an inhibitor of platelet aggregation.

From a philogenetic point of view, the presence of NO producing neurons has been described in vertebrates such as rat, crab, frog and fish, and in invertebrates like crayfish and snail. Thus, the aim of this study was to report the presence and localization of NO producing neurons in the CNS of the mexican amphibian axolotl, *Ambystoma mexicanum*. The axolotls (n=6) were anaesthetized with benzocaine and intracardially perfused with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The brains were dissected and post-fixed for 3-12 h, and were transferred to a sucrose solution (15 %). Parasagittal and coronal sections (20 μm) were cut and mounted on gelatined coated slides. Vincent and Kimura's histochemical reaction was done (*Neurosci.*46:775, 1992). Our results show a wide distribution of positive neurons and fibers, mainly in olfactory bulb, comissures, striatum and pallio-dorsal nuclei, in striatum-amygdaline zone, cerebellum and rombencephalon, hypothalamus and fibers in median eminence. These data show the existence on NO producing neurons in *A. mexicanum*.Further studies should be carried out to elucidate the role of this molecule in this amphibian. This work was partially supported by CONACyT grants MLO 1183-N9203.

479.14

SEROTONINERGIC BUT NOT DOPAMINERGIC FIBERS IN THE STRIATUM OF THE RAT ARE NITRIC OXIDE (NO)-RECEPTIVE. POSSIBLE CO-LOCALIZATION OF 5-HT AND cGMP.
H.W.M. Steinbusch[1]*, J. Pasterkamp[1], D. Hopkins[2] and J. De Vente[1]. [1]Department of Psychiatry and Neuropsychology, Faculty of Medicine, University of Limburg, Maastricht, The Netherlands and [2]Department of Anatomy and Neurobiology, Dalhousie University, Halifax, NS, Canada.

Within the striatum several efferent and afferent transmitter systems can be demonstrated of which serotoninergic and dopaminergic varicose fibers are most prominent. Recently the NO-system in the brain has been visualized using either antibodies to nitric oxide synthase (NOS) or NADPH-diaphore histochemistry. Within the striatum both NOS- and NADPH-diaphorase positive cells and fibers were localized. However, it is yet not clear which proportion of these NO-producing fibers are arising from intra- or extrastriatal origin. In this study we were interested which elements are also receptive for NO, using cGMP as parameter for NO-production. We have found that the produced NO will diffuse from the cell /fiber and can activate in neighbouring neurons guanylate cyclase. This leads to the production of cGMP, and using cGMP-immunofluorescence in conjunction with a slice-procedure we have the possibility to visualize NO-receptive elements. Using 6-OHDA lesions in the ventral tegmental area and the substantia nigra complex, or 5,7-dihydrotryptamine lesions in the medial forebrain bundle we could demonstrate that a reduction of the dopaminergic fibers does not lead to a decrease in the number of cGMP-positive fibers, while 5,7-DHT lesions induces a diminishing of the serotonergic fibers and cGMP fibers in the striatum. Further studies are in progress to show the possible co-localization between 5-HT- and cGMP-immunofluorescence, and the absence of co-localization between cGMP and NOS in the striatum. This co-localization could provide further support for a functional connection between the NO and the 5-HT system. (Supported by NWO of The Netherlands).

479.15

NADPH DIAPHORASE ACTIVITY IN OLFACTORY RECEPTOR NEURON AXONS CONFORMS TO A RHINOTOPICALLY-DISTINCT DORSAL PROJECTION ZONE IN THE MOB OF HAMSTERS. T. K. Knott and T. A. Schoenfeld*. Depts. of Psychology and Biology and the Neuroscience Program, Clark University, Worcester, MA 01610.

NADPH diaphorase histochemical activity is distributed widely in the CNS, where it is most commonly localized to short-projecting neurons whose axons do not collect, for the most part, in the major white matter tracts such as the corpus callosum or cerebral peduncles (Vincent and Kimura, 1992, Neuroscience, 46:755). By contrast, the olfactory and vomeronasal nerves show intense NADPH diaphorase activity in the main olfactory bulb (MOB) and accessory olfactory bulb (AOB), respectively. Moreover, the olfactory nerve activity patterns are also topographically restricted to the dorsal and medial MOB (Scott et al., 1987, J. Comp. Neurol., 260:378; Croul-Ottman and Brunjes, 1988, Brain Res., 460:323; Davis, 1991, J. Comp. Neurol., 314:493). This pattern conforms almost precisely to an obliquely oriented, rhinotopically-distinct dorsal zone known to receive projections from mucosal segments that line a relatively smooth central channel within the nasal cavity (Schoenfeld et al., 1994, Brain Res. Bull., 34:183; Clancy et al., 1994, Brain Res. Bull., 34:211). Thus, NADPH diaphorase activity, putatively indicative of nitric oxide activity as well, may play a role in the spatial coding of odorant molecules.

(Supported by an NSF REU site grant, the Colin Research Award and the Dept. of Psychology, Clark University.)

479.16

MAPPING OF NADPH DIAPHORASE-CONTAINING NEURONS IN THE BUDGERIGAR BRAIN: EVIDENCE FOR A SPECIFIC SYSTEM. B.Cozzi[(1)]*, R.Massa[(2)], and G.C.Panzica[(3)], Inst.of Anat.of Dom. Animals[(1)], and Dept.of Biol.[(2)], Univ.of Milan 20133; and Dept. of Human Anat.&Physiol.[(3)], Univ. of Turin 10126, ITALY

The presence of NADPH diaphorase activity in neurons is believed to be indicative of the presence of nitric oxide synthase in the CNS. In some avian species (quail, chicken), NADPH diaphorase is present in a series of neurons located in specific areas of the brain. This study is directed to investigate whether the presence and systemic distribution of NADPH diaphorase in the budgerigar (*Melopsittacus undulatus*) CNS show any peculiar specific segregation. To this effect, the brains of a series of *M.ondulatus* have been studied for the presence of NADPH diaphorase.

In the telencephalon, the paleostriatal-paraolfactory lobe complex showed the presence of positive neurons and a diffuse network of axons. Diaphorase-containing elements were observed also in the neostriatum, in several nuclei of the archistriatum (including the nucleus taeniae) and in the hyperstriatum (accessory, dorsal and ventral). In the diencephalon, positive neurons were present both in the lateral hypothalamic and periventricular areas, and in a segregate area at the confluence of the anterior commissure and the lateral prosencephalic bundle. A group of positive perikarya was located lateral to the dorsal part of the III ventricle, and continued laterally into the thalamus. In the mesencephalon, diaphorase-containing elements were placed in the pretectal area, reticular formation (pars lateralis and pars medialis), nucleus ruber and nucleus interpeduncularis. Several positive elements were located in the nucleus tegmenti pedunculo-pontinus, and nucleus vestibularis (pars medialis and pars superior). In the cerebellum, large stained neurons were evident in the nucleus cerebellaris internus.

NADPH-containing neurons in the budgerigar brain show a specific distribution, only in part overlapping with what already observed in other avian species. In particular, NADPH-containing neurons in *Melopsittacus undulatus* are largely distributed in several striatal areas except the ectostriate. Differences with what observed in the chicken and quail are evident especially in the hypothalamus and midbrain.

Supported by Grants from the Italian National Research Council to the Authors.

479.17

NEURONAL NITRIC OXIDE SYNTHASE IN THE BRAIN OF THE SALMON. DISTRIBUTION, SUBCELLULAR LOCALIZATION AND COLOCALIZATION
Bo I. Holmqvist*, Per Alm and Peter Ekström *Dept. Zoology, University of Lund, Sweden.*
The distribution of the neuronal nitric oxide (NO) converting enzyme, NO synthase (NOS), its subcellular localization and its relation to dopaminergic neurons are being investigated in the brain of the Atlantic salmon by means of immunocytochemical, single and dual, labeling techniques. Studies include analyses with confocal laser scanning microscopy and transmission electron microscopy.

Our current results show that NOS immunoreactive (NOSir) neuronal populations are present in all major brain areas, including in the olfactory bulb, telencephalon, preoptic/hypothalamic areas, thalamus, posterior tuberculum, optic tectum, torus semicircularis, midbrain tegmental areas, cerebellum and vagus motor nuclei. The viscerosensory root of the vagus nerve is strongly labeled and scattered NOSir nerve fibers course in various parts of the brain. A distinct granular labeling is strongly associated with specific neuronal populations, among others with specific tyrosine hydroxylase immunoreactive (THir) neuronal populations and neurosecretory neurons in preoptic/hypothalamic areas. On the ultrastructural level, NOSir axons and axon terminals were detected which exhibited membrane specializations in close apposition to processes and cell bodies, indicating that the granular staining represent NOSir synaptic contacts. In several cases, NOSir neurons are located together with THir neurons, within the same area or nuclei. NOSir is colocalized with THir in a distinct population of hypophysiotrophic neurons located within the retinohypothalamic termination field.

Our data stress that NO producing neuronal systems are widespread in brain of the salmon. NO may serve multiple functions in neural mechanisms and may, via both inter- and intracellular actions, influence a variety of brain functions in teleosts. Here we show that NO may possess neuromodulatory influence on specific hypophysiotrophic dopaminergic systems.

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479.18

Immunohistochemical Distribution of Calretinin in the Primate Amygdala. C.H. Lam[1], A.F. Sadikot[1]*, M. Fortin[2], A. Olivier[1], A. Parent[2] 1. Division of Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, P.Q.; 2. Laboratoire de Neurobiologie, Enfant-Jésus Hospital, Laval University, Quebec City, P.Q.

The immunohistochemical distribution of the calcium binding protein calretinin was examined in the amygdala of the squirrel monkey (Saimiri sciureus). A complex heterogeneous pattern of cell and neuropil straining is found in the amygdala. The basal nucleus contains a light to moderate density of triangular shaped cells in its magnocellular component, with light staining of neuropil. The parvocellular part of the basal nucleus contains a moderate density of cells with light to moderate staining of neuropil. The accessory basal nucleus stains moderately for neuropil, and contains a moderate density of medium-sized multipolar cells. The lateral nucleus contains a light density of cells in its dorsomedial portion, with light to moderate cell density in its parvocellular portion. The central nucleus shows a moderate to dense population of bipolar and tripolar cells, with a higher cell density medially. Neuropil staining is moderate in the lateral part of the central nucleus as compared to dense staining medially. The periamygdaloid cortex and medial nucleus contain a moderately dense population of small to medium-sized cells, and stain moderately for neuropil. Calretinin has a distinct pattern of distribution as compared to other calcium binding proteins (calbindin, parvalbumin), suggesting the use of disparate calcium-related physiological mechanisms by distinct cell groups in the amygdala.

479.19

TRANSFERRIN RECEPTOR ANALYSIS IN HYPOTRANSFERRINEMIC (Hp) MOUSE BRAIN. **TK Dickinson* & JR Connor, Penn State Univ, Hershey Medical Center, Hershey, PA 17033**

The hypotransferrinemic (Hp) mouse has a point mutation or small deletion in the transferrin (Tf) gene causing a defect in splicing of precursor Tf mRNA. This results in production of <1% of the normal circulating level of Tf. These animals are small, pale and severely anemic at birth and die within 7 days unless given supplemental i.p. injections of mouse serum or purified Tf. We have previously analyzed the brains of these animals and shown morphological alterations, especially in areas of significant postnatal development. We have also studied the cellular and regional distribution of iron, Tf and ferritin in the Hp brain. An appreciation of the cellular and regional distribution and levels of Tf receptor in these animals is critical to complete our understanding of the mechanisms of iron transport and storage in the Hp mouse brain. Polyclonal and monoclonal antibodies to Tf receptor were applied to 40μm brain sections from wild type (HH), heterozygote (Hh) and mutant (hh) Hp animals. The overall staining for Tf receptor using these antibodies is similar for all three types and staining is generally more robust using the polyclonal antibody. The predominant cell type staining positively for Tf receptor are neurons. The Tf receptor positive neurons are seen throughout the brains of all three types. White matter astrocytes stain Tf receptor-positive but white matter itself reacts poorly. Perivascular astrocytes also stain Tf receptor-positive. Blood vessels themselves, known to contain high levels of Tf receptor, stain positive in all three animals, but react more intensely with the monoclonal antibody. Oligodendrocytes, the cells responsible for myelin production in the brain and the predominant iron-positive and Tf-positive cells, do not stain positively for Tf receptor using our antibodies.

479.20

IMMUNOLOCALIZATION OF THE CEK8 RECEPTOR TYROSINE KINASE IN THE CEREBELLUM. A. Bayardo, J.A. Holash+, E.B. Pasquale+, M.E. Martone, M.H. Ellisman*. Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093; +La Jolla Cancer Research Foundation, La Jolla, CA 92037

Cek8 is an avian member of the Eph subclass of transmembrane integral tyrosine kinases. Many tyrosine kinases, including the Eph subclass, are thought to contribute to the growth and development of the nervous system through the mediation of cell signaling. The presence of high levels of Cek8 both during neural development and in adulthood (Sajjadi and Pasquale, *Oncogene* 8:1807, 1993) suggest that it could play a role in neuronal plasticity in the adult brain.

In the present study, we examined the localization of Cek8 in adult cerebellum using a polyclonal antibody raised against a synthetic carboxyl terminal peptide. Cek8 was localized in frozen sections of adult rat cerebellum using indirect immunofluorescence and confocal microscopy. The most intense fluorescence was observed in Purkinje neurons and their processes. Other cell types in the molecular and granule cell layers were unlabeled or only lightly labeled. Within Purkinje cells, intense labeling was observed throughout the cell body, dendritic tree and dendritic spines. Labeled processes in the granule cell layer that appeared to be the axons of Purkinje neurons were also observed. Preabsorption of the primary antibody with excess peptide or omission of the primary antibody from the immunolabeling sequence resulted in no fluorescent signal in the cerebellum. This staining pattern is distinct from that of the closely related tyrosine kinase Cek5, which we have previously reported to be concentrated in axons of granule cells in the cerebellum (Pasquale et al., *J. Neurosci.* 12:3956, 1992). An ultrastructural analysis will be employed to determine the subcellular distribution of Cek8. We are currently mapping the distribution of Cek8 in the remainder of the rat brain and plan to investigate its distribution in the chicken brain as well.

SECOND MESSENGERS: KINASES AND PHOSPHATASES

480.1

METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED POTENTIATION OF CYCLIC AMP RESPONSES DEPRESSES EXCITATORY SYNAPTIC TRANSMISSION BY A PROTEIN KINASE-INDEPENDENT MECHANISM. R.W. Gereau IV* and P.J. Conn. Department of Pharmacology and Program in Neuroscience. Emory Univ. Sch. of Med. Atlanta, GA 30322.

Coactivation of metabotropic glutamate receptors (mGluRs) and β-adrenergic receptors causes a synergistic increase in cAMP formation in the rat hippocampus. Increases in cAMP are known to have many actions in the hippocampus via activation of cAMP-dependent protein kinase. We now report that coactivation of mGluRs with 1S,3S-ACPD and β-adrenergic receptors with isoproterenol induces an acute depression of excitatory postsynaptic currents (EPSCs) at the Schaffer collateral-CA1 synapse. Neither 1S,3S-ACPD nor isoproterenol depresses EPSCs in area CA1 of the adult rat hippocampus when added alone. Pharmacological studies indicate that this depression of EPSCs is dependent upon increases in cAMP levels but is independent of protein kinase activity. This cAMP-mediated depression of EPSCs appears to be dependent on metabolism of cAMP and release of adenosine or 5'-AMP into the extracellular space with resultant activation of presynaptic adenosine receptors. These studies suggest that cAMP can have local hormone-like effects in the hippocampal formation that are independent of cAMP-dependent protein kinase.

480.2

METABOTROPIC GLUTAMATE RECEPTOR STIMULATED cAMP IS IMPLICATED IN VISUAL CORTEX PLASTICITY. H.J. Flavin*, N.W. Daw, D. Gregory and S. Reid Dept. of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT 06520.

Metabotropic glutamate receptor (mGluR) stimulation can activate phosphoinositide (PI) hydrolysis and can increase and decrease cAMP. The mGluR's linked to PI hydrolysis have been implicated in visual cortex plasticity (Bear and Dudek, Ann. NY Acad. Sci. 627: 42-56, 1991). The role of the cAMP mGluR's in visual cortex plasticity has not yet been addressed, although the cAMP second messenger system has been implicated in hippocampal plasticity. This study was undertaken to examine the role of cAMP dependent mGluR's in visual cortex plasticity. The critical period in the rat begins at approximately 14 days of age and extends approximately one month. Consequently, cortical slices (400 μm) were obtained from male Long Evans rats approximately 22 and 50 days of age. After a 90 minute preincubation in a static chamber, slices were transferred and given a 10 minute experimental incubation. Basal cAMP levels were determined from slices incubated in extracellular buffer. Quisqualate (100 μM) was used to stimulate mGluR1 and mGluR5. cAMP was quantitated via RIA. Basal cAMP levels were slightly, but not significantly higher in slices from 22 versus 50 days of age rats, with 41.5 ± 25 and 20.6 ± 11 pmol cAMP / mg protein, respectively. Stimulation by quisqualate increased cAMP levels in slices from 22 days of age animals by 232.9 ± 117 pmol cAMP / mg protein. This was significantly greater ($p < 0.002$, unpaired t-test) than the corresponding values for slices from 50 days of age animals (28.1 ± 10). These results suggest that mGluR1 and mGluR5 stimulation of the cAMP second messenger system is implicated in rat visual cortex plasticity.

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480.3

DIFFERENT ACTIVATION OF PKA IN CORTICAL NEURONS AND CEREBELLAR GRANULES. C. Ventra*, M. Paolillo, A. Porcellini^, A. Feliciello^, V.E. Avvedimento^, & G. Schettini. Dept. of Neurosci., Sect. of Pharmacol.; ^Dept. of Mol. and Cell. Biol. and Pathol., CEOS-CNR, II School of Medicine, University of Naples "Federico II", Italy.

Cortical neurons display a notable amount of type-II holoenzyme compared to cerebellar granules, virtually containing only type-I holoenzyme and devoid of AKAP 150, which specifically anchors type-II PKA holoenzyme. The cortical holoenzyme poorly dissociates under stimulation (5 min) by forskolin (10μM+IBMX 50μM), while completely reassociates after 15 min; nonetheless, a consistent nuclear accumulation of PKA is found. Conversely, cerebellar holoenzyme is highly sensitive to stimulation by forskolin (>60%), does not reassociate at 15 min, and the catalytic subunit does not accumulate into the nucleus. Immunofluorescence studies using a policlonal antibody for PKA catalytic subunit and band-shift experiments using radiolabelled CRE are allowing us to further unravel this issue. In cortical neurons, the cytosolic holoenzyme is dissociated at 15 min, while the particulate counterpart dissociates between 2.5 and 5 min and fully reassociates at 15 min; negligible levels of particulate holoenzyme are found in cerebellar granules, and most of cytosolic holoenzyme dissociates under forskolin stimulation (60-70%, 1-15 min). Our results suggest that relevant functional implications could underlye the different subcellular distribution and response of PKA to cAMP, with particular regard to the cAMP-dependent modulation of gene transcription, which plays a major role in the modulation of long-term synaptic events. (Supported by TP Aging and CNR 93.00417.CT04 grants to G.S.)

480.4

MODULATION OF PKA ACTIVITY BY PHORBOL ESTERS IN RAT CORTICAL NEURONS. M. Paolillo*, C. Ventra, A. Feliciello^, A. Porcellini^, V.E. Avvedimento^ and G. Schettini. Dept. of Neuroscience, Sect. of Pharmacology, and ^Dept. of Cellular and Molecular Biology and Patology, II Fac.of Medicine, Naples, Italy.

In cerebellar granules we found a remarkable dissociation of the PKA holoenzyme (70%) following a forskolin stimulation (10 μM+IBMX 50 μM). In contrast, in primary cultures of rat cortical neurons we found that 15 min activation by forskolin does not result in the dissociation of cAMP-dependent protein kinase (PKA) holoenzyme. Thus we evaluated whether the previous exposition of the cells to pharmacological agents activating PKA (forskolin) or protein kinase C (PMA) could modify the response of PKA to the next forskolin challenge. We found that a pretreatment with forskolin did not affect the PKA response to cAMP, while a 2 and 4 hr PMA pretreatment reduced the amount of particulate holoenzyme (20 %), with a consequent increase of the cytosolic holoenzyme amount. Furthermore, after 4 hr PMA, a 15 min forskolin stimulation resulted in a 25 % dissociation of the holoenzyme. We suggest that a PMA-induced phosphorylation could affect the binding of the PKA holoenzyme to the particulate fraction, either influencing the affinity of the type-II regulatory subunits or of the anchoring protein (AKAP 150). In conclusion, our data show that in cortical neurons phorbol esters determine delocalization of PKA holoenzyme and modulate its dissociation following a cAMP pulse.

480.5

PROTEIN KINASE A (PKA) INDEPENDENT EFFECTS OF IONTOPHORETICALLY APPLIED cAMP ANALOGS ON RAT SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS. J-C. Liu*, L.P. Martin and B.L. Waszczak. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

We have previously shown that iontophoresis of the dopamine D_1 agonist SKF 38393 acts at D_1 receptors on striatonigral terminals to increase firing rates of SNpr neurons, and to enhance their inhibitory responses to applied GABA. To determine if cAMP might mediate these effects, subsequent studies examined effects of cAMP analogs using extracellular single unit recordings in male rats anesthetized with chloral hydrate. Iontophoresis of several membrane permeable analogs (8-bromo-cAMP, dibutyryl-cAMP and chlorophenylthio-cAMP) failed to consistently mimic the rate-increasing effect of the D_1 agonist. Moreover, these compounds, as well as the non-permeable cAMP, *lessened* rather than enhanced the responsiveness of SNpr cells to applied GABA. The decreases in GABA potency by both permeable and non-permeable analogs prompted further studies to assess whether activation of PKA was required for the cAMP effects. Sp- and Rp-cAMPS (membrane-permeable activator and inhibitor of PKA, respectively) were next examined. Iontophoresis of Sp-cAMPS (-5 to -30 nA) caused current-related increases in firing of SNpr cells to 145 ± 12% and attenuated inhibitory responses to applied GABA to 58 ± 10%, similar to other "active" cAMP analogs previously tested. However, Rp-cAMPS caused effects indistinguishable from Sp-cAMPS; at -30 nA, firing was increased to 138 ± 14% and GABA potency was reduced to 68 ± 15%. Co-iontophoresis of Rp-cAMPS (-30 nA) did not block responses to Sp-cAMPS (-5 to -30 nA). On the contrary, the effects were roughly additive. Thus, it appears that the effects of iontophoretically applied cAMP analogs in SNpr involve a PKA independent action, perhaps via an extracellular site on the $GABA_A$ receptor/Cl^- channel of SNpr neurons. In view of this result, caution is advised in interpreting results of iontophoresis studies using cAMP analogs. Further studies are needed to elucidate the role of cAMP in mediating the D_1 agonist effects in SNpr. (Supported by NS 23541.)

480.6

Chronic Haloperidol Treatment Has No Significant Effect on Cyclic AMP Concentration in Rat Brain. W.Feng, R.Li, J.Elkes* and R.S.El-Mallakh. Mood Disorders Research Program, Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, Louisville, KY 40292.

It has been assumed that the therapeutic effect of haloperidol in the treatment of schizophrenia is related to its blockade of dopaminergic (D_2) receptors in the central nervous system. Our previous data has shown that chronic haloperidol treatment attenuated receptor-mediated phosphoinositide turnover in the frontal cortex, striatum and hippocampus. To investigate the influence of haloperidol treatment on another second messenger, cyclic adenosine monophosphate (cAMP), in the central nervous system, we examined the cAMP concentration in the different brain regions of rats treated with haloperidol (1.5mg/kg/day IM) for 4 or 6 weeks. The cAMP concentration was measured by dual range cAMP enzyme immunoassay kits. Our results indicates that cAMP level was slightly increased in the hypothalamus while no alteration was observed in the frontal cortex, striatum and hippocampus after 4 or 6 weeks treatment with haloperidol. However, all these changes are not significant. Chronic haloperidol treatment does not appear to be associated with the changes in cAMP that have been previously reported with acute treatments. Our results provide additional support for the notion that chronic treatment experiments are necessary to understand the mechanism of clinical action of haloperidol.

480.7

SARCOMA PROTO-ONCOGENE c-LYN (P-56) IS HIGHLY EXPRESSED IN THE RAT BASAL FOREBRAIN S. Chen*, R. Bing and D. E. Hillman, Dept. of Otolaryngol. and Phys./Biophys., New York Univ. Med. Center, New. York., N. Y. 10016

The sarcoma proto-oncogenes are second messengers for phosphorylation of tyrosine kinases which are essential in processes of neuronal plasticity and learning. Computer 3D mapping and reconstruction of lyn immunoreactivity (IR) revealed impressive bilateral cores of intensely labeled neurons surrounding the anterior commissure and extending along the base of the striatum from the head of nucleus accumbens caudally through the strial fundus. There was a compartmentalization of accumbens nucleus displaying multiple fascicles consisting positive cells within a negative matrix. Rostrally in this region, intensely labeled small cells were grouped along the margins of the accumbens nucleus. More caudally, these lyn-IR neurons surrounded the anterior commissure forming distinct fascicles. The dendrites of these neurons within the fascicles were oriented parallel forming discrete dorsolateral fascicles. These fascicles were also prominent in the shell of accumbens and bed strial nuclei. The fundal cells were compact dorsally with a ventral reticular extension. The lateral and medial septal nuclei were separable by intense IR somata and overlapping dendritic fields. Additionally, moderate lyn-IR cells having small to medium sizes were evenly scattered throughout the neostriatum. Double labeling revealed that a small percentage of lyn cells colocalized with parvalbumin neurons but none colcoalized with large acetylcholine cells. The lyn core in the basal forebrain may represent a basic system of neurons involved in learning homeostatic functions of behavior. Supported by NS 13742 and AG 09480.

480.8

MULTIFUNCTIONAL CALCIUM /CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII) IN BOVINE CHROMAFFIN CELLS: CHARACTERISTICS AND STIMULATION BY POTASSIUM DEPOLARIZATION. N. Verma and J. C. Waymire*, Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030.

CaMKII is a multifunctional protein kinase that has been found to exist in a variety of tissues and cell types. We examined CaMKII activity in bovine adrenomedullary chromaffin cells using a CaMKII specific substrate. After discerning its presence, we investigated the time course of CaMKII activation as determined by incubations at incremental time points with nondepolarized and high potassium depolarized (55 mM KCl) cells. We found that CaMKII activity in chromaffin cells differs both temporally and quantitatively from that in other cell types, such as PC12 cells and hippocampal slices. In chromaffin cells, the activation is almost immediate (as early as 10 seconds) and rapidly decays by 2 minutes.

CaMKII is known to activate tyrosine hydroxylase by phosphorylating serine residues 19 and 40 in chromaffin cells. The rapid phosphorylation of these residues (maximum phosphorylation within 10 seconds) correlates with the activation of CaMKII. Therefore, in chromaffin cells, the time course of CAMKII activation directly predicts the rapid phosphorylation and activation of tyrosine hydroxylase, and thereby catecholamine synthesis. Supported by NS 11061-16 JCW.

480.9

CALCIUM , BUT NOT GLUTAMATE STIMULATES THE GENERATION OF A CA-INDEPENDENT FORM OF CAM-KINASE II IN CULTURED RAT HIPPOCAMPAL PYRAMIDAL NEURONS. W.K. Scholz*, C.K. Christian and H.C. Palfrey. Univ. of Chicago, Dept. Pharmacol./Physiol. Sci., Chicago, IL 60637

Some glutamate-mediated effects on synaptic plasticity may involve the generation of a Ca-independent form of Ca/calmodulin-dependent protein kinase II (CaM-KII). Glutamate regulation of CaM-KII activity in cultured hippocampal pyramidal neurons was assessed by stimulating intact neurons and then measuring the ability of extracts to phosphorylate the selective CaM-KII substrate peptide, autocamtide-2. Ca-independent activity in neurons equilibrated in 1.26 mM Ca-buffer was about 10% of the total CaM-KII activity. Glutamate (100 uM) and A23187 (2 uM) had no effect on Ca-independent CaM-KII activity in pyramidal neurons equilibrated in 1.26 mM Ca-buffer. However, these agents significantly decreased total kinase activity resulting in an apparent increase in Ca-independent CaM-KII activity of 7-10% of total. Since pyramidal neurons spontaneously release glutamate in buffer containing 1.26 mM Ca it is possible that the neurons were already in a stimulated state. However, the addition of 25 uM APV, an NMDA receptor blocker, or 1-10 mM Mg or 2 mM Co, to inhibit Ca influx, did not reduce Ca-independent CaM-KII activity. Equilibration of neurons in 50 nM Ca/ 0 Mg-buffer reduced the Ca-independent kinase activity to about 5% of total. When extracellular Ca was elevated from 50 nM to 1.26 mM there was a rapid increase in Ca-independent activity that peaked at 5-30sec, reaching 50-100% of the total CaM-KII activity. By 10 min, the kinase activity returned to levels observed in neurons equilibrated in 1.26 mM Ca. The addition of glutamate with the Ca did not potentiate the response and APV did not block it. The mechanism by which extracellular Ca elevation generates the Ca-independent form of CaM-KII may occur by autophosphorylation since ^{32}P-incorporation into CaM-KII doubled at 5 sec following the shift from 50 nM to 1.26 mM Ca, as determined by immunoprecipitation. Transient phosphate incorporation into 2 phosphopeptides of the 50 kDa subunit correlated with the burst of Ca-independent CaM-KII activity.

480.10

TRANSLOCATION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII) IN RAT HIPPOCAMPAL SLICES IS INDUCED BY SIMULATED ISCHEMIC INSULT S.J.Kolb and M.N.Waxham* Dept. of Neurobiol. and Anatomy, Univ. of Texas Med. Sch. Houston, TX 77030.

CaMKII has been shown to lose activity and to translocate from supernatant to pellet fractions of cortical and hippocampal homogenates in a global model of ischemia (Aronowski et al., 1992, J. Neurochem.).

To investigate the mechanisms underlying this translocation, a hippocampal slice model was developed. Slices were exposed to conditions that simulate an ischemic insult, and the subcellular distribution and enzymatic activity of CaMKII were monitored in homogenates prepared after increasing durations of the insult. Semi-quantitative Western Blots using a monoclonal antibody to the alpha subunit showed that there was a 70% decrease in CaMKII in the supernatant fraction and a 150% increase in CaMKII of the pellet fraction after 20 min. of ischemia. Shorter periods of ischemic insult produced a graded change in redistribution. There was little or no change detected in CaMKII in the total homogenates at any time suggesting that little proteolysis of CaMKII was evident at up to 20 min. in this model. CaMKII activity decreased in the supernatant in a parallel fashion with translocation. Activity increased by 20% in the pellet fractions at 5 and 10 min. of ischemia and then decreased back to pre-ischemic levels by 20 minutes.

We conclude that redistribution of the enzyme occurs in the hippocampal slice model and that this system may be useful in elucidating the mechanisms underlying CaMKII alterations and its impact on ischemia-induced neuronal injury.

480.11

DEVELOPMENTAL AND REGIONAL EXPRESSION OF MULTIFUNCTIONAL CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE ISOFORMS IN RAT BRAIN. L. Brocke, M. Srinivasan and H. Schulman*. Dept. of Neurobiology, Stanford University Medical Center, Stanford, CA 94305-5401.

Multifunctional calcium/calmodulin-dependent protein kinase (CaM kinase or CaM kinase II) is a major mediator of calcium signaling involved in diverse neuronal functions. All known subunits of CaM kinase consist of a catalytic domain at the N-terminal half of the protein followed by a regulatory domain and an association domain.

Multiple CaM kinase isoforms can be generated by putative alternative splicing at the variable domain, a region at the border of the regulatory and association domains. We have utilized the RT-PCR technique to amplify the variable domains of α- and β-CaM kinase and examine the diversity of isoforms present in rat brain.

Sequencing of a PCR fragment of α-CaM kinase from midbrain, termed α_B-CaM kinase, revealed an insertion of 11 amino acids at the variable domain identical to α33, a PCR fragment previously obtained from monkey frontal cortex (Benson et al., J. Neurosci. 11:31-47, 1991). Interestingly, this insertion generates a nuclear localization signal (KKRK) that targets α_B-CaM kinase to the nucleus in transfected neuroblastoma cells.

In addition, we isolated two new isoforms of β-CaM kinase from E18 rat brain, termed β_e- and β'_e-CaM kinase, which lack the 24 amino acids encoded by exon IX of the β-CaM kinase gene. The deletion probably involves a three base pairs shift in the position of the 3' splice site before exon VIII. In addition, β'_e-CaM kinase lacks 15 amino acids encoded by exon V. The expression of β-CaM kinase isoforms is developmentally regulated, with a pronounced switch towards the adult isoforms during the second postnatal week.

480.12

Differential Regulation of Multiple Calcium/Calmodulin-Dependent Protein Kinase Type II Subunit mRNA's. K.D. Murray*, C.A. Rosasco, and P.J. Isackson Department of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville, FL, 32224.

Calcium/calmodulin-dependent protein kinase type II (CamKII) is a multifunctional enzyme comprising as much as 2% of total forebrain protein and playing a critical role in excitatory neuronal processing. Originally identified as a multimeric holoenzyme composed of α and β/β' subunits, recent cloning analysis has identified two additional subunits CamKIIδ and γ as well as a series of alternatively spliced isoforms deriving from these four subunits. Earlier studies have shown that hybridization to CamKIIα mRNA is down-regulated throughout hippocampus and neocortex following hilus lesion induced seizures and up-regulated when afferent input is removed (Benson et al 1991, Murray et al 1993). To investigate whether the other CamKII subunits undergo a similar regulation we performed analysis using in situ hybridization, northern blots and S1 protection assays on tissue from adult male Sprague-Dawley rats sacrificed at various time-points following the onset of behavioral seizures induced by intraperitoneal injection of kainic acid (KA; 10mg/kg). The pattern of hybridization to CamKIIα,β,γ and δ subunit mRNA in saline injected animals was consistent with previous reports in untreated control animals. One notable difference was the presence of low, but detectable hybridization of CamKIIδ mRNA within the hippocampus. Consistent with hilus lesion induced seizures, KA treatment produced a stark reduction in hybridization to CamKIIα mRNA (90%) throughout the hippocampus and neocortex. Hybridization to CamKIIγ mRNA was also reduced within hippocampus and neocortex, but to a lesser extent. In contrast, hybridization to CamKIIδ mRNA was unaffected within hippocampal subfields but dramatically increased throughout the superficial layers of neocortex (5 fold). All mRNA changes were maximal 24 hrs following KA injection. CamKIIβ mRNA levels were not significantly altered following KA treatment. These observations provide evidence for the regulation of CamKII at the RNA level and suggest it is more complicated than previously thought.

480.13

INVESTIGATIONS OF KINASE ACTIVATION IN CORTICAL SYNAPTONEUROSOMES. I.J. Weiler *, S. Ahmari, Y. Ahmed, C.F. Lee, and W.T. Greenough, Depts. Psychol and Cell & Struc. Biol., Neurosci. Prog. and Beckman Inst., Univ. Illinois, Urbana-Champaign, IL 61801.

We have shown that metabotropic agonists stimulate polyribosomal aggregation in synaptoneurosomes. We are now using this system to analyze the rapid effects of glutamate agonists on receptor-coupled substrate phosphorylation. Synaptoneurosome suspensions are stimulated by addition of NMDA or quisqualate (with CNQX); at intervals, samples are lysed in the presence of 0.2 M EDTA and EGTA, 500 µM pNPP, 100 µM vanadate, 0,75 mM DTT, then flash-frozen on dry ice in 10 µl aliquots.

The basal phosphorylation activity is high; nevertheless we observe increased activity of PKC and PKA in the presence of either NMDA or quisqualate. MAP kinase activity, measured by MBP phosphorylation seems to be stimulated preferentially by quisqualate. We observe activation of CaMKII by either NMDA or ionophore A23187, more than by quisqualate. However, there seems also to be rapid calcium activation of a phosphatase, which we are currently investigating. Supported by MH 35321.

480.14

CARBACHOL-INDUCED PHOSPHORYLATION AND DEPHOSPHORYLATION OF PERCHLORIC ACID SOLUBLE PROTEINS IN HUMAN NEUROBLASTOMA SK-N-SH CELLS. A.M. Goldsmith and M.E. Gnegy*, Dept. Of Pharmacology, Univ. of Michigan, Ann Arbor, MI 48109.

We found that in SK-N-SH cells calmodulin (CaM) is redistributed from membrane to cytosol in response to the muscarinic agonist carbachol (CARB) or the phorbol ester TPA. This response could be due to phosphorylation of a membrane CaM-binding protein, releasing CaM to cytosol. We examined CARB-stimulated phosphorylation of proteins to correlate the time course of phosphorylation with that of CaM translocation. $^{32}P_i$ pre-equilibrated SK-N-SH cells were treated with 100 µM CARB or 100 nM TPA. Cells were lysed in 2.5% perchloric acid (PA) as some PA-soluble proteins (i.e. GAP-43, MARCKS, and neurogranin) release CaM upon phosphorylation by protein kinase C (PKC). CARB and TPA significantly increased phophorylationin of PA-soluble proteins of approximately 86kDa (p86) and 43kDa (p43). CARB-induced phosphorylation was maximal by 30 sec and lasted 30 min. TPA-induced phosphorylation was significantly greater than that with CARB while CaM translocation was comparable. Phosphorylation of p86 and p43 may be only a part of the CaM translocation process. Pretreatment with the calcineurin inhibitor FK506 (100 nM) increased the phosphorylation of p86 and p43 at all time points including their basal phosphorylation. A 5 min pretreatment with 1 µM OA, inhibiting PP1 and PP2A, had minimal effect on phosphorylation until 15 min after CARB. Staph. aureus V8 protease digestion and Western blotting suggested a 50kDa protein may be GAP-43. Phosphorylation of p50 was unaffected by CARB or TPA suggesting that GAP-43 is not PKC phosphorylated in these cells, that there is more than one form of GAP-43 in the cells which is not recognized by this antibody, or multiple phosphorylated proteins exist at p50. p86 may be MARCKS protein based on its M_r, phosphorylation by TPA, and solubility in PA. These results suggest that if phosphorylation is involved in the translocation of CaM multiple proteins, including p86 and/or p43, may be involved. Supported by NIMH grant MH36044.

480.15

A NOVEL NEURON-SPECIFIC MAP KINASE IN ADULT AND DEVELOPING MOUSE. J.H. Martin, A.A. Mohit and C.A. Miller*, Dept. of Pathology, Univ. Southern Calif. Sch. of Med., Los Angeles, CA 90033

In Alzheimer's disease (AD), the lack of an animal model complicates the analysis of early pathological changes. Mitogen-activated protein (MAP) kinases (also known as ERKs) have been implicated as agents of abnormal phosphorylation of microtubule-associated protein tau, the major component of AD neurofibrillary tangles (NFT). ERKs are thought to function as key regulatory molecules in the signalling pathways regulated by growth and differentiation factors. We have previously described a neuron specific human MAP kinase expressed in a subset of neurons in the human brain that undergo degeneration in AD. Here, we describe the cloning and molecular characterization of the mouse homolog. Immunocytochemically, MAb 3F12 identified a cytoplasmic antigen expressed almost exclusively in neurons. The human $p49^{3F12}$ kinase cDNA was used to isolate a 2.5 kb mouse cDNA clone. The deduced amino acid sequence was novel and shared 99% identity with that of the human p49 kinase. Moreover, the 3' untranslated region showed 88% nucleic acid identity with the human cDNA. This suggests a conserved regulatory role for this region. Northern blot analysis showed a 2.7 kb mRNA that was exclusively expressed in the brain and a 2.4 kb mRNA expressed only in testis. All other tissues showed no expression. *In situ* hybridization showed a similar pattern of expression as that of 3F12 antigen. Developmentally, the $p49^{3F12}$ kinase was turned on at embryonic day 11 (E11), and was expressed only in post-mitotic neurons. At E17.5, the mRNA was also present in the dorsal root ganglion as well as neurons in the CNS. These findings are in agreement with the suggested role of ERKs in neuronal differentiation. The distribution of the $p49^{3F12}$ protein along with evidence suggesting its involvement as a pathogenic agent in AD, may lead to development of a possible model for NFT formation.

480.16

TK/PTP MODULATION OF $[Ca^{2+}]_i$ RISE INDUCED BY NORADRENALINE IN PC Cl3 RAT THYROID CELLS. O. Meucci*, A. Scorziello, A. Avallone, T. Florio and G.Schettini Dept of Neurosci. Sect. Pharm. Univ. of Naples ITALY.

We studied the effect of genistein (an inhibitor of tyrosine-kinases, TKs) and vanadate (an inhibitor of phospho-tyrosine-phosphatases, PTPs) on noradrenergic-induced $[Ca^{2+}]_i$ rise in PC Cl3, a rat thyroid cell line, by means of fura 2 microfluorimetry and video imaging analysis. As previously showed in these cells, in presence of calcium in the extracellular medium, NE-stimulated $[Ca^{2+}]_i$ rise is characterized by a transient spike followed by a sustained plateau phase, mainly due to the IP3-linked mobilization of $[Ca^{2+}]_i$ which follows the activation of $\alpha 1_B$ adrenergic receptrors. A minor component of the noradrenergic response is also due to the activation of $\alpha 1_A$ receptor subtype which partecipate to the $[Ca^{2+}]_i$ increase evoked by NE through the stimulation of calcium influx from the extracellular environment. In this study we investigated the role of TK/PTP pathway in the modulation of calcium entry following noradrenaline (NE) receptor activation. Our data show that vanadate pretreatment of PC Cl3 cells was able to modify the plateau phase of NE-induced $[Ca^{2+}]_i$ increase keeping it higher than the control cells either in continue presence or after removal the agonist from the perfusion medium. Similarly, genistein caused a dramatic drop of the plateau phase in presence of NE in the extracellular enviroment, and a complete abolishment after NE-withdrawal. To rule out the possibility that the effect of vanadate was due to the blockade of ATPase pumps, we performed the same experiments in the absence of $Ca^{2+}$and in presence of Ba^{2+}in the extracellular medium. Ba^{2+}enters the cells via Ca^{2+} channels and is sensed by fura-2 but, since it cannot be pumped out of the cell or into the calcium stores, the entering $Ba^{2+}$is trapped in the cytoplasm. In these experimental conditions NE-induced Ba^{2+}influx was still potentiated by vanadate. These results suggest that the TK/PTP pathway is involved in the modulation of noradrenergic effects on calcium mobilization (AIRC 1993 to G.S.).

480.17

THE γ1 ISOFORM OF PROTEIN PHOSPHATASE 1 IS CONCENTRATED IN DENDRITIC SPINES. C.C. Ouimet*, E.F. da Cruz e Silva° and P. Greengard°. Department of Psychology, Florida State University, Tallahassee, FL 32306, °Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Ave., New York, NY 10021.

Protein kinases and phosphatases regulate diverse neuronal processes. The subcellular localization of the γ1 isoform of protein phosphatase 1 (PP1γ1) was examined in rat brain by immunocytochemistry using an antibody raised against a synthetic peptide derived from the PP1γ1 C-terminal sequence. At the light microscopic level, immunoreactivity was present in small puncta (1 μm or less in diameter) in the neuropil, but not in dendrites or somata. When the caudatoputamen was examined at the electron microscopic level, strong immunoreactivity was observed primarily in dendritic spines. Moderate immunoreactivity was present in myelinated and unmyelinated axons, and weak immunoreactivity was present in dendrites and occasional axon terminals. The localization of PP1γ1 to dendritic spines suggests its involvement in spine function, possibly in the regulation of synaptic plasticity. (Supported by USPHS grant MH40899).

480.18

THE MAJOR OKADAIC ACID- / CALYCULIN A-SENSITIVE PHOSPHATASE ACTIVITY IN LEECH CNS BELONGS TO THE TYPE I PHOSPHATASE FAMILY. A.L. Kleinhaus*, S. Ding., and K.N. Lerea. Dept. of Cell Biology & Anatomy, New York Medical College, Valhalla, N.Y. 10595.

Okadaic acid (OA) and calyculin A (CalA), specific inhibitors of the protein phosphatase types 1 (PP-1) and 2A (PP-2A), slow the recovery of elevated $[Ca^{2+}]_i$ caused by K⁺-depolarization and prolong the divalent cation-dependent action potential in the serotonergic Retzius cells of the leech (Kleinhaus & Zeman, Brain Res. 1994). These studies suggest that inhibition of PP1 and/or PP2A may directly modulate the kinetics of the voltage-dependent Ca^{2+}-conductance and/or alter other mechanisms related to Ca^{2+} movements or buffering.

To identify and quantitate the relative amounts of PP-1 and PP-2A found in these neurons protein phosphatase activity from extracts of leech segmental ganglia and isolated Retzius cells was measured using [^{32}P]phosphorylase-a as substrate. The major phosphatase activity in both preparations showed similarities to the mammalian form of PP-1. The dephosphorylation of phosphorylase-a by extracts from segmental ganglia, isolated Retzius cells and purified mammalian PP-1 was inhibited by okadaic acid with similar IC_{50} values (~50 nM). In addition, enzymatic activity intrinsic to each leech neuronal preparation was inhibited 80% by addition of inhibitor 2, a heatstable inhibitor protein specific for PP-1. The enzyme was also inhibited by calcyculin A, but the IC_{50} value of 20 - 30 nM is higher than that of the purified mammalian PP-1 and PP-2A. Taken together the results indicate that phosphatase activity in leech nervous tissue is mostly due to an enzyme of the type 1 family.

Studies to characterize the subcellular distribution and composition of this PP-1-like enzyme are in progress.

480.19

CLONING OF PARTIAL cDNAs FOR THREE PROTEIN TYROSINE PHOSPHATASES FROM *APLYSIA CALIFORNICA*. F.C. Richardson* and L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06518.

Patch clamp experiments of a voltage-dependent cation channel in *Aplysia* bag cell neurons have shown that the gating mode and response of this channel to protein kinase A (PKA) are determined by a PKA regulated protein-tyrosine phosphatase (PTPase)(G.F. Wilson and L.K. Kaczmarek, 1993, *Nature* vol. 366:433-438). As a first step towards a molecular characterization of this response, a PCR based cloning strategy was employed to isolate potential PTPase isoforms. cDNA was made by reverse transcribing total RNA isolated from abdominal ganglia and bag cell neurons. This cDNA was then amplified using degenerate primers designed to the conserved catalytic domain of published PTPase sequences. Products of predicted molecular weight were directly ligated into a TA vector and sequenced. Based on protein sequence homology to the catalytic domain of cytoplasmic and receptor type PTPases, three clones, named APTPα, APTPβ, and APTPγ, were identified as PTPases. APTPβ and APTPγ are 36.3% identical and are most similar to Human PTPδ (48.7%) and Rat LAR(67.4%), respectively. APTPα, which is 27.9% identical to APTPβ and 23.1% identical to APTPγ, is the most novel of the three isoforms. It's highest similarity to any published PTPase (Human LCA) is only 34.2%. *In situ* hybridization studies and further cloning will help determine whether any of the APTP isoforms are regulated by PKA in the bag cell neurons.

480.20

CLONING AND GENE EXPRESSION OF PHOSPHATASE INHIBITOR-2 (I-2) IN THE RAT BRAIN. H. Sakagami*, and H. Kondo. Dept. of Anatomy, Tohoku Univ. Sch. of Med., Sendai, 980, Japan.

Inhibitor-2 is the regulatory subunit of the ATP-Mg-dependent protein phosphatase, a cytosolic form of type 1 protein phosphatase (PP-1). Our in situ hybridization study has recently demonstrated that the mRNAs for three catalytic subunits of PP-1 and inhibitor-1(I-1) were differentially distributed in the rat brain (H. Sakagami et al., Mol. Brain Res., in press). However, no information has been available concerning the distribution of the gene expression and the functional significance of I-2 in the brain. We, therefore, cloned cDNA encoding the rat I-2 and examined the distribution of the gene expression for I-2 in the rat brain by in situ hybridization histochemistry. The cDNA encoding I-2 was isolated from a rat brain cDNA library using ^{32}P-labeled cDNA probe for rabbit I-2 ampified by polymerase chein reaction (PCR). The cDNA contained a complete open reading frame of 615 nucleotides, corresponding to 205 amino acids. The deduced amino acid sequence was 86 % homologous to previously identified rabbit skeletal muscle I-2 (Park et al., 1994). By in situ hybridization histochemistry using oligonucleotide probes, intense expression signals were detected in the hippocampal pyramidal and dentate granule cell layers, and olfactory tubercle. Moderate expression signals were detected in the olfactory neuronal layer, caudate putamen, neocortex, and cerebellar granule cell layer. When compared this expression pattern with that of I-1mRNA, several differences were found: the homogeneous expression of I-2 mRNA versus the laminar expression of I-1 mRNA in the neocortex; low expression of I-2 mRNA versus high expression of I-1 mRNA in the arachnoid membrane. These findings suggest that the activities of PP-1 may be differentially regulated by I-2 and I-1 in various brain regions. (Supported by grants 04404020 and 0526023 from the Ministry of Education, Science and Culture of Japan)

481.1

CHARACTERIZATION AND REGULATION OF GABA$_A$ RECEPTOR SUBUNITS ON NEURONES IN THE HYPOTHALAMIC SUPRAOPTIC (SON) AND PARAVENTRICULAR (PVN) NUCLEI. V.S. Fenelon and A.E. Herbison. (SPON: Brain Research Association) Lab. of Neuroendocrinology, Babraham Institute, Cambridge CB2 4AT, U.K.

GABA is known to regulate oxytocin (OT) and vasopressin (VP) secretion via the occupancy of GABA$_A$ receptors. As the different GABA$_A$ receptor isoforms have different pharmacodynamic properties, the present study was undertaken in order to characterize the GABA$_A$ receptor subunit types expressed by magnocellular hypothalamic neurones and determine whether changes in specific subunit mRNAs occur during pregnancy and lactation.

Double labelling studies using antisera specific for either OT or VP and antisera directed against α_1, α_2 or $\beta_{2/3}$ subunits of the GABA$_A$ receptor showed that all OT and VP neurones located in the SON express α_1, α_2 and $\beta_{2/3}$ subunits. However, cells in the PVN only expressed the α_2 subunit. *In situ* hybridization experiments using ^{35}S-labelled oligonucleotides complementary to α_1, α_2 or β_3 subunits confirmed that neurones in the SON expressed α_1, α_2 and β_3 subunit mRNAs while those in the PVN only highly expressed the α_2 subunit. Silver grain analysis for α_1 and α_2 subunits were carried out in the SON, PVN and cortex of ovariectomised (OVX), pregnant (day 10 and 19), parturient and lactating rats (day 7 and 14). α_1 mRNA expression in the SON increased during pregnancy, peaking at day 19, and fell to its lowest level on the day of parturition. α_1 mRNA expression in the cortex and α_2 mRNA expression in SON, PVN and cortex did not show such variations.

Together, these results provide a partial characterization of the GABA$_A$ receptor isoforms expressed by rat magnocellular neurones and show that the GABA$_A$ receptors synthesized by magnocellular neurones in SON and PVN are different. Furthermore, it appears that expression of an α_1 subunit-containing GABA$_A$ receptor is altered during pregnancy suggesting that GABA influences on OT and/or VP secretion at this time may be determined, in part, by receptor expression.

481.2

CONOPRESSIN, A VASOPRESSIN-LIKE PEPTIDE REGULATING MALE BEHAVIOUR IN LYMNAEA STAGNALIS: CHARACTERIZATION OF THE PEPTIDE, PRECURSOR, GENE AND RECEPTORS. R. E. Van Kesteren, A. B. Smit, C. P. Tensen, J. Van Minnen, F. Van Golen and W. P. M. Geraerts*. Institute of Neuroscience, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

We used antisera raised against vasopressin to identify a vasopressin-like peptide, named conopressin, from the brain of the mollusk *Lymnaea stagnalis*. cDNA encoding conopressin was isolated using PCR and the amino acid sequence of the conopressin prohormone was deduced. The precursor is very similar to the vasopressin precursor of vertebrates, containing conopressin and a highly conserved neurophysin domain together with a less conserved copeptin domain. In *Lymnaea*, these two peptide domains probably remain uncleaved, as has been observed in lower vertebrates. The conopressin gene has also been cloned and shown to contain two introns. Expression of the conopressin gene was detected in two neurons of the pedal ganglia and in neurons of the anterior lobes of the cerebral ganglia. The neurons in the anterior lobe of the right cerebral ganglion are part of a neuronal network that regulates male copulation behaviour in *Lymnaea*. They project via the penis nerve towards the penial complex and the vas deferens. Conopressin has an excitatory effect on the vas deferens. This effect is antagonized by APGWamide, a peptide that is co-localized with conopressin. Conopressin affects also identified central neurons, indicating that it acts as a neurotransmitter. Two putative conopressin receptors were cloned from the vas deferens. These receptors have high sequence identity with the phospholipase C coupled vasopressin V1 and oxytocin receptors, which mobilize intracellular Ca^{++} upon activation. The conopressin receptors are differentially expressed in the sex organs, with one receptor being expressed in the ovotestis, the prostate and the posterior part of the vas deferens, and the other in the anterior part of the vas deferens. Both receptors are also present in the brain. The exact localization of central expression is currently under investigation.

481.3

OVARIAN HORMONE INDUCES PLASTICITY OF OXYTOCIN NEURON ACTIVITY OF SUPRAOPTIC NUCLEUS IN THE FEMALE RAT. T.KIYOHARA*, T.NAKASHIMA and S.MIYATA. Dept. of Applied Biology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606 Japan

It has been demonstrated that oxytocin (OXT) neurons in the supraoptic nucleus of lactating rats are facilitated by exogenously applied OXT while that of virgin females are inhibited. The present study was carried out to investigate whether OXT increases the OXT neuron activity in hypothalamic slices prepared from virgin female, pregnant, delivering, lactating, and ovariectomized rat by recording extracellular single-unit activity. Most OXT neurons of virgin female and pregnant rats showed inhibitory responses to OXT. In contrast, majority of OXT neurons tested in delivering and lactating rats exhibited excitatory responses to OXT. This excitation reversed to inhibition again after the lactating period had ended. OXT neurons in ovariectomized virgin female rats showed excitatory responses to OXT. This excitation was also reversed to the inhibition by estrogen treatments. It was also founded that morphological changes of OXT neurons associated with the lactation were unaffected by ovariectomization suggesting that other stimuli such as nipple sucking may be crucial in maintaining the changes. These findings suggest that ovarian hormones might play an important role in induction of neural plasticity in rat hypothalamic OXT neurons.

481.4

Intracerebroventricular (icv) injection of an inhibitor of nitric oxide synthase attenuates drinking and release of vasopressin (VP) while stimulating secretion of oxytocin (OT) in conscious rats. M. Kadekaro*, M.L. Terrell, E. Koehler[a], S. Gesti[a], J.Y. Summy-Long[a]. Div. of Neurosurgery, Univ. of Texas Medical Branch, Galveston, TX, [a]Dept. of Pharmacol., Penn. State Univ., Hershey, PA.

We have shown that N^G-monomethyl-L-arginine (NMMA), an inhibitor of nitric oxide (NO) synthase, attenuates water intake (Neurosci. Lett., 1994, in press) and enhances plasma levels of OT (Neurosci. Lett. 152:190, 1993) after icv injection to rats dehydrated for 24h. Because NMMA can be metabolized to L-arginine, the substrate for NO synthase, the effects of another selective inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (NAME), on water intake and plasma VP and OT was examined in conscious adult male albino rats. For drinking studies, CSF (5μl) or NAME (10μg, 250μg or 500μg) was injected icv 10 to 12 min before water was available a) to 24h dehydrated rats or b) after angiotensin II (ANG II; 250 ng/2μl, icv). Water intake (ml/60 min; $\bar{x}$ ± SEM, n=8) induced by ANG II was less ($p<0.02$) after all but the lowest dose of NAME [CSF (9.1 ±0.9) & NAME 10μg (9.4 ±0.9) > NAME 250 μg (6.0 ±0.6) & NAME 500μg (5.5 ±0.7)]. Only 500μg of NAME attenuated drinking (ml/2h) in dehydrated animals (CSF > NAME; 9.3 ±1.1 > 6.0 ±0.9; n=10). Plasma [OT] (pg/ml; $\bar{x}$ ± SEM; n=5-9) elevated after 24h of dehydration (CSF 14 ±2) increased ($p<0.05$) further 15 min after 500μg of NAME (25 ±5), L-(46 ±7) or D-(67 ±8) arginine, whereas plasma [VP] decreased ($p<0.05$; n=5-9) only after NAME (12 ±1 > 6 ±1). In control rats basal [OT], but not [VP], was similarly affected ($p<0.05$) by NAME, L- and D- arginine (CSF < NAME < L- & D-arginine; 7 ±1 < 23 ±3 < 30 ±5 & 40 ±9). Thus, NO promotes positive water balance during dehydration by stimulating drinking and release of VP while attenuating secretion of OT.

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481.5

HYPERTONIC SALINE-INDUCED CHANGES OF THE OXYTOCIN CONTENT OF THE THORACIC SPINAL CORD: REGULATION BY OPIOID PEPTIDES. V. Votin & J. Haldar*. Department of Biological Sciences, St. John's University, Jamaica, NY 11439.

Previously we have demonstrated that oxytocin content of the spinal cord changes in response to various stressors. For example, following immobilization stress of one minute, oxytocin content increases (Brain Res. 17:137-141, '88) whereas following hypertonic and isotonic saline injections, oxytocin content decreases (Life Sc. 53:579-584, '94). The current experiments were designed to determine what factor or factors regulate spinal cord oxytocin content. Since opioid peptides are known to regulate oxytocin release from the neurohypophysis, it was logical to investigate whether spinal cord oxytocin is also under opioid regulation. Male Sprague Dawley rats were divided into 5 groups and injected with (i) isotonic saline (0.85% ip), (ii) hypertonic saline (1M ip), (iii) Naloxone (5mg/Kg BW, sc), (iv) Naloxone + isotonic saline and (v) Naloxone + hypertonic saline. Injection volume always was 0.2 ml/100 gm body weight. For Control group, rats were pin-pricked twice, once subcutaneously, matching with Naloxone injection and second time ip, matching with saline injection. Five minutes after Naloxone injection, the rats were injected with saline and were sacrificed fifteen minutes thereafter. Following decapitation, thoracic part of the spinal cord was isolated from the vertebral column and was separated into dorsal and ventral half. Oxytocin content of the spinal cord was determined by a specific oxytocin radioimmunoassay in a Sep-pak extracted sample. Our results demonstrate that (i) dorsal half of the spinal cord contains ten fold more oxytocin than that of the ventral half, (ii) both isotonic and hypertonic saline decrease oxytocin content of the spinal cord and (iii) naloxone reverses saline-induced decrease of the spinal cord oxytocin content. In conclusion, these results suggest that spinal cord oxytocin is at least partly regulated by opioid peptides.

481.6

INTRACEREBROVENTRICULAR ADMINISTRATION OF INTERLEUKIN-1ß SUPPRESSES REFLEX MILK EJECTION IN URETHANE-ANAESTHETIZED RATS. B.C. Wilson* and A.J.S. Summerlee. Dept. of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G-2W1.

It has been shown that icv IL-1ß stimulates oxytocin release in male (Neumann et al., 1993, Soc. for Neurosci. Abst. 19(1): 97) and female (Wilson and Summerlee, unpublished data) rats and that IL-1ß excites supraoptic neurones (Li et al., 1992, Neuroreport 3: 91-93). Experiments were done using the methodology of Summerlee et al., 1984, Nature 309: 372-374 to investigate the effect of icv IL-1ß on the pattern of reflex milk ejection (RME) in rats at Day 10 of lactation. Urethane-anaesthetized rats were implanted with cannulae in the left saphenous vein and a caudal mammary gland and then placed in a stereotaxic head frame. A microsyringe was placed with its tip in a lateral cerebral ventricle for icv injection. Ten rat pups were placed to suck at the nipples of each dam to induce RME. After 6 milk ejections, rats were treated with IL-1ß (1ng/µL PBS-BSA icv) or PBS-BSA (1µL icv) and the effect on the pattern of RME observed. IL-1ß suppressed RME in 10 out of 12 (83%) rats tested compared with 0 out of 9 (0%) for those treated with the PBS-BSA vehicle. It is possible that in female rats, IL-1β stimulates basal oxytocin release while inhibiting suckling-induced OT release involved in milk ejection. The data suggest that IL-1ß plays a role in mediating suckling-induced neuroendocrine events in the rat.

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481.7

INTRACEREBROVENTRICULAR INJECTION OF RELAXIN AFFECTS THE RELEASE OF BOTH VASOPRESSIN AND OXYTOCIN IN ANESTHETIZED RATS. B.J. Geddes and A.J.S. Summerlee*. Dept. of Biomedical Sciences, University of Guelph, Guelph, Ont. CAN, N1G 2W1.

Injections of exogenous relaxin either IV or ICV produce a marked and sustained rise in systemic arterial blood pressure (Parry LJ & Summerlee AJS, 1991). Intravenous relaxin administration has also been shown to substantially affect the release of vasopressin (AVP) and oxytocin (OX) (Way & Leng, 1992; Geddes BJ, Parry LJ & Summerlee AJS, 1994). There is preliminary evidence that ICV relaxin also affects magnocellular peptide secretion (Jones, 1986) so the present study was conducted to make a more thorough examination of the influence of ICV relaxin on vasopressin and oxytocin release. Urethane-anesthetized female SD rats were cannulated such that blood samples (≤1mL) and fluid replacement could take place simultaneously. Blood samples were removed before and at 1, 2.5, 5, 10 and 30 min after ICV injection of 500ng of relaxin (in 1.0μL physiological saline) and the plasma assayed for AVP and OX by specific RIA (University of Bristol, Bristol, UK). Relaxin treatment caused a substantial (≅250.0%, $p<0.05$) elevation in plasma AVP levels above baseline 1 and 2.5 min post-treatment, and an immediate increase (110.0% at 1min, $p<0.05$) in plasma OX that was sustained for the duration of the experiment. The findings of the present study extend our current understanding of the central actions of relaxin by demonstrating that the effects of relaxin injected ICV and IV are ostensibly identical which supports the hypothesis that relaxin affects cardiovascular function through a central mechanism. Supported by NSERC Canada and OMAF Canada.

481.8

EFFECT OF OVARIAN STEROIDS ON HYPOTHALAMIC PULSATILE OXYTOCIN RELEASE EVOKED BY SUCKLING IN THE ANAESTHETISED RAT. Q.B.Jiang and J.B.Wakerley. (SPON: Brain Research Association). Dept. of Anatomy, Sch. of Medical Sciences, Univ. of Bristol, Bristol, BS8 1TD, U.K.

Effect of ovarian steroids on pulsatile oxytocin (OT) release during suckling was examined in late pregnancy by ovariectomy (OVX) and steroid replacement. Intramammary pressure recordings of milk-ejection responses were used to detect pulsatile OT release from the magnocellular OT neurones in the hypothalamus. OVX on day 20 of pregnancy significantly increased the frequency of milk ejection responses on day 22 of pregnancy, compared with sham-OVXed controls (3.7 ± 0.4/20min,n=7, versus 2.4 ± 0.4/20min,n=6, $P<0.05$, t-test). Replacement with either progesterone (5mg per day, s.c.) or estradiol (5μg per day, s.c.) in day 20 pregnant OVXed rats each reduced milk-ejection frequency to the sham-OVXed level. However, these two different steroid regimes caused a remarkable difference in the facilitatory response to intracerebroventricular (i.c.v.) injection of OT. Milk-ejection frequency in progesterone replaced rats was decreased significantly from 2.0 ± 0.3/20min before i.c.v. OT to 0.8 ± 0.2/20min after i.c.v. OT (n=8, $P<0.05$), whereas in estradiol replaced animals there occurred a significant increase of milk-ejection frequency from 2.0 ± 0.5/20min to 3.6 ± 0.3/20min (n=9, $P<0.05$). In OVX oil treated controls, frequency of milk ejection before and after i.c.v OT were 3.6 ± 0.6/20min and 3.7 ± 1.0/20 min (n=7). These results suggest that ovarian steroids may play a role in determining the frequency of pulsatile OT release towards the end of pregnancy. Furthermore, the rising level of oestradiol and fall of progesterone towards the end of pregnancy may be a necessary prerequisite for allowing the facilitatory action of centrally-released oxytocin on the milk-ejection reflex.
Supported by AFRC AG7/563

481.9

INCREASED VASOPRESSIN (VP) SECRETION FROM HYPOTHALAMIC CULTURES FOLLOWING EXOGENOUS VP mRNA ADMINISTRATION. J.R. Mathiasen*[1], D. Maciejewski-Lenoir[2], F.E. Bloom[2], and C.D. Sladek[1]. [1]Dept. of Physiology and Biophysics, Finch University of Health Sciences/Chicago Medical School, North Chicago IL 60064, [2]Dept. of Neuropharmacology, Scripps Research Institute, LaJolla CA 92037

Previous studies have shown that exogenous administration of vasopressin (VP) mRNA to Brattleboro rats significantly increases serum VP and urine osmolality (Science,1992 225:996-998). As a first step towards understanding the mechanisms by which cells take up and process exogenous mRNA, we have administered VP mRNA to long term (30 day) primary dispersed hypothalamic cell cultures from 14 day old rat (Sprague Dawley) fetuses. Hypothalamic cells were cultured either in control medium or medium containing the cAMP elevating agents 3-isobutyl-1-methyl-xanthine (IBMX 0.5 mM) and forskolin (25 μM, together designated IF) for 23 days or 30 days in vitro to maintain the VP phenotype (VP synthesis and secretion) (Endocrinology, 1993 133:1320-1330). VP secretion into the medium of cells treated with IF for 23 days declined to baseline by 30 days in vitro, but administration of VP mRNA to these cultures on day 30 resulted in a ~5-fold increase in VP content in the medium ($p = 0.005$) after 6 hrs and a ~2.5-fold increase after 24 hrs ($p = 0.002$). Administration of VP mRNA to the cultures treated continuously with IF also resulted in a small increase in VP secretion which did not reach significance after 6 or 24 hrs. Autoradiography of cultures administered ^{3}H or ^{32}P-VP mRNA for 24 h revealed silver grains indicating VP mRNA located in or on varicosities as well as on the perikarya and neuritic processes of rat neurophysin immunocytochemically identified neurons. These results suggest that exogenously administered mRNA has access to cell translation systems in cultured cells and that translation of exogenously applied mRNA may occur as early as 6 h after treatment.

481.10

TESTING THE ROLE OF NEUROHYPOPHYSEAL OXYTOCIN IN PARTURITION, LACTATION AND MATERNAL BEHAVIOR IN GOLDEN HAMSTERS. Y. Delville*, E.W. Quan, K.M. Mansour, and C.F. Ferris. Behavioral Neuroscience Lab., Psychiatry Dept., Univ. of Mass. Med. Ctr., Worcester, MA 01655.

Recent evidence have questioned the source of oxytocin in the control of parturition, as the uterus has been found to produce the peptide. We tested the presumed roles of neurohypophyseal oxytocin by destroying all neurons projecting to the neurohypophysis through micro-injection of volkensin, a neurotoxic retrograde lectin, within the gland itself. This treatment produced polydipsia and polyuria, indicating that the neurosecretory vasopressinergic system was destroyed. All animals followed regular 4-days estrus cycles, and became sexually receptive on the day of estrus. Sixteen days after mating, on the predicted day of parturition, volkensin-treated animals gave birth to their pups, producing regular size litters (7 to 16 pups). The animals were then observed for occurrences of lactation and maternal behavior. One week later, all female were sacrificed, their blood collected, and their brain fixed with acrolein. Oxytocin-immunoreactive neurons were observed and mapped in these brains, and levels of oxytocin were assayed in the blood samples. Some animals were deprived of blood levels of oxytocin and oxytocinergic neurons within neurosecretory cell groups such as the supraoptic nucleus. Interestingly, these animals did not lactate, nor show maternal behavior. These result suggest that, while neurohypophyseal oxytocin is critical for lactation, it is not necessary for parturition. Supported by NS30199 from the NINDS awarded to CFF.

481.11

RESTORATION OF VASOPRESSIN RELEASE FOLLOWING HYPOPHYSECTOMY IN RATS.
R.H. Melloni, Jr.*, J.A. Brewer, Y. Delville, L.J. DeGennaro[§], and C.F. Ferris Laboratory of Behavioral Neuroscience, Dept. of Psychiatry, University of Massachusetts Medical Center, Worcester, MA 01655. [§]Wyeth Ayerst Research, Princeton, NJ 08543.

Synaptic reorganization in the median eminence (ME) following hypophysectomy has been extensively studied morphologically, although, relatively little is known regarding the molecular events that underlie the recovery of water balance. In pilot studies, male rats (n=9) were hypophysectomized and observed for the restoration of water balance over the next 33 days. Animals were sacrificed at weekly intervals following surgery, the blood measured for vasopressin (AVP) and the brains examined for AVP immunostaining in the ME. Urine output and water intake was variable over the first week as were the blood levels of AVP. Examination of the ME revealed an aggregation of AVP immunoreactivity with little organization. During weeks two and three the plasma levels of AVP peptide were stabilized at about 30 pg/ml while urine and water volumes remained at about 40 ml/day. The AVP immunoreactivity has increased in density and organization around multiple layers of capillaries that form a new mini neural lobe in the ME. By the fourth week urine and water volumes decline in correlation with an increase in plasma AVP levels to approximately 220 pg/ml. At this time point, the pattern of AVP immunostaining appeared to be less intense but better organized around the new capillary plexus. Studies are underway to examine the restoration of AVP function in animals maintained for longer periods, and to correlate recovery with the expression of proteins implicated in the regulation of synaptic function. Supported by NIH NS25050 and NS27833 to L.J.D.

481.12

IMAGING SYNAPTIC CONNECTIVITY WITH WIDEFIELD DIGITAL MICROSCOPY. C.F. Ferris*, Y. Delville, J. A. Collins and L. Lifshitz. Behav. Neurosci. Labs., Psychiatry Dept., University of Massachusetts Medical Center, Worcester, MA 01655.

We are developing the technology to visualize and quantify synaptic connections at the light microscopic level. This technology is being validated by examining the synaptic connections of glutamate decarboxylase (GAD) terminals on oxytocin (OXY) neurons in the supraoptic nucleus of rats, a system reported to be highly plastic following salt loading. OXY neurons are labeled with FITC and GAD boutons with Texas Red in 50 μm thick sections. The sections are examined under a Nikon Diaphot inverted microscope mounted with a Photometrics CCD camera. Sections are excited for each fluorochrome, optically sectioned at 250 nm, digitized and restored. Each data set is analyzed on a Silicon Graphics Indigo Elan workstation with the interactive software DAVE. By combining immunofluorescent labeling for OXY and GAD, it is possible to examine the synaptic connections between the two systems. A synapse is identified when both fluorescence signals colocalize in a voxel (187 X 187 X 250 nm) in a set of contiguous voxels. DAVE eliminates all immunoreactive elements (e.g., spurious staining, synaptic boutons, and fibers of passage) that do not colocalize with the membrane of the OXY neuron. All putative synaptic connections that do not fall within the dimensions of a GAD synaptic bouton programmed into the computer are deleted and the remaining putative synapses are displayed on the surface of the postsynaptic membrane.

481.13

IONIC DEPENDENCE OF THE DEPOLARIZING AFTERPOTENTIAL (DAP) IN VASOPRESSIN NEURONS: ENHANCEMENT BY HISTAMINE . W.E. Armstrong* and B. N. Smith**, Depts. of Anat. Neurobiol., Univ. Tenn., Memphis, TN 38163*, and Colorado State Univ., Ft. Collins, CO 80523**.

Acting through H_1 receptors, histamine depolarizes vasopressin neurons and augments the Ca^{++}-dependent DAP to promote phasic bursting activity (Smith and Armstrong, Neurosci. 53: 855, 1993). The enhancement of the DAP persists when blocking the afterhyperpolarization, is independent of $[Cl^-]_i$ and is TTX-insensitive. Conventional intracellular recordings from supraoptic nucleus (SON) neurons in the rat hypothalamic explant were used to further assess the ionic dependence of histamine's effect. Neurons recorded with electrodes containing the Ca^{++} chelator BAPTA (100-500 mM) failed to exhibit a DAP and also failed to depolarize to histamine, suggesting its actions may depend upon increased $[Ca^{++}]_i$. Histamine broadened action potentials slightly in normal cells and more markedly in cells loaded with the K^+ channel blocker Cs^+, indicating it may also promote increased Ca^{++} influx. Raising $[K^+]_o$ depolarized SON neurons and elevated proportionately the threshold for the DAP. When measured at the same membrane potential, the DAP was consistently smaller in high $[K^+]_o$, and was only weakly enhanced by histamine. The *apparent* conductance of the membrane is slightly but consistently decreased during the DAP when tested with small, brief current pulses which do not alter its amplitude or time course. While the conductance data could be interpreted as arising from deactivation of a voltage-dependent inward current (Bourque, Neurosci. Lett. 70: 204, 1986), combined with the K^+-dependence of the DAP they are also consistent with the idea that the DAP, and histamine's effects, could be mediated in part by Ca^{++}-dependent inhibition of a slowly or non-inactivating K^+ current which is activated at depolarized potentials. Since an inward current is nevertheless likely to be involved in the DAP, experiments in progress are testing its sensitivity to altered $[Na^+]_o$.

Supported by NIH NS23941 (WEA) and NIMH MH0993 (BNS).

481.14

VASOPRESSINERGIC INNERVATION TO SUBSTANTIA NIGRA (SN) AND VENTRAL TEGMENTAL AREA (VTA) IN THE RAT. T. Yamamoto* and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Vasopressin (VP) fibers are widely distributed in the brain in addition to a hypothalamo-hypophysial system. In addition to classically known antidiuretic and vasoconstrictive actions, VP affects memory process, cardiovascular function, thermoregulation and motor behavior. SN and VTA in the midbrain are known to receive VP innervation, but the cells of origin of these VP fibers are unknown. In this study, we examined the VP innervation to SN and VTA in the rat using a retrograde fluorescence tracing technique and immunohistochemistry.

Ten to 14 days following pressure injection of Fluoro-Gold (FG) (2.5 to 4%, 25 to 45 nl) into the SN/VTA region, colchicine (120 µg/kg) was injected into the lateral ventricle. After a 2 day survival period, the rats were transcardially perfused and sacrificed. The brains were removed, post-fixed and stored in phosphate buffer. Using a vibratome 50 µm sections were cut for immunohistochemistry.

The neurons in the bed nucleus of the stria terminalis (BST), medial amygdaloid nucleus (MeA), locus coeruleus and paraventricular hypothalamic nucleus (PVN) were retrogradely labeled by FG. In BST there were many double labeled neurons, but there were only a few in the MeA, PVN, and hypothalamic area. These observations indicate that SN and VTA receive VP afferents mainly from BST, but some originate from the hypothalamic area, MeA and PVN.

Supported by NIH Javits Award NS 20702 and the Human Frontier Science Program.

481.15

GLUTAMATE MICROSTIMULATION OF LOCAL INHIBITORY CIRCUITS IN RAT SUPRAOPTIC NUCLEUS J.-P. Wuarin* and F.E. Dudek Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

GABA provides most if not all of the inhibitory synaptic input throughout the neuroendocrine hypothalamus. Half of the synaptic boutons are immunoreactive for GABA in supraoptic (SON), paraventricular, suprachiasmatic and arcuate nuclei. Neurons immunopositive for GABA and glutamic acid decarboxylase have been shown in and around SON and other hypothalamic nuclei, suggesting that inhibitory input to hypothalamic neuroendocrine cells might originate predominantly from local GABAergic neurons. Using whole-cell recordings and glutamate microdrops in hypothalamic slices, we tested the hypothesis that local GABAergic neurons provide inhibitory synaptic input to supraoptic magnocellular neuroendocrine cells. Microdrops of glutamate (20 mM, 50-150 µm in diameter) were applied in the periphery of the SON while frequency and amplitude of synaptic currents in supraoptic magnocellular neurons were monitored (n=23). In 4 cells, glutamate microdrops dramatically increased frequency and average size of inhibitory postsynaptic currents (IPSCs) while no change in excitatory postsynaptic currents was detected. Glutamate microdrops increased IPSC frequency from 0.5-2 Hz to 3-20 Hz. Glutamate microstimulation also increased the average amplitude in all 4 cells by increasing the proportion of the largest IPSCs, suggesting that more IPSCs were action potential-mediated. These results support the hypothesis that GABAergic neurons located in the periphery of SON provide inhibitory input to supraoptic magnocellular neuroendocrine cells.

481.16

MANNITOL AND ANGIOTENSIN II MODULATE SPONTANEOUS SYNAPTIC CURRENTS IN SUPRAOPTIC NEURONS OF RAT HYPOTHALAMUS. T. NAGATOMO, K. INENAGA*, L.-N. CUI, H. TANAKA and H. YAMASHITA. Dept. of Physiol., Univ. of Occup. and Environ. Health, Kitakyushu 807, Japan

Spontaneous synaptic currents were recorded in the supraoptic (SON) magnocellular neurosecretory cells using whole-cell patch-clamp techniques in rat hypothalamic slice preparations. Excitatory postsynaptic currents (EPSCs) were almost completely blocked by the non-NMDA receptor antagonist CNQX (10 μM). Inhibitory postsynaptic currents were blocked by the $GABA_A$ receptor antagonists picrotoxin (50 μM) and bicuculline (1 μM). Mannitol and NaCl (30-60 mOsm/kg), and angiotensin II (1 μM) increased the number of EPSCs without changing the mean amplitude of EPSCs. The AT_1 receptor antagonist losartan potassium (10 μM) decreased evoked postsynaptic current but little affected the spontaneous EPSCs. These results support evidence that 1) glutamate and GABA are main synaptic transmitters in *in vitro* preparations, acting through non-NMDA receptors and $GABA_A$ receptors, respectively, and suggest that 2) hyperosmotic stimulation increases the synaptic transmission, affecting presynaptically and 3) angiotensin II modulates neurotransmission in the SON neurons of rat hypothalamic slice preparations.

NEUROENDOCRINE REGULATION: OTHER II

482.1

EXPRESSION OF THYROID HORMONE RECEPTORS (THRs) IN GH3 AND B103 CELLS. C-G Hahn, A. Pawlyk, P.C. Whybrow* & S. M. Tejani-Butt. Dept. of Psychiatry, University of Pennsylvania School of Medicine, Phila., PA, USA.

The association of altered thyroid axis in affective illness and its modulation in treatment responsiveness is well established. Recent clinical studies suggest that the availability of thyroid hormones (TH) to the CNS is critical for the treatment responsiveness of lithium (Li), a mood stabilizer, in bipolar affective disorder. This study examined the effects of Li on THR-α2 transcript in GH3 cells, a pituitary adenoma cell line. Cells were grown in resin stripped TH deficient media and treated with triiodothyronine (T3, 10 and 100 nM) up to 5 days, in the presence or absence of Li (1 mM). 20 ug of total RNA from each sample was analyzed by Northern hybridization. The results indicated that the expression of THR-α2 was decreased (75%) by T3, when compared to cells that were grown in T3 deficient media for 2 days, as has been previously reported. In cells that were analyzed at 5 days, the expression of THR-α2 was also found to be decreased in comparison to cells grown in T3 deficient media. Addition of Li did not appear to alter the expression of THR-α2 in GH3 cells. The present study also examined whether B103 cells, a neuroblastoma cell line, would express THRs. B103 cells were found to express both THR-α2 and THR-α1, suggesting that B103 cells may be a potentially useful model for the study of neuron specific regulation of THRs. (Research funds from USPHS grant MH 44210).

482.2

POSTNATAL DEVELOPMENT OF THE RAT ADRENAL MEDULLA

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The functional innervation of the rat adrenal medulla matures postnatally. In the present study in situ hybridization and indirect immunohistochemistry were employed in order to study this process. Neurofilament 10 (NF10)- and growth associated protein 43kD-immunoreactive (IR) nerve fibers were present in the medulla on day 2, and the gradual onset of a functional innervation correlated to increasing acetylcholinesterase (AChE) and enkephalin (ENK) (and ACh?) levels in splanchnic preganglionic fibers. The maturation of these fibers seemed to exert a suppressive effect on the expression of ENK, calcitonin gene-related peptide (CGRP) and neurotensin in chromaffin cells, while galanin (GAL) was low also in neonatal rats. The catecholamine synthesizing enzymes were little affected during this period. The large, neuropeptide tyrosine containing ganglion cells developed in parallel to the preganglionic innervation, and gradually exhibited mature properties, i.e. strong AChE- and NF10-like immunoreactivities, and expression of nerve growth factor receptor and tropomyosin receptor kinase mRNAs. In contrast, the assumed sensory CGRP- and GAL-IR innervation, and the vasoactive intestinal polypeptide-containing ganglion cells were almost fully developed on day 2. IN CONCLUSION, the postnatal development of the rat adrenal medulla is paralleled, and most likely influenced by the maturation of splanchnic preganglionic fibers, both with regard to enzyme and peptide regulation, and to maturation of the ganglion cells. However, other factors such as thyroid hormone, glucocorticoids and neurotrophins may also influence these processes.

482.3

ANTIBODY-INDUCED SYMPATHECTOMY OF THE RAT ADRENAL GLAND INCREASES PEPTIDE LEVELS MAINLY IN ADRENALINE PRODUCING CELLS. Å. Dagerlind*, M. Pelto-Huikko, S. Brimijoin and T. Hökfelt. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Systemic administration of monoclonal acetylcholinesterase antibodies has been shown to cause selective degeneration of sympathetic preganglionic neurons. In the present study rats were subjected to a single i.v. injection of these antibodies. Exophthalmos, piloerection and ptosis were observed within 1 h after administration. Rats were sacrificed at different time-points, and the adrenal glands were analysed by means of indirect immunohistochemistry and in situ hybridization histochemistry. As soon as 3 h after the antibody treatment a marked increase in the number of chromaffin cells expressing mRNA encoding, respectively, enkephalin, calcitonin gene-related peptide, galanin, neurotensin and substance P was seen. At 12 h the peptide mRNA levels were still elevated and there was a concomitant increase in the number of peptide-immunoreactive cells. All petide levels remained high for at least 48 h, however, seventy-seven days after the antibody treatment only enkephalin-immunoreactive cells could be encountered. A disappearance of acetyl-cholinesterase- and enkephalin-positive fibers was seen already 3 h after the antibody treatment, and after 24 h no fibers were encountered. In contrast, up till 48 h there was no apparent change in the number or intensity of immunofluorescent fibers expressing calcitonin gene-related peptide, galanin, neurotensin or substance P. However, 77 days after the antibody treatment the number of calcitonin gene-related peptide- and substance P-immunoreactive fibers was increased as compared to controls. In addition, reappearance of acetylcholinesterase- and enkephalin-immunoreactive fibers was seen at 77 days, although their number still was low as compared to controls. Double-labeling immunohistochemistry revealed that the cells expressing peptides preferentially were adrenaline storing cells. The majority of these cells expressed only one peptide. Both transection of the splanchnic nerve as well as treatment with acetylcholine receptor antagonists mimicked the effects seen after the AChE-antibody treatment, although changes were less pronounced.

482.4

ANTAGONISM OF ANGIOTENSIN TYPE$_1$ RECEPTORS STIMULATES PLASMA RENIN ACTIVITY IN PREWEANLING SHR AND WKY RATS. R. F. Kirby*, K. A. Marshall and A. K. Johnson. Departments of Psychology and Pharmacology and The Cardiovascular Center, University of Iowa, Iowa City, IA 52242-1407.

Blockade of the angiotensin type 1 receptor (AT_1) with losartan potassium has been found to stimulate plasma renin activity (PRA) by interupting negative feedback within the renin-angiotensin system. This response is largely dependent upon sympathetic innervation of renal juxtaglomerular cells, because the stimulation of PRA by losartan is abolished or severely inhibited by blockade of beta$_1$ adrenoceptors. In a previous study, we found the ability of sympathetic activation to stimulate PRA was minimal during early preweanling development (prior to postnatal day 10) but matured rapidly during the late preweanling period in the rat. Therefore the present study examined the developmental time course of losartan-induced PRA stimulation during the period in which functional sympathetic control of the renin-angiotensin system emerges.

Normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) pups born in our laboratory were administered losartan potassium (10 mg/kg) or vehicle on postnatal days 5, 10, 15, 20 or 35. Additional groups of 20 and 35 day old animals were tested for their response to losartan with sympathetic activity to the juxtaglomerular cells blocked by the beta$_1$ adrenoceptor antagonist atenolol.

Blockade of AT_1 receptors significantly increased PRA in both WKY and SHR. Pretreatment with atenolol diminished the PRA response to losartan in 20 and 35 day old animals, indicating a contribution of the sympathetic nervous system to the response. However, losartan significantly increased PRA even at the youngest ages examined. These data suggest that AT_1 receptor activity may act independently of the sympathetic nervous system during early development to regulate activity of the renin-angiotensin system.

482.5

STREPTOZOTOCIN-INDUCED DIABETES AND VITAMIN A METABOLISM. Shahed Ziari, Andrew T.C. Tsin*, Tapan K. Basu[1], Elena Rodriguez, Yolanda Moreno, and Nickie Crocket. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249 and [1]The Department of Foods and Nutrition, The University of Alberta at Edmonton, Alberta, Canada T6G 2M8.

Purpose: To investigate the effect of streptozotocin-induced diabetes on concentration of vitamin A (retinol) and retinyl ester in the plasma, liver and ocular tissue of Wistar rats. To relate any changes in vitamin A metabolism to retinyl ester hydrolase (REH) activity in hepatic microsomal membranes. **Methods**: Twenty four animals were divided equally into experimental and control groups. Diabetes was induced by a single i.v. injection (tail vein) of streptozotocin (55 mg/kg) dissolved in acetate buffer, pH 4.5. Control group received only the vehicle. Plasma glucose concentrations were determined by glucose oxidase method. Both groups were fed *ad libitum* with a synthetic diet. Animals were sacrificed 42 days post injection. Retinoid identity and concentration was determined by normal phase HPLC. The hydrolysis of [^{3}H]all-*trans*-retinyl palmitate by microsomal membranes of liver was measured by a radiometric assay which quantitates liberated [9,10-^{3}H]palmitic acid. Apparent kinetic constants (K_m and V_{max},) were determined by Lineweaver-Burk transformation of the kinetic data. **Results**: In diabetic rats the plasma retinol concentration was significantly lower than those in normal rats. Conversely, hepatic retinol concentration of diabetic rat was significantly higher than that in normal rat. The retinyl ester level in plasma and liver was not notably different between the diabetic group and control. In ocular tissues the retinyl ester of diabetic rats was significantly increased in comparison to control group while retinol level was not perturbed. The all-*trans*-REH of liver microsome ($K_m \approx 35\ \mu M$, $V_{max} \approx 102$ pmol/min/mg) were not significantly different between the experimental and control groups. **Conclusion**: Our results show that streptozotocin-induced diabetes influenced vitamin A metabolism in the Wistar rat without alteration in storage of vitamin A. However, the transport of retinol seemed affected by diabetes. In the present study the liver REH was not affected by diabetes, suggesting that the elevation of liver retinol may be due to factors other than ester hydrolysis. Furthermore, the increase of retinyl ester storage in the eye suggests the possibility of ocular pathology(ies) whereby diabetes may be related to some form of retinopathy in the eye.

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482.6

DIFFERENCES IN PHARMACOKINETICS OF LIDOCAINE IN THE PERIPHERAL NERVE OF GRAVID RATS. FA Popitz-Bergez*, GR Strichartz, JG Thalhammer. Anesth. Res. Labs. HMS. BWH. Boston MA 02030.

The mechanisms underlying the decrease in drug requirements for central and peripheral anesthesia during pregnancy in humans are not yet understood. Sex hormone effects on excitable membranes have been suggested as a possible cause. Intravenous progesterone induces sleep in man, and decreases seizure activity in experimental models of epilepsy. Furthermore, long-term progesterone exposure in rabbits enhances the effect of local anesthetics (LA) in peripheral nerve block in vitro.

The present study was undertaken to examine 1) if the gravid rat was a suitable model to study this phenomenon, 2) the possibility of altered pharmacokinetics of LA in the gravid rat by studying lidocaine uptake in a peripheral nerve in vivo.

Pregnant (P; n=3) and non pregnant female (NP; n=3) rats were briefly anesthetized with Sevoflurane. A unilateral sciatic nerve block was performed with 0.1 ml of 1% radiolabelled lidocaine (*LID). After recovery from general anesthesia (~1min.), proprioception, motor function and responses to superficial (skin pinch) and deep (digit squeeze) pain were tested. As soon as deep pain sensation returned, the animal was sacrificed, the sciatic nerve rapidly removed, frozen, cut in 5 mm segments, desheated, weighed, digested and the uptake of *LID measured by liquid scintillation counting of the different segments.

A significant difference in the time for return of deep pain sensation was observed between the 2 groups ; 37, 25, 21 minutes for the NP rats and 50, 50, 47 minutes for the P rats. The fraction of the total *LID injected dose found in the nerves was 0.49± 0.14 % for the NP rats and 0.39± 0.08 % for the P rats. *LID distribution along the the nerve was not significantly different between the 2 groups.

In summary, the duration of anesthesia was prolonged in P rats as was observed in humans, but the amount of *LID in the nerve when deep pain recovered was the same in both groups. This effect might be due to 1) an increase in uptake of LA (e.g, due to an increase in nerve sheath permeability) 2) a decrease in clearance of the LA from the nerve or 3) both.

482.7

ELECTROPHYSIOLOGICAL EFFECTS OF NORADRENERGIC AGONISTS ON NEURONES OF THE BED NUCLEI OF THE STRIA TERMINALIS AND CAUDAL MEDULLARY ORIGINS OF THE CATECHOLAMINERGIC INNERVATION. M.G.Terenzi and C.D.Ingram* Department of Anatomy, School of Medical Sciences, University of Bristol, BS8 1TD, UK.

The bed nuclei of the stria terminalis (BST) are a limbic collection of neurones at the junction of the hypothalamus and septal area. The BST are important for autonomic functions and reproductive activity associated with maternal behaviour and the milk-ejection reflex. The BST are innervated by medullary noradrenergic groups (A1, A2 and A6) and high turnover of noradrenaline occurs in the BST during suckling.

We investigated the topography of the connections from the A1/A2 groups to the BST using fluorescent retrograde tracing and tyrosine hydroxylase (TH) immunocytochemistry. The results showed that the projection from the caudal medulla originates in the ventrolateral medulla and the nucleus of the tractus solitarius (NTS). Most of the input is ipsilateral and terminates in the BST ventral to the anterior commissure. Almost no labelling is observed in the caudal medulla when the injection was in the caudal BST. TH immunoreactive retrogradely labelled neurones were observed in A1/C1 and A2/C2 and a group of non-catecholaminergic neurones was observed in the NTS in the midline, dorsolateral to the central canal.

Electrophysiological studies showed that the basal firing of neurones in the BST is inhibited by iontophoresis (*in vivo*) or superfusion (*in vitro*) of clonidine and excited by phenylephrine. These neurones were also inhibited by electrical stimulation of the A2/NTS area *in vivo*. The inhibition resulting from iontophoresis of clonidine and the electrical stimulation of the dorsomedial medulla was reduced by iontophoresis of the alpha-2 antagonist yohimbine. The BST receive strong catecholaminergic innervation from the medulla which appears to exert an inhibitory effect via alpha-2 receptors and an excitatory effect via alpha-1 receptors.

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482.8

INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) PROTECTS GT1-7 CELLS FROM OXIDATIVE INJURY. M.A. Sortino*, U. Scapagnini and °P.L. Canonico Institute of Pharmacology, University of Catania School of Medicine and °Chair of Pharmacology, University of Pavia School of Dentistry, Italy

The neuroprotective action of Insulin-like growth factor-1 (IGF-1) has been recently demonstrated in different experimental models of neurodegeneration. Immortalized hypothalamic, gonadotropin-releasing hormone-(GnRH) secreting, GT1-7 cells display a marked sensitivity to toxicity induced by reduced glutathione- (GSH) depleting agents that in these cells cause necrotic death. GT1-7 cells express IGF-I receptors whose activation causes a bimodal modulation of GnRH secretion. On these bases we have studied the potential protective effect of IGF-I in GT1-7 cells. Subconfluent GT1-7 neurons cultured for two days were exposed to different concentrations (50-500 μM) of buthionine sulfoximine (BSO), which reduces the intracellular levels of GSH, for 24-48 hours or to diethyl maleate (DEM; 1 mM) which binds sulfhydryl groups of GSH, for 1 to 3 hours. Both treatments produced extensive neuronal death that however was more pronounced in DEM-treated cells. Concomitant exposure of GT1-7 cells to IGF-I (at the maximal concentration of 25 ng/ml) resulted in a remarkable reduction of neuronal death as assessed either by trypan blue exclusion or by staining with fluoresceine diacetate and propidium iodide. The neuroprotective effect of IGF-I has been compared to that induced by the potent antioxidant idebenone that, in the low micromolar range, provided a virtually complete protection of the oxidative stress produced by either BSO or DEM. These results suggest that IGF-I, as already proposed in different cellular systems for various growth factors, may exert its neuroprotective action through antioxidant mechanisms.

482.9

FUNCTIONAL DEVELOPMENT OF NEUROENDOCRINE BAG CELLS OF *APLYSIA*. N. Wayne*[1], T. Nick[2], T. Carew[3], L.K. Kaczmarek[4]. Dept Physiol[1], UCLA Sch Medicine, Los Angeles, CA 90024, Interdept Neurosci Prgm[2], Depts Psychol[3] and Pharm[4], Yale Univ, New Haven, CT 06510.

Egg-laying behavior in mature *Aplysia* occurs in response to egg-laying hormone (ELH) released from neuroendocrine bag cells; ELH release is triggered by a prolonged pattern of action-potential firing (afterdischarge). Electrical stimulation of bag cells from immature *Aplysia* does not trigger afterdischarges. However, prolonged depolarization (plateau potentials) can be elicited in the presence of the K^+ channel blocker TEA (Nick et al., Soc Neurosci, Abst 708.7, 1993). In the present study, we sought to determine whether immature bag cells that are incapable of showing afterdischarges contain the functional mechanisms required for secretion. Suction electrodes were used for both electrical stimulation and extracellular recording from bag-cell preparations dissected from mature (avg body wt=256 g) or immature (avg body wt=10 g) *Aplysia*. Medium bathing the preparations was collected every 5 min before, during and after stimulation for a total of 125 min. Samples were frozen at -20 °C until radioimmunoassay for ELH was performed. Immature preparations (n=3) showed ~9 plateau potentials (no afterdischarges), while mature preparations (n=3) showed afterdischarges lasting ~18 min with ~650 action potentials fired during that period. Both immature and mature bag-cell preparations secreted ELH above baseline, although the total amount (~36 ng/ml vs ~2400 ng/ml) and duration (~25 min vs >105 min) of secretion was significantly lower from immature preparations. These results indicate that immature bag cells that do not show afterdischarges can nonetheless be stimulated to release ELH, and therefore must contain the necessary cellular machinery for secretion. Supported by NIH-HD 28336 (to NW), NSF-BNS 8614961 (to TC) and NIH-NS 18492 (to LKK).

482.10

VARIATIONS OF MEMBRANE CURRENTS DURING SOMATOSTATINE RELEASE AS MEASURED ON IDENTIFIED *HELIX* NEURONS BY AN IMMUNOMICROELECTRODE.

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Helix neurons of the visceral ganglia were studied in-situ using simultaneous current clamp and somatic somatostatin release using a micro-immunoelectrode. The results have shown it exists a somatic release of somatostatin in the extracellular spaces. This release was potential-dependent since it appears for membrane potentials higher than + 20 mV. Nevertheless, the release intensity seemed to voltage-independent but was delayed when membrane potential increase. Calcium channels blockade suppressed the release. Under slow stimulations (10 hz, +20 mV) the release became cyclic during some minutes but higher frequency increased the somatostatin relase in a dependent manner. Even-though a recent demonstration of a noticeable regional difference of calcium channels density, it seemed that somatic exocytosis characteristics were a good model for synaptic release.This is in good agreement with previous works wich have demonstrated that exocytosis was not only related to calcium channels but also to sodium channels.

482.11

EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES ON NADPH-DIAPHORASE POSITIVE NEURONES IN THE RAT PANCREAS. S.S.W. Tay* and G. Burnstock+. *Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511 & +Department of Anatomy and Developmental Biology, University College, London, WC1E 6BT, U.K.

In saline-injected rats, NADPH-diaphorase positive neurones were dispersed in the interlobular septa, near blood vessels and ducts as well as within pancreatic lobules. NADPH-diaphorase activity was localized in the cell cytoplasm but not in the nucleus. Most of the neurones were heavily labelled while others were moderately to lightly stained. Some labelled neurones sent processes that ramified amongst the exocrine acinar cells. Numerous fine varicose NADPH-diaphorase positive nerve fibres formed networks around the individual islet cells. At 8 weeks post-diabetes, the number of NADPH-diaphorase positive neurones and nerve fibres was reduced. Labelled neurones that persisted were solitary or in small groups (2-5 cells). They were heavily labelled for NADPH-diaphorase activity, some appeared rounded and possessed few short and stubby processes. Islets of Langerhans were not easily distinguishable and their associated nerve networks seemed to have disappeared. NADPH-diaphorase positive macrophages were also found in the interlobular spaces. These results suggest that diabetes has a drastic effect on NADPH-diaphorase positive neurones and nerve fibres in the rat pancreas.

482.12

NEW EVIDENCE ON THE DIFFERENCES BETWEEN THE LEFT AND RIGHT OVARY ON THE NEURAL CONTROL OF OVULATION. **R.Domínguez*, M.E.Cruz, and C.Morán. UIBR FES Zaragoza. AP9-020, CP15000 México.**

There is evidence on the existence of different ovulatory ability between the right and left ovary [RO, LO] depending on ovarian innervation; which is very significant in hemiovariectomized [Hovx] animals. Present study was conducted to analyze if the blockade of ovarian innervation induced by lidocaine+adrenaline [LiA] injection into the ovarian bursa, previous to the extirpation of contralateral ovary, affects the ovulatory ability by the *in situ* ovary, performed at 13.00 h in the day of proestrus of 4-day cyclic rats. The animals were sacrificed on the next expected day of estrus. When LiA was injected to the LO and the RO extirpated 0/6 rats ovulated, while when the RO was injected and the LO extirpated 8/8 rats ovulated [p<0.01]. When Hovx by the LO or RO was performed without LiA injection, 8/8 and 9/9 rats ovulated. 6/6 rats treated at 18.00h with LiA on the LO and the RO extirpated ovulated next morning.
Present results support the hypothesis that the LO is more dependent on neural information than the right one. **Supported by DGAPA grant IN210893 and PUIS**

482.13

DECREASED GLUCOSE, BUT NOT FATTY ACID AVAILABILITY INCREASES FOS-LIKE IMMUNOREACTIVITY IN THE CAUDAL BRAIN STEM OF FEMALE SYRIAN HAMSTERS

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In Syrian hamsters, estrous cycles are inhibited by food deprivation or by treatment with 2-deoxy-d-glucose (2DG), a drug that inhibits glucose utilization, and methyl palmoxirate (MP), a drug that inhibits fatty acid oxidation. For example, hamsters become anestrous when treated with high doses of 2DG alone (1750 mg/kg), or with low doses of 2DG and MP given concurrently (750 mg/kg and 20 mg/kg respectively), but not with either drug alone at low doses. Systemic administration of 2DG at high doses (<1750 mg/kg) stimulates expression of the proto-oncogene *c-fos* in the area postrema (AP), and the nucleus of the solitary tract (NTS). These and other data suggest that the effects of 2DG on estrous cycles may occur via a glucoprivic signal detected in the AP/NTS. The present study investigated whether other metabolic treatments that induce anestrus also stimulate neural activation in the AP/NTS. Hamsters received one of the following treatments: (1) 1750 mg/kg (high dose) (2) 750 mg/kg 2DG (low dose) (3) 20 mg/kg of MP (low dose) (4) low doses of both 2DG and MP, or (5) the appropriate vehicle. Cells stained for FOS were observed in the AP/NTS only in groups that were treated with high doses of 2DG. This suggests that glucoprivation (decreased glucose availability) but not lipoprivation (decreased fatty acid availability) affects neural activation in the AP/NTS. Therefore, the signal detection for the availability of metabolic fuels necessary for reproduction may be specifically related to glucose availability. MP may have effects on estrous cycles which are independent of the metabolic effects via the AP/NTS. Supported by research grant BNS9121056 from NSF.

482.14

Neuroendocrine control of plasma extravasation in rats.

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Inflammation produced by complete Freund's adjuvant injection in the rat hindpaw potently inhibits plasma extravasation produced by the inflammatory mediator, bradykinin (160 nM), perfused through the knee joint. This negative feedback process is abolished in rats that have been neonatally treated with capsaicin (100 mg/kg) to deplete most of their unmyelinated primary afferent fibers, which suggests that this negative feedback process is mediated by activation of primary afferent C-fibers. Excitation of C-fibers with electrical stimulation (25mA, 3Hz) of the hindpaw also inhibited bradykinin–induced plasma extravasation in the knee joint.

Acute surgical interruptions of lumbar sympathetic afferents to the hind limb did not attenuate the depression of bradykinin-induced plasma extravasation produced by C-fiber stimulation, indicating that the depression is not mediated by the sympathetic outflow. However, transection of the spinal cord, hypophysectomy, inhibition of corticosterone synthesis, and adrenalectomy all prevented the inhibition of BK–induced plasma extravasation by electrical stimulation, indicating that the negative feedback inhibition on plasma extravasation is dependent on intact neuraxis and an intact hypothalamic-pituitary-adrenocortical axis.

Our data demonstrate a negative feedback inhibition of an inflammatory process, which is elicited by stimulation of C-fiber afferents. It is mediated by ascending pathways in the spinal cord, and probably the hypothalamic-pituitary-adrenocortical axis.
Supported by NIH grant AM32634

483.1

AFFERENT PROJECTIONS TO THE ROSTRAL NUCLEUS RAPHE PALLIDUS BY IONTOPHORETIC APPLICATION OF CHOLERA TOXIN B. D.M. Hermann, P. Hinckel*, P.H. Luppi and M. Jouvet. Physiol. Institut, D-35392 Giessen, Germany; CNRS URA 1195, F-69373 Lyon, France

In order to investigate its role in thermoregulation, the afferents to the rostral nucleus raphe pallidus (rNRP) were examined by extremely restricted injection sites of cholera toxin b, which seemed necessary, since the rNRP is wrapped both dorsally and laterally by the caudal nucleus raphe magnus (cNRM).

Substantial bilateral afferents were received from the prelimbic, the medial precentral, the infralimbic, the insular and the perirhinal cortex for the rNRP. Strong afferents from the periventricular preoptic area, the paraventricular and the dorsal hypothalamic area could be confirmed in this study; additional afferents from the caudal preoptic area and the adjacent bed nucleus, the central amygdala and the perifornical lateroposterior hypothalamic area were shown. Compared with the cNRM, very few afferents were observed from the fields of Forel, the midbrain and pontine periaqueductal gray and the deep mesencephalic reticular formation. Specific afferents for rNRP, but not for cNRM, were found close to the dorsal raphe nucleus in the ventromedial pontine periaqueductal gray and laterally to the third nucleus inside the midbrain reticular formation. Afferents from the nucleus Kölliker-Fuse and the lateral medullary reticular formation were stronger for rNRP than cNRM, afferents from the subcoeruleus area, the lateral parabrachial area and the caudal pontine reticular formation less intense for rNRP than cNRM. By highly restricted injection sites, substantial afferents could be shown for rNRP from key structures of central nervous thermoregulation.

483.2

THE EFFECTS OF IL-1β ON THE INTRACELLULAR ACTIVITY OF HYPOTHALAMIC THERMOSENSITIVE AND TEMPERATURE INSENSITIVE NEURONS. J.D. Griffin* and C.B. Saper. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115

The production of endogenous cytokines within the CNS may play a role in the induction of a fever during the acute phase response. Recently, it was shown that IL-1β may have a direct effect on the inherent activity of thermosensitive neurons within the preoptic and anterior regions of the hypothalamus. These neurons are known to directly influence thermoregulatory responses. To identify the intracellular mechanisms of IL-1β induced changes in activity, tight-seal, whole-cell recordings were made of neuronal intracellular activity in rat hypothalamic tissue slices. Based on their slope of firing rate as a function of tissue temperature, neurons were classified as either warm sensitive (high slope) or temperature insensitive (low slope). Neurons were then treated with IL-1β (0.4μg/20ml) while temperature was held at a constant level (~36°C). Temperature insensitive neurons showed no change in their spontaneous firing rates or intracellular characteristics during treatment with IL-1β. Neurons classified as warm sensitive showed a decrease in spontaneous firing rate activity during treatment with IL-1β which may be linked to specific conductance changes inherent to the neurons' activity.

(Supported in part by NIH NS09466, NS07291 & AHA 91011)

483.3

PREOPTIC PROSTAGLANDIN E_2 (PGE_2) AND CORE TEMPERATURE (T_c) RESPONSES OF GUINEA PIGS TO LPS i.v., INDOMETHACIN i.m., AND/OR PGE_2 i.a. ADMINISTRATION. E. Sehic, M. Szekely, A.L. Ungar and C.M. Blatteis*. Dept. of Physiology & Biophys., Univ. of TN, Memphis, TN 38163.

PGE_2 has been postulated to be a central mediator of fever. It is generally believed that PGE_2 is produced in the preoptic area (POA) because its level increases both in the third ventricle and the POA in response to i.v. pyrogen. However lately, the question has arisen whether PGE_2 may in fact be formed outside the brain substance and then diffuse into it across cerebral vessels. If produced outside the brain substance, the blockade of its synthesis should prevent LPS-induced fever, whereas the intracarotid infusion of PGE_2 should produce an increase in T_c as well as in preoptic PGE_2. To verify this hypothesis, continuous measurements of PGE_2 levels in the POA were made (sampling by microdialysis and analysis by RIA). T_c was monitored continuously during the following experiments: 1) Following a 90-min stabilization period, indomethacin (10 or 50 mg/kg) was administered i.m. 30 min before *S. enteritidis* LPS (2 μg/kg, i.v.); or 2) PGE_2 (1 and 10 μg/μl) was infused at 2 μl/min into a carotid artery for one hour. LPS induced a biphasic 1.2 °C fever which was consistently associated with an increase in the level of PGE_2 in the POA. Indomethacin at 10 mg/kg attenuated the LPS-induced fever and prevented the associated increase in PGE_2 for 90 min after fever onset; thereafter, PGE_2 was significantly reduced by comparison to controls. Indomethacin at 50 mg/kg completely abolished both the fever and the increase in preoptic PGE_2. Intracarotid infusion of PGE_2 produced a dose-dependent hypothermia without any increase in preoptic PGE_2. The blockade of fever and inhibition of the increase in preoptic PGE_2 levels further substantiate the presumptive link betwen preoptic PGE_2 and fever. The failure of exogenous PGE_2 intracarotid infusion to induce increases in T_c and preoptic PGE_2 excludes the possibility that PGE_2 formed outside of the brain diffuses into the POA across cerebral vessels and induces fever. (Supported by NIH grant NS 22716.)

483.4

SUPPRESSION OF INTERLEUKIN-1ß FEVER AT PARTURITION IN RATS. S.M.Martin*, I.Neumann, T.J.Malkinson and Q.J.Pittman. Biology Department, Mt. St. Vincent University, Halifax, NS B3M 2J6 and Neuroscience Research Group, The University of Calgary, Calgary, AB T2N 4N1 Canada.

We have previously shown that pregnant rats at or near term show a significantly reduced fever to intravenous bacterial pyrogen. It is not known if pregnant rats have a decreased central sensitivity to pyrogens or if there is a change in the production of one of the cytokines, interleukin-1ß (IL-1ß), which is thought to mediate some of the effects of pyrogen. These experiments were undertaken to compare the effects of intravenous administration of IL-1ß in pregnant rats at or near term.

Timed pregnant and virgin Sprague-Dawley rats were anesthetized for jugular vein cannulation and implantation of telemetry thermistors. Following recovery, a dose response curve for IL-1ß in virgin female rats was ascertained. On the basis of this, a dose of 0.1 μg/kg iv was chosen for subsequent experiments. Pregnant rats showed an attenuated fever response as early as 72 h preparturition and the response was significant ($p \leq 0.05$) 24 h prior to (0.6 ± 0.11°C) and 24 h postparturition (0.76 ± 0.14°C) when compared to that of virgin females (1.54 ± 0.09°C).

The results indicate that IL-1ß fever is suppressed at the time of parturition. The mechanism for this suppression remains unknown but may involve some central component of the fever response.

Supported by MRC and Mt. St. Vincent University.

483.5

BOMBESIN-INDUCED HYPOTHERMIA IN ADRENALECTOMIZED RATS. C. Barton*, D.A. York and G.A. Bray. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808.

Bombesin (BOM) is a tetradecapeptide known to produce hypothermia when administered centrally in rats tested at normal ambient temperatures. Previous studies have demonstrated bombesin-induced hypothermia is requires a state of reduced sympathetic nervous system activity. The importance of adrenal activity on this phenomenon is unknown. The present study examines the impact of bombesin injection (100ng/ 5μl) or vehicle (VEH) into the lateral ventricles on core body temperature of food-deprived (20hr) adrenalectomized (ADX) and sham operated (SADX) rats with peripheral corticosterone (CORT) replacement (0.1 mg/kg, ip) or vehicle injection 17 hrs prior to testing. Completeness of ADX was assessed by serum aldosterone assay at the completion of testing. This produced a between subject design with four experimental conditions: (central inj/peripheral inj/surgical treatment) VEH/VEH/SADX, BOM/VEH/SADX, BOM/VEH/ADX, & BOM/CORT/ADX (n=7/condition). Core temperatures were recorded at time of central injection and 60 min later. Central bombesin induced hypothermia in all conditions as compared to central vehicle with a significantly greater hypothermia seen in ADX + CORT (-4.13±0.19 °C) & ADX + VEH (-3.18±0.38 °C) conditions versus SADX+VEH (-1.79±0.31 °C). This data demonstrates that adrenalectomy potentiates bombesin-induced hypothermia, and suggests that corticotrophin-releasing hormone and adrenal catecholamines modulate this phenomenon.

483.6

DISSOCIATION OF BROWN FAT SYMPATHETIC NERVOUS SYSTEM (SNS) ACTIVITY FROM BASAL HYPOTHALAMIC-PITUITARY ADRENAL (HPA) AXIS FUNCTION. A.M. Strack*, C.J. Horsley, and S.F. Akana. Dept. Physiol. UCSF, San Francisco, CA, 94143-0444

We have used the nonshivering thermogenic action of brown adipose tissue (BAT) as a measure of SNS activity. Generally SNS activity and the HPA axis, as measured by the release of the stress hormones, ACTH and corticosterone, increase in parallel (e.g. hemorrhage, cold). We tested whether a hypercaloric load (which increases BAT SNS activity, but presumably not HPA activity) would dissociate these two systems. Rats were allowed 30% sucrose to drink in addition to their chow diet for 5 or 10 days. We measured plasma ACTH and corticosterone as indices of HPA activity. As a measure of BAT SNS activity, the BAT specific thermogenic protein, uncoupling protein (UCP) was assayed. A subset of rats was instrumented with temperature probes for chronic remote measurement of BAT and core temperature (Minimitter Co.).

At 5 and 10 days, all HPA measures were the same between sucrose and control rats. As early as 5 days, UCP levels in interscapular BAT were increased. BAT temperature was elevated by the 3rd day of sucrose intake and remained elevated through all portions of the circadian rhythm throughout the remainder of the 10 day period. These results show a long term increase in SNS activity independent of that of the HPA axis. This dissociation of SNS activity from that of the HPA axis will allow differential dissection of the efferent pathways regulating these systems. (Supported by DK28172 to MF Dallman and AHA93-42 to AMS)

483.7

NONSHIVERING THERMOGENIC RESPONSE TO COLD IS GREATER IN AGED MICE THAN IN ADULTS. S.A. Kirov* and M.I. Talan. Laboratory of Behavioral Sciences, National Institute on Aging, NIH, Gerontology Research Center, Baltimore, MD 21224.

Efferent sympathetic nervous activity (SNA) from a small branch of the intercostal nerve, which ended in the interscapular brown adipose tissue (IBAT), and metabolic heat production (MHP) were measured in C57BL/6J male adult and aged mice under urethane/ isoflurane anesthesia and vecuronium bromide myorelaxation. MHP was calculated on the basis of O_2 and CO_2 concentrations measured in expired air taken directly from the trachea through a mechanical ventilator. Nerve recording and MHP measurements were conducted before and during rapid (15-18 min) whole body cooling until colonic temperature dropped to 7°C below control level (37-38°C) and during rewarming.

SNA to IBAT and MHP increased during cold stress and returned to the baseline level during rewarming in both age groups. MHP and SNA responses to cold stimulation were significantly greater in aged mice than in adults. Maximal responses of aged mice exceeded those of adults by 70% for MHP and by 50% for SNA. Since all other sources of facultative thermogenesis were suppressed by anesthesia and myorelaxation, we conclude that nonshivering thermogenesis in BAT is responsible for greater MHP during cold exposure in old animals.

CARDIOVASCULAR REGULATION: MEDULLARY RETICULAR RESPONSES

484.1

NEUROTRANSMITTERS IN THE VENTROLATERAL MEDULLA MEDIATING CORTICAL AND HYPOTHALAMIC SYMPATHETIC RESPONSES. K.S. Butcher* and D.F. Cechetto. Robarts Research Institute, Univ. of Western Ontario, London, Ontario, Canada N6A 5K8

Our previous investigation demonstrated that sympathetic nerve responses to insular cortical (IC) stimulation are mediated by synapses within the lateral hypothalamic area (LHA) and ventrolateral medulla (VLM). The neurotransmitter(s) in the VLM for IC and LHA responses are unknown. Twenty male Wistar rats were instrumented for renal nerve, arterial pressure and heart rate recording. The IC or LHA were stimulated with a bipolar electrode (200-1000 μA; 2 ms; 0.8 Hz) to elicit sympathetic nerve responses. Antagonists were then pressure-injected into the VLM (300 nl). Bilateral and unilateral kynurenate (25 mM) resulted in 100% block of IC and LHA stimulated sympathetic nerve responses. Bilateral injection of the non-NMDA receptor antagonist 6-cyano-7-niroquinoxaline-2,3-dione (CNQX;200 μM) also resulted in 100% block of IC and LHA sympathetic responses. Unilateral injection of CNQX in two animals resulted in 100% and 83% block of LHA sympathetic responses. Bilateral injection of the NMDA receptor antagonist D,L 2-amino-5-phosphonopentanoic acid (AP5; 200 μM) did not affect the response to IC or LHA stimulation. Kynurenate produced a 363 ± 78% increase in baseline activity, while CNQX and AP5 resulted in 183 ± 19% and 191 ± 21% increases respectively. Bilateral injections of antagonists for GABA (bicuculline; 0.1 μM), acetylcholine (atropine; 0.1 μM) and catecholaminergic alpha and beta receptors (phentolamine and propranolol: 0.1 μM) had no effect on LHA sympathetic responses. These results indicate IC and LHA responses are mediated specifically by non-NMDA receptors in the VLM, although NMDA and non-NMDA receptor mediated inputs have tonic effects on RVLM neurons. (Supported by the he Heart and Stroke Foundation of Ontario)

484.2

DECREASE IN BLOOD PRESSURE AND HEART RATE (HR) ELICITED BY INJECTIONS OF GLUTAMATE (GLU) INTO THE LOCUS COERULEUS (LC) ARE MEDIATED BY α_1-ADRENERGIC RECEPTORS IN THE NUCLEUS AMBIGUUS/VENTROLATERAL MEDULLARY DEPRESSOR AREA (NA/VLDA). V. Malhotra* and H.N. Sapru., Depts. of Neurosurgery & Pharmacology, New Jersey Medical School, newark, NJ 07103.

Experiments were carried out on pentobarbital-anesthetized, artificially ventilated male Wistar rats. Microinjections of L-GLU (1.77 nmol/20 nl) into the LC (n = 5) elicited a decrease in mean arterial pressure (MAP; 45 ± 12 mmHg) and HR (31 ± 4 bpm). An area encompassing NA/VLDA was identified by injections of L-GLU (1.77 nmol/50 nl). Muscimol (150 pmol/50 nl) was injected into the NA/VLDA (n = 5) to inhibit the neurons. Muscimol injections into the NA/VLDA blocked the bradycardic and depressor responses induced by injections of L-GLU into the LC. Injections of norepinephrine (NE; 0.75-6 pmol/50 nl) into the NA/VLDA elicited a decrease in MAP (15-30 mmHg) and HR (15-55 bpm). Maximum effect was elicited by a dose of 1.5 pmol/50 nl; this effect was completely blocked by prazosin (PRZ; 150 pmol/50 nl). Injections of PRZ into the NA/VLDA blocked the depressor and bradycardic effects elicited by injections of GLU into the LC. PRZ did not exert any local-anesthetic effect in the NA/VLDA because the bradycardic and depressor responses to L-GLU remained unchanged. These results indicate that: (1) the depressor and bradycardic effects elicited by stimulation of neurons in the LC are mediated by NA/VLDA, (2) α1-adrenergic receptors in the NA/VLDA mediate the cardiovascular effects of the stimulation of LC-neurons, and (3) norepinephrine may be the transmitter released in the NA/VLDA following the stimulation of LC-neurons which are preponderantly noradrenergic in the rat.
Support: NIH: HL24377 and AHA (NJ).

484.3

RELATIONSHIP OF THE NUCLEUS RETICULARIS PARVOCELLULARIS (NRP) TO CARDIOVASCULAR REGIONS OF LOWER BRAIN STEM AND SPINAL CORD. L.D. Mitchell and M.A. Nathan*. Depts. of Physiology and Pharmacology, CUNY Medical School, NY 10031.

Regions of the lower brain stem and spinal cord traditionally considered important in controlling resting vascular tone are the rostral ventrolateral medulla (RVLM), caudal ventrolateral medulla (CVLM), which project directly or indirectly to the intermediolateral cell column (IML) (Chalmers & Pilowsky, J. Hypertension, 9:675-694,1991). The rostral ventromedial medulla (RVMM) also appears important in establishing vascular tone (Vamer et al., Hypertension 19:193-197, 1992). We recently showed that the NRP is another major area contributing to control of the circulation (Cochrane & Nathan, J. Autonom. Nerv. Syst., 43:69-82, 1993) and that it projects to the RVLM and CVLM (Mitchell & Nathan, Soc. Neurosci. Absts., 17:993, 1991).

Projections to the NRP from the RVLM, RVMM and CVLM and projections from the NRP to the IML were studied in male, Long-Evans rats. A micropipette (20-25 μM tip) was used to inject HRP-WGA (Sigma, 1% in 0.5 M NaCl, 10.0 nL) into the NRP. Survival times after the injections were 48-72 hrs. The tissue was processed according to the method of Gibson et al. (Brain Res., 298:235-241, 1984).

We found that the NRP receives massive ipsilateral projections from cells in the RVLM and RVMM. Lighter contralateral projections were observed as well. The CVLM also projects to the NRP. In addition, the NRP projects to the ipsilateral, lower thoracic IML.

These results, in conjunction with our earlier anatomical study, demonstrate that the NRP is reciprocally linked to other principal cardiovascular areas of the brain stem. Moreover, the NRP has a direct connection to the IML.
(Supported by a grant from PSC-CUNY.)

484.4

CATECHOLAMINERGIC MECHANISMS INVOLVED IN THE INHIBITION OF PACEMAKER-LIKE TYPE II NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) *IN VITRO*. A. R. GRANATA*. Department of Pharmacology, The University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163.

In previous studies we identified *in vivo* a group of bulbospinal neurons in the rat RVLM with chloride-dependent IPSP synchronized to the cardiac cycle. During constant hypotension and hence baroreceptor inactivation, the pattern of discharge of these neurons (barosensitive type II) was very regular and had pacemaker-like properties. In addition, the IPSP synchronized to the cardiac cycle vanished. Electrical stimulation of the ipsilateral aortic depressor nerve evoked IPSP on these neurons. Using an *in-vitro* slice preparation, we characterized a group of spontaneously active neurons in the RVLM with features very similar to barosensitive type II *in vivo*. These features included (1) regular pattern of discharge, (2) absence of EPSP during hyperpolarization by negative current pulses below spike threshold level, (3) resetting of neuronal activity by interrupting the spontaneous activity with pulses of hyperpolarizing current, (4) slow depolarizing and afterhyperpolarization potentials, (5) action potential < than 2.2-ms duration, (6) anomalous rectification developed by passing hyperpolarizing current pulses. Tyramine (0.5-1.0 mM) reversibly suppressed the firing of these neurons. This suppression was accompanied by an increase in membrane input resistance, abolition of anomalous rectification and a minor change in membrane potential. The alpha-2 adrenergic agonist clonidine (10 μM) reversibly reduced the firing frequency in neurons with barosensitive type II characteristics. Conidine increased the firing rate of other RVLM neurons with different electrophysiological characteristics.

484.5

PRE-SYMPATHETIC NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) OF THE RAT 'IN VIVO': MORPHOLOGICAL PROPERTIES AND RELATIONSHIP TO C1 CATECHOLAMINERGIC NEURONS. B. Kruszewska*, J. Lipski, R. Kanjhan and M. Smith. Dept. of Physiology, Univ. of Auckland, Auckland, New Zealand.

The activity of preganglionic sympathetic neurons is maintained by an excitatory input from neurons located in the RVLM. Previous studies conducted in tissue slices (Sun et al. *Brain Res.* 1988, 451:345; and 1991, 556:61) examined the morphology and presence of catecholamine-synthesizing enzymes in RVLM neurons. However, due to functional and anatomical heterogeneity in this medullary region, it is important to examine neurons which can be directly classified as barosensitive and reticulospinal. This was possible in the present experiments conducted *in vivo*, in pentobarbital anaesthetised adult rats (see Lipski et al., this meeting). Recordings were made with microelectrodes filled with 1% Neurobiotin or 3% Lucifer Yellow. RVLM neurons which responded with IPSPs following stimuli applied to the aortic nerve were intracellularly labeled. Some of these neurons could be excited antidromically following stimulation in T2 segment. Filled somas were found ventromedial to the compact formation of nucl. ambiguus. Dendrites extended up to 800 μm from cell bodies. Some main axons bifurcated in the dorsomedial tegmentum, and a few had axon collaterals which displayed bouton-like varicosities in the ipsilateral RVLM. C1 adrenergic cells were revealed by immunofluorescence using antibody to tyrosine hydroxylase. Some injected neurons were shown to be double labeled.

484.6

NEURONS IN THE C1 AREA OF THE ROSTRAL VENTROLATERAL MEDULLA ARE NECESSARY FOR THE ARTERIAL CHEMORECEPTOR PRESSOR REFLEX. P.S. Clifford*, S.W. Mittelstadt, and K.J. Dormer. Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53295 and University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Neurons of the C1 area of the rostral ventrolateral medulla project to the intermediolateral cell column of the thoracolumbar spinal cord and contribute to the maintenance of vasomotor tone by providing tonic excitation to sympathetic preganglionic neurons. Previous studies have demonstrated that C1 area neurons are activated by stimulation of arterial chemoreceptors. The hypothesis of this study was that neurons in the C1 area are essential for the expression of the pressor response to stimulation of arterial chemoreceptors. Experiments were performed on five mongrel dogs anesthetized with alpha-chloralose/urethane and mechanically ventilated with room air. Selective stimulation of arterial chemoreceptors was accomplished by ventilation with 100% N_2 for 6 breaths and by intravenous infusion of sodium cyanide (NaCN, 50 μg/kg). C1 area pressor sites were first identified by microinjection of l-glutamate (100nl, 150mM) and then lesions were created with bilateral microinjections of ibotenic acid (100 nl, 75mM). The following mean arterial pressures (mmHg, mean ± SEM) were observed:

	CONTROL	100% N_2	CONTROL	NaCN
PRELESION	120±7	141±8	119±5	147±8
POSTLESION	77±9	69±9	80±8	72±9

Stimulation of arterial chemoreceptors by brief periods of hypoxia (100% N_2) or intravenous infusion of NaCN produced marked increases in blood pressure. Following placement of lesions in the C1 pressor area, stimulation of arterial chemoreceptors resulted in depressor responses in all 5 dogs. These results indicate that, in the dog, the C1 pressor area of the rostral ventrolateral medulla is the essential brainstem site for expression of the blood pressure response to stimulation of arterial chemoreceptors.

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484.7

MORPHOLOGY OF IDENTIFIED BULBOSPINAL NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) OF THE RAT. D. Huangfu*, W.B. Goodwin, G.F. Alheid, R.L. Stornetta and P.G.Guyenet. Depts. of Pharmacology and Psychiatric Medicine, Univ. of Virginia, Charlottesville, VA 22908.

RVLM bulbospinal neurons were identified by the presence of retrogradely transported green latex microspheres injected under anesthesia in the spinal cord at T_3, 4-7 days prior to sacrifice. Then these cells were labeled intracellularly in fixed 100 μm slices (coronal, horizontal or parasagittal) with Neurobiotin that was visualized with an avidin-HRP reaction. Drawings and quantitative analysis of morphological features were done with the Neurolucida software. Some of the sections were doubly labeled for tyrosine hydroxylase (TH) and Neurobiotin using fluorescent tags.

In coronal sections, 83 well-filled cells were analyzed. They had 2 to 11 dendrites (mean 5.2±0.2) that extended 212±10 μm (up to 690 μm) and branched up to five times. Their perikarya were fusiform (6%), round (16%), triangular (30%) or multipolar (48%) and had a mean cross sectional area of 417±18 μm^2, corresponding to a roughly computed diameter of 23±1 μm (range: 14 to 33 μm).

Comparing parasagittal (23 cells injected), horizontal (N=21), and coronal (N=83) sections, we failed to find a predominant orientation in any plane for the extention of the dendritic tree in the whole population of the injected RVLM cells.

Forty five percent of the RVLM neurons examined in the coronal plane (37/83) had sparsely distributed spines and protuberances on their soma and dendrites. In double stained cases, TH-positive cells, which account for 67% of the injected retrogradely labeled RVLM cells, contained both neurons with and without spines. (Grant: HL 28785 to PGG and NS 17743 to GFA).

484.8

EFFECTS OF $GABA_B$ RECEPTOR AGONIST BACLOFEN ON NEURONS IN ROSTRAL VENTROLATERAL MEDULLA (RVLM): AN "IN VITRO" AND "IN VIVO" ELECTROPHYSIOLOGY STUDY. Yu-Wen Li* and P.G. Guyenet. Department of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

We studied the effects of baclofen, a $GABA_B$ receptor agonist, on neurons in the RVLM of the rat. In 17 neurons recorded intracellularly with sharp electrodes, bath application of baclofen (1-3 μM) produced: 1) a membrane hyperpolarization of 8±2 mV (baseline 58±2mV, n=17), 2) a 15±4% decrease in the membrane input resistance (baseline 138±17MΩ, n=16), 3) a decrease in firing rate (n=14) and 4) a decrease in spontaneous postsynaptic potentials (n=9). Of 83 single units recorded extracellularly in the RVLM *in vitro*, all but 2 were inhibited by baclofen (1-3 μM). The inhibition was concentration dependent (0.1-3.0 μM, max. inh. =94±4%, n=16), and persisted in low-Ca^{2+}/high-Mg^{2+} medium (n=19). The $GABA_B$ receptor antagonists CGP54626A (1 μM, n=19), CGP55845A (1μM, n=15) or 2-hydroxysaclofen (0.5 mM, n=3), attenuated the inhibitory effect of baclofen (1-3 μM), but not that of GABA or muscimol ($GABA_A$ receptor agonist). *In vivo*, iontophoresis of baclofen (40-120 nA) inhibited most RVLM neurons including 15 out of 16 bulbospinal barosensitive cells. This effect was attenuated by iontophoresis of the $GABA_B$ antagonist, CGP55845A (60-80 nA, n=6), but not the $GABA_A$ antagonist, bicuculline (40 nA, n=5). These results suggest that 1) most RVLM neurons including sympathetic premotor neurons have somato-dendritic $GABA_B$ receptors, 2) $GABA_B$ receptors in the RVLM may play a role in central regulation of cardiovascular function.

484.9

GABA AND GLUTAMATE INPUTS TO BULBOSPINAL NEURONS IN RAT ROSTRAL VENTROLATERAL MEDULLA. I.J. Llewellyn-Smith*, J.B. Minson, P.M. Pilowsky, L.F. Arnolda and J.P. Chalmers. Department of Medicine and Centre for Neuroscience, Flinders University, Bedford Park, SA., 5042, AUSTRALIA.

A potent vasopressor area coincides with the C1 group of catecholamine neurons in the rat rostral ventrolateral medulla (RVLM). Bulbospinal RVLM neurons participate in the baroreflex and receive an afferent inhibitory input from the caudal ventrolateral medulla and an afferent excitatory input from the nucleus tractus solitarius. Both pathways probably use amino acid neurotransmitters. To study amino acids in synapses on bulbospinal RVLM neurons, we injected cholera toxin B-gold into the intermediolateral cell column. Bulbospinal neurons were visulaized with silver-intensification. Immunoreactivity for tyrosine hydroxylase (TH) was detected by pre-embedding immunocytochemistry; and GABA or glutamate, by post-embedding immunogold. Only the inputs to bulbospinal RVLM neurons forming a tight cluster ventral to the nucleus ambiguus compacta were examined. The cell bodies and dendrites of these neurons received synapses and contacts from GABA and glutamate nerve fibers. GABA occurred in 43% (23/54) and glutamate in 73% (51/70) of inputs to bulbospinal TH neurons. GABA was present in 39% (59/150) and glutamate in 54% (74/136) of inputs to non-TH bulbospinal neurons. Studies are underway to determine the origin of the amino acid synapses on bulbospinal RVLM neurons and whether GABA and glutamate are co-localized in any of these inputs.

484.10

DEVELOPMENTAL CHANGES IN EXCITATORY RESPONSES OF VENTROLATERAL MEDULLARY NEURONS TO HYPOXIA ARE NOT DUE TO DIFFERENCES IN TISSUE PO_2 LEVELS. P.C. Nolan* and T.G. Waldrop. Dept. of Physiology & Biophysics, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Developmental differences in neuronal responses to hypoxia in several brain sites have been reported previously by other investigators. The purpose of the present study was to determine if there are developmental changes in the excitatory response to hypoxia of neurons in the ventrolateral medulla (VLM). In addition, tissue PO_2 measurements were made to quantify the hypoxic stimulus *in vivo* and *in vitro*. Whole cell recordings were made from neonatal (<16 days) and juvenile (>16 days) VLM neurons both in bottom perfused (Hatton-style) and in inclined plane (Haas-style) brain slice chambers. A platinum polarographic electrode with a 15 μm tip was used to measure tissue oxygen tensions during control and hypoxic periods in both brain slice chambers and in the VLM of anesthetized, spontaneously breathing rats. With 95% O_2/5% CO_2 aerating the brain slice chambers, basal oxygen tensions ranged from 518 ± 18 torr immediately below the surface of the tissue to 282 ± 18 torr at a depth of 200 μm. Aerating the chamber for 90 seconds with severe hypoxic (0% O_2/5% CO_2/95% N_2) or moderate hypoxic (10% O_2/5% CO_2/85% N_2) gas stimulated a higher percentage of the neurons from the older (>16 days) rats. In addition, hypoxia elicited a depolarization of 4.8 ± 0.5 mV in neonatal and 8.2 ± 1.3 mV in juvenile neurons. Oxygen tensions at a depth of 100 μm decreased to similar levels in neonatal and juvenile VLM tissue (5.1 ± 3.4 torr and 18.0 ± 8.4 torr during moderate and severe hypoxia). No differences in the PO_2 levels during hypoxia were seen between the two types of brain slice chambers. *In vivo*, VLM tissue PO_2 was 51.7 ± 0.7 torr (room air) and 66.6 ± 7.8 torr (100% O_2) and fell to 31.5 ± 6.8 torr during a 60 sec. inhalation of 10% O_2. These findings indicate developmental changes in the responses to hypoxia of VLM neurons which cannot be explained by differences in the level of tissue PO_2.

(Supported by NIH 32876, AHA-IL Affiliate and HD-07333)

484.11

DIFFERENTIAL TIME- AND DOSE-RELATED EFFECTS OF HEMORRHAGE ON TYROSINE HYDROXYLASE (TH) AND NEUROPEPTIDE Y (NPY) mRNA LEVELS IN MEDULLARY CATECHOLAMINERGIC NEURONS. R.K.W. Chan* and P.E. Sawchenko. The Salk Institute, La Jolla, CA 92037.

Hypotensive hemorrhage induces cellular activation and up-regulation of TH mRNA in medullary catecholaminergic cell groups. To shed light on the significance of the widespread coexistence of NPY in aminergic neurons, quantitative hybridization histochemical methods were used to compare the impact of graded doses of hemorrhage (15, 20 and 25%) on the time course of changes in relative levels of TH and NPY mRNA; concurrent staining for nuclear Fos-immunoreactivity (Fos-ir) permitted comparisons between cells that ostensibly were and were not targeted by the challenge. The major results may be summarized as follows: (1) Hemorrhage induced significant up-regulation of TH mRNA in all medullary aminergic cell groups, and of NPY transcripts in all but the A2 region. At later time points (2-4 hrs) these changes were predominantly seen in Fos-ir neurons. (2) Elevated levels of hemorrhage provoked only slight increases in the total number of Fos-ir neurons and in the percentage hybridized for TH or NPY mRNA in each aminergic region, but more pronounced increases in the magnitude and duration of both mRNA responses. (3) Noradrenergic cell groups displayed more robust maximal increases in relative levels of TH mRNA, while the reverse was true of the adrenergic cell groups. (4) Hemorrhage-induced up-regulation of TH and NPY mRNAs in Fos-ir neurons displayed differential time courses, with NPY responses peaking more rapidly, particularly in the C1 and C2 adrenergic cell groups. (5) At early time points (0.5-1.0 hr), a majority cells displaying increased levels of each transcript in the aminergic regions of interest were non-Fos-ir. Sampling at early time points, however, may precede the ability of cells to mount a detectable Fos protein response. These findings indicate that hemorrhage differentially affects relative levels of TH and NPY mRNA in medullary catecholaminergic cell groups that are known to participate in central neuroendocrine and/or autonomic control mechanisms. The surprisingly robust NPY mRNA responses in adrenergic cell groups suggests a mechanism by which the peptide content of the projection fields of these cell groups is defended.

484.12

DIMINISHED RESPONSES OF SPONTANEOUSLY HYPERTENSIVE RATS TO LESIONS OF THE CAUDAL VENTROLATERAL MEDULLA. H.M. Wilfehrt* and S.F. Morrison. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611-3008.

Elevated sympathetic nerve activity (SNA) and exaggerated sympathetic responses to posterior hypothalamic (POH) stimulation in the spontaneously hypertensive rat (SHR) suggest that central nervous system dysfunction may contribute to the pathogenesis of hypertension in this model. In the present study, we examined the hypothesis that the activity of vasodepressor neurons in the caudal ventrolateral medulla (CVLM) is diminished in SHR. Prior studies have demonstrated that excitotoxic lesions in CVLM in Sprague Dawley rats increase both SNA and mean arterial pressure (MAP). In urethane-anesthetized SHR and WKY rats (12-16 wks old), we examined the effects of kainic acid lesions in CVLM on spontaneous SNA, resting MAP and the evoked nerve responses to POH stimulation. In all rats, CVLM lesions produced significant increases in both SNA and MAP. Peak post-lesion MAP was no longer significantly different between strains (140 mmHg SHR vs. 129 mmHg WKY). However, the increase in total power (RMS) in SNA after the lesions was significantly smaller in SHRs (111%cnt) than in WKYs (302%cnt; $P < 0.01$). In both strains, post-lesion evoked responses to POH stimulation were greater than control, although this enhancement of evoked sympathetic responses was smaller in SHRs than in WKYs. We conclude that CVLM neurons may be less active in SHRs, resulting in diminished sympathoinhibition that could contribute to the pathophysiology of hypertension in the SHR. Supported by NIH HL47196.

484.13

CAUDAL VENTROLATERAL MEDULLARY (CVLM) NEURONS: ELEMENTS OF THE NETWORK RESPONSIBLE FOR THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND). H.S. Orer*, S.M. Barman, and G.L. Gebber. Dept. Pharmacol. & Toxicol., Michigan State Univ., E. Lansing, MI 48824.

Spike-triggered averaging and coherence analysis were used to study the relationship between the discharges of 246 CVLM neurons and the 10-Hz rhythm in SND of 17 urethane-anesthetized cats. Spike-triggered averaging showed that the naturally occurring discharges of 66 of these neurons were correlated to the 10-Hz rhythm in SND. The interval between unit spike occurrence and the peak of the 10-Hz slow wave in inferior cardiac SND averaged 59±2 ms for these CVLM neurons. Frequency domain analysis was used to characterize further the relationships between SND and the discharges of 45 of these CVLM neurons. The autospectra of the discharges of 22 of these neurons contained a sharp peak near 10 Hz (corresponding to the peak in the autospectra of SND), although the mean firing rate of these neurons was only 5.9±0.5 spikes/s. The peak coherence value relating the 10-Hz discharges of these CVLM neurons and the inferior cardiac nerve was 0.42±0.03. The autospectra for the other 23 CVLM neurons did not contain a peak near 10 Hz. Their mean firing rate (2.3±0.5 spikes/s) and the peak coherence value relating their discharges to the 10-Hz rhythm in SND (0.08±0.01) were significantly lower than the corresponding values for the other group of CVLM neurons. In five baroreceptor-denervated cats, muscimol (1 nmol/100 nl) was slowly microinjected at four sites in the CVLM ipsilateral to the nerve recording to chemically inactivate neurons in this region. This procedure reversibly eliminated the 10-Hz rhythm in SND. Thus, we propose that CVLM neurons are elements of the network responsible for the 10-Hz rhythm in SND. (Supported by NIH grants HL-33266 and HL-13187.)

484.14

DIFFERENT POOLS OF BRAINSTEM NEURONS GENERATE THE 10-HZ RHYTHM AND LOW FREQUENCY COMPONENTS IN SYMPATHETIC NERVE DISCHARGE (SND). S.M. Barman*, H.S. Orer, and G.L. Gebber. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

A 10-Hz rhythm is ubiquitous to the discharges of sympathetic nerves with different targets in urethane-anesthetized or decerebrate cats. This rhythm can coexist with a low frequency component (a cardiac-related rhythm in cats with intact baroreceptor nerves or an irregular 2- to 6-Hz oscillation after baroreceptor denervation). Spike-triggered averaging was used to study the relationship between the discharges of individual medullary neurons and SND. Data from these studies support the hypothesis that the generators of the 10-Hz rhythm and low frequency components in SND are comprised of separate pools of brainstem neurons. First, in baroreceptor-denervated cats, caudal ventrolateral medullary (CVLM) neurons with activity correlated to the 10-Hz rhythm did not have activity correlated to the irregular 2- to 6-Hz oscillations. Second, in baroreceptor-innervated cats, CVLM neurons with activity correlated to the 10-Hz rhythm did not have discharges correlated 1:1 to the cardiac-related rhythm when both rhythms were prominent in the autospectra of SND. Third, for CVLM neurons with activity correlated to the 10-Hz rhythm, the relationship between their discharges and SND was eliminated when the predominant pattern in SND was changed to a cardiac-related rhythm by raising arterial pressure. Fourth, lateral tegmental field neurons had activity correlated to the low frequency components but not to the 10-Hz rhythm in SND. Some rostral ventrolateral medullary and raphe neurons had activity correlated to both the 10-Hz rhythm and low frequency components in SND. Thus neurons in these regions receive convergent inputs from the two generators. (Supported by NIH grants HL-33266 and HL-13187.)

484.15

BRAIN STEM MECHANISMS ARE RESPONSIBLE FOR DIFFERENTIAL RELATIONSHIPS AMONG THE 10-HZ RHYTHMIC DISCHARGES OF SYMPATHETIC NERVES WITH DIFFERENT TARGETS. S. Zhong*, G.L. Gebber, and S.M. Barman. Dept. Pharm./Tox., Michigan State Univ., E. Lansing, MI 48824.

We previously demonstrated differential relationships among the 10-Hz rhythmic discharges of the cardiac, renal, and splenic postganglionic sympathetic nerves by using partial coherence analysis in baroreceptor-denervated, urethane-anesthetized cats (Gebber et al., **Soc. Neurosci. Abstr.**, vol. 19., Part 2, p. 1078, 1993). Partialization mathematically removes the portion of the coherence of two signals attributable to the sources of a third signal. For example, we found that partialization using splenic sympathetic nerve discharge (SND) usually reduced the coherence of the 10-Hz discharges of the left and right cardiac nerves to a significantly greater extent than did partialization using renal SND. Such results might reflect among other things 1) nonuniform coupling of multiple brain stem oscillators, each controlling a different nerve or 2) nonuniform crosstalk among spinal circuits that control different nerves but share inputs from a common source. In the current study, we compared the ordinary and partial coherence functions relating left and right cardiac SND before and after midsagittal section of the spinal cord from the seventh cervical (C7) through the eighth thoracic (T8) segments combined with right hemisection at T8. These sections failed to change the ordinary coherence value (near one) relating the 10-Hz discharges of the two cardiac nerves or the nonuniform reductions in the coherence value after partialization using splenic SND versus left renal SND. This also was the case after midsagittal section from the C1 through C8 spinal segments. These results suggest that the differential relationships of the 10-Hz rhythmic discharges of sympathetic nerves with different targets arise in the brain stem. (Supported by NIH grants HL-13187 and HL-33266.)

484.16

MATURATIONAL CHANGES IN COHERENCES OF EFFERENT SYMPATHETIC (SYMP) ACTIVITY. P.M. Gootman*, B.W. Hundley, A.L. Sica. Dept. of Physiology, SUNY-Hlth. Sci. Ctr., Brooklyn, NY 11203.

Simultaneous recordings of cervical SYMP, splanchnic and phrenic (PHR) activity along with aortic pressure, EKG and end-tidal CO_2 were obtained in Saffan-anesthestized, paralyzed and artificially ventilated (100 % O_2) 1-40 days old swine. Power spectra and coherence estimates between SYMP nerves and between SYMP and PHR discharge were obtained by a FFT routine. PHR discharge defined the inspiratory (I) and expiratory (E) epochs used for gating spectral estimates. Coherence between SYMP nerves was significant at birth in the 3-5 Hz range; the value of coherence was not age-related. In the 8-12 Hz range, coherence revealed age-related maturation attaining significance circa 2 wks. At about 16 days, higher frequencies (> 12 Hz) were present in the preganglionic SYMP power spectra. Coherence estimates of these higher frequencies were significant until about 4 wks. In both power spectra and coherence estimates, these higher frequencies disappeared after 4 wks of age. Coherence between PHR and SYMP was not significant until circa 3 wks. By 1 mo, SYMP activity resembled that observed in adult mammals (Gootman & Cohen, *Acta Physiol. Polon.* 1973, 24:97). The results suggest that there is a period of reorganization within SYMP rhythm generating circuits. (Supported by NIH grants HD-28931 and HL-20864.)

484.17

BRAIN STEM ROSTRAL DEPRESSOR AREA UNIT ACTIVITY IS CORRELATED TO THE 2- TO 6-HZ RHYTHM OF THE SYMPATHETIC NERVE DISCHARGE IN CAT. C. W. Dempsey*, D. E. Richardson, C. J. Fontana, and C.R. Burkeens . Lab. of Neurosurgery, Tulane University School of Medicine, New Orleans, LA 70112.

We have previously shown by chemical microinjection methods (Brain Research, 548:279-286, 1991) that the rostral depressor area (RDA) of the brain stem in cat contains sympathoinhibitory neurons involved in cardiovascular regulation. We now report in pentobarbital anesthetized cats a study of 55 cells in the RDA whose spontaneous activities, detected extracellularly with a metal microelectrode, were found to be correlated with the tonically active 2- to 6-Hz sympathetic discharge of the inferior cardiac nerve. The method employed was that developed by Barman & Gebber (Progress in Brain Research, 81:117-129, 1989), in which the RDA unitary action potentials serve as triggers for averaging 1 sec segments of the sympathetic discharge envelope wave. The activities of 17 cells yielded sympathetic slow wave averages of 2- to 3-Hz (synchronized with the heart rate), while the activities of 38 cells failed to correlate with the sympathetic discharge. Baroreceptor reflex activity (induced with IV phenylephrine or balloon-inflation in the aorta) revealed that, of these 17 cells, 8 were sympathoexcitatory, 5 were sympathoinhibitory, and 4 were cardiac-unrelated. This indicates that the RDA is a heterogeneous population of cells, with cardiac-related sympathoinhibitory neuronal activity not as predominate as our previous methods suggested.

484.18

DETECTION OF LOW FREQUENCY RHYTHM IN THE DISCHARGE OF CARDIOVASCULAR MEDULLARY NEURONS IN CATS. N. Montano, S.M. Barman, A. Porta, L. Imeri*, T Gnecchi-Ruscone, A. Malliani, G.L. Gebber. Medicina Int. II, Osp. L.Sacco & Fisiol. Umana II, University of Milan, Italy; Dept. Pharmacol. & Toxicol., Michigan State University, East Lansing, MI.

Spectral analysis of heart rate (HR) and arterial pressure (AP) variabilities in man as well as in experimental animals displays two major components: a low frequency (LF; 0.04-0.15Hz) and a high frequency respiratory related (HF; 0.25 Hz) rhythm. HF is considered a marker of vagal modulation while LF is mainly a marker of sympathetic modulation. In a previous study (Montano et al, JANS, 40:21-32, 1992) spectral analysis of cardiac sympathetic efferent discharge, in the range between 0 and 0.5 Hz, showed a predominant LF oscillation, highly correlated with the LF detectable in HR and AP. This study was undertaken to evaluate whether LF rhythmicity could also be present in the variability of discharge of single medullary neurons involved in cardiovascular regulation. We analyzed, by means of autoregressive spectral analysis algorhythms, AP and impulse activity of single units recorded from lateral tegmental field, medullary raphe and rostral ventrolateral medulla in decerebrate and in urethane anesthetized, baroreceptor-denervated cats. The activity of medullary neurons was correlated to higher frequency components (2- to 6- Hz or 10 Hz) in sympathetic nerve discharge. Power spectra of single neurons discharge displayed an LF oscillation, highly coherent (between 0.6 and 0.8) with the LF detectable in systolic AP. In conclusion we described for the first time that the impulse activity of single medullary neurons contains an LF rhythmicity similar to that detectable in spectral analysis of the cardiovascular variables, thus raising the possibility of an involvement of these nuclei in generating this oscillation.

484.19

MEDULLARY NEURONS HAVING FAST SYMPATHETIC-RELATED RHYTHMS SHOW A HIGH INCIDENCE OF RESPIRATORY MODULATION. Q.P. Yu, M.I. Cohen*, W.-X. Huang. Dept. of Physiol., Albert Einstein Col. Med., Bronx NY 10461.

Sympathetic nerve discharges have both fast sympathetic-specific rhythms (2-6 Hz and 10 Hz) and respiratory rhythm. In 18 midcollicular decerebrate, paralyzed cats, we recorded phrenic and cervical sympathetic (CS) nerve discharges together with discharges of neurons in ventral medullary regions that may contain vasomotor-related systems (rostral and caudal ventrolateral medulla, lateral tegmental field, and raphe). Correlations between fast rhythms in unit and CS discharges were ascertained by coherence analyses, and respiratory rhythms in these discharges were identified by cycle triggered histograms. Of 208 recorded units, 26 had distinct and significant unit-CS coherence peaks (range 2.1-17.8 Hz). Of these, 12 had peak coherence values > 0.1 (range 0.11-0.71, mean 0.28±0.23SD). The other 14 units had peak coherence values < 0.1 (range 0.05-0.09, mean 0.07±0.01SD). Most of the neurons (21/26) had a respiratory modulated rhythm (usually tonic I or E modulated) as determined by the eta-square test (Orem & Dick, J. Neurophysiol. 50:1098-1107, 1983). These results suggest that the combination of fast and slow (respiratory) rhythms seen in CS discharges originates in the medulla. (Supported by N.I.H. Grant HL-27300.)

484.20

RHYTHMIC DISCHARGES IN THE SYMPATHETIC SUPPLY TO THE CAUDAL VENTRAL ARTERY OF THE ANAESTHETIZED RAT. C.D. Johnson, H.A. Futuro-Neto and M.P. Gilbey*. Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF UK.

Recently we reported our observations regarding the rhythmic discharges of sympathetic postganglionic unit activity recorded from the caudal ventral artery of the rat tail using an extracellular focal recording technique (Johnson and Gilbey, J.Physiol. 476, 437-442, 1994). Units fired bursts of action potentials during normocapnia which were shown by autocorrelation analysis to have a frequency coincident with the phrenic rhythmic discharge. In the present series of experiments we examined the effects of hypocapnia and hypercapnia on this burst rhythm in chloralose anaesthetized, paralysed, vagotomized and artificially ventilated rats. Autocorrelation analysis revealed that a rhythmic discharge remained during hypocapnia when there was an absence of a regular phrenic discharge. The burst frequencies were in the range 1-1.5 Hz and were similar but different to those seen during normocapnia and hypercapnia. When the respiratory rate approached that of the burst frequency seen during phrenic silence burst frequency appeared to lock onto the phrenic rhythm. Bursts of action potentials occurred on variable levels of tonic discharge. Consequently, it appears that the generation of burst frequencies around 1 Hz may involve factors other than central respiratory drive. We hypothesize that the rhythmic burst activity seen during phrenic silence is generated by the postganglionic neurones in response to tonic drives and that phasic inputs in a similar frequency range are transmitted faithfully across the ganglion.
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SUBCORTICAL VISUAL PATHWAYS: MIDBRAIN

485.1

MEDIUM-SIZED RETINAL GANGLION CELLS PROJECTING TO THE GROUND SQUIRREL SUPERIOR COLLICULUS. N. Rivera, R.E. Blanco and N. Lugo*. Inst. of Neurobiology and Dept. of Anatomy, U.P.R., R.C.M., San Juan, P.R. 00901.

In the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), retinal ganglion cells have been classified into 3 groups on the basis of their soma cell diameter: large (>14 μm), medium (10-14 μm) and small cells (6-10 μm; Flores, 1983). Of these, mostly small and medium-sized cells project to the superior colliculus (SC) and mostly large cells project to the dorsal lateral geniculate nucleus (Lugo-Garcia and Kicliter, 1988). In order to characterize further the ganglion cells projecting to the SC, a retrograde tracer, cholera toxin subunit B (CTB), was injected into the superficial layers of the SC. After a survival period of 5-7 days, the animal was perfused, the retina dissected and the CTB immunohistochemically detected with a goat anti-CTB antiserum (List Biologicals; 1:2,500). Labeled cells were drawn and measured with Neurolucida and ZIDAS image analysis systems. Our results confirm that retinal ganglion cells projecting to the SC are medium and small-sized. Most medium-sized cells had diameters of 10-11 μm. Their dendritic trees were extensively labeled. They differed in soma shape, number of primary dendrites, density of branching, symmetry of branching and pattern of dendritic ramification within the inner plexiform layer. This morphological diversity suggests that medium-sized cells are composed of more than one sub-population. (Supported by ONR grant N00014-89-J-3070 & NIMH Grant MH-48190).

485.2

DIFFERENTIAL EXPRESSION OF FOS-LIKE IMMUNOREACTIVITY IN BRAINSTEM PROJECTIONS OF RAT SUPERIOR COLLICULUS AFTER ELECTRICAL STIMULATION
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It has been proposed, in rodent, that the orienting and approach elicited by collicular stimulation is mediated by the contralateral descending pathway of superior colliculus (SC), and avoidance-type behaviour is mediated by some targets of the ipsilateral descending projections. The c-fos immediate early gene is expressed polysynaptically in neurons in response to a wide range of extracellular stimuli, and hence has been proposed as a technique for mapping functional pathways. The purpose of this study was, therefore, to investigate further the behavioural specificity of the target zones of the two main descending projections from SC by analyzing the brainstem patterns of fos-like immunoreactivity [FLI] after electrically stimulating the tectal efferents which mediate either approach or avoidance-type behaviours.

The main results of this experiment were; (i) animals in which the stimulation elicited defensive behaviour had significantly higher levels of immunostaining in specific terminal areas of the ipsilateral descending projections, for example, the ventrolateral midbrain/pontine reticular formation, the cuneiform area and rostral periaqueductal grey; (ii) there was no FLI expression in any of the terminal areas of the crossed descending projection, even in animals where the electrical stimulation elicited approach. Control studies showed that the lack of expression in the crossed descending pathway was not due to the restricted range of stimulation parameters used in the main study, or to the effects of anaesthetic. In conclusion, this experiment was able to identify likely substrates for the mediation of defensive reactions elicited by electrical stimulation of the colliculus. However, given the total lack of expression in targets of the contralateral pathway, which are known to have been activated, it also provides further evidence that c-fos cannot simply be used as a high resolution neuronal activity marker for mapping functional pathways. Supported by a Royal Society Fellowship (SK) and an SERC Research Studentship [SK].

485.3

NON-SPECIFIC EFFECTS OF ANGIOTENSIN II AND ITS AGONISTS AND ANTAGONISTS IN THE SUPERFICIAL LAYERS OF THE RAT SUPERIOR COLLICULUS. L. Merabet[1,2]*, H. McLelland[3], C. Casanova[1,2]; Depts. of Ophthalmology[1], Physiology and Biophysics[2], Neurosurgery[3]; Faculty of Medicine, University of Sherbrooke; Sherbrooke, Quebec, Canada.

We have reported in a previous study (Merabet et al.;ARVO,1994) that activation of angiotensin II (AngII) receptors in the superficial layers of the rat superior colliculus (SC) have a profound suppressive effect on visual evoked potentials (VEP). Some of our initial observations have suggested that the "early" and "late" components of the VEP complex (likely corresponding to the "on" and "off" responses respectively) were differentially affected by the peptide. In the attempt to further characterize these effects, we investigated the impact of various concentrations of AngII and its specific receptor ligands (DUP 753, CGP 42112A). We also studied the effects of AngII on separate "on" and "off" evoked potentials. Experiments were carried out on anesthetized adult rats (13-15 weeks). A recording-injecting microelectrode filled with the peptide or selective ligand was lowered into the superficial layers of the SC to record VEP. Visual stimulation was provided by either a diffuse flash or a small flashing spot (duration of 500ms) placed in the corresponding receptive field to evoke "on" and "off" responses. The substance was injected at various concentrations (10^{-4}M to 10^{-10}M). Inhibitory effects of AngII were observed at concentrations greater than 10^{-10}M and were shown to be concentration dependent. Injection of the AT_2 agonist CGP 42112A showed differential effects depending on its concentration. At lower concentrations (10^{-9}M), there were no significant changes in the amplitude of the evoked potentials. However at 10^{-8}M, a concentration known to activate AT_1 receptors preferentially, there was a reduction in the amplitude of the potential (≈35%) similar to that observed with AngII. Injection of the AT_1 antagonist DUP 753 together with AngII yielded no significant changes in the amplitude of the response. Finally, contrary to our original observation, AngII equally reduced the amplitude of both the "on" and the "off" potentials suggesting a non-specific effect for the peptide. These results contribute to earlier findings that AngII has a suppressive effect on the superficial collicular layers in the adult rat. These responses appear to be mediated by the sole activation of AT_1 receptors and tend to be nonspecific. *Supported by MRC of Canada and CIBA-GEIGY Ltd.*

485.4

ADRENERGIC MODULATION OF RETINOTECTAL INPUT TO THE HAMSTER'S SUPERIOR COLLICULUS *IN VITRO*. H. Tan*, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Previous work from our laboratory suggested that multiple adrenergic receptor subtypes may be present in the superficial layers of the hamster's superior colliculus (SC), and that substantial numbers of α_2 and β_2 receptors may be located presynaptically on retinotectal and/or corticotectal axon terminals. The functions of these receptors were tested by intracellular or whole-cell recording in a perfused SC slice that included most of the optic tract (OT) to permit stimulation of retinotectal fibers. Addition of norepinephrine (NE) to the bath or application by micropressure hyperpolarized 55.5% and depolarized 13.5% of 207 SC neurons tested; the remaining 31% showed no change >1 mV. On average the membrane potential hyperpolarized from -70.7 ± 16.5 mV to -74.3 ± 9.6 mV. When voltage clamped at their resting potentials, hyperpolarized neurons had net outward currents of 18 to 50 pA and depolarized cells had inward currents of 26 to 38 pA. The OT-evoked EPSP was reduced from 10.8 ± 3.7 mV to 6.7 ± 3.7 mV by NE, but the decrease was not correlated with any change in membrane potential. To date, tests with more selective ligands have shown that *para*-aminoclonidine (α_2), dobutamine (β_1), and isoproterenol ($\beta_{1\text{ and }2}$) have effects on SC cells similar to those of NE. In contrast, neither phenylephrine nor methoxamine (α_1) produced significant effects on the membrane potentials of SC neurons;

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485.5

EFFECTS OF ADRENERGIC AGENTS ON NEURONAL RESPONSES IN THE HAMSTER'S SUPERIOR COLLICULUS. Y. Zhang*, R.W. Rhoades, and R.D. Mooney. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Radioligand binding experiments have demonstrated the presence of multiple adrenergic receptor subtypes in the hamster's superior colliculus (SC) and the present experiments were undertaken to assess the pharmacology of noradrenergic effects on the visual, optic chiasm (OX)-, and visual cortex (VCTX)-evoked responses of SC neurons. Of 40 neurons tested visually, 90% were suppressed by NE to 39.2 ± 28.4% of control responses and 10% were excited. Similar results were obtained during OX-stimulation (N=28 cells). Both isoproterenol (N=52 cells) and *para*-aminoclonidine (N=24 cells) had effects on SC cells similar to those of NE. Dobutamine (N=36 cells) had slightly weaker effects, while methoxamine suppressed 4 cells and excited 8. The effects of NE were strongly antagonized by α_2 antagonists and β-adrenergic antagonists (β_1 or $\beta_{1\text{ and }2}$) generally reduced NE-evoked effects by about 50%; β_2 antagonists were ineffective in nearly all cells. The α_1 antagonist prazosin completely blocked NE-evoked excitation, but did not reduce the suppressive effects of this amine. Thus, the suppressive effects of NE upon SC responses appear to be mediated primarily by α_2 and β_1 receptors while excitation involves α_1 receptors. No noradrenergic receptor subtype was specifically associated with either the retinotectal or corticotectal pathway and experiments carried out to date have not allowed us to determine whether any of the effects of NE are exclusively pre- or postsynaptic.

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485.6

NMDA receptors and cortical influences on visual responses in cat superior colliculus (SC). K.E.Binns & T.E. Salt*. Dept Visual Science, Inst. Ophthalmology, Bath St. London. ECIV 9EL. UK.

Neurones in the superficial grey layer (SGS) of the SC are innervated by the retina and visual cortex. SGS has 3 sublayers SGS_{1-3}. Retinal inputs dominate in SGS_1 & SGS_3, while in SGS_2 (120-500μm from the surface) cortical inputs are prevalent. The NMDA antagonist AP5 reduces visual responses in 60% of SGS neurones indicating that NMDA receptors participate in these responses[1]. Many of these cells are in SGS_2. To test whether the contribution of NMDA receptors to visual responses is derived from cortical input to SGS, multibarreled pipettes were used for single-unit extracellular recording and iontophoresis of NMDA, AMPA and AP5 in cats anaesthetized with halothane/N_2O/O_2 and paralysed with gallamine.

The effects of NMDA receptor selective applications of AP5 on visual responses were studied before and during inactivation of the visual cortex with topical lignocaine. In 8 neurones where AP5 reduced the response to visual stimulation, the visual response was resistant to the effects of AP5 following cortical inactivation. Since the cortex is considered to influence the directional bias of SGS neurones, the effects of AP5 (n=17) and cortical inactivation (n=10) on directional responses were compared. Neither procedure altered the directional bias of SGS neurones.

These data imply that, considerable NMDA receptor mediated input to SGS is from the cortex. However, the cortex may not influence the directional bias of SGS neurones.

(Supported by the MRC).

1. KE Binns and TE Salt, Eur. J. Neurosci. 6: 161-169. 1994.

485.7

GAMMA-AMINOBUTYRIC ACID (GABA) IS EXPRESSED EARLY IN PRENATAL DEVELOPMENT IN THE CAT SUPERIOR COLLICULUS. J.P. Hewitt*, M.T. Simon, F. T. Banfro, and R. R. Mize, Dept. of Anatomy and Neuroscience Ctr., Louisiana State Univ. Med. Ctr., New Orleans, LA 70112

GABA is expressed precociously in some neurons during CNS development. GABA cells are found throughout the superior colliculus (SC) in the adult cat, but the time of expression of GABA in this structure is not known. We therefore studied the expression of GABA in kittens ranging in age from embryonic day 24 to postnatal day 60 using antibody immunocytochemistry. Labeled cell position and density were determined using a neuron tracing system. At E24, GABA-immunoreactive (ir) cells were scattered throughout the tectal plate as well as within the subventricular zone. By E28-30, GABA-ir cells and neuropil were more densely concentrated within the superficial layers of SC than deeper. Some cells in the deeper layers had bipolar morphologies with vertically oriented dendrites, reminiscent of cells in the process of migration. The number of GABA-ir cells increased roughly eight fold from E30-E46, and the adult pattern of cell distribution was clearly established during this prenatal period. There was a two-fold decrease in GABA-ir cell number between E46 and P60. Cells also increased in maturity during this period. Based upon labeling of adjacent sections with antibodies to vimentin and glial fibrillary acidic protein (GFAP), we believe that most of the GABA labeling is in neurons and not glia throughout this developmental period. In summary, GABA is expressed very early in prenatal development in the cat SC, corresponding to the early expression of the calcium binding protein calbindin in this structure. GABA is expressed in some cells before they migrate into SC and clearly precedes the formation of the adult pattern of GABA neurons. The decrease in number of GABA-ir cells may be due to loss of expression of the neurotransmitter or to cell death. Supported by NIH EY02973.

485.8

PARVALBUMIN AND CALBINDIN HAVE DIFFERENT DEVELOPMENTAL HISTORIES IN THE PRE- AND POSTNATAL CAT SUPERIOR COLLICULUS. R.R. Mize* and F.T. Banfro, Dept. of Anatomy and Neuroscience Ctr., Louisiana State Univ. Med. Ctr., New Orleans, LA 70112

Parvalbumin (PV) immunoreactive neurons in the cat superior colliculus (SC) are projection neurons that form a dense sublaminar tier in the deep superficial gray and upper optic layers. Calbindin (CB) neurons are mostly interneurons that form three sublaminar tiers, with the PV tier lying between the two upper CB tiers. We have examined kittens aged E24 to P60 to determine when these tiers develop. Sections were immunocytochemically treated with monoclonal antibodies directed against PV and CB. Labeled cell depth and number were measured from computer plots of the labeled cell distributions. PV labeled cells were first observed at E59 and were located mostly within the periaquaductal gray. A few scattered PV labeled neurons were found in the optic, intermediate, and deep gray layers by P1. By P7, the dense sublaminar tier was visible and many PV cells had mature morphologies. Dense neuropil labeling was found in the tier by P14-21. The adult pattern of labeling was complete by P35. PV neurons increased continuously in number from P1-P35. By contrast, CB was first expressed at E24. Between E28-40, CB cells migrated from the subventricular zone into the tectal plate. Sublaminar tiers were first visible at E40 and were well-established by E46-48. Cell morphology matured from E46-E59. CB cell number increased from E28-E59, then decreased three-fold by P60. In summary, PV is expressed late in development, after cells have migrated. CB is expressed early, before cells have migrated and matured. Thus, CB may play a role in afferent-target interactions while PV is more likely involved in the final stages of synaptogenesis. Supported by NIH EY02973.

485.9

CALCIUM BINDING PROTEIN CALBINDIN D-28K IN THE PRETECTUM AND ACCESSORY OPTIC SYSTEM OF THE RABBIT. B. Nunes Cardozo[1], J.J.L. van der Want[1], G. Butler[2] and R.R. Mize[2] SPON: European Neuroscience Association* [1]Dept. of Morphology, The Netherlands Ophthalmic Res. Inst., 1100AC Amsterdam and [2]Dept. of Anatomy and Neuroscience Ctr., Louisiana State University Med. Ctr., New Orleans, LA 70112.

The calcium binding protein calbindin-D 28K (CB) is a highly selective marker of specific cell types in the CNS. In the cat pretectum (PT), CB cells form four cell clusters, two of which lie partially within the nucleus of the optic tract (NOT). CB cells are mostly large projection neurons that precisely overlap the retinal input to PT and cross nuclear borders. In this study, we determined whether these same cell clusters are present in the rabbit, a lateral-eyed species with a well developed PT. Sections through the NOT and accessory optic system (AOS) nuclei of three rabbits were incubated with an antibody directed against CB and visualized using silver-gold enhanced DAB. A few neurons in the rostral NOT were CB positive, while the caudal NOT was devoid of CB neurons. Unlike the cat, separate dense clusters of neurons were not observed in the PT of rabbit. However, clusters of CB neurons were observed in the caudal portion of the dorsal terminal nucleus (DTN) and in the transition zone of the DTN and interstitial nucleus of the superior fasciculus posterior fibers (inSFp). However, the medial terminal nucleus (MTN) was completely free of CB neurons. In contrast to cat, the clusters of CB neurons in the AOS fell within the established borders of these nuclei. In addition, there was no precise overlap between CB cells and retinal afferents in the rabbit PT and AOS. CB appears to label different cell classes in the two species. Supported by NIH EY02973.

485.10

POSTNATAL DEVELOPMENT OF EXCITATORY SYNAPTIC FUNCTION IN DEEP LAYERS OF SUPERIOR COLLICULUS

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Despite the rapid maturation of neurons in superficial layers of the cat superior colliculus (SC), those in deep layers are not visually-responsive for several postnatal weeks. The present study sought to determine the ontogeny of synaptic responses in deep layer neurons using whole cell patch recordings in coronal slices. Excitatory synaptic responses were recorded in response to local electrical stimuli, and the sensitivity of postsynaptic currents/potentials (EPSC/Ps) to the NMDA antagonist APV was studied at different developmental stages. Thirty percent of 1-8 DPN neurons exhibited stimulation-evoked EPSCs, compared to 73% of 31-34 DPN neurons. Not only did the proportions of activated neurons increase during maturation, but the nature of the EPSCs changed as well. APV- sensitive components of the EPSCs did not appear until 9 DPN, and their incidence gradually increased until 33 DPN when 88% of the neurons recorded were APV-sensitive. Thus, excitatory synaptic function shows a protracted postnatal development in deep SC and may contribute both to the delay in the maturation of visual responsiveness (Kao et al., 1994), and the delay in the ability to integrate information from different sensory modalities (see Wallace et al., 1993).

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485.11

CROSS-MODAL INHIBITION IN MULTISENSORY NEURONS IS BASED ON UNIMODAL RECEPTIVE FIELD ORGANIZATION. D.C. Kadunce*, M.T. Wallace, G. Benedek, and B.E. Stein. Dept. Neurobiology & Anatomy, Bowman Gray School of Medicine/Wake Forest University, Winston-Salem, NC 27157

A fundamental principle of multisensory integration is that spatially coincident stimuli from different modalities enhance one another's effectiveness, whereas spatially disparate stimuli may depress one another's effectiveness. The present experiments in cat superior colliculus (SC) were designed to examine the basis for this cross-modal inhibition, and to determine whether the excitatory-inhibitory organization of multisensory RFs is significantly different from that of unimodal RFs. A total of 114 (55 unimodal; 59 multisensory) neurons were examined. Surprisingly, each multisensory RF was organized in fundamentally the same manner as its unimodal counterpart. However, the incidence of inhibitory border regions differed among modalities. The majority (81%) of auditory RFs had inhibitory regions, but far fewer visual (22%) and somatosensory (15%) RFs did. In many multisensory neurons RF inhibitory borders were not modality-specific (e.g. auditory stimuli would suppress visual excitation); in others, inhibitory regions were modality-specific and this was most common for visual inputs (e.g. visual inhibits only visual). These data indicate that while cross-modal inhibition in SC is due to the presence of nonspecific inhibitory regions, inhibitory regions themselves do not guarantee such effects. In these cases the inhibitory effect is on the input rather than on the target multisensory SC neuron. Supported by NIH grant NS22543.

485.12

SENSORY REPRESENTATIONS AND THE INTEGRATION OF MULTIPLE SENSORY CUES IN MONKEY SUPERIOR COLLICULUS. M.T. Wallace*[1], L.K. Wilkinson[2] and B.E. Stein[1]. Dept. Neurobiology & Anatomy[1], Bowman Gray School of Medicine/Wake Forest University, Winston-Salem, NC 27157 and Department of Psychology[2], Virginia Commonwealth University, Richmond, VA 23298

A set of principles governing the neural integration of stimuli from the different senses have been found in cat superior colliculus (SC). We sought to determine the applicability of these principles to the rhesus monkey. Single unit extracellular recordings were conducted in the deep SC of anesthetized monkeys. Of the sensory responsive neurons, 73% were unimodal and 27% were multisensory. Our first question concerned the intermodal register between the visual, auditory and somatosensory representations. The second concerned the nature of the integration exhibited by multisensory SC neurons. The data suggest that, as in the cat, each modality has a well defined representation, and that these representations are in good register with one another (e.g., the rostral body surface is represented in the same areas as nasal visual space). Exceptionally good register was noted among the different receptive fields of individual multisensory neurons, and, as in the cat, dramatic response enhancements were evoked by stimuli that were spatially coincident, and response depressions were evoked by stimuli that were spatially disparate. These data are consistent with the hypothesis that many of the principles of sensory representation and multisensory integration in the SC are operative in widely divergent species, and are adaptive to a wide variety of ecological niches. Supported by NIH grant NS22543 and the James S. McDonnell Foundation.

485.13

SIMULTANEOUS RECORDINGS OF MULTI-SINGLE NEURONAL ENSEMBLES IN THE SUBCORTICAL VISUAL SYSTEM REVEAL PROPAGATION OF OSCILLATORY WAVES. Rowshanak Hashemiyoon* and John K. Chapin, Department of Physiology & Biophysics, Hahnemann University, Philadelphia, PA

We have previously reported that dark-spontaneous oscillations (10-40Hz) are ubiquitous in the subcortical rat visual system. These oscillations are broadcast from the retina to synchronize unit activity across multiple visual pathways. These were initially investigated through the simultaneous recording of multiple single units in the visual midbrain, thalamus and optic tract. To further investigate the spatiotemporal structure of these oscillations, electrode arrays consisting of 16-microwire electrodes (1.8 x 0.5 mm) were chronically implanted across the stratum griseum superficiale (SGS) of the superior colliculus. Under anesthetized and awake conditions dark-spontaneous synchronous oscillatory discharges recorded from these electrodes exhibited variable phase angles of up to 15ms, indicating propagation of activity waves across the SGS. A small (14°) flashing light (1s ON, 1s OFF) in the contralateral visual field suppressed the oscillatory activity of the SGS neurons during LIGHT-ON. LIGHT-OFF produced a phase resetting oscillatory rebound, with response latencies varying by up to 15ms, suggesting propagation of waves away from the LIGHT- OFF point. Other studies investigated the effects of patterned vs. non-patterned stimuli. Whereas non-patterned flashing circles of light produced simple LIGHT-ON suppression, addition of a dark bar to the middle of the circle produced a more complex response, consisting of an additional oscillatory peak during the LIGHT-ON period. In conclusion, the propagating spatiotemporal pattern of these oscillations may not only integrate sensory processing across the subcortical visual system, but might also carry a distributed code for visual sensory information. *Supported by NS26722 to JKC.*

485.14

GABAa MEDIATED DISINHIBITION OF THE SUPERIOR COLLICULUS RESTORES VISUAL ORIENTING RESPONSES IN THE PREVIOUSLY HEMIANOPIC VISUAL FIELD OF THE CORTICALLY BLIND CAT

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Following unilateral removal of all known visual cortical areas, a cat is rendered hemianopic in the contralateral visual field as measured by visual perimetry and other behavioral tests. It has been shown that visual orientation behavior can be restored to the previously blind hemifield by transection of the commissure between the two colliculi, or by destruction of the contralateral substantia nigra. (Sprague, J.M., *Science* 153:1544-1547, 1966; Wallace, S. F. et.al., *J. Comp. Neurol.* 296:222-252, 1990) It was hypothesized that the mechanism mediating recovery is disinhibition of the SC ipsilateral to a cortical lesion.

We directly tested this hypothesis by reversibly disinhibiting the SC ipsilateral to a cortical lesion with bicuculline, a GABAa antagonist. In accordance with the model, disinhibition of the SC ipsilateral to the cortical lesion restored visual orienting responses in the hemianopic visual field for a period of several hours. Control injections of saline had no effect on visual orienting behavior. Conversely, inhibition of the SC with muscimol, a GABAa agonist in a normal animal renders the animal unable to detect and orient to visual stimuli in the hemifield contralateral to the SC.

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485.15

RECOVERY OF VISUAL ORIENTING IN THE HEMIANOPIC CAT AFTER SMALL SUBSTANTIA NIGRA LESIONS IS NOT ABOLISHED BY SUBSEQUENT ENLARGEMENT OF THE INITIAL LESION.

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Dept. of Neuroscience, Univ. of Penn, Phila., PA 19104

Unilateral removal of all known visual cortical areas in the cat renders the animal hemianopic in the contralateral visual field as measured by visual perimetry and other behavioral tests. Visual orientation behavior can be restored in the previously blind hemifield by ibotenic acid destruction of a critical zone in the substantia nigra pars reticulata (SNpr) contralateral to the cortical lesion (Wallace, S.F. et. al., *J. Comp. Neurol.* 296:222-252, 1990). Paradoxically large lesions to the SNpr which include but extend beyond this critical zone fail to restore visual orienting behavior.

In an attempt to understand this paradoxical finding, we made unilateral visual cortical lesions followed by ibotenic acid lesions of the contralateral SNpr critical zone in 5 cats. All cats recovered visual orienting behavior in the hemianopic field. In these recovered animals, the original SNpr critical zone lesion was subsequently enlarged with ibotenic acid an average of 5.4 weeks after the initial lesion. None of the 5 animals showed a loss of recovery. This finding argues strongly for the role of plasticity in nigrotectal circuitry and visual orienting behavior. The time between a critical zone lesion and an enlargement of this lesion appears to be sufficient for compensatory mechanisms to maintain recovery.

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485.16

IBOTENIC ACID LESIONS OF THE PEDUNCULOPONTINE NUCLEUS RESTORE VISUAL ORIENTING RESPONSES IN THE PREVIOUSLY HEMIANOPIC VISUAL FIELD OF THE CORTICALLY BLIND CAT

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The unilateral removal of all known visual cortical areas in a cat results in an enduring hemianopsia as measured by visual perimetry and other behavioral tests. By transecting the commissure of the superior colliculus (CSC) or destroying a critical zone of the contralateral substantia nigra pars reticulata (SNpr), visual orientation behavior returns in the previously hemianopic field (Sprague, JM, *Science* 153:1544-1547, 1966; Wallace, SF et al., *J. Comp. Neurol.* 296:222-252, 1990). Disinhibition by disruption of contralateral SNpr or its projections to the superior colliculus (SC) has been proposed as the mechanism for this recovery. However, large lesions of the SNpr which include but extend beyond the critical zone do not result in recovery, but transection of the CSC in these animals does. Thus, it is clear that other projections through the CSC mediate this effect. Retrograde transport studies indicate that fibers crossing in the CSC arise from the SNpr and the pedunculopontine nucleus (PPN) (Edley, S et al., *J. Comp. Neurol.* 217:187-215, 1983; Hall, W et al., *J. Comp. Neurol.* 287:495-514, 1989).

We tested the hypothesis that projections originating from the contralateral PPN mediate the recovery of visual orienting behavior by destroying this nucleus with ibotenic acid in cortically blind cats. Results indicate that recovery of the visual orienting response is mediated by the PPN.(*Supported by EY02654 and 5-T32-MH-18902*)

485.17

SUBSTANTIA NIGRA AFFERENTS INNERVATE THE CAT SUPERIOR COLLICULUS DURING THE SECOND HALF OF GESTATION.

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Most afferents to the cat superior colliculus (SC) are present at birth, including those from the substantia nigra (SN). It is not known when these reach the SC during prenatal development. We have therefore studied the development of innervation of the SC by SN fibers in fetal tissue using the carbocyanine dye DiI. DiI crystals were placed within the SN in blocks of midbrain perfused at ages E40, 46, 51, 55, 57, P1 and P7. The blocks were stored in fixative in an oven at 37°C for 4-5 weeks, then sectioned and examined with an epi-fluorescence microscope. Labeled fibers were traced with a neuron tracing system. No DiI fibers were seen in SC at E40, although they were present in the mesencephalic tegmentum. By E46, DiI fibers were present in the deep layers of the caudal SC, but none were found in the superficial layers. The fibers were thin and had few varicosities. Putative growth cones were seen on some of the labeled fibers. Few fibers were found in the rostral SC. The labeled fibers were confined primarily to the ipsilateral side. By E51, the number of labeled fibers in the SC had increased. By E55, numerous labeled fibers were found in the intermediate gray layer (IGL) of the caudal SC. The fibers at this age were thicker and some had numerous varicosities. Fiber density increased at E57 and P1, but no fibers were found above the IGL even at P1. Thus, a sharp border between the superficial and deep layers is maintained at this age. No obvious fiber patches were observed at any age. The patch-like organization of SN fibers seen in the adult must therefore develop postnatally. SN fibers reach the SC about two weeks after retinal afferents have innervated SC and after SC lamination is complete. Supported by NIH EY-02973.

485.18

EVIDENCE THAT AREA 17 CORTICAL SYNAPSES IN THE CAT SUPERIOR COLLICULUS ARE GLUTAMATERGIC.

G. D. Butler, R. H. Whitworth* and R. R. Mize, Dept. of Anatomy and the Neuroscience Center, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

We have previously shown that antibodies to the excitatory amino acid neurotransmitter, glutamate, label at least two types of synaptic terminals in the cat superior colliculus (SC). One type contains large round synaptic vesicles and pale mitochondria and is of retinal origin (RT). The second type contains smaller round vesicles and dark mitochondria (RSD). The origin of the latter type is unknown. To determine if RSDs arise from visual cortex, we made unilateral aspiration lesions of area 17 in three cats and waited 5, 14, and 28 days before sacrifice. We then examined the distribution of glutamate in the SC of these cats using electron microscopic post-embedding immunocytochemistry and colloidal gold-conjugated antibodies. On the side contralateral to the lesion, both RT and RSD axon terminals were labeled by anti-glutamate, as in the normal SC. Some conventional dendrites and myelinated axons were also labeled. On the side ipsilateral to the lesion, morphologically normal RSD terminals were infrequently observed. However, synaptic terminals in the initial stages of electron dense degeneration that had a dark ground and irregularly shaped swollen vesicles were labeled by anti-glutamate. Axon terminals and myelinated axons undergoing neurofilamentous hypertrophied degeneration also were often labeled. By contrast, terminals in advanced stages of degeneration with no synaptic vesicles contained little or no glutamate. These results confirm that both major sets of afferents to the cat SC contain glutamate; RT terminals from the retina and RSD terminals from visual cortex. It is possible, however, that not all terminals from these sources contain glutamate because a few RT and RSD terminals were not labeled by the antibody. Supported by NIH EY-02973.

BASAL GANGLIA AND THALAMUS IX

486.1

CANNABINOID AGONISTS ATTENUATE DOPAMINE-MEDIATED ROTATION IN RATS WITH UNILATERAL 6-HYDROXYDOPAMINE LESIONS.

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The presence of cannabinoid receptor mRNA in striatal neurons and dense cannabinoid receptor binding in substantia nigra pars reticulata, globus pallidus, and striatum, suggests that cannabinoids influence basal ganglia motor function by actions on the striatal output pathways. Contralateral rotation produced by dopamine D1 and D2 receptor stimulation in rats with unilateral nigrostriatal dopamine cell lesions is a behavioral measure of striatal outflow through the striatonigral and striatopallidal pathways. We have evaluated the effect of cannabinoid agonists on this rotation in rats with unilateral 6-hydroxydopamine lesions. Rotation was monitored for 4 hours following administration of the D1 agonist SKF 38393 (1.5 mg/kg s.c.) or the D2 agonist quinpirole (0.1 mg/kg i.p.), in combination with the cannabinoid agonist WIN 55,212-2 (1, 2.5, or 10 mg/kg i.p.; n = 8-11) or vehicle (60% DMSO; n = 19-20). WIN 55,212-2 produced a dose-related attenuation of D1-mediated rotation that was significant at the 2.5 and 10 mg/kg doses, decreasing rotation to 16% of control at 10 mg/kg. WIN 55,212-2 was less potent in attenuating D2-mediated rotation, producing a significant decrease at the 10 mg/kg dose only, to 57% of control. The cannabinoid agonist CP 55,940 (0.1 mg/kg i.p.) decreased both D1 and D2 rotation, although D1 rotation was suppressed to a greater extent (16% of control; n = 6) than D2 rotation (53% of control; n = 7). These results suggest that cannabinoid receptor stimulation influences both D1- and D2-mediated processes, although D1 processes may be affected to a greater degree.

486.2

EFFECT OF SUBSTANCE P (NK_1) RECEPTOR BLOCKADE ON CATALEPSY INDUCED BY DOPAMINE ANTAGONISTS.

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Systemic administration of dopamine D_1 or D_2 receptor antagonists induces catalepsy, a behavior defined as the long-term maintenance of an abnormal posture. Catalepsy is often considered an animal model of neuroleptic-induced parkinsonism. Substance P, a neurokinin found in striatonigral neurons, acts preferentially at the NK_1 receptor. Receptor binding studies suggest that the striatum contains a moderate density of NK_1 receptors, while the substantia nigra pars reticulata contains relatively few NK_1 receptors. Hence, striatonigral substance P may act preferentially in the striatum via local axon collaterals of striatonigral neurons. In this study, the ability of NK_1 receptor blockade to influence catalepsy produced by D_1 and D_2 antagonists was examined. Male Sprague-Dawley rats were injected with the non-peptide NK_1 antagonist CP 99,994 (0.5, 2.5, or 10 mg/kg sc) or saline 15 min after administration of either the D_1 antagonist SCH 23390 (0.5 mg/kg sc) or the D_2 antagonist raclopride (2.5 mg/kg ip). Catalepsy was evaluated by the vertical grid test every 20 min for 160 min. CP 99,994 (2.5 and 10 mg/kg) decreased the catalepsy produced by raclopride by 46 and 78%, respectively. In contrast, CP 99,994 did not diminish the catalepsy induced by SCH 23390. These findings suggest that blockade of NK_1 receptors reduces catalepsy produced by D_2 but not D_1 antagonists and indicate that NK_1 receptors may be important in modulating behavioral responses mediated by D_2 receptors. In addition, NK_1 antagonists may have therapeutic potential in alleviating motor side effects associated with neuroleptic treatment.

486.3

AN INVESTIGATION OF D1 AND D2 DOPAMINE AGONIST-INDUCED CHANGES IN THE ACTIVITY OF SUBSTANTIA NIGRA PARS RETICULATA NEURONS FOLLOWING LOCAL INFUSION OF SKF 38393 IN EITHER THE STRIATUM OR SUBSTANTIA NIGRA. M. J. Twery*, D. A. Bergstrom and J. R. Walters. ETB, NINDS, Bethesda, MD 20892.

Concurrent stimulation of D1 and D2 receptors by systemically administered dopamine agonists inhibits the activity of substantia nigra pars reticulata (SNpr) neurons in rats with 6-hydroxydopamine (6OHDA)-induced lesions of midbrain dopamine cells. In order to investigate possible sites of action, the D1 agonist SKF 38393 was locally administered in the striatum (15 pmol-34 nmol) or the substantia nigra (9-17 nmol) of gallamine-paralyzed rats with unilateral 6OHDA lesions (6-9 wk post-lesion) while the activity of individual SNpr neurons was recorded extracellularly. Rats were tested for apomorphine (0.05mg/kg, s.c.)-induced contralateral rotation 1-2 wk prior to study. An infusion of SKF 38393 in the striatum (n=7) or the substantia nigra (n=7) produced no significant changes in the firing rate of SNpr neurons. The D2 agonist quinpirole (0.3 mg/kg, i.v.) administered 3 or 10 min after the start of a nigral SKF 38393 infusion decreased the firing of SNpr neurons (46±5%, ANOVA, p=0.02, n=6). However, quinpirole administered after striatal infusion of SKF 38393 (n=7) or after nigral infusion of vehicle (n=6) produced no net change in the firing rate of SNpr neurons. These findings suggest that the response to intravenous quinpirole depends on whether D1 receptors are stimulated by SKF 38393 in either the striatum or the substantia nigra. However, the stimulation of only D1 receptors by SKF 38393 at either striatal or nigral sites is not sufficient to alter the activity of SNpr neurons. Explanations include the possibility that D1 receptors at another site or at a combination of sites including the striatum and substantia nigra are required to produce changes in the activity of SNpr neurons.

486.4

AP-1 DNA BINDING ACTIVITY IN STRIATUM: DOPAMINERGIC REGULATION K.-X. Huang* and J.R. Walters. ETB, NINDS, NIH, Bethesda, MD 20892.

Stimulation of dopamine (DA) receptors in the basal ganglia affects expression of c-*fos*, while interruption of c-*fos* expression in the striatum with antisense DNA reduces behavioral response to DA agonists[1,2]. Since Fos and Jun proteins influence cellular function by forming AP-1 which binds to DNA sites regulating gene transcription, effects of DA receptor stimulation on striatal AP-1 DNA binding activity were examined using a gel shift assay. Nuclear protein extract (10 µg), prepared from homogenized striatum, was incubated with ^{32}P-labeled oligonucleotides containing AP-1 or CREB consensus sequence. In normal rat striatum there was a high level of AP-1 binding. I.p. administration of (+) SKF 38393 (5 mg/kg, n = 9) or quinpirole (0.25 mg/kg, n = 8) did not significantly affect AP-1 binding 2 hr later, nor did a combination of these drugs (n = 7). In striata of rats treated with reserpine (10 mg/kg, s.c.) for 4-7 h, AP-1 binding was increased by 90% (n = 8, $p<0.01$). AP-1 binding was also increased by 80% (n = 7, $p<0.01$) 12 weeks after 6-OHDA-induced lesion of the nigrostriatal pathway and by 45% (n = 5, $p<0.05$) 8-9 months later. In 6-OHDA-lesioned rats, a combination of (±) SKF 38393 (10 mg/kg) and quinpirole (0.25 mg/kg) further increased AP-1 binding by 76%(n = 5, $p<0.01$). Striatal CREB DNA binding activity was not significantly affected in reserpinized or lesioned rats. Thus, changes in Fos synthesis critical to DA agonist behavioral effects[1,2] are not reflected in total striatal AP-1 binding 2 hr after agonist treatment in normal rats, but are evident in 6-OHDA-lesioned rats. The observation that striatal AP-1 DNA binding is enhanced by DA depletion as well as by DA receptor stimulation after DA cell lesion suggests that different underlying mechanisms are involved. [1]Sommer et al., Neuroreport 5: 277, 1993; [2]Dragunow et al., Neuroreport 5: 305, 1993.

486.5

THE MITOCHONDRIAL TOXIN 3-NITROPROPIONIC ACID INDUCED ORAL MOVEMENTS IN RATS. O.A. Andreassen[1]* and H.A. Jørgensen[2]. Depts. of Physiology[1] and Psychiatry[2], Univ. of Bergen, N-5009 Bergen, Norway.

Tardive dyskinesia (TD) is a serious side effect of long-term neuroleptic treatment. The mechanism of TD development is still unknown. It has been proposed that TD may be a result of neuroleptic-induced excitotoxic neuronal damage in the striatum. To investigate this hypothesis, the effect of 3-nitropropionic acid (3-NP), an inhibitor of energy metabolism which has been shown to induce striatal lesions due to glutamate receptor stimulation (indirect excitotoxicity), was studied in a rat model of TD. Three groups of rats (female Sprague-Dawley rats, n = 14-15 / group) were treated continuously for 1 month with 3 doses of 3-NP (4, 8 and 12 mg/kg/day) administered SC with osmotic minipumps. Empty plastic tubes were inoperated SC to control rats. The behavior was videotaped regularly and vacuous chewing movements (VCM), a putative analogue to human TD, as well as the total behavior, were recorded.

3-NP induced a dose-dependent increase in VCM in the period from 2 to 4 weeks after start of treatment. Rats receiving the highest dose of 3-NP had significantly more VCM than controls and the rats receiving the lowest dose were at control level. 3-NP also dose-dependently decreased the general motor activity.

The effect of 3-NP on VCM suggests that striatal glutamate receptor stimulation may underlie the development of VCM in rats and maybe TD in humans.

486.6

REGULATION OF NEOSTRIATAL NEURONS BY cAMP-DEPENDENT MECHANISMS. C.S.Colwell* and M.S.Levine. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of the present study was to examine the possibility that cAMP-dependent regulation of synaptic transmission occurs in the neostriatum. Intracellular recordings were obtained from neostriatal neurons in slices using standard techniques. Depolarizing postsynaptic potentials (DPSPs) were evoked by local electrical stimulation. Bath application of forskolin (FKN), an activator of adenylate cyclase, enhanced DPSP amplitude [155±8% (mean±se) of controls] and duration (122±6%). This potentiation was dose-dependent, occurred in presence of the GABA receptor antagonist bicuculline, and was not seen with the inactive FKN analog 1,9-dideoxyforskolin. Rp-cAMPS, an inhibitor of cAMP-dependent protein kinase (PKA), was applied in addition to FKN. Rp-cAMPs (100μM) significantly attenuated FKN's enhancement of DPSP amplitude. Moreover, the PKA activator Sp-cAMPS (100μM, 10 min) also enhanced DPSP amplitude. These results suggest that FKN is acting through a PKA-dependent mechanism to enhance DPSP amplitude. Interestingly, in some neurons, treatment with Rp-cAMPS clearly depressed the evoked response. This inhibitory effect was also seen when the intracellularly active PKA inhibitor PKI was applied via the recording electrode. In contrast, bath application of the phosphatase inhibitor, okadaic acid (10μM), increased DPSP amplitude. These results indicate that, at least under these experimental conditions, tonic kinase and phosphatase activity regulates DPSP amplitude. Taken together, these results suggest a role for the adenylate cyclase cascade in the regulation of synaptic transmission in the neostriatum. Supported by USPHS HD 05958.

486.7

DOPAMINE RECEPTOR AGONISTS AND FORSKOLIN SIMILARLY POTENTIATE RESPONSES EVOKED BY EXCITATORY AMINO ACID RECEPTOR AGONISTS IN RAT NEOSTRIATAL NEURONS. M. Day, C. Colwell, N.A. Buchwald*, and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Our laboratory has studied the physiological interactions between dopamine (DA) and excitatory amino acids (EAAs) in the neostriatum. We have shown that DA potentiates responses mediated by activation of N-methyl-D-aspartate (NMDA) receptors but attenuates responses mediated by glutamate or activation of non-NMDA receptors. The present experiment was designed to examine the possibility that DA's ability to potentiate responses mediated by activation of NMDA receptors involves alterations in the adenylyl cyclase transduction cascade. Intracellular recordings were made from neostriatal neurons in brain slices using standard techniques. Similar to our previous findings, iontophoretic or bath application of the DA D_1 receptor agonist SKF 38393 potentiated NMDA-evoked responses by increasing the maximum amplitude, half amplitude duration and number of action potentials evoked. SKF 38393 also enhanced glutamate-evoked responses, but to a lesser degree. Receptor antagonists were used to evaluate the specificity of the agonist's actions. Bath application of forskolin, an activator of adenylate cyclase which increases cAMP, mimicked the effects of D_1 DA receptor activation and enhanced responses evoked by iontophoretic application of NMDA. Forskolin also enhanced responses evoked by glutamate or AMPA. These results are consistent with a model in which DA, acting through activation of D_1 receptors, enhances EAA-evoked responses through a cAMP-dependent mechanism. Supported by USPHS HD 05958.

486.8

WHOLE CELL CURRENTS MODULATED BY DOPAMINE IN PATCH-CLAMP RECORDINGS FROM VISUALIZED NEOSTRIATAL NEURONS IN SLICES USING INFRARED VIDEOMICROSCOPY. C. Cepeda*, Q. Yu, S.H. Chandler[1], N.A. Buchwald and M.S. Levine. Mental Retardation Research Center and Department of Physiological Sciences[1], UCLA, Los Angeles, CA 90024.

We have shown that the direction of the neuromodulatory actions of dopamine (DA) is dependent upon the specific subtype of excitatory amino acid receptor activated. DA enhances responses evoked by N-methyl-d-aspartate (NMDA) but attenuates responses induced by glutamate or activation of non-NMDA receptors. To provide information on the ionic mechanisms and membrane currents responsible for these actions of DA, whole-cell patch-clamp recordings from visually identified neurons (Nomarski optics, infrared video-microscopy) were used. Thin (120-200 μm) or thick (up to 400 μm) slices were obtained from rats 6-20 days of age. Visualized cells (50-100 μm below the surface of the slice) had small to medium (5-15 μm) or large diameter (20-30 μm) somata. The effects of DA were examined on currents evoked by slowly depolarizing ramp commands. A persistent inward current was evident after application of the command voltage. It activated at about -45 mV and was followed by an outward current. The inward current was blocked by application of TTX (1 μM) suggesting it was mediated by Na^+ ions. DA (20-100 μM) also reduced the current. These results indicate that the inward rectification observed in neostriatal neurons is the result of activation of a TTX-sensitive persistent Na^+ current that is modulated by DA. It is possible that this Na^+ current may be partly responsible for DA's ability to attenuate responses evoked by glutamate. The present findings also underscore the feasibility of using infrared videomicroscopy to identify recorded cells in thick slices in which local connectivity is intact. Supported by USPHS HD 05958.

486.9

DIZOCILPINE MALEATE DIFFERENTIALLY INFLUENCE THE POSTSYNAPTIC EFFECTS OF NIGROSTRIATAL DOPAMINE DEAFFERENTATION AMONG SUBPOPULATIONS OF STRIATAL NEURONS.

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This study examined the effects of systemic treatments with dizocilpine maleate (MK-801; 0.2mg/kg, i.p., twice a day for 8 days) either alone or in combination with unilateral 6-hydroxydopamine-induced lesion of the nigrostriatal dopamine neurons on neuropeptide Y(NPY) and substance P (SP) immunostaining in the rat striatum. MK-801 by itself elicited an increase in the striatal number of neuropeptide Y-immunodetectable cells, paradoxically concomitant with a decrease in the levels of intraneuronal labelling, and a decrease in SP immunostaining in both the striatum and substantia nigra. As reported previously, unilateral lesion of the nigrostriatal dopamine pathway elicited an increase in the number and staining intensity of NPY-immunoreactive neurons, out a decrease in SP immunoreactivity. MK-801 treatment of animals with unilateral nigrostriatal dopamine lesion totally reversed the changes in NPY immunoreactivity related to the dopamine lesion without modifying those in SP. These data indicate that both NPY- and SP-containing neurons in the striatum undergo tonic glutamatergic influence through NMDA receptors. They further suggest that the mechanisms underlying the effects of dopamine deafferentation may differ among the striatal neuronal populations.

486.10

GAMMA-AMINOBUTYRIC ACID (GABA) TURNOVER IN MOUSE THALAMUS FOLLOWING GABA-T INHIBITION; EFFECTS OF DOPAMINERGIC MANIPULATIONS.

P. Martin*, M.L. Carlsson and A. Carlsson. Dept. of Pharmacology, Medicinareg.7, 413 90 Goteborg, Sweden.

The accumulation of gamma-aminobutyric acid (GABA) in the mouse thalamus was measured after inhibition of GABA-transaminase (GABA-T) by means of systemic administration of the irreversible inhibitors gamma-acetylenic GABA (GAG, 200 mg/kg i.p.) or gabaculine (150 mg/kg).

After treatment with reserpine and alfa-methyltyrosine, the accumulation of GABA in both thalamus and cerebellum was decreased to 73 % of controls. Likewise, when gabaculine was administered locally in the thalamus, the GABA turnover in thalamus was decreased to 75 % of controls.

The GABA turnover following an i.p. injection of apomorphine (0.5-1.5 mg/kg) was decreased to 82 or 67 % of control, after systemic GAG or gabaculine, respectively.

Results of ongoing microdialysis studies will be reported.

The data are discussed in relation to the proposed existence of positive and negative cortico-striato-thalamo-cortical feedback loops.

486.11

NEUROTENSIN AND FOS IMMUNOREACTIVITIES IDENTIFY TWO SUBPOPULATIONS OF STRIATAL NEURONS FOLLOWING ACUTE 6-HYDROXYDOPAMINE LESIONS AND RESERPINE ADMINISTRATION.

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It was previously reported that following dopamine (DA) D_2 receptor blockade or DA depletion, neurotensin (NT) immunoreactivity (IR) is elicited in at least two distinct subpopulations of rat striatal neurons. Colocalized Fos-IR, interpreted as an indicator of enhanced neuronal activity, was found in only one of these subopulations following D_2 blockade (Neurosci. 57: 649-60, 1993). In this study, the degree of colocalization of Fos- and NT-IR in striatal neurons in response to acute midbrain 6-hydroxydopamine (6-OHDA) lesions and reserpine administration were studied. It was observed that a subpopulation of smaller neurons exhibiting light NT-IR and frequent colocalization with Fos-IR was predominant after reserpine administration, mainly in the dorsolateral quadrant of the striatum. Another subpopulation of larger, intensely NT-immunoreactive neurons that was rarely colocalized with Fos-IR was observed following all the drug treatments, but was present almost to the exclusion of the smaller cell type three days following 6-OHDA lesions, mainly in the dorsomedial and ventrolateral portions of the striatum. The present data are consistent with two subpopulations of striatal neurons that accumulate NT following DA depletion. The possibility is considered that one subpopulation accumulates NT in response to coordinate increases in neuronal activity and NT synthesis, and the other as a result of decreased release. Support: NIH NS-23805, T32 NS-07254, and the United Parkinson Foundation.

486.12

DIFFERENTIAL MODULATION OF DOPAMINE D1- AND D2-RECEPTOR BINDING IN RATS TREATED WITH A CLASSICAL NEUROLEPTIC.

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Classical neuroleptics are often employed in the treatment of schizophrenia. These compounds are known to act on the dopaminergic system primarily by blocking dopamine (DA) D2-receptors. In the following experiments, we evaluated the consequences of exposure to the classical neuroleptic, fluphenazine decanoate (FLU), on DA D1- and D2- receptor binding in several regions of the basal ganglia. Adult female Sprague-Dawley rats were treated either chronically or acutely with FLU (30mg/kg) or with vehicle. Animals were killed by decapitation and brain sections through the striatum (STR), entopeduncular n. (EP), subthalamic n. (STh) and substantia nigra (SN) were collected and processed for receptor autoradiography. D1-receptors were labelled with [^{125}I]-SCH23982 in the presence or absence of unlabelled SCH23390 and D2- receptors were labelled with [^{125}I]-NCQ298 ± Etiocloprìde. Analysis of autoradiograms showed that *in vivo* exposure to FLU significantly decreased specific [^{125}I]-NCQ298 binding by up to 70% relative to controls in all brain regions measured probably by competing with [^{125}I]-NCQ298 binding *in vitro*. With regard to D1-receptors however, FLU treatement decreased [^{125}I]-SCH23982 binding in the dorsal STR (35%), but increased binding in EP (38%) and was without effect in the SN. *In vitro* competition assays performed on sections from untreated rats showed that FLU is a potent competitor of binding at both receptor subtypes with an affinity of about 10nM for D1-receptors in the dorsal STR and EP and 2.5nM for D2-receptors in the dorsal STR and STh. These results suggest that although FLU is an effective competitor of both D1 and D2 compounds *in vitro*, it acts primarily as a D2 antagonist *in vivo*. These data suggest that even for ligands with high affinity for both D1 and D2 receptors *in vitro*, these compounds may act primarily at D2 binding sites *in vivo*. Supported by grant #8318 from the Swedish MRC.

486.13

ELECTROPHYSIOLOGICAL EFFECTS OF A CANNABINOID ON NEURONS IN THE SUBSTANTIA NIGRA PARS RETICULATA.

A.S. Miller* and J.M. Walker. Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

Binding studies have demonstrated that cannabinoid receptors are densely distributed in the outflow nuclei of the basal ganglia and are especially abundant in the substantia nigra pars reticulata (SNR). Cannabinoid receptors in the SNR have been localized on the striatonigral terminals. Single unit electrophysiology was used to explore the role of cannabinoid receptors in the SNR. Intravenous or intraperitoneal injection of WIN 55,212-2 in anesthetized rats produced a significant but modest increase in the basal firing rate of SNR neurons. A second set of experiments was performed in order to assess whether this increase represented a disinhibition of SNR activity. Striatal stimulation produced a brief inhibition of neural activity in the SNR. WIN 55,212-2 (up to 1 mg/kg i.v.) dose-dependently reversed the inhibition of cell firing in the SNR which was produced by striatal stimulation. These results suggest that cannabinoid receptors on the striatonigral terminals may regulate movement by disinhibiting the activity of SNR neurons, perhaps by inhibiting the release of GABA into the SNR.

486.14

PRE- AND POSTSYNAPTIC MEASURES OF SHORT-TERM SYNAPTIC PLASTICITY AT THE CORTICOSTRIATAL SYNAPSE OF THE AGED RAT.

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The corticostriatal synapse of the aged rat expresses a decrease in paired-pulse facilitation, without an associated change in short-term synaptic depression (Ou and Walsh, 1994). It is not known, however, whether age affects the kinetics of recovery from synaptic depression. Synaptic depression was induced at the corticostriatal synapse by delivering 15 stimuli (ISI of 50 msec) to the corpus callosum in an *in vitro* brain slice. Recovery from the depression was monitored by responses to a single test stimulus occurring 0.2 to 5 seconds after conditioning. Our data indicates that there is a significant difference between the two-age groups in the kinetics of recovery from synaptic depression, with aged cells showing slower recovering from depression than young cells.

We next examined the effect of age on the contribution of NMDA receptor activation to the synaptic response. Paired synaptic responses (ISI of 60 msec) were evoked before and after addition of the NMDA receptor antagonist APV (30 μM) in Mg^{2+}-free ACSF. Subtraction of synaptic responses evoked under these two conditions indicates that neurons from aged rats expressed a decrease in the NMDA component of the synaptic response. All slices were treated with 20 μM bicuculline to block $GABA_A$ receptor mediated inhibition.

Research was supported by a grant from the NIA (5 P01 AG0979).

486.15

EFFECTS OF 5-$HT_{1A/1B}$ AND D_1 RECEPTOR ANTAGONISTS ON THE INDUCTION OF STRIATAL JUN B AND FOS-LIKE IMMUNOREACTIVITY BY DEXFENFLURAMINE. A.M. Gardier*[1], R. Moratalla[2], and A.M. Graybiel[2]. [1]Dept. Pharmacol. JE 92-372, Fac. Pharmacie, Chatenay-Malabry 92296, France & [2]Dept.of Brain and Cognitive Sciences, MIT, Cambridge MA 02139, USA.

The indirect serotoninergic agonist fenfluramine {20 mg/kg i.p.} induces Fos-like immunoreactivity in the caudoputamen (CPu) and in other brain regions {Richard et al., *Brain Res.*, **594** (1992) 131-137}. However, fenfluramine is a racemate in which the d-isomer (d-fen) acts on the serotoninergic system by increasing the extracellular levels of serotonin (5-HT) at nerve terminals (Samanin and Garattini, In *Nutrition and The Brain, Vol.8* , Raven Press, New York, 1990), whereas the l-isomer increases the turnover of dopamine (DA) in rat CPu {Invernizzi et al., *Eur.J.Pharmacol.*, **120** (1986) 9-15}. Here, the induction of c-*fos* and *jun B* protein products was studied in rats 2 hrs after administration of d-fen (0, 5, 10, 20 and 40 mg/kg, i.p.). D-fen induced a dose-dependent increase in both Fos-like and Jun B-like immunoreactivity (F-LI, Jun B-LI) in the CPu, and in other sites including the central amygdaloid nucleus and layer 4 of frontoparietal cortex. The CPu induction was most intense centrally and medially and, at caudal levels, in localized dorsal and ventral zones. The induction of F-LI and Jun B-LI by d-fen (40 mg/kg) was attenuated in CPu, but not in the cortex, by pretreatment with low doses (0.2 and 0.3 mg/kg, i.p.) of the D_1 DA receptor antagonist SCH 23390. Furthermore, the induction of F-LI and Jun B-LI by d-fen (40 mg/kg) was attenuated in the cortex, but not in CPu, by prior administration of a drug known as one of the most effective 5-$HT_{1A/1B}$ autoreceptor antagonists, methiothepin. The latter drug was administered at a dose (10 mg/kg) that has been shown to block d-fen's effect on brain 5-HT metabolism {Gardier et al., *Brain Res.*, **588**(1992)67-74}. Our results suggest that an indirect 5-HT agonist d-fen may stimulate F-LI and Jun B-LI in the rat striatum at least in part indirectly through striatal DA release, leading to an activation of postsynaptic D_1 DA receptors.

486.16

FOS-LIKE-IMMUNOREACTIVITY IN BASAL GANGLIA OUTPUTS FOLLOWING ADMINISTRATION OF DOPAMINE AGONISTS. David Wirtshafter* Dept. Psych., Univ. Illinois at Chicago, Chicago, Il 60680.

The pars reticulata of the substantia nigra (SNpr) and the entopeduncular nucleus (EPN) tonically inhibit cells in a number of structures including the lateral habenula, the pedunculopontine region and certain thalamic nuclei. Many workers have theorized that stimulation of dopamine receptors leads to inhibition of cells in the SNpr and the EPN which, in turn, results in a disinhibition of neurons in the the structures listed above. Since many neurons respond to excitation by expressing the immediate-early gene c-*fos*, we attempted to evaluate this model of basal ganglia organization by using immunocytochemistry to examine the distribution of Fos like immunoreactivity following systemic injections of either amphetamine (5 or 10 mg/kg), amfonelic acid (2.5 or 5 mg/kg) or apomorphine (4 mg/kg). Following all of these treatments, many labeled neurons were observed in the lateral portion of the lateral habenula, in the ventromedial thalamic nucleus and the rostral pole of the ventrolateral thalamic nucleus and in the pedunculopontine region. In subjects with unilateral 6-OHDA lesions of the ascending dopamine pathways, injections of amphetamine resulted in labeling of all of these structures on the intact, but not the deinnervated side. Simultaneous processing of tissue for NADPH-diaphorase revealed that staining in the midbrain tegmentum was concentrated in the so called "midbrain extrapyramical area" just medial to the the cholinergic cells of the pedunculopontine nucleus proper.

486.17

STIMULATION OF ADENOSINE A_{2a} RECEPTORS BY CGS 21680 INDUCES C-FOS EXPRESSION IN THE STRIATUM OF DOPAMINE DENERVATED RATS Morelli M.*, A. Pinna, G. Di Chiara Dpt. Toxicology Univ. of Cagliari (Italy)

The induction of the early-gene c-fos after the adenosine A_{2a} receptor agonist CGS 21680, was studied in the striatum of normal rats or after a unilateral 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic nigro-striatal neurons. CGS 21680 (5 mg/kg) induced c-fos expression in the 6-OHDA lesioned striatum while in normal rats CGS 21680 failed to induce c-fos expression even at doses of 10-20 mg/kg. The dopamine D_2 agonist quinpirole reversed CGS 21680-induced c-fos expression, while blockade of muscarinic receptors by scopolamine partially prevented c-fos expression in the dorso-lateral part of the striatum. The results are consistent with the coexistence of A_{2a} and D_2 receptors on striatal neurons and with their opposite influence on adenylate cyclase. However the data also suggest that stimulation of cholinergic transmission by CGS 21680, might play a role in the induction of c-fos expression.

VESTIBULAR: VESTIBULOOCULAR REFLEX PHYSIOLOGY

487.1

VESTIBULARLY-EVOKED ACTIVITY OF SINGLE NEURONS IN THE DORSOMEDIAL CELL COLUMN OF THE INFERIOR OLIVE IN RABBIT. N.H. Barmack* and M.H. Fagerson. R.S. Dow Neurological Sciences Inst., Portland, OR 97209 and Dept. of Cell Biol., Oregon Health Sciences University.

GABAergic pathways originating from the medial and descending vestibular nuclei (DVN, MVN) as well as the nucleus prepositus hypoglossi convey vestibular information to various divisions of the inferior olive. Previously we have demonstrated a topographic representation of both the vertical semicircular canals and utricular otoliths in one of the subdivisions of the inferior olive, the β-nucleus. In the present experiment we have characterized the inputs, projections, as well as the encoding characteristics of neurons in another subdivision, the dorsal medial cell column (dmcc). We have recorded extracellularly from dmcc neurons in chloralose-urethane-anesthetized rabbits placed in a three axis rate table, permitting static and sinusoidal vestibular stimulation, as well as the determination of a vestibular null point.

All neurons were driven by static tilt or sinusoidal rotation about the longitudinal axis. These neurons responded in phase with contralateral side-down head position during sinusoidal stimulation. Measured null points did not correspond to the orientation of pairs of vertical semicircular canals. These neurons appeared to be driven primarily from the utricular otoliths. These vestibularly responsive neurons were located in the dorsal half of the dmcc. An immunohistochemical stain for glutamic acid decarboxylase demonstrates that the dmcc, as well as the β-nucleus is innervated by GABAergic fibers originating from the DVN and MVN. Injections of horseradish peroxidase (2-4 µl, 30%) into both the nodulus-uvula and flocculus demonstrate that the dorsal half of the dmcc projects to the nodulus, whereas the ventral half projects to the flocculus. In sum, the dmcc accounts for some of the otolithic input to nodulus-uvula and may contribute to the topographic modulation of postural control about the longitudinal axis.

487.2

NEURAL RESPONSES TO VESTIBULAR PARADIGMS IN THE MACAQUE NODULUS. A.F. Rosenberg' and C.A. Scudder. Dept. of Otolaryngology, Univ. of Pittsburgh, Pittsburgh PA 15213.

The goal of this study is to investigate the involvement of the nodulus in vestibular processing by characterizing its 1) input and output signals, and 2) responses during off-vertical axis rotation (OVAR). Both granular layer units (GLUs) and Purkinje cells (PCs) were recorded from the cerebellar cortex of an awake, trained, head-fixed monkey during passive vestibular stimulation in both horizontal and vertical planes, and during eye movements.

Most neurons were sensitive to vestibular stimulation. Of these neurons that were tested in three orthogonal planes, the vast majority were sensitive to movement in a vertical plane whereas few neurons responded exclusively to yaw rotation. GLUs that responded to vertical motion were sensitive to head velocity (indicating canal or dynamic otolith input) or to static tilt (indicating otolith input). Most responsive PCs were sensitive to some combination of vertical head velocity and tilt, which in some cases, depended on the particular plane of stimulation. The responses of these PCs suggest that they receive convergent signals originating from multiple types of vestibular afferents.

PCs were recorded during OVAR, which presumably invokes velocity storage. Some PCs responded with a constant change in firing rate, a modulated firing rate related to instantaneous direction of tilt, or both. The peak firing rate of units that had a modulated response component sometimes corresponded to different tilt directions for different senses of rotation.

Some neurons (GLUs and PCs) possessed eye movement sensitivity, including bursts (GLUs). Others, mostly PCs, had responses related to body movement, and appeared to be restricted to the ventral nodulus. GLUs and PCs tested with optokinetic stimulation were unmodulated.

It is concluded that the nodulus contains reflex circuitry utilizing vertical motion and static tilt information from the labyrinth. Neural signals in the nodulus may be involved in the generation of eye movements during OVAR.

487.3

FUNCTIONAL INDEPENDENCE OF NODULAR AND UVULAR MICROZONES CONTROLLING SPATIAL ORIENTATION OF VELOCITY STORAGE. B.Cohen, S.Wearne, T.Raphan, H.Reisine* Mt Sinai School of Medicine and Brooklyn College of CUNY

Tilting the gravitoinertial acceleration (GIA) vector during yaw-axis vestibular or optokinetic nystagmus reorients the compensatory eye velocity vector toward the spatial vertical (axis reorientation). Two processes are associated with this: reduction of the horizontal time constant (dumping) and generation of vertical and/or torsional components in the eye velocity vector (cross-coupling). Axis reorientation was studied in rhesus monkeys following discrete lesions of the nodulus and uvula, and after midline medullary section to abolish velocity storage. The GIA vector was tilted in three paradigms: OKAN with static head tilts, centrifugation and dynamic head reorientation during postrotatory nystagmus. Midline medullary section abolished velocity storage, reducing the horizontal time constant from 19.1±2.1s to 5.6±1.2s, without affecting the VOR gain. This abolished eye velocity reorientation, showing that it depends upon velocity storage, not on the direct vestibular pathways. After nodulo-uvulectomy, the horizontal time constant was insensitive to static or dynamic head tilts and monkeys could generate neither vertical nor torsional components to shift the eye rotation axis. Vertical and torsional VOR time constants, tested in on-side or supine positions, fell to ≈2 sec. Selective ablation of the medial nodulus and uvula, sparing the most lateral nodular microzones, eliminated vertical and torsional cross-coupling but not the ability to reduce the horizontal time constant. Thus, processes associated with axis reorientation can be dissociated structurally in the nodulus and uvula. This suggests independence of the nodulo-uvular microzones controlling cross-coupling and dumping. **Supported by NS00294, EY04148, EY01867.**

487.4

RECOVERY OF ANTERIOR CANAL AFFERENT SPONTANEOUS ACTIVITY AND RESPONSES TO MECHANICAL STIMULATION FOLLOWING AMINOGLYCOSIDE-INDUCED HAIR CELL LOSS IN CHICKS. R. Boyle[1]*, J.P. Carey[2], S.M. Highstein[3], E.W Rubel[2], and R.D. Rabbitt[4]. Depts. of Otolaryngology[1,2,3] and Bioengineering[4]; Oregon Health Sci. Univ., Portland, OR 97201[1]; Univ. Washington, Seattle, WA 98195[2]; Washington Univ., St. Louis, MO 63110[3]; Univ. Utah, Salt Lake City, UT 84112[4].

The chicken vestibular sensory epithelium is capable of regenerating new hair cells after ototoxic drug injury; the time course of anatomical recovery is complete after 60 days (Weisleder and Rubel, *J. Comp. Neur.* 331: 97-110, 1993). To determine the events, time course, and functional status of this recovery, extra- and intracellular recordings were made from anterior canal afferents in treated (streptomycin, 1200 mg/kg/day x 5d starting at 5-7 days post-hatch) and age-matched control anesthetized White Leghorn chicks. A piezoelectric micro-actuator was used to adequately stimulate, by mechanical indentation, the exposed anterior semicircular canal. At 3 wk post-treatment, spontaneous rates of afferents are zero or low, and indentation responses are mostly absent. By 5-6 wk post-treatment, spontaneous rates increase, but on average are less than those of control chicks, and indentation responses are present in some, but not all, spontaneously active afferents. The results suggest that afferent spontaneous activity and responses to mechanosensory stimulation are progressively reestablished in the regenerating crista ampullaris. The anatomical correlates of this functional recovery is under study using a morphophysiological analysis. (Supported by PHS Grant DC00520 and 00018)

487.5

L-ACETYL-CARNITINE TREATMENT INFLUENCES THE AGE-RELATED ADAPTATION OF BENZODIAZEPINE RECPTORS AFTER UNILATERAL LABYRINTHECTOMY. M., Zanni , L. Giardino, M.T. Ramacci* and L. Calzà Inst. of ORL, University of Modena and Milano, Italy; Ist. Ricerca Senescenza, Sigma Tau, Pomezia, Italy; Inst. of Human Physiol., University of Cagliari, Italy.

The functional recovery from peripheral and central nervous lesions is modified by aging. Also the vestibular compensation after hemilabyrinthectomy (HLTX) follows an age-related profile. The early phase of vestibular compensation involves a rearrangement of cerebellar GABAergic input to the lateral vestibular nucleus (LVe). In this paper, we investigated the modification of benzodiazepine binding sites in the LVe after 28 days from the HLTX in 3- and 24 months old rats *(male Sprague Dawley)*. We also studied the effect of 28 days treatment with L-acetyl-carnitine *(50mg/kg/day, ip)* . The experiments have been performed by means of quantitative autoradiography using ^{3}H-flunitrazepam (^{3}H-FLU) as ligand. In the LVe of old unlesioned rats, an higher density of ^{3}H-FLU binding sites than in adult rats is found *(+14.3 +- 2.6%)*, which is reversed by L-acetyl-carnitine treatment. The L-acetyl-carnitine treatment is ineffective in adult unlesioned rats. In adult hemilabyrinthectomized rats, ^{3}H-FLU binding sites in both lesioned and unlesioned sites of the LVe are not modified after 28 days from the lesion. The L-acetyl-carnitine treatment did not modify this result. The same experimental condition induces a decrease of benzodiazepine receptor density in LVe of both sides of old rats *(lesioned side: -33.1 +- 2.5; unlesioned side: -29.8 +- 2.6)*. The L-acetyl-carnitine treatment performed for 28 days starting from the day of surgical hemilabyrinthectomy induces a bilateral increase in receptor density of LVe, the level of which reachs those found in old unlesioned untreated rats. These data indicate: 1. the GABAergic involvement in vestibular compensation at LVe level shows an age-dependent profile, at least for the regulatory site of $GABA_A$ receptor labeled by ^{3}H-FLU; 2. the L-acetyl-carnitine treatment affects the ^{3}H-FLU receptor modifications observed in LVe of old rats and also those related to a rearrangement of the synaptic function during vestibular compensation.

487.6

PHYSIOLOGIC CHARACTERISTICS OF REGENERATED VESTIBULAR CANAL AFFERENTS. J. Hernandez, L. Hoffman , V. Honrubia* Victor Goodhill Ear Center, UCLA School of Medicine, Los Angeles, CA 90024.

Canalicular primary afferent neurons were investigated *in vivo* for spontaneous and evoked activity 4 months following peripheral axotomy of the anterior vestibular nerve in the bullfrog. Glass micropipettes filled with 1M KCl were used to obtain intraaxonal recordings from anterior and horizontal canal units central to the site of transection. These data were compared to data from normal afferents (Honrubia et al., J. Neurophys. 61:688, '89; Hoffman and Honrubia, Abs. Soc. Neurosci. 15:517, '89).

Spontaneous activity recorded in the post-transected nerve exhibited normal characteristics exemplifying regularly and irregularly discharging units, as determined by the coefficient of variation (CV) of the interspike interval (ISI). CVs ranged from 0.10 to 1.20 for anterior units (mean: 0.59 ± 0.40, n = 18), and from 0.07 to 1.01 for the horizontal units (mean: 0.53 ± 0.33, n = 19).

Canalicular afferents responded unambiguously to sinusoidal angular accelerations of 0.05Hz and peak velocity of 15°/sec. Response gains ranged from 1.73 to 12.57 spikes•s^{-1}/°•s^{-2} for anterior units (mean: 5.51 ± 3.19, n = 15) and from 0.45 to 10.45 spikes•s^{-1}/°•s^{-2} for horizontal units (mean: 3.62 ± 3.51, n = 16). For horizontal and anterior units with spontaneous firing CVs < 0.5, gains were comparable to the distribution of normal afferents with similar CVs. However, units with CVs > 0.5 exhibited lower gains compared to normal afferents (Mann-Whitney U test, p<0.001). There was no difference between horizontal and anterior gains among the regenerated afferents (t-test, p>0.2). Phase measurements (re: head acceleration) ranged from +5.0° to -70.2° and -11.6° to -74.3° for anterior and horizontal units, respectively. These measures were comparable to that found for normal horizontal and anterior afferents.

Thus, the appearance of spontaneous activity and responsiveness to sinusoidal rotations among horizontal and anterior afferents in the post-transected nerve indicates that the regenerated primary afferents observed morphologically in a previous study have established functional synapses. However, the reduction in response gains from normal suggests that these regenerated afferents have diminished synaptic inputs. Supported by GM08042 and DC01404.

487.7

EVIDENCE FOR A STOCHASTIC MECHANISM IN COMPENSATION FOR VIII$_{TH}$ NERVE SECTION IN THE VESTIBULO-OCULAR REFLEX OF THE GOLDFISH. R. Ratnam and T. J. Anastasio*. Beckman Institute, Center for Biophysics, and Department of Physiology and Biophysics, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

To study compensation in the period immediately following loss of vestibular input to one side in the goldfish, a preparation was developed to quickly cut the stato-acoustic (VIIIth) nerve while the animal was set-up for recording eye movements. Unilateral nerve section produces intense, nystagmic, spontaneous vestibulo-ocular reflex (VOR) eye movement, averaging in the acute stage about 35°/*sec* to the ipsilateral side (from a normal of less than 1°/*sec* directed nasally). VOR gain in the acute stage is harder to estimate, although it very quickly drops to values below normal. Spontaneous VOR (SVOR) is eliminated by the compensatory process within 10 minutes to 1 hour; some gain recovery also occurs in this period. This is the most rapid SVOR compensation yet reported, and greatly facilitates observation and analysis of the process. SVOR elimination appears to occur in two phases. The first phase is characterized by high variability, in which SVOR fluctuates in a random fashion with eye velocities ranging from 5−60°/*sec* to the ipsilateral side. The second phase is marked by an abrupt decrease in SVOR to less than 5°/*sec* to the ipsilateral side with an associated decrease in variability. Gain recovery in the first phase is more complex, but generally follows that of compensation for SVOR. In the second phase variability in gain is also much reduced. Gain increases to subnormal levels after about 1 hour, and continues to increase over an ensuing period of weeks. These results suggest that SVOR compensation may involve a stochastic process that results in a symmetry breaking transition to the compensated state. (This study is supported by grants from the Whitaker foundation and the Beckman Institute.)

487.8

EFFECTS OF BLOCKADE OF NMDA RECEPTORS AND UNILATERAL VESTIBULAR DAMAGE ON THE DYNAMICS OF THE VOR. D. M. Broussard* and J. K. Bhatia. Playfair Neuroscience Unit, Univ. of Toronto, Toronto, ON M5T 2S8, Canada.

Motor learning in the mammalian VOR, like compensation for vestibular lesions, may involve changes in the efficacy of the vestibular commissure. Synaptic transmission via the commissure has been shown, in frogs, to be mediated in part by N-methyl-D-aspartate (NMDA) receptors. To explore the role of commissural synapses in generating the mammalian VOR, we have compared the effects of the NMDA receptor antagonist ketamine, optically-induced VOR plasticity, and peripheral vestibular damage on the steady-state and peak responses of the VOR of young cats to a rapid horizontal rotational acceleration followed by a constant velocity. Ketamine caused a decrease in both the peak and the steady-state response of the VOR and an increase in the ratio of the peak to the steady-state eye velocity (dynamic index). We also changed the gain of the VOR using magnifying or miniaturizing spectacles, which altered both the steady-state and peak gain. After the horizontal semicircular canal was unilaterally inactivated, steady-state VOR gain for rotation in both directions recovered to near the normal value. The contraversive peak gain and dynamic index were considerably greater than ipsiversive throughout compensation. Our results are consistent with a role of the vestibular commissure in generating the low-frequency components of the VOR. The relationship between steady-state gain and dynamic index during compensation differed from the relationship during optically-induced motor learning. The relative contributions of different parts of the VOR pathway to motor learning in the VOR may, therefore, be altered by a complete unilateral lesion of the horizontal semicircular canal.

487.9

SIGNAL CONVERGENCE AND DYNAMICS RECORDED FROM VESTIBULO-OCULOMOTOR NEURONS IN THE ALERT CAT. S.C. Brettler* and J.F. Baker. Northwestern University, Chicago, IL 60611

Vestibulo-oculomotor neurons contribute to the dynamics and spatial organization of compensatory eye movements produced by the vestibulo-ocular reflex (VOR). We have recorded the responses of 96 vestibular nucleus neurons in alert cats to investigate canal convergence, eye position sensitivity, response dynamics, and otolith input. Neurons were classified according to latency of orthodromic responses to labyrinth electrical stimulation and antidromic responses to oculomotor nucleus stimulation.

Extensive spatial testing (14+ runs, 20 neurons), including several sinusoidal rotations about axes near response nulls, confirmed canal convergence on oculomotor-projecting neurons. Six of 9 neurons receiving primarily anterior canal input and 2 of 9 posterior canal neurons showed horizontal canal convergence (>20°). Maximum activation direction vectors of anterior canal neurons (AC) varied more widely than those of posterior canal neurons (PC). Vertical and horizontal eye position sensitivity of 28 vestibular nucleus neurons (16 oculomotor-projecting) varied from 0 to 3.1 spikes/sec/° and generally corresponded to vertical and/or horizontal vestibular sensitivity, with some striking exceptions. During sinusoidal rotations at low frequencies, subtraction of the eye position component from the total neuronal response advanced the response phase.

Responses of 26 neurons to sinusoidal rotations were nearly in phase with velocity (89° ± 18° s.d. at 0.5 Hz) with advanced phases at high (129° ± 10° at 4 Hz) and low (horizontal canal, 104° ± 21°; PC, 114° ± 19°; AC, 78° ± 16° at 0.05 Hz) frequency extremes. Comparison of responses to horizontal and vertical axis pitch rotations at 0.05 Hz revealed modest phase changes for some neurons (-13° to +27°) while others had large phase shifts (+91° to +98°) indicating otolith input. Supported by EY07342, DC01559.

487.10

PVP CELLS RECORDED DURING VERTICAL VISUAL-VESTIBULAR INTERACTION, IN THE ALERT SQUIRREL MONKEY J.D. Dimarogonas*, Y.Zhang, and S.M. Highstein, Dept. of Otolaryngology and Program in Neurosciences, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Extra cellular records were taken from the region of the medial (MVN) and ventro-lateral (VLVN) vestibular nuclei in the alert squirrel monkey. Responses were recorded during (i) visual following (of a full-field optokinetic drum), (ii) VOR in the dark, (iii) VOR in the light, and (iv) VOR suppression. Two types of neurons were identified, namely those that had up-eye position and down head velocity sensitivity, and those that had down eye position and up head velocity sensitivity. Both types paused for saccades and are thus classified as classic position-vestibular pause (PVP) neurons, previously reported. We conclude that some MVN & VLVN PVP cells receive input from the anterior canal (AC) and others from the posterior canal (PC). Therefore it appears that there are two components of the 3 neuron arc from the AC to the third nucleus: 1 via the superior vestibular nucleus that conveys head and eye velocity information and a second via the MVN & VLVN conveying PVP type of information to the IIIrd nucleus. The extent of flocculus control of each of these two pathways is under investigation.

487.11

CONNECTIONS BETWEEN THE SACCULAR NERVE AND NECK EXTENSOR AND FLEXOR MOTONEURONS IN THE DECEREBRATE CAT. H. Sato[2]*, M. Imagawa[1], M. Sasaki[1], H. Ikegami[1] and Y. Uchino[1]. [1]Dept. of Physiol., Tokyo Medical College, Tokyo 160; [2]Dept. of Anat., Nippon Medical School, Tokyo 113.

The saccular (SAC) receptors in the otolith organs are sensitive to vertical linear acceleration of the head. We studied the sacculo-neck connectivities by means of intracellular recording from the extensor and flexor motoneurons in C_2-C_3 segments. Bipolar tungsten electrodes (inter-electrode distance, 0.8 mm) were fixed in place on the SAC nerve; the other branches of the vestibular nerve were transected. Dorsal rami (DR; extensor) and longus capitis (LC; flexor) motoneurons in C_2-C_3 were identified antidromically. Stimulation of the SAC nerve evoked excitatory postsynaptic potentials (EPSPs) in bilateral DR motoneurons. The latencies of EPSPs in ipsi- and contralateral motoneurons ranged from 1.8 to 2.6 ms (2.2 ± 0.2 ms; mean ± SD, n=26) and from 1.8 to 4.0 ms (2.9 ± 0.7 ms; mean ± SD, n=17), respectively. The SAC nerve stimulation evoked inhibitory postsynaptic potentials (IPSPs) in almost all bilateral LC motoneurons. The latencies of IPSPs ranged from 2.1 to 3.6 ms (2.5 ± 0.4 ms; mean ± SD, n=18) ipsilaterally and from 2.7 to 4.0 ms (3.1 ± 0.4 ms; mean ± SD, n=13) contralaterally. The results demonstrate the presence of reciprocal connections from SAC to extensor and flexor muscles which may play a role in fixing the head and neck to the body under resting condition and during vertical linear acceleration.

487.12

VESTIBULAR INPUTS TO THE DORSAL Y GROUP NUCLEUS, IN THE SQUIRREL MONKEY P.M. Blazquez, A.M. Partsalis and S.M. Highstein* Dept. of Otolaryngology, Washington University, St. Louis, MO 63110.

Dorsal Y group (Y) neurons in the squirrel monkey carry visual following and vestibular signals, and the nucleus has been implicated in adaptation of the vertical VOR (Partsalis et al 1993). Y neurons are monosynaptically inhibited by the flocculus; pharmacological inactivation of flocculus removes the visual following signal but spares a head velocity signal on Y cells. To examine the origin of this signal, we studied responses of Y cells to electrical stimulation of the labyrinths in alert squirrel monkeys.

Stimulating electrodes were implanted in the labyrinth bilaterally in two animals. Y cells were recorded extracellularly in the alert animal and identified by their characteristic response patterns during visual following and visual-vestibular interaction paradigms, as previously described (Partsalis et al 1993). The responses of cells to electrical pulse stimulation of the labyrinths were examined. Peristimulus time histograms revealed that the vast majority of Y cells were activated at disynaptic latencies following stimulation of either (both) labyrinths.

Taken together with previous evidence, our results suggest that the Y group, an immediate premotoneuronal center, is a site of interaction between vestibular signals from both labyrinths and flocculus output signals. The interneuron between the primary afferent and the Y group probably lies in the vestibular nucleus. Preliminary anatomical data indicate that the Y group receives a projection from the superior vestibular nucleus.

487.13

LINEAR AND ANGULAR MOTION MAPPED INTO THE VESTIBULAR NUCLEI. J.E. Holly*, R. Boyle, and G. McCollum, R.S. Dow Neurological Sciences Institute, Portland, OR 97209 and Oregon Health Sciences Univ., Portland, OR 97201.

Cells in the vestibular nuclei discharge in relation to motion factors including angular head velocity, linear head velocity or acceleration, the position and/or velocity of the eyes in orbit during ocular tracking and gaze holding, and events associated with rapid eye movements. Each responding cell discharges either in relation to a single factor or to a certain combination of two or more factors. The number of possible combinations of converging factors grows exponentially with the number of factors considered. The ensemble response across the vestibular nuclei during head and/or eye movement reflects the multitude of combinations of convergence.

We have developed a formal framework that identifies patterns of convergence within the vestibular nuclei and relates these patterns to ensemble response during movement. Each movement of the head maps formally to a certain status of the sensors, one relevant set of sensors being the hairs of the semicircular canals and otolith organs. Deflection of the vestibular hairs evokes a range of primary afferent responses, reflected in the afferents' sensitivity to velocity and/or acceleration. Convergence within the vestibular nuclei determines the ensemble response of secondary vestibular neurons. In the framework, we relate the identified patterns of convergence with the full map from head movement to cell discharge in the vestibular nuclei, thus identifying patterns available to the nervous system for responses such as the vestibuloocular reflex, the vestibulocollic reflex, and self-motion perception.

487.14

A COMPARISON OF THE RESPONSES OF RAT AFFERENT VESTIBULAR NEURONS AND VESTIBULAR NUCLEI NERVE CELLS EVOKED BY SINEWAVE POLARIZATION OF THE LABYRINTH. O.-J. Grüsser* and J. Kleine, Department of Physiology, Freie Universität, 14195 Berlin, Germany

Sinewave galvanic stimulation (0.1-100 Hz, 2-200 uA amplitude) was applied in pentobarbital-anaesthetized pigmented Norwegian rats. The responses of *Ggl. Scarpae* nerve cells were uniform and independent of their peripheral origin (semicircular canals-SC, otoliths-O). Within the vestibular nuclei complex (*VNC*) there was differentiation:

(a) Negative ipsilateral labyrinth polarization led to an activation of units driven by one or two SC-inputs or by SC- and O-inputs.

(b) Some VNC neurons were activated by contralateral negative labyrinth polarization.

(c) Some neurons responded with a slow delayed activation to ipsilateral, some to contralateral negative polarization.

The neurons of category (a) exhibited similar response characteristics as Ggl. Scarpae neurons and followed up to a frequency range between 60 and 100 Hz. Owing to the lower spontaneous discharge rates of VNC neurons, however, non-linear response components that consisted of a "silencing" during the positive phase of ipsilateral labyrinth polarization tended to emerge at lower stimulus frequencies. The lowest thresholds at 1-2 Hz galvanization did not differ significantly from those of the afferent fibers (~5 uA). The convergence of many fairly uniformly responding afferent vestibular fibers seems to determine the response characteristics of this subgroup of VNC cells. Sinewave galvanic stimulation of the labyrinth is a powerful tool to synchronize vestibular input. (Supported in part by a BMFT-grant).

488.1

OFFSET OF HUMAN SMOOTH PURSUIT: EFFECTS OF TARGET VELOCITY, POSITION, AND PRESENCE. J. Pola* and H. J. Wyatt. SUNY College of Optometry, New York, NY 10010.

We have been studying the offset response of the pursuit system as part of an effort to determine some of the system's dynamic features. **Methods.** Subjects observed a target moving horizontally at 15 deg/sec. When pursuit velocity became steady, the target either: (1) suddenly disappeared; (2) jumped to the fovea with target velocity and feedback becoming 0 (target stabilized at the fovea); or (3) stepped to a retinal location 3 deg ahead of the fovea with target velocity of 0 and feedback of either 0 (target stabilized 3 deg from fovea), -0.2, -0.4, or -1 (target fixed in space). **Results.** When the target disappeared, the eye decelerated rapidly (time constant T ≈ 0.1s), but with the target stabilized at the fovea, deceleration was slower (T ≈ 0.6s). With the target stabilized 3 deg ahead of the fovea, the eye continued at moderate velocity, a response to target position (since target retinal velocity was zero). As feedback increased from 0 to -1.0, eye deceleration systematically increased. **Modelling and Conclusions.** All of the experimental results can be simulated by a model with target velocity and position inputs, and an internal positive feedback loop. A key feature of the model is that the positive feedback loop is enabled by the presence of a visual target. Thus, long-time-constant offset reflects the action of the positive feedback loop, whereas short-time-constant offset is a result of a loss of positive feedback following target disappearance. The model also suggests that the systematic change in deceleration with increasing feedback depends on the effects of a pursuit position mechanism — the same mechanism that drives the eye when the target is stabilized 3 deg ahead of the fovea.

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488.2

ATTENTION AND TARGET SELECTION IN SMOOTH PURSUIT. Vincent P. Ferrera* and Stephen G. Lisberger, UCSF Dept. of Physiology and Keck Center for Integrative Neuroscience, San Francisco, CA 94143

The selection of appropriate sensory inputs to guide movement is a major issue in sensory-motor integration. We have developed a behavioral paradigm for studying the representations and cognitive decisions involved in input selection for smooth pursuit eye movements. When multiple inputs (targets) are present, selection may be achieved by shifting attention to a particular feature or spatial location. We investigated the role of attention in initiating smooth pursuit in monkeys trained to track moving targets presented on a CRT display. In the first experiment, we adapted a familiar attention paradigm (Posner) by preceding the target with a valid cue indicating the location or direction of the target, or an invalid cue indicating a different location or direction. A single target then appeared randomly in one of four positions around a central fixation mark and moved either towards or away from the fovea. When the target was preceded by a location or direction cue there was no difference in performance (pursuit latency or initial eye acceleration) for valid vs. invalid cues. In contrast, saccade latencies may be up to 50 ms shorter with valid location cues as compared to invalid cues. In the second experiment, two targets (red and green, equated for luminance) were presented and the monkey was instructed which one to track by the color of the fixation mark. If the two targets moved in the same direction, pursuit latencies were as short or shorter than latencies for single targets. If they moved in opposite directions, monkeys were able to choose the correct target >90% of the time, but latencies were 20-40 ms longer than for single targets. Latencies were reduced only slightly (~5 ms) if a cue was given that anticipated the position of the correct target. These observations are consistent with the hypothesis that pursuit initiation uses a velocity-space representation that preserves motion, but not position information. *Supported by the McDonnell-Pew Program in Cognitive Neuroscience Fellowship JSMF 92-38.*

488.3

Craniotopic smooth pursuit deficits in humans. MJ Morrow*, Olive View-UCLA Department of Neurology, Sylmar, CA 91342

Neural circuits arranged in a head-centered (craniotopic) framework influence smooth pursuit eye movements. Craniotopic ocular motor organization is demonstrated in patients with acute cerebral hemispheric damage causing ipsilateral gaze deviation. These patients typically cannot generate pursuit movements across the orbital midline, away from the side of the lesion. Smooth pursuit was recorded in 5 patients with acute frontal or parietal lobe infarction. By comparing responses to target motion within the right and left gaze fields, craniotopic pursuit deficits were quantified. Responses were also compared in rightward and leftward directions to identify directional pursuit asymmetry.

One patient had left hemispheric infarction with right spatial neglect and left gaze deviation. He could not make any eye movements across the orbital midline to the right, but smooth pursuit had lower velocities to the *left* within the left gaze field. Three patients without gaze deviation or limited ocular motor range generated worse smooth pursuit in the gaze field contralateral to cerebral damage; each of these patients had lower velocities of pursuit for targets moving in the ipsilateral direction. Craniotopic pursuit defects are distinct from pursuit impairment based on the direction or retinal locus of motion. These defects comprise a spectrum in which the most severe examples are associated with complete inability to generate smooth pursuit in the contralateral field of gaze. The craniotopic pattern of impairment implies that orbital position is taken into account by cerebral mechanisms that govern smooth pursuit in humans.

488.4

ADAPTATION OF OPEN-LOOP SMOOTH PURSUIT RESPONSES IN THE RHESUS MONKEY. Maninder Kahlon and Stephen G. Lisberger*, Dept. of Physiology and Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

During the initiation of smooth pursuit eye movements, there is a clear relationship between eye acceleration in the first 100 ms (the open-loop period) and target velocity. We have developed an adaptation paradigm for changing the eye acceleration response to a particular velocity. Adapting trials required an animal to track a target that began to move at an adapting velocity, but accelerated or decelerated, after 100 ms, to a higher or lower velocity. Thirty minutes of adaptation caused as much as two-fold changes in the eye acceleration evoked either by the adapting stimulus or by test stimuli consisting of prolonged target motion at the adapting velocity. The largest change in eye acceleration occurred in the interval 50-80 ms after the onset of pursuit, with less change occurring in the initial 0-30 ms interval. The adaptation did not transfer to the opposite direction, but did transfer in an unexpected way to other target velocities. With a stimulus that started at 10 deg/sec and accelerated to 30 deg/sec, the greatest changes in eye acceleration responses occurred at the adapting velocity. However, with a stimulus that started at 25 deg/sec and decelerated to 5 deg/sec, the greatest changes occurred at test velocities lower than the adapting velocity.

This paradigm will allow us to study neuronal changes that underlie adaptation of a relatively well-defined voluntary behavior that requires the cerebral cortex. *Supported by NIH grant EY03878.*

488.5

TWO DIMENSIONAL SMOOTH PURSUIT EYE MOVEMENTS DURING TRACKING OF DOUBLE SINE WAVES IN MONKEY RE Kettner*, H-C Leung, ME Giswold & BW Peterson. Dept of Physiology, Northwestern University Medical School, Chicago, IL 60611

We examined smooth pursuit in the rhesus monkey along simple 2D trajectories by moving a back-projected laser spot sinusoidally along horizontal and vertical axes. Component waveforms had a frequency ratio of 2:3 and were equated for velocity. Single-sine and sum-of-two-sine tracking along the single axes were studied at the same frequencies. A computer rewarded correct eye position via a scleral search coil. Gain and phase were computed by comparing sine regression fits to saccade-edited velocity components.

Above about 0.6 Hz, the gain of all components decreased with increasing frequency. Phase differences were always less than ±20° indicating good predictive pursuit. There were several consistent trends in the interactions between components on both the same axis and on opposite axes. (1) The addition of a higher frequency component reduced the gain of a component presented alone. (2) Reductions were greater for components added to the same axis versus the opposite axis. (3) Horizontal gains were larger than vertical gains for comparable stimuli. (4) For combination stimuli, the high frequency component had a higher gain and a phase lag, while the low frequency component had a lower gain and a phase lead. Analogous results were obtained for stimuli generated from three sinusoids presented along the same and different axes. These findings indicate nonlinear interactions between components both within and across axes, and suggest distinct roles for high and low frequency components during pursuit. Others have reported similar data for 1D pursuit in humans. We also studied correlations between kinematic variables and tracking performance. Corrective saccades were most commonly clustered around points of peak velocity. Pursuit gain was correlated with measures of peak velocity, acceleration and jerk. This explains why pursuit was better for cross-axis combinations that appeared subjectively smoother than the same components acting along the same axis. (Supported by grant MH 48185)

488.6

TWO DIMENSIONAL SMOOTH PURSUIT EYE MOVEMENTS DURING CIRCULAR TRACKING AND PERTURBATIONS IN MONKEY H-C Leung*, RE Kettner, ME Giswold & BW Peterson. Dept. Physiology, Northwestern Univ. Med. School, Chicago, IL 60611

We studied smooth pursuit along three trajectories: circles, circles with occasional perturbations along horizontal or vertical midlines, and horizontal and vertical sinusoids. Reliable behavior developed after repeated sessions with reward delivered contingent upon correct fixation. Horizontal and vertical eye and target velocity curves were estimated using a least square error fit after removing all the saccades. Eye velocity gain was defined as eye velocity divided by target velocity.

Eye velocity gain and phase response to a 5° circular trajectory with a frequency range of 0.2 to 1.8 Hz was examined. Gain and phase curves of horizontal and vertical components initially showed unity gain and zero phase below 0.6 Hz, followed by decreasing gain and increasing phase lag at higher frequencies. Comparison analyses of horizontal and vertical sinusoidal pursuit showed similar trends. In both cases, corrective saccades were preferentially clustered near high vertical and horizontal velocity points, especially for the sinusoids. Because a number of kinematic variables including peak velocity and acceleration vary systematically during these tracking behaviors, this result suggests that there are preferred parameters controlling saccade initiation during pursuit. Collewijn and Tamminga (1984) have obtained similar gain and phase results in humans.

Horizontal and vertical perturbations produced consistent corrective saccades after a delay of 120-180 ms. After perturbations and before corrective saccades, pursuit followed the curved trajectory of the non-perturbed waveform. This continuation of pursuit is caused by the prediction of the circular waveform instead of the maintenance of velocity in a single direction. Tracking along the perturbed axis showed attenuation in amplitude at all frequencies. However, pursuit along the non-perturbed axis only showed attenuation at higher frequencies. This indicates cross-axis interactions under these conditions. (Supported by grant MH48185).

488.7

VISUAL TRACKING OF ILLUSORY MOTION IS AFFECTED BY TARGET WAVEFORM. A.Z. Zivotofsky, L. Averbuch-Heller, C.W. Thomas, V.E. Das, A.O. DiScenna, and R.J. Leigh* Ocular Motor Lab., VA Medical Center and Case Western Reserve University, Cleveland OH 44106

We studied ocular motor responses to a variant of the Duncker illusion, in which the perception of motion of an object is induced by moving its frame of reference. Using the magnetic search coil technique, we measured head-fixed and head-free tracking responses of 10 normal subjects to vertical movement of a target (laser spot). Movement of the target was synchronized to horizontal movement of a background Amsler grid subtending 20 X 20 deg, producing a strong illusion of diagonal motion, the horizontal component being opposite to the direction of the grid. Both visual stimuli moved ± 9.2 deg at 0.35 Hz, either sinusoidally or in square-waves. In response to sinusoidal target movement, gaze always followed the target vertically, with a small horizontal component (mean gain 0.05) that was phase lagged compared with grid movement by a mean of 37 deg. When the head was free to move, it usually (85%) showed a diagonal trajectory with a horizontal component (mean gain 0.32) that was phase lagged compared with grid movement by a mean of 156 deg. In response to stepping target movement, inappropriate horizontal saccades were made for 71% of steps. When these saccades were anticipatory (58%), they were always (100%) in the direction of the illusion; when not (latency >90 msec), 49% of saccades were still in the direction of the illusion. The head tracking movements in response to stepping targets were also (100%) in the direction of the illusion. Thus, ocular tracking was programmed differently for sinusoidal and stepping target waveforms, the former response being to actual target motion, the latter to illusory target motion. Head tracking, however, was always of illusory movement of the target, irrespective of its waveform.

488.8

SPATIOTEMPORAL VOR ADAPTATION AFTER SEMICIRCULAR CANAL INACTIVATION. D. E. Angelaki*, B. J. M. Hess and J.-I. Suzuki, Dept. of Surgery (Otolaryn.), Univ. of Mississippi, Jackson MS 39216; Dept. of Neurol., Univ. of Zurich, Switzerland; Dept. of Otolaryn., Teikyo Univ, Tokyo Japan.

Selective semicircular canal inactivation by plugging and three-dimensional eye movement recordings were used in rhesus monkeys to address the following questions: 1) What is the 3d structure of the VOR matrix which relates semicircular canal inputs to oculomotor output? 2) How is the VOR matrix modified during recovery from inactivation of either both lateral or a coplanar pair of vertical canals? To address these questions, horizontal, vertical and torsional eye movements were recorded during sinusoidal oscillations at different frequencies (0.01-1.1 Hz) and head orientations. The VOR matrix was computed based on data acquired before and acutely after canal plugging. Similarly, the evolution of the VOR matrix elements during recovery was computed from data acquired two weeks, as well as one, three and eight months after canal plugging.

The VOR matrix elements were frequency dependent. At mid and high stimulus frequencies, inputs from the lateral and vertical canals contribute to torsional and horizontal eye movements. At low frequencies, however, the VOR matrix was diagonal. After semicircular canal inactivation, the VOR matrix in the mid and high frequency range changed continuously while gain and phase in the plugged canal plane progressively recovered. No recovery was ever observed at frequencies below approximately 0.05-0.1 Hz. These results suggest a frequency-selective central reorganization of vestibulo-ocular connectivity after selective semicircular canal inactivation. In addition, the data strongly suggest that the spatio-temporal organization of the VOR in the mid and high frequency range differs distinctly from that in the low frequency range. This observation raises the possibility that the "velocity storage mechanism" is not simply a parallel loop in the VOR circuitry but that it might represent a distinct entity with different organization and function.

488.9

OTOLITH DETECTION OF ANGULAR VELOCITY IN THE VOR. B. J. M. Hess*, D. E. Angelaki and J.-I. Suzuki, Dept. of Neurology, Univ. of Zurich, Switzerland; Dept. of Surgery (Otolaryn.), Univ. of Mississippi, Jackson MS; Dept. of Otolaryn., Teikyo Univ, Tokyo Japan.

It has been shown previously that in the presence of simultaneous dynamic otolith input the vestibulo-ocular reflex (VOR) represents more faithfully head angular velocity in the low frequency range (Rude and Baker 1988; Tomko et al. 1988). These observations have raised two yet unresolved questions. First, do these results reflect the sensitivity of the otolith system to head position or to centrally computed otolith-born angular velocity? Second, what is the functional significance of such a property? We have addressed these questions by examining the horizontal, vertical and torsional VOR in rhesus monkeys before and after plugging of either the lateral or one pair of coplanar vertical canals, using sinusoidal low frequency oscillations (0.1-0.01 Hz, ±58 °/s) and constant velocity off-vertical axis rotations (±58°/s).

Our results in the intact animals indicate that the low frequency VOR enhancement during simultaneous dynamic otolith stimulation reflects the sensitivity of the otolith system to head angular velocity. A surprising observation was made in canal plugged animals: Acutely after canal plugging, the otolith contribution to the VOR was unchanged, however, it progressively decreased thereafter. Two months after the surgery, the low frequency and steady-state responses to sinusoidal and constant velocity off-vertical axis rotations were completely abolished in the plane of the plugged canals. Our findings in canal plugged animals do not support the current belief that the main function of canal/otolith interactions through the velocity storage mechanism is to improve the low frequency VOR dynamics. Rather the low frequency dynamic otolith contribution to the VOR could reflect the inertial representation of vestibular angular motion signals in the velocity storage network (Angelaki and Hess 1994).

488.10

DIFFERENTIAL EFFECTS ON COMPENSATORY GAZE OF INTERAURAL (IA) LINEAR ACCELERATION IN MONKEY AND MAN S. Wearne, T.Raphan, B.Cohen*. Brooklyn College of CUNY, Mt. Sinai School Medicine

Contributions of linear and angular vestibulo-ocular reflexes (LVOR & AVOR) to gaze stabilization were determined in macaque monkeys during centrifugation, off-vertical axis rotation (OVAR) and optokinetic after-nystagmus (OKAN). Animals were centrifuged either facing or back to motion with velocity trapezoids (10-40°/s^2 to 400°/s) producing peak IA linear accelerations of 1.24g. Peak horizontal eye velocity ($\dot{E}_H$) was invariant for the two orientations and beating fields remained close to the midline. Horizontal time constants (Tc) fell faster with GIA tilt when facing than back to motion (.13s/°vs.07s/°). This corresponded to Tc asymmetries during yaw axis OKAN for head tilts associated with upward or downward cross-coupling. Dynamic parameter modification as a function of tilt was incorporated into our velocity storage model, using the OKAN Tc's from each monkey. These simulations accounted for all asymmetries in $\dot{E}_H$ during centrifugation. Despite this, these monkeys had a direct horizontal translational LVOR during OVAR at velocities of 5°/s to 360°/s (≡.01-1Hz). Plugging the 6 semicircular canals moreover abolished $\dot{E}_H$ during eccentric rotation, leaving ocular torsion and the response to OVAR intact. Thus, in macaque monkeys, the translational LVOR does not contribute significantly during sustained linear acceleration. In contrast, humans had large facing/back beating field asymmetries and differences in peak $\dot{E}_H$ during centrifugation with similar GIA magnitudes (Wearne, 1993). A direct contribution from the translational LVOR and consequent shift in the beating field was necessary to model the human response. Thus, LVORs both modify the Tc's of velocity storage, reorienting AVOR coordinates, and generate direct compensatory responses to translation. Differentially weighting these two mechanisms accounts for different effects of linear acceleration on compensatory gaze in man and monkey during centrifugation. SUPPORT: EY04148, EY01867, NS00294, PSC-CUNY 664230.

488.11

TEMPORAL CONSTRAINTS ON THE MECHANISM OF LEARNING IN THE VOR. Jennifer L. Raymond* and Stephen G. Lisberger, UCSF Dept. of Physiology and Keck Center for Integrative Neuroscience, San Francisco, CA 94143.

Changes in the amplitude and dynamics of the vestibulo-ocular reflex (VOR) can be induced by the association of vestibular and visual (image motion) stimuli. We examined the dependence of these learned changes in the VOR on the temporal properties of the sensory signals used to induce learning. First, rhesus monkeys were adapted for 3 hours with sinusoidal vestibular and visual stimuli at a single frequency from 0.5 to 10 Hz (±10°/s). The stimuli for adaptation were provided by passive head turns either with magnifying or miniaturizing spectacles or with optokinetic stimuli that mimicked the effects of magnifying or miniaturizing spectacles. Adaptation with stimuli at frequencies up to 5 Hz induced large, adaptive changes in the VOR. Adaptation with frequencies of 8 or 10 Hz resulted in smaller and less consistent changes in the VOR. A second set of experiments examined the changes in the VOR induced by short (300-ms) pulses of vestibular and optokinetic stimuli, presented either simultaneously or at some constant relative delay during a 3-hour adaptation period. When the visual and vestibular stimuli were presented simultaneously during adaptation, large adaptive changes in the amplitude of the VOR occurred. The adaptive changes were not specific to the vestibular stimulus used during adaptation, but generalized to vestibular stimuli of different amplitudes and durations and to sinusoidal vestibular stimuli. When the onset of the visual stimulus was delayed relative to the vestibular stimulus during adaptation, striking changes in the dynamics as well as the amplitude of the VOR occurred. When the onset of the visual stimulus preceded the vestibular stimulus during adaptation, the changes in the VOR were small relative to the case of simultaneous onset or delayed visual stimulus onset. These temporal constraints on the stimuli that induce learning can guide efforts to identify the neural mechanism(s) of plasticity in VOR pathways. *Supported by the NASA Space Biology Research Associate Program and NIH grant EY10198.*

488.12

MODELLING THREE DIMENSIONAL VELOCITY-POSITION INTEGRATION IN THE VESTIBULO-OCULAR REFLEX (VOR) C. Schnabolk* and T.Raphan, Institute of Neural & Intelligent Systems, Dept. of CIS, Brooklyn College of CUNY, 2900 Bedford Ave, Brooklyn New York, 11210

A recent model of velocity-position transformation has focused on the torque-orientation properties of the eye, and the dynamical system representing the eye dynamics (Schnabolk & Raphan, 1994). The final common velocity-position integrator is represented as a dynamical system with a matrix feedback which is an extension of the one dimensional integrator. Because of the nonlinear dynamics of the torque-orientation equations associated with the plant, it was of interest to utilize this three dimensional model to examine the predicted behavior of the canal mediated rotational VOR. When the eye was initially in the primary position, changes in head orientation about an axis in Listing's plane induced both eye velocity and position compensation which was maintained along the axis of rotation. If the head was first moved about a yaw axis, held for a second, and then rotated about a pitch axis, eye velocity compensated accurately. Eye orientation compensated accurately for this sequence for 1-2 seconds following the second movement. If the head was first pitched and then rotated about a yaw axis, eye velocity and orientation behaved similarly. In both instances, the eye slowly rotated to an orientation aligned with the torque axis of the velocity-position integrator which was in Listing's plane. Additional compensatory components generated from the otoliths would enhance the compensation over time. Thus, the model is consistent with the compensatory function of the VOR. It generates appropriate roll components for 1-2 seconds despite the fact that the torque is in Listing's plane and has no roll component. It supports the idea that velocity-position integration in three dimensions is an additive feedback system which is a three dimensional extension of the one dimensional integrator model.
Supported by: EY04148, PSC-CUNY Award 664230, 662480.

488.13

MODULATION AND BIAS OF THE VESTIBULO-OCULAR REFLEX (VOR) DURING LOW VELOCITY HORIZONTAL AXIS PITCH ROTATIONS. S.A. Rude* and J.F. Baker. Northwestern University, Chicago, IL 60611.

We used electromagnetic search coil techniques in darkness to assess at equilibrium the sinusoidally varying eye velocity (modulation) and average eye velocity (bias) of the VOR in cats during ±2-32°/s constant velocity horizontal axis pitch rotations.

Modulation of vertical VOR eye velocity increased with head velocity, and was greater at all velocities for backward (nose up) than forward (nose down) rotations. Minimum modulation responses shifted toward forward rotations, occurring at +4°/s forward pitch. Slow phase eye velocity bias increased nonlinearly with head velocity and was greater for forward than backward rotations at all velocities. Minimum average eye velocity shifted toward backward rotations, occurring at -2°/s (backward).

We propose that three mechanisms are responsible for this behavior. (1) A central velocity estimate, generated from otolith signals as proposed by others, which produces an eye velocity bias that increases with head velocity in the range tested. (2) An afferent or central "jerk" signal, generated from the rate of change of an anterior-posterior otolith stimulus, which produces modulation of eye velocity increasing with head velocity. (3) A gravity-dependent downbeat nystagmus (GDDN), as we reported in cats (NS '91), generated by a saccular response to tilt, summing with the central velocity estimate to affect bias while also summing with the jerk signal to affect modulation of vertical eye velocity. The static position GDDN is near zero in the upright position and peaks with an upward slow phase at 180° tilt. During rotation the upward slow phase velocity of GDDN adds to the central velocity estimate, subtracting during backward rotation when the velocity bias is downward. Analogously the peak upward slow phase velocity of the GDDN signal at 180° tilt subtracts from the peak modulation signal only during forward rotations when, at 180° tilt, the eye velocity is downward. Thus the GDDN signal is of the correct direction and magnitude to account for the observed shifts in minimum slow phase eye velocity modulation and bias at low rotation velocities. Supported by EY07342.

488.14

CEREBELLAR β-NORADRENERGIC RECEPTOR ANTAGONISM BY PROPRANOLOL INHIBITS THE VESTIBULO-OCULAR REFLEX ADAPTATION IN GOLDFISH. L.Williams* and J. G. McElligott. Dept. of Pharmacology, Temple University School of Medicine, Phila., PA 19140.

Adaptation of the vestibulo-ocular reflex (VOR) in cats has been shown to be inhibited after norepinephrine (NE) depletion by the neurotoxin 6-hydroxydopamine (6-OHDA) within the central nervous system. Cerebellar NE blockade in rabbits by sotalol (a β-adrenergic antagonist) has also been shown to inhibit an adaptive VOR gain increase. In this study cerebellar β-adrenergic antagonism in the goldfish using propranolol was investigated to confirm and extend these findings. After the initial calibration of the VOR in light and dark, goldfish received a bilateral vestibulo-cerebellar injection (0.25 ml/side @ 0.025 ml/min) of either artificial cerebral spinal fluid or propranolol. VOR measurements were taken after the injections to assure that the pre-adapted VOR was unchanged. VOR gain modification began approximately 30 minutes after the injection and continued for 3 hours. Training towards a gain increase (or decrease) was accomplished by presenting visual stimuli 180 degrees out of phase (or in phase) with the vestibular table rotating about the vertical axis (1/8 Hz @ +/-20 degrees). Our results showed a dose-dependent (14, 108 and 270 mM) reduction of adaptive VOR gain increase in the light and dark without any effect on pre-adapted VOR gain. For VOR gain adaptation towards 0X, propranolol exhibited no effect. Vestibulo-cerebellar injection of propranolol after the completion of 3 hours of VOR adaptive increase showed an initial rapid decay of the VOR adaptation toward pre-adapted levels compared to controls. The present study agrees with previous studies conducted in the cat and rabbit implicating goldfish cerebellar β noradrenergic receptors in VOR adaptation and post-adaptational processes. (Supported by a grant from NIDCD-NIH # DC 01094)

488.15

STATIC AND DYNAMIC EFFECTS OF GRAVITO-INERTIAL ACCELERATION (GIA) ON SPATIAL ORIENTATION OF VELOCITY STORAGE T.Raphan*, S.Wearne, B. Cohen. Brooklyn College of CUNY, Mt. Sinai School of Medicine

We compared axis orientation during off-axis rotation and dynamic head tilt during post-rotatory nystagmus (PRN), i.e., tilt dumping, to that during OKAN. We tested reorientation from pitch to roll/yaw and roll to pitch/yaw. Absence of the latter was a key aspect in model-based studies of OKAN axis reorientation (Raphan & Sturm, 1991). Monkeys were rotated off-axis at 10°/s² & 40°/s² to velocities of 200-400°/s, tilting the GIA vector from 17°-52°. Orientation vectors were computed using a model-based approach. During acceleration, the GIA vector had pitch and roll components and both pitch and roll cross-coupled eye velocities were generated. At constant velocity, the GIA pitch component remained, inducing continued pitch eye velocity that decayed with a 5-8s Tc. This was close to the yaw Tc. The roll component of the GIA dropped rapidly and roll velocity decayed with a short time constant (Tc ≈ 1 s). Off-axis rotations of monkeys such that the GIA vector was solely in the yaw plane elicited no pitch-roll or roll-pitch axis reorientation. Yaw tilt dumping during pitch/roll PRN with peak tilt velocities < 60°/s elicited no significant horizontal velocity and no pitch-roll or roll-pitch cross-coupling. Thus, the reported 'pitch-yaw' and 'pitch-roll' cross-coupling (Angelaki & Hess 1994) is likely due to high velocity pulses along the yaw axis that charged the roll state with a small time constant which then discharged with a longer time constant when the animal was supine/prone. It is not due to additional off-diagonal terms in the system matrix. The reorientation of eye velocity trajectories was the same during dynamic head movements and centrifugation as during OKAN (≈ 10° from the spatial vertical), and was predicted by dynamic parameter changes in the system matrix of velocity storage. We conclude that the yaw axis vector codes the spatial orientation of velocity storage during vestibular activation as during OKAN. Only the yaw axis reorients toward the spatial vertical when the GIA vector is shifted; the pitch and roll axes move with the head. EY04148, NS00294, EY01867, CUNY-PSC 664230

OCULOMOTOR: CLINICAL STUDIES

489.1

CHANGES IN OCULOMOTOR CONTROL WITH AGE IN HUMANS. H. Cohen*, L. Heaton, S.L. Congdon, H.A. Jenkins. Dept. of Otorhinolaryngology, Baylor College of Medicine, Houston, TX 77030.

We examined oculomotor responses in elderly people and compared them to responses in younger adults. Twenty-two asymptomatic, community-dwelling subjects aged 70-79 and 11 subjects aged 80-89 were tested on a standard battery of sinusoidal vestibular, optokinetic, saccadic and pursuit eye movements, in a servo-controlled rotatory chair, while eye movements were recorded with electrooculography. Their data were compared to those from 137 subjects aged 18 to 44 and 44 subjects aged 45 to 69 years.

As reported previously, vestibuloocular reflex (VOR) gain and phase in darkness changed significantly with age. Also, the gain of optokinetic responses was significantly higher in the younger subjects, and they had shorter phase lags than elderly subjects. On VOR tests in light, young and middle-aged subjects had higher gains than elderly subjects, and shorter phase lags, but younger subjects had only minimal differences in gain from older subjects on VOR fixation/ suppression in light. Subjects in their 70s had less variability than subjects in their 80s in response times and accuracy in making saccades to randomized stimuli. Young adults had significantly higher pursuit gains and shorter phase lags than other subjects.

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489.2

REDUCTION OF VOR TIME CONSTANT WITH MAINTAINED VOR GAIN IN A GROUP OF ELDERLY SUBJECTS. M. Dai*, B. Cohen, T. Raphan. Mount Sinai School of Medicine, Booklyn College of CUNY

Some elderly patients have short bilateral vestibulo-ocular reflex (VOR) time constants (Tc) but normal or high VOR gains. They generally complain of imbalance rather than vertigo. The anatomic basis for these findings is unknown. We studied 14 subjects, ages 75-93, with short VOR Tc and with no history of ototoxicity. VOR gains, determined from the initial jump of slow phase velocity elicited with velocity steps (60°/s, 200°/s²), ranged from 0.5-1.0 (mean 0.74±0.15). VOR Tc ranged from 3-5 s (mean 4.2±0.6 s), compared to Tc of ≈ 15-25 s in normals. The gains of optokinetic nystagmus (OKN) induced by full field motion were normal (gain 0.9 for 20 & 40°/s), and there was no optokinetic after-nystagmus (OKAN). Subjects were rotated in a lighted earth-fixed surround for 15 sec and stopped in darkness. The post-rotatory VOR gain was the same as after they had been rotated in darkness, showing that velocity storage was not activated during OKN. This is in contrast to the normal reduction of post-rotatory eye velocity by 40-100% after rotation in light. The short VOR Tc and the absence of OKAN as well as the inability to reduce post-rotatory eye velocity could be modelled by a reduced canal Tc and a deactivated velocity storage integrator. Aging is associated with degeneration of hair cells and a loss of fibers in the vestibular nerve. The selective impairment of the VOR Tc with preservation of gain may be secondary to selective hair cell or nerve fiber loss. The imbalance noted by these elderly people may be related to inactivation of velocity storage, which is important for coding spatial orientation and stabilizing gaze and posture during movement. Support: NS00294 DC01705 EY04148 EY201867

489.3

HIGH FREQUENCY, PITCH AND YAW VESTIBULO-OCULAR REFLEX (VOR) IN VERY ELDERLY PEOPLE. J. L. [1,2]Demer* and Robert W. [2]Baloh. [1]Jules Stein Eye Institute & [2]Department of Neurology, University of California, Los Angeles, 90024.

Advanced age is known to result in a shortening of the time constant of the yaw VOR at high velocities, resulting in reduced low frequency gain and increased phase lead. Since these conditions are not representative of physiologic head rotations during everyday life, we investigated the VOR of very elderly subjects during high frequency rotations in both pitch and yaw.

Eye movements were recorded using magnetic search coils for pitch and electro-oculography for yaw in groups ($N \geq 9$) of young subjects (mean age 30 yrs) and very elderly subjects (mean age 80 yrs). Subjects underwent passive, sinusoidal whole-body rotations (peak velocity 20-30°/sec) in yaw at 0.8 and 1.25 Hz, and in pitch at multiple frequencies from 0.8-3.2 Hz. Gain (eye velocity/stimulus velocity) of the VOR was obtained from digitally sampled signals following saccade removal.

For both young and very elderly subjects, VOR gain was greater for pitch than for yaw, and gain increased with increasing frequency. Gain in the very elderly was never significantly less than that in the young. The pitch VOR gain of the elderly was actually significantly greater that that of the young at 0.8 Hz (0.94±0.04 vs. 0.83±0.11, mean ± standard deviation, $P < 0.05$).

Very elderly people have no functionally significant deterioration of pitch and roll VOR at velocities ≤30°/sec. Possible velocity saturation in the high frequency VOR of the elderly, such as occurs at low frequencies, cannot yet be excluded and is being studied in our ongoing experiments. However, these data suggest that the rotational VOR of elderly people is normal in the important functional range of frequencies and velocities.

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489.4

THE EFFECT OF HEAD PITCH ON HORIZONTAL VOR GAIN DURING ROTATION AT HIGH SPEEDS M.P. Grant, R.J. Tusa*, and D.S. Zee The Johns Hopkins University School of Medicine, Baltimore, MD 21287

The VOR serves to maintain a stable retinal image during rapid changes in head position by producing slow-phase eye movements to offset the change in head position. Previous studies suggest that at low velocities (< 100 °/s) head pitch does not influence the gain of the horizontal VOR. We recorded horizontal VOR gain using EOG in 7 normal subjects during constant velocity chair rotations (60-300 °/s accl 80 °/s^2) in yaw with the head pitched up or down 30°. The mean VOR gain with the head pitched down was 0.56, independent of speed. With the head pitched up, the gain decreased from 0.58 at 60 °/s to 0.40 at 300 °/s.

During the normal VOR at high velocities, central mechanisms must compensate for the nonlinear behavior of the primary vestibular afferents since the vestibular afferents are driven into inhibitory cutoff. This study demonstrates an unexplained decrease in the gain of the horizontal VOR in the head up position, suggesting the central mechanisms are not completely compensating for the nonlinear behavior. Two hypothesis which could explain our observations are: 1) Otolithic afferents may modulate the "antinonlinearity" mechanism. 2) The inputs from the horizontal and vertical semicircular canals may effect the "antinonlinearity" mechanism in different ways.

489.5

THE EFFECT OF HEAD PITCH ON HORIZONTAL GAIN IN PATIENTS WITH UNILATERAL VESTIBULAR ABLATION. R.J. Tusa, M.P. Grant, D.S. Zee, S.H. Herdman*, U. Buettner, J.K. Niparko. The Johns Hopkins Univ School of Medicine, Baltimore MD, 21287.

Following unilateral vestibular loss, high speed rotation towards the intact ear results in a higher gain than rotation towards the defective ear, resulting in a directional preponderance (DP)((GainR-GainL)/(GainR+GainL)X100)). This occurs because of the inability of inhibitory stimuli to decrease vestibular nerve firing rates to < 0. We examined the DP in 4 patients following unilateral vestibular nerve section, and in 2 patients with partial labyrinthectomy with absent caloric but spared vertical SCC on CT scan and compared it to 6 normal subjects. The horizontal VOR was measured by EOG during constant-velocity step rotations (300°/s) while the head was pitched 30° up, kept level or pitched 30° down.

The DP in the controls was less than 5% regardless of head pitch. The DP in patients with unilateral nerve section varies according to pitch in a manner expected by the degree in which the H_ and V_SCC sense head acceleration. When the head is pitched down, head acceleration is maximally sensed by the H_SCC and DP is maximum (23%). When the head is pitch 30° up only 61% of the head acceleration is sensed by the H_SCC[1], 25% is sensed by the V_SCC, and there is little DP (4.2%). In the patients with spared V_SCC, there is little to no DP when the head is level or pitched up.

These results suggest that rotary chair with the head pitched up and down may be a useful technique for assessing V_SCC function.

[1]Fetter et al., Exp Brain Res 1986;64: 208-216.

489.6

FRACTAL ANALYSIS OF READING EYE MOVEMENTS. A.D.Epstein*, E.T.Schmeisser, J.M.McDonough, Q.Dai. Depts of Ophthalmology, Mechanical Engineering, and Computer Science; Univ of Kentucky, Lexington KY 40536-0284.

We recorded eye position during free text reading in a 48 year old man with clinically undiagnosable reading discomfort. Position traces were digitized at 200 Hz with a resolution of 1.52 arc min. Nonlinear dynamical systems analysis by the Grassberger-Procaccia algorithm demonstrated a correlation dimension (Dc) less than 3.0. In this patient, gross inspection of position and delay map traces during sinusoidal pursuit testing showed satisfactory pursuit right of the midline and saccadic pursuit left of it. This asymmetry persisted at all velocities tested.

Previous analysis of reading eye movements showed a Dc near 3.3 for 2 normal subjects, near 2.1 for a slow reader and a dyslexic, and indeterminate for a patient with acquired nystagmus. All non-dyslexic subject data showed evidence of multifractal behavior.

These findings suggest that fractal analysis of unconstrained eye movements alone cannot explain the differences between normal and abnormal reading eye movements. Experiments using a forcing function and more sophisticated multifractal analysis may be more sensitive.

489.7

DIFFERENCES BETWEEN NORMALS AND PATIENTS WITH UNILATERAL VESTIBULAR DEAFFERENTATION DURING ON-CENTER ROTATION AND CENTRIFUGATION. T. Haslwanter[1], I.S. Curthoys[1]*, R.A. Black[2], A.N. Topple[1], G.M. Halmagyi[2]. [1]Dept. of Psychology, University of Sydney, NSW, 2006. [2]Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia.

Using dual search coils we have recorded 3-dimensional eye position in normal subjects and in patients after unilateral vestibular deafferentation on the right (RUVD) or left (LUVD) side. We used two different paradigms. In a first set of experiments, subjects were exactly aligned with the axis of rotation, and then accelerated at 20°/s^2 about an earth vertical axis to a steady state velocity of 250°/s. These experiments produced two interesting results: first, even in normals, rotation about an earth vertical axis elicits torsional eye movements; and second, the torsional eye velocity components in LUVDs, normal subjects, and RUVDs are clearly different. In a second set of experiments, subjects were placed 1m out on the arm of the centrifuge, facing either towards the center of the rotation or away from it. Then they were accelerated at 10°/s^2 to 200°/s. The results from eccentric rotation show the relative contributions of the semicircular canals and the otoliths, and an analysis of these experiments is presented.

489.8

ANALYSIS OF EYE TRACKING DYSFUNCTIONS IN SCHIZOPHRENIA

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Although many studies exist on smooth pursuit eye movement (SPEM) dysfunctions in schizophrenic patients, the specifity of the defect remains unclear.

We examined SPEM performance of 39 schizophrenic in-patients and compared them with 39 healthy volunteers. Eye movements were recorded with a high resolution infrared scleral reflection technique. We counted the number and amplitudes of saccades during SPEM and determined the average gain.

Mean number of saccades was significantly increased and mean gain was reduced in schizophrenic patients. There were no significant correlations to psychopathology or previous doses of neuroleptic medication. We found strong negative correlations between frequency of saccades and gain and between gain and amplitude of saccades.

The reduced mean gain in the schizophrenic group could indicate a dysfunction of the smooth pursuit system or could be an effect of anticipatory saccades with large amplitudes and following reduction of gain. Since anticipatory saccades are rather infrequent, we think that our results rather indicate a functional impairment of the smooth pursuit system.

489.9

FAST SACCADIC RESPONSES IN PARKINSON'S DISEASE

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Saccadic reaction times of 14 patients with mild to moderate Parkinson's Disease (PD) were compared to those of 9 elderly control subjects (CS). The target appeared 8° left or right from the central fixation point at the time when the fixation point was switched off (gap 0) or 200 ms later (gap 200). An experimental session consisted of 200 trials in which directions and gap intervals were randomized. Only primary saccades were considered. We found no significant differences in the mean saccadic reaction time between the PD and control group in either gap condition. The mean saccadic reaction time was shorter for gap 200 (PD 166 ms; CS 179 ms) than for gap 0 (PD 221 ms; CS 246 ms); this difference (gap-effect) was not statistically different in between groups. There was a tendency toward shorter reaction times and a higher proportion of anticipatory saccades in the PD group. Express saccades were present in some gap 200 trials of several subjects in both groups. Therefore, we conclude that express saccades may be mediated by pathways which do not involve the basal ganglia.

In some subjects anticipatory responses, identified by directional error, extended to longer reaction times and the entire saccadic reaction time distribution was shifted along the time axis. To allow for these effects we proposed 1) correction of the bin value in the saccadic reaction time distribution based on the statistical estimation of the proportion of anticipatory saccades; 2) flexible boundaries for visually guided saccade types (express, fast regular, slow regular) by introducing an adaptive classification schema.

489.10

IMAGE PROCESSING MEASUREMENT OF EYE MOVEMENTS IN HUMANS AS A VIABLE ALTERNATIVE TO SCLERAL SEARCH COILS.

S.T. Moore[2], T. Haslwanter[1], I.S. Curthoys[1], G.M. Halmagyi[2]*. [1]Dept. of Psychology, University of Sydney, NSW, 2006. [2]Royal Prince Alfred Hospital, Camperdown, NSW 2050. Australia.

Up to now, scleral search coils have been the only way to measure 3-dimensional eye position in human subjects accurately. The main disadvantages of search coils are the need to wear a contact lens, and the cost of the coils. To overcome these disadvantages, our group has developed an image processing technique for measuring 3-dimensional eye position *"VTM"*, which is based on commercially available video cameras and personal computers. Horizontal and vertical eye position are determined by tracking the center of the pupil; torsion is obtained by measuring the amount of rotation of a segment of the iris about the pupil center. We found that it is possible to express eye position accurately in a manner analogous to coils, i.e. in rotation vectors or Euler angles, if we take into account the geometric distortion of the video image of the eye when calculating eye position. Neglecting this image distortion leads to large errors in measured ocular torsion. We present a summary of the techniques used, the effects of the correction algorithms, and show experimental data, for example Listing's Plane, obtained with this video system.

CONTROL OF POSTURE AND MOVEMENT VII

490.1

THE INFLUENCE OF TARGET MOVEMENT ON REACHING AND GRASPING IN LEUKOTOMIZED AND UNLEUKOTOMIZED ADULTS WITH SCHIZOPHRENIA.

H. Carnahan[1], D. Elliott[2], & V.R. Velamoor[3]. Dept. of Kinesiology Univ. of Waterloo[1], Waterloo, Ont., Dept. of Kinesiology, McMaster Univ.[2], Hamilton, Ont., & Victoria Hospital[3], London, Ont. Canada.

Seven leukotomized adults with schizophrenia (LS), eight unleukotomized adults with schizophrenia (ULS), and eight healthy control (C) individuals were required to reach toward and grasp a small object that was either stationary or moving. Reflective markers were placed on the subject's index finger, thumb and wrist, and movements were videotaped. As expected the LS and ULS groups moved more slowly than the C group when the target was stationary. However, when the target was moving, all three groups moved faster, with the LS and C groups having the same movement times, and the ULS group having the fastest movement time. When the timing of the reaching trajectory was assessed, the LS group spent less time decelerating toward the object, indicating their movements were controlled with less precision. When grasp formation was analyzed, for the stationary condition, the maximum apertures of the LS and ULS groups were not different, and both were larger than those of the C group. For the moving target condition, aperture increased for all groups but was smallest for the C group, intermediate for the LS group and largest for the S group. There was actually less within subject variability in peak aperture for the LS and ULS groups in comparison to the C group, perhaps indicating a limited repertoire of potential motor responses. These results are discussed in terms of the role of the frontal lobes in reaching and grasping, and the ability of schizophrenic individuals to use redundant information. (Supported by NSERC).

490.2

THE INFLUENCE OF GRASPING SURFACE CONTOUR ON PREHENSION.

P.L. Weir*, & S. Desjardins-Denault, Dept. of Kinesiology, University of Windsor, Canada, N9B 3P4.

Altering the size of the grasping surface has shown that smaller contact surfaces require greater precision (Weir, Desjardins-Denault, & Gyurcsik; 1993). However, kinetic measures remain unchanged with the exception of peak load force over the transport phase which decreased with surface size. Weir (1991) reported that changing the contour of the grasping surface from flat to curved effectively reduced the surface area contacted. This decrease in surface area produced kinematic differences, but the forces associated with the movement were not examined. The question then becomes: Does changing the contour of the grasping surface alter the forces associated with the reach to grasp movement?

Subjects were required to reach over a 20 cm distance to contact and grasp a force transducer dowel, and transport it an additional 20 cm to place it centred over a target. The contact plates were 2.5 cm^2 and 2 cm apart with one set of the plates being flat and the other curved. The movement was divided into four phases: free motion, dowel acquisition, dowel transport and release (Weir, 1991).

The contour of the grasping surface had no effect on the free motion phase. However, over the dowel acquisition phase, the rate of both load and grip force application was lower for the curved plate than the flat plate. This paralleled the finding that subjects contacted a smaller surface area on the curved plate. Peak velocity and deceleration were lower for the curved plate over the dowel transport phase, suggesting that a less stable grasp had been effected. The findings during the dowel release phase paralleled those of the acquisition phase. Subjects released the curved plate over a longer period of time, with a lower rate of grip and load force release. These results suggest that altering the contour more profoundly influences the performance of the movement in the phases that occur after contact.
(Supported by NSERC)

490.3

UPPER EXTREMITY RHYTHMIC MOVEMENTS: EFFECTS OF ARM POSITION AND FATIGUE ON DYNAMICAL PATTERN SWITCHING AND MUSCLE COACTIVATION.

S.M. Tuel*, W.B. McKay, D.N. Surber, and A.M. Sherwood, Department of Physical Medicine and Rehabilitation and Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX, 77030.

Dynamical analysis of coordinated movements of the fingers have shown evidence of multistability and nonlinearity. As rate of cycling increases, loss of stability and pattern switching occur. We examined how these effects occur in whole arm movements during two different forearm positions, pronated and supinated, and in the rested and fatigued states.

Subjects were asked to trace a figure-8 on a digitizing tablet using a writing cuff to stabilize pen position. Surface EMG was obtained from the pectoralis, infraspinatus, biceps, triceps, wrist flexors and extensors. Subjects smoothly increased the rate of tracing until pattern switching occurred.

The critical frequencies for pattern switching depended on the position of the forearm, the orientation of the figure-8, and the direction of movement. EMG analysis indicated that the majority of control occurred in the proximal muscles at slow speeds, but distal activity increased with speed. Coactivation reached a maximum just before the pattern switch, followed by a change of EMG activity from tonic to more phasic. Fatigue decreased the critical frequencies and shortened the latency of coactivation, but does not appear to alter the relationship between those characteristics.

490.4

A METHOD OF ANALYSIS TO DETECT AND CHARACTERIZE UPPER LIMB CONTROL DEFICITS IN SUBJECTS WITH STROKE.

PA Genova, CA Giuliani*, KE Light. Motion Analysis Laboratory, UNC CH, Chapel Hill, NC 27499-7153

The purposes of this study were to develop measurements based upon dynamic systems analysis and nonlinear dynamics for quantifying trajectory dynamics of rapid reciprocal limb movements and to characterize limb control deficits in subjects with stroke. Twenty five subjects with CVA (12 left, 13 right) and 25 age and gender matched non-disabled (ND) subjects were tested performing a vertical tapping task. All subjects were videotaped for 2-D analysis tapping as fast as possible with a stylus on a 3 inch target for 10 seconds. The trajectory of the stylus was digitized at 60 Hz and the data were 4 times upsampled through Fourier interpolation. Variables included: cycle period, peak vertical position, up and down velocities, the ratio of amplitude/velocity slopes, the ratio of acceleration to deceleration duration for upstroke and downstroke, the ratio of maximum to average velocity for upstroke and downstroke, Pearson's r coefficient for return maps of cycle period and peak amplitude, and harmonic magnitude ratio (HMR). Subjects with CVA had lower tapping frequencies ($p < 0.003$) than ND subjects and exhibited spatial and temporal asymmetries between upstroke and downstroke that were markedly different than those of the ND subjects. Return maps of peak position showed significant ($p < 0.003$) differences in the control of successive peak vertical positions due to dominance, and to the presence of right and left brain lesions. Significantly higher values for HMR were seen for dominant limbs of ND subjects, and for ND subjects compared to subjects with CVA. High values for HMR may represent minimum jerk movements. The results of this study suggest that these analyses captured the movement dynamics of the stylus trajectory and are sensitive to subtle differences in upper limb control between dominant and non-dominant limbs in ND subjects and between involved and uninvolved limbs of subjects with CVA.
Supported in part by a grant from the Foundation for Physical Therapy.

490.5

FRICTION, NOT TEXTURE, DICTATES GRASP FORCE USED IN OBJECT MANIPULATION. Geneviève Cadoret* and Allan M. Smith, Centre de Recherche en Science Neurologique, Université de Montréal, Québec, Canada, H3C 3J7

A study of grasping and lifting in 10 subjects indicated a capacity to detect the friction of surfaces of a grasped object against the skin and adjust dynamic and static grip forces accordingly. Test surfaces, were composed of smooth or 1.0 mm raised beads spaced at 2.0 or 3.0 mm intervals etched in polyamide plastic. In addition, the surfaces were treated with lubricant (e.g. talc). or adhesive (e.g. water or sucrose) coatings which either increased or decreased the coefficient of friction. Changes in friction were associated with appropriate and inversely scaled changes in grip force. Both the dynamic grip force used to lift an object, as well as the static force used to hold it against gravity were negatively correlated ($r = -0.70$) with the coefficient of friction. Surface texture was only effective in changing grip force insofar as it altered the surface friction. Lubricants had less effect on the coefficient of friction of textured surfaces because of the smaller area in contact with the skin. Conversely, adhesives increased the friction of the smooth surface more than the beaded surfaces. The grip forces for all subjects reflected changes in the coefficients of friction and ignored the changes in surface texture. When coatings were used to equate different textures to the same coefficient of friction, the grip force profiles were nearly identical. This research was supported by the Medical Research Council of Canada and a fellowship from FCAR groupe de recherche sur le système nerveux central.

490.6

THE EFFECTS OF GRADED TACTILE ANESTHESIA ON THE CONTROL OF GRIP FORCE. K.J. Cole*. The University of Iowa, Iowa City, IA 52242.

Tactile sensory information from the hand appears to provide information about discrete mechanical events during manipulation of objects (Johansson, R.S. In Humphrey, D.R., Freund, H-J. Motor Control: concepts and issues. Wiley, New York, pp. 331-355, 1991). One prediction from this theory is that altered fingertip forces are unlikely at mild levels of tactile anesthesia. A sufficient population of afferents should remain to encode the necessary mechanical information. Tactile anesthesia of the hand was induced by variably compressing the median nerve at the palm in three subjects. Median nerve function was monitored by recording the compound sensory nerve action potential (SNAP) at the finger from electrical stimulation of the nerve at the wrist. The forces at the fingertips (normal and tangential, corresponding the grip and lift forces) were recorded as subjects lifted an object using the thumb and index finger. Reductions in SNAP amplitude of 20-30% yielded small but significant decreases in tactile sensibility. Fingertip forces were normal in their size and timing in all subjects. Substantial increases in grip force occurred when SNAP amplitudes were reduced by 50% or more. No subject could detect cotton stroked on the fingertips and all reported that their hand felt 'thick'. Grip forces returned to precompression levels when SNAP amplitudes returned to 70% of normal. These observations are consistent with the theory of discrete event sensory-driven control of grasp.

490.7

DISCRETE POINTING MOVEMENTS IN ALZHEIMER'S DISEASE: EVIDENCE OF PRESERVED AND IMPAIRED MOTOR CONTROL J.D. Fisk*, K. Rockwood, C.E. Maxner, K. Stadnyk, and D. Tripp, Dalhousie University and Camp Hill Medical Centre, Halifax, N.S., Canada

This study employed a kinematic analysis of visually directed upper limb movements in an unconstrained pointing task, to see if characteristic deficiencies in the performance of Alzheimer's disease (AD) patients could be identified. Groups of young controls, elderly controls, and patients with clinically probable AD were asked to point to small or large light targets, that were presented randomly in one of 4 locations, and that were illuminated either for 300 ms or until the target screen was contacted. For some blocks of trials the subjects were asked to point "as quickly as you can" while for others, they were asked to point "as accurately as you can". All groups showed the same pattern of changes in their motor behaviour in response to the trial-by-trial changes in target characteristics. Although both control groups demonstrated changes in peak velocity, duration of deceleration, and accuracy with changes in the instructions, the AD group failed to modify their performance in accordance with the changes in the objectives of the task. This study illustrates that impairments analogous to those revealed on cognitive neuropsychological tasks may be evident in the motor planning and execution of relatively simple reaching tasks.

490.8

DETERMINING CONSISTENCY OF ELBOW JOINT THRESHOLD ANGLE IN SPASTIC MUSCLES. S.L. Wolf*, P.A. Catlin, R.L. Segal, P. Pate, T. Raleigh, J. Tschorn, and H. Watson. Div. Physical Therapy, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Valid measurements of spasticity are needed to evaluate effectiveness of therapeutic interventions. The angle at which EMG onset to passive muscle lengthening (threshold angle) occurs has been suggested as a measure of spasticity. However the conditions under which consistency in threshold angle responses within subjects occur must be determined. This study determined the effect of starting joint position and velocity of stretch (provided from a torque motor) on threshold angle within and between subject. Two starting angles and 2 stretch velocities (the conditions) were randomly assigned at each of three testing sessions among 5 patients with spastic hypertonia. Threshold angle was determined from biceps and brachioradialis EMG responses. Starting angle, subject, and session produced a significant effect on threshold angle ($p<.05$). A 90° starting angle and a 1.0 rad/s velocity stretch yielded the most consistent threshold angles between sessions within subjects. If data from these subjects can be extended to more patients with spastic hypertonia, then ability to use threshold angle to measure spasticity consistently should depend on: comparison within individuals, use of a consistent starting angle, and use of condition 90° and 1.0 rad/s across sessions.

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490.9

SYNCHRONIZATION AMONG MOTONEURON POOLS DURING DIFFERENT PHASES OF CAT REACHING. M. L. McCurdy*, T. M. Hamm, and K. M. Horn. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85015.

Coherence spectra reveal the strength of synchronization between coactive neurons or nerves and are used as evidence of common synaptic input (Christakos et al., 1991; Farmer et al., 1993). Coherence analysis of electromyographic records collected from elbow, wrist and digit muscles during a reaching task from one cat was used to determine synchronization during reach, grasp, hold and stance phases. Significant coherence was observed at lower (<20Hz) and higher frequencies. Stronger coherence at high frequencies was present between proximal and distal muscles during the reach phase than during the grasp phase. Coherence at high frequencies was also found in comparisons among digit muscles or digit and wrist muscles during reach, grasp and hold phases, but not the stance phase. These findings suggest that there are common inputs to motor pools involved in the reaching task. In addition, inputs may differ during various phases of movement. Direct descending projections from the red nucleus to digit pools (McCurdy et al., 1987) may account for coherence among distal limb pools while interneuronal pools projecting to both proximal and distal pools may be important during the reach phase. Supported by NS22454 and NS30013.

490.10

IS ACCURACY IN POINTING INFLUENCED BY DISTURBANCES TO POSTURAL KINESTHESIS?

S.Prentice and J.S.Frank*. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Vibration of the posterior postural muscles is known to cause illusions of falling forward. In a previous study, we demonstrated that this illusion affects one's ability to locate a target in space: the arm's position became elevated in the presence of Achilles tendon vibration. This study further investigates the relationship between postural kinesthesis and the performance of a goal-directed arm movement. Subjects ($N=7$) stood in the dark and pointed to a 5x5 matrix of diodes. Only those diodes located in the vertical midline were analysed. Bilateral vibration of the Achilles tendon was initiated after the target diode had been extinguished. In the previous study, vibration was present during target illumination. All movements were initiated with the arm at the subject's side, and targets were located 20° and 5° above and below a shoulder angle of 90°. Trials were randomized with respect to presentation of target diode and vibration, with each combination being presented only once. Joint angles (Optotrak) and center of pressure data confirmed that subjects tended to lean backwards in response to the illusion of falling forward, which was induced by the vibration. The trunk orientation remained unchanged in the presence of vibration. Vibration also had no effect on the final position of the arm or on the accuracy of the pointing movement. The absence of any arm adjustments may be attributed to the effects of vibration on the perceived target location rather than postural orientation. Since vibration was not on during target illumination, there was not a false impression of target location and an accurate pointing movement was achieved. Thus, it appears that postural kinesthesis has a limited contribution to pointing movements when gravitational cues are present, and perception of target location dictates the pointing movement.

490.11

RELATIONSHIP BETWEEN THE SITE OF LESION AND THE REGULATION OF STANDING POSTURE DURING REACHING MOVEMENTS IN STROKE PATIENTS. M.C. Verrier*, D.R. Fogal Smith, C.D. MacKinnon, S.H. Dick, J.A. Howe, and C. Diep. Departments of Physical Therapy and Physiology and the Queen Elizabeth Hospital, University of Toronto, Toronto, Ontario, Canada M5T 1W5.

Previous work in our lab has shown a predictable pattern of ground reaction forces (GRFs), center of pressure (CP) and postural muscle activity (EMG) during visually cued fast-paced focal reaching movements in normal subjects. Following stroke, GRFs were prolonged and decreased in amplitude, CP excursions were both decreased and abnormal and postural muscle activation was delayed and had increased variance. In contrast to the literature, we found that patterns of muscle activation were variable and subject-specific in stroke. Moreover, muscle activation patterns were not related to pre-established clinical postural measures, but appeared to relate to the site of lesion (cortical (C) versus basal ganglia (BG)). We postulated that C lesions would have decreased reaction time/movement time ratios (RT/MTs) compared to normals indicative of disordered movement execution whereas BG lesions would have increased RT/MTs indicative of disordered movement preparation.

Current studies show that RT/MTs are 14% lower for the C's and 11% higher for the BG's in comparison to controls. C's are unable to activate contralateral distal postural muscles whereas BG's are able to activate bilateral postural muscles but with inappropriate temporal sequencing. These findings support lesion specific sensorimotor processing deficits in postural regulation associated with goal-directed movements. Subject specific 3-D lesion reconstructions using MRI and differences in postural muscle activation strategies are presented for subjects with C and BG lesions during both cued and self-initiated fast reaching movements.

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490.12

EFFECTS OF PRACTICE ON THE CONTROL OF RECIPROCAL AIMING MOVEMENTS POST-STROKE. P.S. Pohl* and C.J. Winstein.
Univ. of S. Calif, Dept. Biokinesiology & Physical Therapy, L.A., CA 90033.

Changes in control of aiming movements as a function of practice have been characterized in healthy, young adults (e.g., Proteau & Cournoyer, 1990), but the literature is silent about practice effects on these movements in adults with brain damage. Subjects with unilateral cerebral lesions have demonstrated differences in performance and control of aiming movements dependent on the side of the lesion, suggesting that each hemisphere has a specialized role in motor control (Haaland et al., 1987). The purpose of this study was to investigate the differential roles of the hemispheres in the control of aiming movements over practice. Two groups of right-handed adults with unilateral stroke (right-RS and left-LS) practiced a reciprocal aiming task in two conditions of task difficulty. Using the ipsilesional arm, subjects performed 10 s trials until they accumulated 500 target hits in each condition. Compared with the LS group, the RS group required more trials due to a higher error rate. Movement time group differences were largest early in practice. Kinematic analyses revealed differences in control strategies over practice. Overall, subjects increased horizontal velocity, especially in the less difficult condition. Control of the vertical dimension revealed group differences which increased over practice. In contrast to the persistent low vertical target impact of the LS group, over practice the RS group appeared to use impact to minimize reversal time. The inability of the LS subjects to optimize the reversal despite practice is consistent with the role of the left hemisphere in the sequencing of actions (Winstein & Pohl, 1994). To address the effect of handedness, healthy controls were tested and their performance was compared to that of those post-stroke.

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490.13

EFFECT OF SURFACE TEXTURE ON THE PERCEPTION OF WEIGHT WHEN LIFTING WITH A PRECISION GRIP. J. R. Flanagan*, A. M. Wing. Teachers College, Columbia University, New York, NY 10027 and MRC Applied Psychology Unit, Cambridge, UK

We investigated the effects of surface texture (satin and sandpaper) on perceived weight in a task in which subjects lifted a reference object and then a test object with the same hand using a precision grip with the tips of the thumb and index finger at the sides. Subjects judged objects covered in satin to be heavier than objects of equal weight covered in sandpaper. Because the grip force required to lift the more slippery satin objects is greater than that required to lift the sandpaper objects, these results suggest that the effect of texture on perceived weight may be due to grip force; the greater the grip force, the heavier the perceived weight. This conclusion was supported by a control study in which subjects grasped objects with the index finger and thumb above and below the object so that different grip forces for the two textures would not be required. In this case, there was no effect of texture on perceived weight. The implications of these results with respect to sensorimotor memory are considered.

490.14

AUTOMATIC ADUSTMENTS OF REACHING MOVEMENTS TO A TARGET THAT MOVES UNPREDICTABLY. BL Day* & IN Lyon. MRC HMBU, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

We have investigated the limb adjustments that occur when reaching for a target that moves and how these responses are modified by the subject's intent.

Subjects (n=8) reached out to touch a disc of light projected on to a screen in front of them. In a third of the trials, approximately 25ms after movement onset, the disc moved either to the left or the right. Subjects were required either to continue trying to point to the disc (P+) or to point in the opposite direction as soon as they perceived it to move (P-). This was contrasted with a reaction task in which subjects held their right index finger stationary in front of them. When the disc moved, they had either to follow it (R+) or move in the opposite direction (R-). The position of the finger tip in space was recorded at 5ms intervals (SelspotII).

In all conditions, when the disc moved, the finger tip began to move in the same direction approximately 130ms later. However, in the P- and R- conditions, this was followed by a second response in the opposite direction, at approximately 200ms. The magnitude of the early response was measured by calculating the lateral deviation of the finger during the period 130-190ms and finding the difference between target left and target right trials. These magnitudes were (mean $\pm$ SEM): P+ 18.1$\pm$4.4mm; P- 9.3$\pm$2.5mm; R+ 2.3$\pm$0.8mm; R- 0.7$\pm$0.2mm.

The results show that arm movements can be adjusted at short latency by a moving visual stimulus. The fact that the early response was not suppressible by voluntary intent may suggest that it is an automatic or reflex component. The early response was much larger during a reaching movement than in the reaction task which indicates that the mechanism is task-dependent.

490.15

CONTROL OF MULTIJOINT ARM MOVEMENTS IN HUNTINGTON'S DISEASE. L.A. Quinn, V. Hamel, J.R. Flanagan, T.R. Kaminski*. Teachers College, Columbia University, NY, NY 10027.

Multijoint arm movements of individuals with Huntington's Disease (HD) were examined using 3D kinematic analysis. Six HD subjects with moderate chorea and 4 age-matched healthy subjects performed rapid pointing movements to 1" target located at 3 distances, 2 of which required trunk motion. HD subjects were divided into high and low functioning groups (HF and LF) based on the Barthel index. Movement times of HD subjects were longer than healthy subjects ($p=.005$). All HD subjects had similar curvilinear hand paths, which were not reflective of an inability to move shoulder and elbow joints simultaneously. However, LF subjects showed greater variability in the hand paths and shoulder/elbow coordination, particularly in the farthest target which required substantial trunk movement. Whereas the movements of healthy subjects featured unimodal hand tangential velocity profiles, the movements of HD subjects were multimodal. For the HF subjects, a large initial movement was followed by smaller submovements (SM's) as the subject honed in on the target. Moreover, the peak velocity and amplitude of the initial movement scaled with target distance. The velocity profiles of the LF subjects were more variable, especially for the farthest distance, where they exhibited a higher number of SM's than the HF subjects. Additionally, the LF subjects did not scale peak velocity to displacement. Presence of SM's in all HD subjects could be due to impaired force modulation, or to factors such as slowness of movement and greater accuracy constraints, as previously observed in healthy subjects (Milner & Ijaz, *Neuroscience*, 1990).

490.16

ADAPTATION TO CORIOLIS FORCE PERTURBATIONS OF REACHING MOVEMENTS IN THE BLIND. J.R.Lackner[1]*, R.Easton[2], E. Bentzen[2], P. DiZio[1] [1]Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254 and [2]Psychology Department and [3]School of Education, Boston College, Chestnut Hill, MA 01267.

Forward reaching movements during passive, constant velocity, counterclockwise body rotation (10rpm) generate Coriolis forces that deviate the arm to the right. As a movement slows down the Coriolis force abates and the arm trajectory bends back toward the intended path but still ends significantly to the right of pre-rotation endpoints. Normal subjects regain straight line trajectories and accurate endpoints, without tactile feedback about target location, within ~20 reaches in darkness and 6 with sight of the arm. We exposed five congenitally blind subjects to Coriolis forces as they attempted to reach forward along their perceived body midline (distance, 32cm; peak velocity, 620mm/s). Relative to pre-rotation their movement trajectories were deviated rightward 55mm, peak, and ended 47mm right. After 20 reaches their endpoints were again accurate and movement trajectories straight. Thus, blind subjects adapted to Coriolis force perturbations of their arm movements as well as sighted subjects denied vision. Vision is clearly not essential for motor adaptation to altered inertial characteristics of arm movements. Brachial proprioceptors, cutaneous receptors and efferent commands must be providing the information used to tune arm trajectory and endpoint.

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490.17

INEQUIVALENCE OF DIRECTION AND EXTENT PRECUE EFFECTS ON REACTION TIME. J.G. Anson,[*1] J.R. Wickens,[2] B.I. Hyland,[3] & R. Kötter.[4] [1]Schl. of Physical Education, [2]Dept. of Anatomy & Structural Biology, [3]Dept. of Physiology & [1,2,3]The Neuroscience Res. Centre, U. Otago, New Zealand, [4]Inst. f. Hirnforschung, U. Düsseldorf, Germany.

In a reaction time task with two possible directions and two possible extents in each direction, direction and extent cues would be considered equivalent in information-theoretic terms: Precue information about direction should have the same effect on reaction time (RT) as information about extent. Eight subjects performed a wrist pronation/supination RT task to one of two possible extents in each direction. One, two or four targets were lit prior to the GO signal to give five different conditions: (i) a single target lit specifying direction and extent; two targets lit specifying (ii) direction only, (iii) extent only, or (iv) a choice of two direction-extent combinations; and (v) four targets lit. Subjects performed 320 trials per condition in four sessions of four blocks (100 trials each, 20 in each condition, randomly mixed) recorded on different days. Results showed that direction precuing was associated with significantly greater RT benefit (shortening) than extent. Precuing extent but not direction was no more beneficial than giving a choice of two different yet completely specified targets, but did offer an advantage compared to conditions in which no precue information was available. These results indicate an inequivalence in the way that information about direction and extent are represented in the brain. They are consistent with a model of motor programming in which movements involving antagonist groups of muscles are represented by different cell assemblies while level of activation within an assembly encodes movement extent.

490.18

BIMANUAL COUPLING IN DIRECTION IS EXHIBITED WHEN AN AMPUTEE PRODUCES CONCURRENT MOVEMENT WITH THE INTACT LIMB AND THE PHANTOM LIMB. E.A. Franz,* R.B. Ivry, and V.S. Ramachandran. University of California, Berkeley, CA 94720.

Normal subjects exhibit spatial coupling in movement trajectories when movements of different directions are combined in a bimanual task. We investigated whether subjects with an amputated limb experience similar coupling in the movement trajectories of their intact limb when making simultaneous imagined movements with the missing limb. Two subjects with amputation of the proximal third of the right humerus were tested. One of the amputees experiences a vivid phantom limb experience, whereas the other has no sensation attributed to the missing limb. The subjects were asked to produce lines in the vertical direction by drawing on a digitizing tablet with the intact limb flexing and extending at the wrist. These lines were produced in single-limb conditions and in combination with an imagined movement which was in the same direction as the intact limb movement, or in the horizontal direction (pronation-supination movements at the wrist). For the amputee with a phantom limb experience, the vertical trajectory of the intact limb deviated from its intended path when the phantom limb produced a horizontal trajectory. In contrast, the amputee without a phantom limb produced no such spatial interference. The results suggest that imagined movements of a phantom limb interact with central pathways in planning the spatial trajectory of the intact limb movement. This coupling can not be explained by interference due to imaging two tasks at once. Phantom limb sensation appears to be necessary for spatial coupling to occur.

490.19

CHORNOBYL VERTIGO: DIAGNOSTICS AND TREATMENT. K. Trinus,* G. Meshcheriakov. BCA, Kiev.

We have examined 167 patients. Almost all the patients have complained of vertigo episodes which lasted more then 1 minute and have appeared more frequently than once per week. The episodes have resulted or been augmented with the head movements and body position changes. Vertigo is accompained with headaches, sweating, vascular disorders, cardiac symptoms. Among frequent complaints there have been nausea with vomiting up to consciousness losses (40%), which might be the index of the seriousness of illness. Specific vestibular tests have shown the sign of alteration: Uemura up to 2.9, Fucuda stepping-1.3, Fucuda writting-1.3, Tracking-1.5, Indication-1.7 and Complaints-2.7. Vestibular evoked potentials have shown the increase of all the peaks latencies up to: P1-44.6 ms, N1-95.6 ms, P2-154.6 ms. We have not found the differencies in the symptomatics between former inhabitants of Pripiat (41 persons), clean-uppers living now in Donetsk (29 persons) and inhabitants of Kiev (32 persons). The treatment with the specific vestibular drugs (Arlevert; EGE-761-Rokan, Tanakan; Vertigoheel) has the positive effect in up to 80% of cases.

CIRCUITRY AND PATTERN GENERATION II

491.1

OPTICAL RECORDING OF THE *TRITONIA* SWIM NEURAL NETWORK. G.D. Brown*, S. Yamada, M. Nakashima, A. Watanabe, and S. Shiono. Central Research Laboratory, Mitsubishi Electric Corporation, Amagasaki 661, Japan.

The escape swim response of the nudibranch mollusc *Tritonia* and the underlying swim neural network (SNN) have been used to study the neural bases of rhythmic movement and of behavioral plasticity. However, some elements of the SNN are still hypothetical (Getting, 1983). We have undertaken a study of the SNN using optical recording to determine if the current model of the SNN is correct and to find missing elements. Previously, the gastropod *Aplysia* has been used in optical recording studies by L. Cohen and coworkers and by us. In general, signals from neurons in the isolated brain of *Tritonia* are bigger and have a better signal to noise ratio than signals from the *Aplysia* abdominal ganglion when using the voltage sensitive dye RH155.

Many *Tritonia* SNN neurons fire periodic bursts of action potentials in the isolated brain preparation and their optical signals are easily recognized. Based on their swim-like bursting patterns, we have identified a set of candidate SNN neurons including most previously identified neuron types. Although the data analysis is ongoing, it is clear that flexion neuron motor pools are bigger than previously reported (Hume et al. 1982). We have also located a neuron which bursts in phase with the swim neural program and which may be an unidentified part of the SNN, perhaps part of the central pattern generator (CPG) for swimming. Another group of neurons bursts strongly at the beginning of the swim program but does not fire in phase with the CPG, a pattern predicted for a group of cells which produce the ramp depolarization in CPG interneurons. We are currently using conventional electrophysiological techniques to determine if these candidates are actually part of the SNN.

491.2

THE EFFECTS OF NEUROMODULATORS ON THE DYNAMIC INTERACTIONS BETWEEN VOLTAGE AND CA^{++} DEPENDENT SYSTEMS IN A MODELED BURSTING NEURON. R. J. Butera, J. W. Clark, C.C Canavier*† and J. H. Byrne†. Dept. of Elec. & Comp. Eng., Rice U., Houston, TX 77251 and †Dept. of Neurobiology & Anat., U. of Tex. Med. Sch., Houston, TX 77225.

A major action of second messenger systems is the modulation of the electrical properties of neurons. The effects can be complex, however. A single second messenger can directly modulate several types of ionic currents in a cell and these currents may indirectly affect other currents via voltage-dependent or Ca^{++}-dependent mechanisms. Thus, in order to understand these global actions of modulatory agents, it is necessary to have a detailed understanding of all of the membrane currents and their modulatory influences. One neuron which meets these criteria is the endogenously bursting neuron R15 in *Aplysia*.

We have implemented proposed mechanisms for the modulation of two ionic currents (I_R and I_{SI}) associated with a model of R15. The model is sufficient to simulate a wide range of endogenous behavior in the presence of various concentrations of serotonin (5-HT) or dopamine (DA). Additional insights into the consequences of modulation of multiple currents were obtained by placing the model into a silent or beating mode by various methods and examining the model's response to extrinsic stimuli. Some of these insights were obtained by examining the Ca^{++}-dependency of I-V plots. Also, by selectively "clamping" specific variables within the model we were able to elucidate mechanisms through which depolarizing and hyperpolarizing pulses can terminate beating and elicit a sustained (8-10 sec) poststimulus hyperpolarization. The results suggest that the actions of modulatory agents and second messengers cannot be understood on the basis of their direct effects alone. It is also necessary to take into account their indirect effects on other unmodulated ion channels which occur through the dynamic interactions of voltage-dependent and Ca^{++}-dependent processes.

491.3

SEROTONIN REGULATES THE ELIGIBILITY FOR MULTIMODAL ACTIVITY IN THE BURSTING NEURON R15 IN *APLYSIA* H.A.Lechner, D.A.Baxter, J.W.Clark and J.H.Byrne* Dept. of Neurobiol. and Anatomy, Univ. of Texas Medical School at Houston, TX 77030, Dept. of Electrical and Computer Engineering, Rice University, Houston, TX 77251-1892.

Simulations of a mathematical model of the bursting neuron R15 in *Aplysia*, revealed that multiple stable modes of oscillatory electrical activity can coexist at a given set of parameters, that transient synaptic inputs can induce a persistent shift from one mode to another and that modulatory transmitters can regulate multimodal activity (Canavier, 1993).

Intracellular recordings from R15 revealed that long lasting changes in spike activity can be induced by brief (0.5 - 1.5 s), depolarizing (5 - 10 nA) or hyperpolarizing (-10 to -20 nA) current pulses, as predicted by the model. Under certain conditions such perturbations were able to switch the electrical activity of R15 between bursting and beating. The beating persisted for varying durations ranging from ≈30 sec seconds up to ≈40 min. The effect of perturbations was dependent on two factors, steady-state bias current and the presence of serotonin (5-HT). Perturbations were ineffective for bias currents of < 0.5 nA, whereas 30 - 40 % of the perturbations triggered transitions from bursting to beating with bias currents >1 nA. In the presence of 5-HT (2.5μM), multistability was observed at lower levels of bias current. With a bias current of -0.5 nA transitions to beating were triggered in 60 % of the cases, and with bias currents >1 nA 40 - 50 % of the perturbations induced transitions.

We conclude that both 5-HT and depolarizing bias currents can regulate the eligibility of R15 for mode shifts, and thus may alter its sensitivity to synaptic input.

491.4

DETERMINATION OF SYNAPTIC POLARITIES USING A COMPUTATIONAL CELLULAR MODEL OF THE NEMATODE TAP WITHDRAWAL REFLEX. C.J. Roehrig,*[1] S.R. Wicks[2], and C.H. Rankin[3]. [1]Dept. of Computer Science, [2]Program in Neuroscience, and [3]Dept. of Psychology, University of British Columbia, Vancouver, B.C., CANADA V6T 1Z4.

The nematode *C. elegans* is a tiny soil-dwelling worm which exhibits many behaviours found in higher vertebrates, such as reflex hierarchies, learning, and memory. Its genetics, neuroanatomy, and physiology are well understood to the point where it is possible to construct a model of a complete behaviour that includes every individual neuron and synapse.

We have previously constructed a computational model of the *C. elegans* tap withdrawal reflex. The model circuit consists of all 7 sensory cells and 6 pairs of interneurons which were found to play a role in the tap withdrawal reflex (Wicks and Rankin, 1992), together with a "gearbox" which models the motorneuron circuitry and mechanics which transduce the interneuron activity into locomotion. Based on biological evidence, we used a non-spiking, isopotential model of the neuron membrane potential and a tonic and graded model of synaptic transmission. Synaptic efficacy was inferred from anatomical data (White *et al*, 1986).

The polarities of the synaptic connections are as yet unknown and we have developed a strategy to use our model to predict these polarities. We computed the probabilities of different polarity combinations by testing the model's predictions against the behaviour of the living animal when several different neurons were ablated using laser microsurgery. In addition, we used a modified factorial design to determine interaction effects between connection polarities and other physiological parameters that describe membrane properties and synaptic activation kinetics.

491.5

EVIDENCE THAT SEROTONERGIC NEUROMODULATION INTRINSIC TO THE *TRITONIA* SWIM CPG IS DUE TO PRESYNAPTIC ENHANCEMENT OF RELEASE. P.S. Katz* and W.N. Frost. Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77030.

One of the neurons in the central pattern generator (CPG) for escape swimming in the mollusc *Tritonia diomedea* evokes neuromodulatory effects upon other members of the CPG. We previously reported that stimulation of one dorsal swim interneuron (DSI) enhances the size of synaptic potentials evoked by neuron C2 onto two followers: the dorsal flexion neurons (DFN) and other DSIs (Katz et al., *Nature* 367:729-731, 1994). Our evidence now suggests that this neuromodulation is mediated by effects on the presynaptic neuron (C2) rather than on its followers. 1) DSI enhanced the chemical synapses (both excitatory and inhibitory) of C2 onto each of its followers which have been tested, including the ventral swim interneurons (VSI), DSI, DFN, and pedal cells not directly postsynaptic to DSI. 2) DSI evoked little or no change in R_{in} recorded in the somata of C2 followers. 3) DSI-evoked modulation did not affect the E_{rev} of the synapses. 4) If the effect were postsynaptic then inputs from other neurons onto the follower might be enhanced, yet DSI stimulation did not enhance the synapses from other DSIs onto the same C2 follower, but instead caused them to decrease.

Although the mechanism producing the presynaptic enhancement is not known, possible mechanisms are suggested by the following observations. Stimulation of the serotonergic DSIs decreased the after-hyperpolarization (AHP) following C2 spikes. Likewise, exogenous serotonin also caused the C2 AHP to decrease. The decrement in AHP may be related to presynaptic currents involved in transmitter release. Preliminary results indicate that DSI stimulation also may interact with the homosynaptic facilitation of transmitter release from C2. (This work was supported by NIH grant MH49563.)

491.6

IDENTIFICATION OF *TRITONIA* "RAMP" INTERNEURON: DRI NEURON RELAYS SENSORY INPUT TO THE ESCAPE SWIM CPG AND ALSO PARTICIPATES IN RHYTHM GENERATION. W.N. Frost* and P.S. Katz. Dept. of Neurobiol. & Anat., Univ. Texas Medical Sch., Houston, TX 77030.

Work by Getting and colleagues concluded that the key driving force for the oscillatory escape swim motor program in the marine mollusc *Tritonia* is a long-lasting declining ramp depolarization in the dorsal swim interneurons (DSIs) of the swim central pattern generator (CPG). A significant component of this depolarization was hypothesized to originate from unidentified "ramp" cells presynaptic to the CPG. Here we describe a neuron which is the source of the ramp depolarization in the DSIs. The dorsal ramp interneuron (DRI) elicited strong, fast epsps onto the DSIs (n=11). In response to a swim-inducing nerve stimulus, DRI fired intensely at the onset, and then at a lower, steadily declining rate throughout the swim motor program (n=8). Hyperpolarization of DRI during the nerve stimulus prevented both the swim motor program and the ramp depolarization in the DSIs (n=5). Tonic DRI depolarization elicited the swim motor program, which then continued for as long as DRI was active (n=6). These observations identify DRI as a "ramp" interneuron, and indicate that this single neuron has a key role in relaying sensory input to the *Tritonia* CPG.

DRI also had features not predicted by previous work. DRI: 1) fired in phase with the DSIs during the dorsal portion of the oscillatory swim motor program; 2) was excited by C2, and in fact appeared responsible for much of C2's polysynaptic excitatory synaptic connection to DSI in normal saline (n=4); 3) could reset the phase of an ongoing motor program (n=1); and 4) quickly halted an ongoing rhythm when hyperpolarized (n=2). These features indicate that DRI not only produces the ramp input to the CPG, it is also a component of the central pattern generator itself.

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491.7

CALCIUM PLATEAUS AND GRADED SYNAPTIC TRANSMISSION IN LEECH HEART INTERNEURONS. Ø.H. Olsen, F. Nadim, and R.L. Calabrese*, Dept. of Biology, Emory University, Atlanta, GA 30322.

Pairs of reciprocally inhibitory neurons in the leech CNS form the oscillator that controls the frequency of the heart rhythm. These pairs oscillate in antiphase, generating bursts with underlying plateaus and both graded and spike-mediated postsynaptic transmission. To find the extent to which low-threshold Ca currents contribute to plateaus and determine the duty cycle of the graded synaptic transmission, we voltage-clamped such a neuron with a voltage waveform that was obtained by removing the spikes from real waveforms. Its post-synaptic cell was simultaneously voltage-clamped at -35 mV and showed the graded postsynaptic current associated with these presynaptic oscillations. The same experiments were also performed in a detailed conductance-based model of the two cells. The results confirmed the predictions of the model; the rise of the plateau cannot be completely accounted for by the Ca currents only. Thus other inward currents like the persistent Na current and the hyperpolarization-activated inward current (h) contribute in the process of building the plateau. The duty cycle of the graded synaptic transmission proves to be smaller than 0.5, thus suggesting that the spike-mediated transmission is necessary to produce the observed prolonged inhibition.

These results are supported by further experiments in Na-free saline where the persistent Na current and h-current were reintroduced through dynamic-clamp (Sharp et al., TINS 16; 389, 1993). To obtain non-spiking oscillations, it was necessary to add these currents, thus demonstrating their role in triggering the plateau. The period of these oscillations is shorter than that observed in normal saline where the graded synaptic transmission is supplemented with spike-mediated transmission.

491.8

MODULATION OF OSCILLATIONS IN A MODEL OF LEECH HEART INTERNEURONS. F. Nadim*, Ø. H. Olsen and R. L. Calabrese. Dept. of Biology, Emory University, Atlanta, GA 30322.

The bursting oscillations underlying the heartbeat of the medicinal leech are generated by pairs of reciprocally inhibitory heart interneurons (HN cells). We have built a conductance-based Hodgkin-Huxley type model of a pair of HN cells using experimentally measured ionic and synaptic currents. The synaptic current in the HN cells has a graded component, which, in our model, is dependent on presynaptic Ca^{++}, as well as a spike-mediated component. Our Lab has previously shown that FMRFamide has neuromodulatory effects on the heartbeat rhythm by changing the shape and amplitude of spike-mediated IPSP's, and by shifting steady-state activation and inactivation curves of the K^+ currents. The effect of changing the shape and amplitude of the IPSP's in the model on modulating the period of oscillations is similar to the modulatory effects observed in the biological cells. We are in the process of analyzing the effect of shifting the activation curves of the K^+ currents in the model.

Even though the model cells are capable of oscillation merely with the graded component of synaptic transmission, the simulated oscillations closest to oscillations of the biological cells are dominated by the spike-mediated component. This suggests that spike-mediated transfer can play a dominant local role in a network of neurons that has large graded synaptic currents. The dominant role of spike-mediated transfer is significant in light of the fact that neuromodulation of the heartbeat cycle rate is directed towards spike-mediated ipsp's and towards K^+ currents that affect spiking.

491.9

A HIGHER ORDER LOCOMOTOR INTERNEURON CAUSES DIRECT INHIBITION OF RESPIRATORY CENTRAL PATTERN GENERATING NEURONS OF *LYMNAEA* T. Inoue, G. Spencer, N.I.Syed ,K. Lukowiak., W. Wildering, S.U.Hassan * and M.Takasaki# Departments of Anatomy and Medical Physiology, University of Caglary, Calgary, Alberta, Canada, T2N 4N1. # Department of Anaesthesiology, Miyazaki Medical Collage, Kiyotake, Miyazaki, Japan, 889-16.

A pair of electrically coupled interneurons termed Left and Right Pedal Dorsal 11(L./RPeD11) was previously shown to control locomotor and /or whole body withdrawal behaviors of *Lymnaea* (Syed and Winlow, 1991, JEB 158, 37-62). Since respiration and locomotion or whole animal withdrawal in *Lymnaea* are antagonistic motor functions, reciprocal inhibitory connections must exist between the CPG neurons controlling these behaviors. This study demonstrates that the electrical stimulation of RPeD11 in an isolated brain preparation causes direct inhibition of respiratory interneurons RPeD1 and IP3I. Whereas, its effects on a third respiratory interneuron VD4, known to be involved in the termination of respiratory episodes, were biphasic. When cell VD4 was hyperpolarized to -70 to -80mV, the electrical stimulation of RPeD11 caused a strong depolarization of its membrane potential (10-20 mV). However, when VD4 was depolarized to its spiking threshold, RPeD11 stimulation induced spiking in this cell following a brief period of inhibition. The respiratory interneurons IP3I and VD4 on the other hand, inhibited the activities of neuron RPeD11. These connections persisted in salines containing high divalent ions. Furthermore, RPeD11 reestablished its specific synaptic connections with a number of cardio-respiratory neurons in culture, demonstrating further that they are monosynaptic in nature. This study provides first direct evidence for mutual inhibitory connections between respiratory and locomotor or withdrawal CPG neurons of *Lymnaea*.

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491.10

INTERACTIONS BETWEEN SEGMENTAL LEG CENTRAL PATTERN GENERATORS IN ISOLATED LOCUST THORACIC NERVE CORDS.

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Rhythmic motor patterns can be induced in leg motor neurons of isolated thoracic ganglia of the locust, *Schistocerca americana*, by bath application of the muscarinic agonist pilocarpine (Ryckebusch and Laurent, *J. Neurophysiol.*, **69**(5), 1993). We have recorded the rhythmic motor patterns evoked in preparations of thoracic ganglia in which the connectives between ganglia remained intact. Cross-correlation analysis of the activities of different motor pools in the three thoracic ganglia revealed that the rhythmic motor patterns in different ganglia are centrally coupled. In particular, trochanteral levator motor neurons were coactive with trochanteral depressor motor neurons in adjacent hemiganglia. Trochanteral levators also tended to be active within a short latency of levator bursts in an ipsilateral adjacent hemiganglion. The coupling we observed, while not sufficient to account for the coordination of the legs during walking, demonstrates that central mechanisms exist which may contribute to leg coordination and the maintenance of stability and posture during walking.

This work was supported by NIH and ONR.

491.11

EFFECTS OF TEMPERATURE ON PROPERTIES OF LOCUST FLIGHT NEURONS: IMPLICATIONS FOR MOTOR PATTERN GENERATION. H. Xu* and R. M. Robertson. Dept. of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Elevated temperature causes a mild increase in wing-beat frequency of flying locusts. This is mainly due to the global effects of temperature on neuronal properties of flight neurons in CPG circuits. Our previous investigation of flight motoneurons showed that an increase in conduction velocity (Q_{10}=1.53) and a decrease of membrane time constant (Q_{10}=0.62) were the major factors which might account for the increase of the central rhythm (Q_{10}=1.28). Hyperpolarization of the resting membrane potential (Q_{10}=1.18) and reduction in input resistance (Q_{10}=0.54) might be involved in automatic compensation for the effects of increased temperature. Also increases in temperature above room temperature decreased the amplitude of postsynaptic potentials (PSPs).

We have investigated how the change of resting membrane potential and the PSP amplitude contribute to the observed temperature effects on the frequency of the central rhythm. The temperature dependent hyperpolarization of flight neurons was mimicked by changing the K^+ concentration of the saline from 10 mM to 2 mM. After 5 mins low-K^+ superfusion, the resting membrane potential of flight motoneurons hyperpolarized from an average of -39.1 mV to -47.8 mV, while the frequency of the central rhythm decreased from an average of 10.1 Hz to 8.8 Hz. The temperature dependent change of PSPs recorded from flight neurons was mimicked by replacing normal saline with zero Ca^{++}/high Mg^{++} saline. Although the amplitude of PSPs was more than halved after 10 mins zero Ca^{++}/high Mg^{++} treatment, the frequency of the central rhythm changed minimally (<1 Hz). These results support the hypotheses that resting membrane potential directly affects the central rhythm, whereas synaptic strength could serve simply to trigger endogenous mechanisms of burst generation.

491.12

QUANTIFICATION OF SYNCHRONY IN NEURAL POPULATIONS BY COHERENCE ANALYSIS. C.N. Christakos*, Med. School, Univ. of Crete, Greece, and Ctr. Neurobiol., Columbia Univ., New York, U.S.A.

A recent study (C.N. Christakos, Neurosci. 58, 43-57, 1994) indicated that for a neural population comprising uncorrelated units and units that are correlated around some frequency Fs, the unit-to-aggregate (UTA) coherence function is near zero for the uncorrelated subset but has a nonzero value at Fs for members of the correlated subset. This value reflects the numerical size of the population and the characteristics of synchrony (extent and strengths of unitary correlations, and phase concentration for the correlated units); and as indicated by simulations of a uniform population, it is substantial for wide ranges of values of these parameters. Thus, UTA coherence computations on a sample of units allow the estimation of the extent of population synchrony, as well as inferences on correlation strengths, i.e., a quantification of neural synchrony. Current mathematical analysis using the same model of synchrony verifies these results for the UTA coherence. It further reveals that the value of the aggregate-to-aggregate (ATA) coherence at Fs between unit (sub)populations reflects the same four parameters, and is also substantial in a wide range of conditions. However, from this value the characteristics of synchrony cannot be separately estimated. Therefore, the ATA coherence at Fs only provides a general measure of synchrony, on the condition that the sizes of the two (sub)populations stay relatively fixed. Finally, the analysis shows that the UTA and particularly the ATA coherence are very sensitive indicators of population synchrony, especially when synchrony is weak (unit-to-unit coherences < 0.3).

491.13

pH SENSITIVITY OF SPONTANEOUS NETWORK ACTIVITY OF MAMMALIAN NEURONAL CULTURES. S. R. Laufer* and G. W. Gross. Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

The association of pH changes with normal and pathological neural activity, as well as the influence of pH on major ligand-gated ion channels, has been established in studies using single cell, slice, and animal models. However, pH effects on the activity of small networks in well controlled culture environments have received less attention. We investigated the effects of pH, close to and within the physiological range, on monolayer networks derived from embryonic mouse spinal tissue. Neurons were cultured on multimicroelectrode plates to permit extensive spatio-temporal monitoring of network activity. The pH was shifted by altering the CO_2 flow to bicarbonate buffered medium in the recording chamber and was continuously monitored. Spike data was stored on 14 channel analog tapes and integrated for chart recorder display. Preliminary observations indicate that *spontaneous native activity* is highly sensitive to pH over the explored range of 7.0 to 8.0. Using activity at pH 7.3 as reference, total spike production, as reflected by integrated burst area, fell as low as 1% at pH 7.2 and increased as much as 665% at pH 7.7. With increasing pH, burst frequencies increased as did the appearance of large complex bursts. This pattern reversed as pH was lowered, with large bursts disappearing below pH 7.2. Under *bicuculline disinhibition* (producing coordinated, regular bursting) a pH decrease from 7.4 to 7.2 caused both burst frequency and duration decreases of up to 40%. pH increases from 7.4 to 7.7 caused frequency decreases of up to 30%, but duration increases of up to 40%. This degree of pH sensitivity necessitates that quantitative studies of network dynamics be performed in a well controlled pH environment. In addition, pH may be a useful variable in determining burst initiation and burst maintenance mechanisms.

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491.14

COMPUTATIONAL MODELS OF THE RESPIRATORY OSCILLATOR IN MAMMALS. J.C. SMITH* Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Computational models for the respiratory oscillator in both the neonatal and adult nervous systems have been developed and used to explore interactions of intrinsic cellular and synaptic properties in the generation of respiratory rhythm and pattern. The model for the neonatal nervous system is an extension of our previous hybrid pacemaker-network model (Smith et al., 1992) which includes voltage-dependent pacemaker neurons as the kernel of the oscillator. The model for the adult is an extension of previous network models (Ogilvie et al., 1992; Balis et al., 1994) and now incorporates intrinsic membrane currents which give the model neurons voltage-dependent bursting and spiking behavior. Modeled membrane currents and synaptic connections are inferred from available intracellular recording and connectivity data from neonatal rat brainstem in vitro and the anesthetized adult cat in vivo. The models incorporate all major classes of neurons currently known in the neonatal and adult ventral respiratory group, including the preBötzinger Complex-the proposed locus for rhythm generation (Smith et al., 1991). Membrane currents are modeled with Hodgkin-Huxley equations and synaptic conductances mimicked fast excitatory and inhibitory interactions. The simulations reproduce spike discharge patterns and membrane potential trajectories of the main classes of respiratory neurons in the neonate and adult. Possible transformations of the oscillator during development were investigated. The oscillatory behavior and discharge patterns in the neonatal model transform into the adult patterns when synaptic inhibition in the network is increased, indicating that the neonatal and adult models are compatible. These models provide tools for investigating the dynamic properties of the respiratory oscillator, including developmental transformations. (Supported in part by HL40959).

491.15

THE USE OF SYNAPTIC POTENTIALS AS THE CHARGE ON PARTICLES IN THE GRAVITATIONAL REPRESENTATION OF NEURONAL ASSEMBLIES: RESULTS FROM SIMULATIONS

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The gravitational representation of simultaneously recorded spike trains (Gerstein et al. 1985) provides a conceptual framework for the analysis of the dynamic organization of neuronal assemblies (Lindsey et al. 1992). Each neuron is represented as a particle carrying a time varying charge that is a filtered version of spike activity. The charged particles exert force on each other; aggregation of particles indicates non-random temporal relationships (effective connectivity) among the spike trains. We have enhanced the gravity method so that fluctuations in membrane potential can be mapped to particles as well, allowing correlation of intracellular and spike train data. Simulated uncorrelated spike trains and an analog signal representing the membrane potential of a target cell were used to evaluate the enhanced gravity algorithm. All of the simulated neurons exhibited similar firing rates but only one in each trial induced inhibitory synaptic potentials in the target cell, resulting in IPSPs with amplitudes and time constants approximating *in vivo* values. The algorithm correctly distinguished the spike train producing the hyperpolarizations. Subsequent tests introduced synaptic noise in the intracellular trace from up to 512 discrete inhibitory synapses unrelated to the analyzed spike trains. The algorithm detected the interaction in the presence of this synaptic noise. These results 1) show that the advantages of the gravity method can be extended to intracellular recordings, and 2) support the idea that other analog signals related to neuronal activity may be used to define the charge on particles in the gravitational representation. Supported by NS19814.

491.16

GRAPHICAL MEASURES OF SPIKE TRAIN SIMILARITY AND COUPLING BASED ON RECURRENCE TIME HISTORY MATCHING (RTHM).

B.K.Rhoades*, J.M. Kowalski, H.J. Mackey and G.W. Gross, Depts. of Biological Sciences and Physics, Univ. of North Texas, Denton, TX 76203.

We have applied the Hausdorff metric and related similarity functions as cumulative and running time estimators of similarity or coupling between two spike trains, based on the local auto-recurrence time history associated with each spike. These new methods have been tested with biological spike trains from cultured mouse spinal cord networks, as well as artificial spike trains from a class of stochastic models. The central algorithm operates on two spike trains and a particular cross pair of spikes A and B, and begins by finding the best match for each of N sequential auto-recurrence times of spike A in the auto-recurrence time history of spike B. This constitutes a set of N minimal A->B distances. A set of N minimal B->A distances is similarly determined. The maxima from each of these sets are then summed to produce the local Hausdorff distance (D_H) between the two spike trains, associated with the cross recurrence time L (lag or lead) between A and B. Accumulating D_H and associated L values across spikes provides a metric for graphical assessment of local spike train similarity and temporal relationships. Alternatively, the minimum distance (D_m) from the A->B and B->A sets provides a very sensitive measure of local spike train coupling. By comparing D_m to a predetermined threshold Θ, we can find the set of associated L values corresponding to cross pairs of spikes with criterion matches in their auto-recurrence time histories. The histogram of this "$RTHM_m$" set reflects defined coupling relationships between artificial spike trains more accurately than does the standard cross-correlogram, which is shown to be a special case of the $RTHM_m$ histogram, where Θ or N is large. $RTHM_m$ is also appropriate as a running-time measure of coupling latencies and strengths, for detecting coupling state transitions in non-stationary data sets. By graphically combining this similarity function with the ranked mean local interspike interval we have produced a clear demonstration that networks can become functionally decoupled during paroxysmal bursts, as a manifestation of nonlinear, activity-dependent gain. Supported by the State of Texas Advanced Technology Program.

491.17

A NEW HIGHER-ORDER INTERSPIKE INTERVAL ANALYSIS FOR DETECTING SEQUENTIAL FIRING TRENDS IN NEURONS.

D. C. Tam.* Center for Network Neuroscience, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

A new spike train analysis method is developed to detect how transitions of firing intervals in neurons are dependent on higher-order interspike intervals (ISIs). A set of "firing trend indices" is introduced to detect trends in firing independent of time scales. The firing trend index h_1 is defined as the ratio between the first-order ISI and the second-order ISI relative to any reference spike. Since the value of h_1 is bounded by 0 and 1, (i.e., $0 < h_1 < 1$) comparison between how firing intervals are changed can be made independent of the absolute time scale of the underlying intervals. The asymptotic value of $h_1 \to 0$ represent the extreme change from short to long ISIs, and vice versa, for $h_1 \to 1$, and $h_1 = 0.5$ represents no change. Similarly, h_2 is defined as the ratio between the second first-order ISI and the second-order ISI relative to a reference spike, with the relationships: $h_1 = 1 - h_2$ and $(0 < h_2 < 1)$. In addition, another firing trend index k can be defined as the ratio of the difference between the two adjacent ISIs to the second-order ISI. The value of k is bounded by -1 and 1, $(-1 < k < 1)$, with the relationship: $k = h_2 - h_1$, where $k = 0$ indicating no change in firing consecutive intervals, $k \to 1$ indicating extreme lengthening in ISI, and $k \to -1$ indicating extreme shortening in ISI. Plotting these indices against the sequential spike number provides clear indication of the evolution of serial trends in firing. Thus, the transition into and out of burst firing, for instance, can be detected easily. Furthermore, plotting these indices against the first-order ISI will provide an estimate of the transitional probability of spike firings. Using these new state transition analyses, the underlying state transition processes, such as Markov processes or other processes, may be revealed in neurons. (Supported by ONR N00014-93-1-0135)

491.18

A FUZZY-CLUSTERING APPROACH TO THE RECOGNITION OF MULTINEURON ACTIVITY.

G. Zouridakis* and D. C. Tam.† *Dept. of Electrical & Computer Engineering, University of Houston, Houston, TX 77204-4793 and †Center for Network Neuroscience, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

Typically, spike discrimination techniques can be used to extract separate spike trains from many neurons in multi-unit extracellular recordings. The performance of these methods often depends critically on the availability of reliable spike templates. The presence of overlapping spikes may introduce misidentification of spikes when conventional template-creation approaches are used. To overcome this problem, we use a fuzzy clustering approach to extract multi-unit activity.

Our procedure employs fuzzy K-means clustering on a data segment containing multi-unit activity (1) to identify the number of neurons contributing to the recorded signal, and (2) to create reliable spike templates for each neuron. For this purpose, a performance index is used as an optimization criterion.

Comparison of performance between the conventional crisp K-means and our fuzzy K-means clustering approaches is done with synthetic spike trains generated using real spikes and segments of background noise recorded from crayfish neurons. Extensive simulations clearly demonstrate the advantage of the fuzzy procedures in identifying the exact number of neurons in the recording and the exact templates over a wide range of signal-to-noise ratios and several "fuzziness indices". They also allow us to determine the optimum "amount of fuzziness" for real-time processing. This method can be applied to other electrophysiological signal analyses, such as EEG and evoked potentials. (Supported by ONR N00014-93-1-0135)

491.19

LEVEL OF ACTIVITY AND INHIBITION EFFECTS IN A SPINAL MOTOR CIRCUIT SIMULATION.

D. P. Bashor* and D. M. McDermott. Dept. of Biology, University of North Carolina at Charlotte, NC 28223.

A computer simulation based on R. J. MacGregor's (1987) SYSTM series was used to investigate the importance of level of network activity on flexor and extensor motoneuron (fMn, eMn) activity in a segmental motor circuit. The spatial distribution of Renshaw cell (Ren) excitation by Mn was also studied. Single fMn and eMn populations (169 cells each) were interconnected with 5 pairs of interneuron populations in a network composed of about 2300 cells. The network was driven by 5 pairs of fibers representing IA and IB afferents, and tonic descending excitation. At low levels of network activity, fIA and fIB input initiated fMn firing which continued following termination of input. At intermediate levels of network background firing, fMn activity was quenched immediately after termination of input. At high levels of background, afferent input made only small differences in frequency and number of active Mn cells, as well as providing little reciprocal inhibition of the antagonist population. The effects of focal activation of Ren by Mn compared with widespread activation demonstrated complex effects in the network. Changing from widespread to focal activation of fRens by fMns had little effect on fMn activity, but a rather large effect on eMn frequency. However, both mean frequency and number of cells active changed in fRen and eRen populations.

491.20

CORRELATION RASTER DISPLAYS OF SPIKE TRAINS: A NEW METHOD OF ANALYSIS OF OSCILLATIONS IN EXPERIMENTAL AND SIMULATED DATA.

A.B. Kirillov and D.J. Woodward. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Standard tools for analysis of spike trains are usually based on averaging techniques. Thus, auto-correlation histogram represents a time-averaged measure of internal structure of a spike train. This and other time-averaged histograms cannot specify, however, the onset and termination of coherent oscillating modes within groups of spike trains. Raster displays of spike trains, that are able to show the patterns in neuronal firing times, are usually used only to demonstrate changes in neuronal activity around a stimulus presentation. We routinely use similar raster displays but with a reference point being a spike instead of a stimulus. This simple generalization of stimulus-related raster displays gives the investigator a powerful tool to analyze detailed pattern structure of spike trains. It is not unusual to display thousands of raster dot lines using this technique and therefore a high resolution monitor and printer as well as some restructuring of the computational routines are required. Auto- and cross-correlation raster displays are especially useful when analyzing spike trains with an oscillatory components. Auto-correlation raster displays allow one to visualize and identify the instances of oscillatory behavior and their structure. Cross-correlation raster displays reveal the time course of synchronization and phase lag changes. We demonstrate typical patterns that emerge in correlation rasters and present several examples of using this technique to study ensemble neuron data recorded from hypothalamus, cortex and neostriatum of rats as well as from neural network models. *Supported by DA2338. Poster is available on WWW server http://biogfx.bgsm.wfu.edu/*

492.1

FROG HINDLIMB MUSCLE FORCE FIELDS *Loeb, E.P.*; Bizzi, E.; Saltiel, P.; Giszter, S.F; Schotland, J.* Dept of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

Using microstimulation, we have previously described a few hindlimb postures encoded in the spinal interneuronal grey. These postures can be described as convergent force fields. We have also previously measured force fields from the major muscles in the frog hindlimb. In order to clarify the relation between muscle force fields and spinal force fields we measured force fields over a large portion of the workspace by electrically stimulating each muscle in the hindlimbs of 10 frogs.

All of the muscles had divergent force fields (because the force never reversed direction. It should be mechanically impossible for a muscle to produce a stable force field). There were three primary directions in which those divergent force fields pointed. Radially away from the body (Pyriformis, Quadratus Femorus, Peroneus, Vastus Externus, Tibialus, and Semimembranosus), Contralaterally behind the body (Semitendinosus, Adductor Magnus, Rectus Internus (major and minor), and toward flexion (Biceps, Ilio-Psoas, Pectinius, Rectus Anticus, Adductor Longus, Gastrocnemius, and Sartorius). Thus there is redundancy in the muscle force fields. In addition, some of the muscles had zero-valued force fields throughout some part of the workspace. The muscles Rectus Anticus, Rectus Internus (major and minor), Gastrocnemius, Adductor Longus, Vastus Internus, and Sartorius all showed a complete or near complete loss of force throughout some portion of the workspace. The utility of this type of muscle force field may lie in its ability to provide position-dependent force field modulation without requiring position (afferent)-dependent EMG modulation.

Because none of the muscle force fields was stable, we conclude that limb stability can only be achieved through appropriate combinations of muscle activations. A primary function of the spinal cord may be to insure stability by serving as a locus for implementing the co-activation of muscles. **ACKNOWLEDGEMENTS: NIH NS09343 and AR26710, and ONR N00014/90/J/1946.**

492.2

EFFECT OF DOUBLE PULSE ACTIVATION ON FORCE ENHANCEMENT IN THE HUMAN PARALYZED SOLEUS MUSCLE. R.K. Shields* and K.C. Shields, Physical Therapy Graduate Program, University of Iowa, Iowa City, IA 52242.

Doublets, or two repetitive stimuli delivered with a short interpulse interval, have been shown to produce a force output that is greater than the linear sum of force produced from one stimulus (Burke et al. Science 1970). In addition, this effect may be motor unit or muscle fiber type specific. We previously showed that the human chronically paralyzed soleus muscle histochemically and physiologically responds as a predominantly fast muscle while the acutely paralyzed soleus muscle (less than 6 weeks) responds as a slow muscle. This study examined the effects of the repetitive doublet on the human chronically and acutely paralyzed soleus muscle both before and after a standard fatigue regimen.

Ten chronic (one year) and two acute (6 weeks) completely paralyzed individuals had their lower leg positioned in a torque measuring device with the knee in 90° of flexion. The soleus muscle m-wave was monitored during all stimulations to ensure supramaximal activation. Three interpulse intervals (6 msec, 12 msec, 18 msec) for the doublet were delivered in six possible combinations via the tibial nerve. Each doublet was preceded by a single twitch. Next, a fatigue protocol consisting of a 20 hz frequency delivered each second for 330 msec was delivered for two minutes. This protocol lead to significant fatigue. Immediately after the fatigue protocol the same doublet protocol was administered. Dependent measures included peak force, force-time integral, and the rate of force production both before and after the fatigue protocol. Following the fatigue protocol, the doublet lead to approximately 3 times the peak force and force-time integral than that of a single stimulus in the chronically paralyzed soleus muscle. Conversely, the acutely paralyzed soleus muscle showed no significant change in the output from the doublet. This study suggests that increased force production from the doublet was muscle fatigue dependent. Mechanisms and implications of these findings will be discussed.

492.3

INFLUENCES OF DAILY SHORTENING OR LENGTHENING CONTRACTIONS OF THE FUNCTIONAL PROPERTIES OF CHRONICALLY INACTIVATED SOLEUS. S. J. Harkema, R.R. Roy*, J.A. Hodgson, V. H. Zhong and V.R. Edgerton. BRI and Physiological Science Dept., UCLA, L.A., CA. 90024.

Spinal isolation (SI, transection of the spinal cord at T12-T13 and at L7-S1 and deafferentation between the two transection sites) was used to produce inactivation of the cat soleus for 4 months. We examined the effects of cyclical activity (1 Hz) mimicking the step cycle with electrical stimulation (40 Hz, 300ms, 30 min/d) during either lengthening (SI-L) or shortening (SI-S) on the functional deficits associated with chronic inactivation: MWt, muscle wet weight, TPT, isometric twitch time to peak tension; Po, maximum tetanic tension; Vmax, maximum rate of shortening; FI, fatigue index (% difference from control).

Group	MWt	Po	TPT	Vmax	FI
SI	-64	-81	-33	+57	-15
SI-S	-45	-68	-41	+29	-12
SI-L	-47	-60	-38	+58	- 5

The force frequency curve was shifted towards that of a faster type muscle after SI. This effect was attenuated by programmed activity. The in vivo soleus tendon force was ~7 and 9 times more during active than passive manipulation in the SI-L and SI-S cats, respectively. SI resulted in a smaller and faster muscle with general daily programmed activity ameliorating the adaptations in MWt, Po, and FI. SI-L had a greater effect than SI-S in Po and FI. The relative nonfatigability of the solcus was maintained in all groups. Thus, as little as 30 min/day of activity had a positive effect in maintaining the mechanical properties of a chronically inactivated muscle. Further, stimulation during lengthening was more effective than during shortening probably reflecting the larger force integrals during lengthening. (Supported by NIH Grants NS16333 and NRSA HD 7416).

492.4

COMPARISON OF FORCE-FREQUENCY RELATIONS IN HUMAN VOLUNTARY AND STIMULATED CONTRACTIONS. C. L. Rice* and B. Bigland-Ritchie. Dept. of Pediatrics, Yale University School of Medicine, New Haven, CT 06510 and The School of Physical and Occupational Therapy, McGill University, Montreal, Canada H3G 1Y5

In voluntary contractions muscle force is modulated by a combination of motor unit recruitment and rate-coding. When either whole muscle or single motor units are stimulated at different rates, a sigmoid force-frequency relation results. In contrast, during voluntary contractions, mean spike frequencies increase linearly with force up to 100% MVC (Bigland-Ritchie, et al. 1991; Thomas et al. 1991); a force which usually matches that from supramaximal stimulation. The purpose of this study was to compare, in the same muscle, forces generated by electrical stimulation with those generated by motor units recorded during voluntary contractions of various intensities.

Muscle force and motor unit firing rates were recorded from the quadriceps muscles of 16 subjects during voluntary contractions varying from 25% to 100% MVC. In separate experiments, quadriceps muscles were stimulated electrically at rates from 1 to 100 Hz. The two force-frequency relationships were compared. For forces up to about 60% of MVC, there was little discrepancy between voluntary and stimulated excitation rates. However, for higher forces the stimulated force increments per Hz became progressively smaller, while this non-linearity was largely avoided during voluntary contractions. Possible explanations for this discrepancy include: 1) the progressive recruitment of larger units at higher forces; 2) that forces from different units add non-linearly when their territories overlap (Clamann & Schelhorn, 1988); and 3) whether or not the CNS can excite all units to respond at maximal rates.

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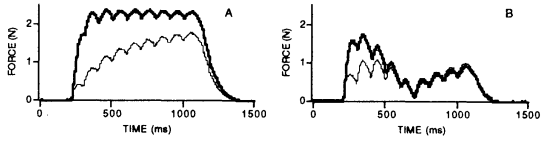
492.5

DOUBLET POTENTIATION IS DIMINISHED DURING SHORTENING MOVEMENTS OF CAT SOLEUS MUSCLE. T.G. Sandercock* and C.J. Heckman. Department of Physiology, Northwestern University School of Medicine, Chicago, IL 60611

When motor units are stimulated with low frequency trains, the addition of a single extra pulse, or doublet, at the beginning of the train can dramatically increase tension during an isometric contraction (Burke et al., Science 168:122-124, 1970). This study investigated the effects of muscle movement, using either isovelocity ramps or simulated locomotor patterns, on doublet potentiation in whole muscle or single motor units in cat soleus. The light line in Fig. A shows the isometric force produced by stimulating a portion of the soleus with a 10Hz train. The heavy line depicts the addition of a doublet (pulse added 10ms after the first pulse). In B, using identical stimulus patterns, the same muscle was shortened at 20mm/s from 0.2 to 0.7s, and then allowed to contract isometrically. Shortening movements diminished the effects of the doublets in whole muscle and motor units. Less dramatic results were seen with slower or shorter isovelocity ramps, or with locomotor type movements. Moderate isovelocity stretches of similar speed and distance had little effect on doublet potentiation.



492.6

EFFECTS OF TRAIN FREQUENCY AND FATIGUE STATE ON THE CATCHLIKE PROPERTY IN THE RAT GASTROCNEMIUS MUSCLE. S.A. Binder-Macleod* and L.J. Landis. Dept. of Phys. Therapy, Univ.of Delaware, Newark, DE, 19716

The catchlike property of skeletal muscle is the tension enhancement seen when an initial, brief interpulse interval (IPI) is added to the beginning of a subtetanic train of pulses. Though the train frequency and fatigue state have been reported to affect the catchlike property of a muscle these factors have never been simultaneously investigated. This study compared the force produced by trains with all pulses within the train separated by the same IPI (CFTs) to trains with the initial IPI = 10 ms and the remaining IPIs equal to the IPIs of the CFTs. These later trains were the catch-inducing trains. The IPIs of the CFTs ranged from 20 to 60 ms. Data were collected prior to fatigue, during repetitive fatiguing contractions, and during recovery from fatigue.

The CFTs with the 25 ms IPIs produced greater integrated forces (impulses) than any of the other CFTs during all fatigue states. This IPI was consistently slightly less than the twitch contraction times (TwCTs) of the muscles. In the nonfatigued state, the catch-inducing trains only augmented forces when the IPI of the trains were ~ 2 times the TwCT of the muscle. However, when the muscle was fatigued, the catch-inducing trains showed marked force augmentation for all trains with IPIs greater than or equal to the TwCT of the muscle. As the muscle recovered from fatigue, this augmentation gradually diminished. These data show for the first time that when a muscle is fatigued catch-inducing trains can produce greater impulses than even the optimal CFT. (Supported by NIH Grant AR41264)

492.7

MODELING ISOMETRIC FES MUSCLE FATIGUE BY ARTIFICIAL NEURAL NETWORKS. M. Dornay*, O. Yadid Pecht, J. Mizrahi, E. Isakov and Z. Susak. Dept. of Biomed. Eng., Technion, Haifa 32000, Israel and Loewenstein Rehabilitation Hospital, Ra'anana, Israel.

This study investigates the relation between the input electrical current applied to the paralyzed human quadriceps muscle in vivo during Functional Electrical Stimulation (FES) and the output muscle force. The electric current is applied by surface electrodes to the muscle, and the force is measured in isometric conditions. The instantaneous input to the muscle during FES consists of the following parameters: 1: Muscle length, described by the knee angle θ (60°); 2: Current frequency, f (20 Hz); 3: Current pulse width, D (0.25 msec) and 4: Current amplitude, A (0 to 130 mA). The output is muscle tension T (N). T at time t is the function of the input history to the muscle. We model T using a sampling rate of 1 Hz to represent the current amplitude A. The output force is very non-linear and it is difficult to estimate its magnitude in advance. We use 2-layer feedforward artificial neural networks simulations to learn the forward dynamics model of the force produced in the muscle. The training stage is done by supervised learning with the backpropagation algorithm. Following the training stage with muscle fatigue data of A = 50 or 70 mA FES, the neural networks predict the output force for the unknown case of muscle fatigue induced by 60 mA FES. The network can also extrapolate the output muscle force during a 50-60 seconds period, after learning the forward dynamics model of muscle force production during the first 50 seconds of FES stimulation. We propose that by using appropriate neural network architectures one can represent knowledge of FES experiments by training the weights of the networks. The network weights could be eventually stored on VLSI chips and help in the design of appropriate controllers aimed to replace the motor brain in activating paralyzed muscles. This study was supported by the Segal Foundation and the Sandra and Walter Kaye fund.

492.8

ELBOW-JOINT IMMOBILIZATION DECREASES FATIGABILITY AND ALTERS THE PATTERN OF ACTIVATION IN HUMANS. G.H. Yue*, M. Bilodeau, and R.M. Enoka. Dept. Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH 44195

The left arm of 8 subjects was immobilized in a fiber-glass cast and a sling for 4 weeks at a elbow joint angle of 90 degrees. Twenty-four-hour chronic EMG recordings indicated that the activity of the biceps brachii muscle was significantly reduced during immobilization. Before and after immobilization, the following variables were measured: (1) elbow-flexion force, and EMG signals from the biceps brachii and brachioradialis muscles during maximal voluntary contractions (MVCs); (2) fatigability of the elbow-flexor muscles when force was sustained at 20 an 65% of the MVC force; (3) cross-sectional areas and volume of the biceps and triceps brachii, and brachioradialis muscles measured with magnetic resonance imaging (MRI); and (4) the degree of muscle (biceps brachii and brachioradialis) activation during an elbow-flexion MVC as evaluated with MRI T2 relaxation time. The immobilization induced a significant decrease in the elbow-flexion MVC force and cross-sectional areas and volume of the elbow flexor muscles, but the reduction in muscle size was less than the decline in MVC force. After immobilization, the fatigability of the elbow flexors was decreased (more resistant to fatigue) at both force levels (20 and 65% MVC). There were also significant changes in the EMG activity and T2-indicated degree of activation during the MVC. The neuromuscular adaptations were diverse and had different effects on the various physiological processes.

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492.9

CHANGES IN MUSCLE MEMBRANE EXCITABILITY FOLLOWING FATIGUE. L.McFadden, V.Galea and A.J.McComas*. Dept.Biomed.Sci., McMaster Univ., Hamilton, ON, Canada L8N 3Z5.

Following either supramaximal electrical stimulation or maximal voluntary contractions sufficient to induce fatigue, a full or near full recovery of the muscle compound action potential (M-wave) has been observed within 3 minutes of cessation of the fatiguing process. (Galea and McComas, J.Physiol. 438:212P, 1991). Little attention has been given to the excitability during recovery, and we have found evidence of a late depression.

Fatigue was induced by 20 Hz supramaximal stimulation of the motor nerve of the biceps brachii muscle in healthy men of varying activity levels, and the recovery period was examined for up to 8 hours post fatigue. Immediately after cessation of the fatiguing stimulus the amplitude and area of the M-wave had returned to control values, whereas the isometric twitch force was significantly reduced in all subjects. The M-waves declined throughout the recovery period, with one group (n=9) reaching a maximum M-wave depression (DEP) between 7 to 30 minutes post fatigue (60.4 $\pm$ 9.0% control); whereas another group showed maximum M-wave depression much later in the recovery period, between 13 minutes and 4 hours post fatigue (DEP= 42.4 $\pm$ 6.2 % control). The isometric twitch force recovered gradually, returning to control values by the end of the 8 hour recovery period. The depression of M-wave activity may be explained by a reduction in Na^+-K^+ pump activity or by increased open times of the sodium channels.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS IX

493.1

A RODENT NAVIGATION MODEL THAT COMBINES PLACE CODE, HEAD DIRECTION, AND PATH INTEGRATION INFORMATION. H.S. Wan, D.S. Touretzky,* and A.D. Redish. Computer Science Dept., Carnegie Mellon, Pittsburgh PA 15213.

This model, utilizing coupled mechanisms for place recognition, maintenance of head direction, and path integration, replicates a variety of rodent behavioral and neurophysiological data. We simulated experiments by Collett, Cartwright, and Smith on gerbils performing a family of open-field landmark-based search tasks, and experiments by Cheng on rats navigating to a goal location in a rectangular arena.

Experiments by Mittelstaedt & Mittelstaedt have shown that gerbils possess a path integration faculty allowing them to follow a direct trajectory back to the nest after performing a random search. Presumably the animal's position in some internal coordinate system is being updated with each motor action. Although the neural basis of this ability is unknown, it clearly requires an accurate sense of head direction. Head direction cells have been found in several areas of the rat brain.

In addition to a path integrator and units coding for head direction, our model contains units with place fields controlled by visual landmarks, reflecting properties of hippocampal pyramidal cells. However, unlike other models of place cells, our model also accounts for the persistence of place fields in the absence of any visual input, by allowing place units to be driven by the path integrator. Thus, in our theory, the role of the hippocampal formation is to learn associations between external state (views of landmarks) and internal state (path integrator coordinates). Visual cues, when available, drive the place units, which can then correct for drift in the path integrator or reset it to a known value when the animal is first placed in a familiar apparatus, such as a radial maze.

Our model shows how allocentric bearings derived from head direction can be used to disambiguate visually identical landmarks, replicating the gerbil and rat data. Conversely, local view information can be used to correct for drift in the head direction estimate. Parallel relaxation among place units produces a consistent place code even when the model is presented with a distorted landmark array, emulating the robustness that animals show in novel situations. Parts of the model have been implemented on a mobile robot.

493.2

AROUSAL DEPENDENT ACTIVATION OF FD MOLECULAR LAYER GLYCOGEN PHOSPHORYLASE-A. A. Uecker*, G. Rao, B.L. McNaughton, C.A. Barnes, T.A. Foley and E.M. Reiman. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

"Patches" of glycogen phosphorylase-a activity observed in the fascia dentata (FD) molecular layer (Harley & O'Keefe, 1992; Wallace, 1982) occur following microinjections of glutamate into the entorhinal cortex (Harley & O'Keefe, 1986) and are correlated with the depletion of glycogen stores in the nervous system during metabolic work (e.g. Wallace, 1983). In addition, *reduced* phosphorylase-a activity in the FD molecular layer after exploration (Uecker et al, 1994) concurs with single cell recording studies (Ranck, 1973; Thompson & Best, 1989) showing that most hippocampal neurons are least active during behaviors typically associated with hippocampal theta rhythm, i.e. active exploration and paradoxical sleep. Place cell activity is maintained when an animal explores or is manually transported to an identified place field, but dramatically reduced after it has been snugly restrained (Foster, et al., Science, 244:1580, 1989), although the theta rhythm is only marginally reduced. We hypothesized that phosphorylase activity would be minimized under restraint, relative to animals either freely exploring, manually moved, or anesthetized with sodium pentobarbital. FD molecular layer glycogen phosphorylase-a activity was measured in 12, 6 month old female Long-Evans rats. Contrary to the firing-rate based expectation, phosphorylase-a activity was least during exploration, higher during passive-movement, higher yet during restrained movement, and highest under anesthesia. The omnibus F was significant ($p < .05$). Phosphorylase-a expression may thus be more correlated (inversely) with hippocampal theta rhythm and/or septohippocampal activity than with principal cell firing-rates *per se*. Supported by MH48824, MH00897 and the Flinn Foundation.

493.3

TOWARDS A COMPUTATIONAL MODEL OF THE HIPPOCAMPAL FORMATION INCORPORATING REALISTIC ANATOMICAL CONNECTIVITY: AMMON'S HORN AREA CA3. P. Patton* and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

A computational model of the rat hippocampal formation incorporating realistic anatomical connectivity is being developed. This report focuses on Ammon's horn area CA3. Points representing neurons were distributed through a rectangular sheet representing this structure. 250,000 pyramidal cells were assumed (West et al., Anat. Rec. 231:482-497). Major extrinsic inputs to CA3 derive from the entorhinal cortex and FD granule cells. Entorhinal input possesses a rough topographic organization, and is divergent, with individual arbors extending up to 2 mm septotemporally (Tamamaki and Nojyo, Hippocampus 3:471-480). Entorhinal arbors are represented as rectangles extending across CA3. Each entorhinal neuron makes about 4700 synaptic contacts onto pyramidal cells (Amaral et al., Prog. Brain Res. 83:1-11). Each granule cell makes about 15 mossy fiber contacts onto pyramidal cells (Claiborne et al., JCN 246:435-458). CA3 pyramidal cells possess a network of recurrent collaterals, represented in the model as diagonal swaths like those described by Ishizuka et al. (JCN 295:580-623), with each cell receiving 6,000 ipsilateral synaptic contacts. These cells also provide a significant input to the inner molecular layer of FD (Li et al., JCN 339:181-208). 5 groups of CA3 non-principal cells were represented: vertical and horizontal basket cells, chandelier cells, spiny non-principal cells, stratum oriens cells, and superficial cells. CA3 contains an estimated 67,000 non-principal cells (Dietz et al., Verh. Anat. Ges. 81:883-884). This anatomical representation will serve as a basis for models of postulated hippocampal operations such as associative memory. Supported by MH46823.

493.4

SIMULATION OF THE SPONTANEOUS REACTIVATION OF EXPERIENCE-SPECIFIC HIPPOCAMPAL CELL ASSEMBLIES DURING SLEEP. B. Shen and B.L. McNaughton*. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

During slow-wave sleep (SWS) following periods of spatial activity, CA1 "place-cells" that were temporally correlated, by virtue of the overlap of their place fields, exhibit enhanced temporal correlations, even though the animal sleeps in a different location (Wilson and McNaughton, Neurosci. Abs., '93, '94). The discharge of cells with overlapped place fields was more correlated in subsequent sleep, particularly during sharp-waves, than in sleep episodes prior to the behavior, or than cell-pairs with non-overlapped place fields. To explore the plausibility that this reflects spontaneous reactivation of cell assemblies in the CA3 recurrent association system, we constructed a simple model in which place fields are represented as 2-D gaussians, and connection weights between cells with place fields in the environment are proportional to the spatial overlap of their firing probability density functions (a Hebbian learning rule), which falls off exponentially with the square of the distance between place field centers. The network was presented with random input patterns to simulate the presumed condition during SWS. Consistent with results of Pavlides and Winson (1989), simulated cells with place fields exhibited higher firing during "SWS" than cells without place fields, and, as observed by Wilson and McNaughton, cells with overlapped fields were highly positively correlated, whereas those with non-overlapped fields were weakly negatively correlated. Moreover, during correlated states, coherent patterns (cell assemblies) corresponding to specific locations were reactivated. The random inputs resulted in all-or-nothing network reactivations of place representations, irregularly distributed in time in a manner reminiscent of hippocampal sharp-wave activity during SWS. Supported by MH46823.

493.5

SPARSE VS. DISTRIBUTED POPULATION CODING IN SUPERFICIAL AND DEEP LAYERS OF RAT NEOCORTEX. W.E. Skaggs*, B.L. McNaughton, C.A. Barnes, K. Moore, C. Duffield, L. Frank and R. D'Monte. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

324 cells were recorded extracellularly from two areas of the rat neocortex (Zilles's areas HL and OC2ml), using a parallel recording method that permitted monitoring of up to 40 individual neurons at the same time. Excluding a small group with distinctive properties (putative interneurons), during sleep, all superficial cells fired at mean rates of 0.1- 0.5 Hz, with little variance across the population. While the rat ran for food reward on a triangle-maze, the population mean rate was not much different, but the population variance was much higher, with many cells shutting off almost completely, while others exhibited behavioral correlates, and fired at instantaneous rates of up to 30 Hz for brief periods of time. In contrast, most cells in the deeper layers fired at mean rates an order of magnitude higher, during both sleep and performance of the task. The depth of modulation varied considerably, but it was very unusual for deep-layer cells to be silent for extended periods of time. Thus the overall pattern of activity appears much more sparse in the superficial neocortical layers than in the deep layers. These findings are of interest in light of the fact that the superficial layers are the predominant source of the cortico-cortical association system. A role in memory has been proposed for this system by theorists including Marr (1970) and Eccles (1978, etc.); and it has been shown mathematically that sparse activity patterns confer special benefits on associative memory networks by reducing interference between jointly stored patterns. Moreover, the majority of neocortical NMDA receptors are located in the superficial layers (Monaghan and Cotman, 1989). Supported by MH46823 and MH00897.

493.6

POPULATION ACTIVITY PATTERNS IN HIPPOCAMPUS AND NEOCORTEX PERSIST MUCH LONGER DURING WAKING AND REM SLEEP THAN DURING SLOW-WAVE SLEEP. K. M. Moore, W. E. Skaggs, B. L. McNaughton, C.A. Barnes*, R. D'monte, C. Duffield. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

To gain insight into the possible role of sleep in memory consolidation, we quantified the structure of population dynamics during different brain states. Using a parallel recording method (Wilson and McNaughton, 1992), populations of up to 40 neocortical cells or up to 70 hippocampal cells were recorded simultaneously in the neocortex or area CA1 of rats, during behavioral states of REM sleep, slow wave sleep (SWS), or performance of a spatial task. The persistence of population activity patterns in each state was quantified by calculating the correlations between population firing rate vectors at different times, with care to exclude high-rate cells (putative interneurons) from the populations. In each behavioral state, the mean correlations decayed more or less exponentially as a function of time interval, and the time constant of decay was taken as a measure of the average persistence of an activity pattern. The approximate time constants observed, both in neocortex and hippocampus, were: SWS, 100-200 msec; REM sleep, 1-2 sec; active locomotion, 400 msec-2 sec. As might be expected, the persistence of correlated states during behavior appears to be a function of the behavior itself. During SWS, correlations persist on a timescale similar to hippocampal sharp-waves, consistent with a possible hippocampal origin of the correlated states. During REM, the persistence is not correlated with any obvious neocortical or hippocampal EEG phenomenon. These findings add to the extensive evidence for similarities between REM sleep and the waking state. Supported by MH46823 and MH00897.

493.7

PRESERVATION OF TEMPORAL ORDER IN HIPPOCAMPAL MEMORY REACTIVATION DURING SLOW WAVE SLEEP. M.A. Wilson*, and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson AZ 85724.

In a previous study, it was found that coactivity of cells during behavior produced an increase in discharge correlation between these cells during subsequent slow wave sleep (Wilson and McNaughton, 1994). This suggested that hippocampal memories for recent experience were recapitulated during these sleep periods. A question which remained unanswered was whether the temporal structure of experience was also preserved and was present during sleep activity. To address this question, 50-100 cells in area CA1 of the hippocampus were recorded simultaneously during spatial behavioral tasks. Individual pairwise correlation histograms taken during these tasks were classified based on their direction of skew. In each pair, if firing of cell 1 (master cell) tended to precede the firing of cell 2, this pair was assigned a positive skew. The converse relationship was assigned a negative skew. During behavior, this skew resulted from the relative spatial locations of the "place fields" for each cell, and the specific pattern of behavior the animal would follow during each task. It was found that the direction of skew of the cross correlation function for pairs examined during behavior (peak shift 1-2 sec) determined the direction of skew of the same pairs during the immediately following slow wave sleep period (peak shift 5-10 msec). This indicates that both the structure and temporally compressed order of recently experienced states are recapitulated in the hippocampus during slow wave sleep. The preservation of both coordinated states and their temporal order suggests a mechanism within the hippocampus which is capable of storage and reactivation of temporally sequenced information. Supported by MH46823 and McDonnell-Pew.

493.8

VARIATIONS IN PLACE-SPECIFIC FIRING OF HIPPOCAMPAL NEURONS ALONG THE SEPTOTEMPORAL AXIS. M.W. Jung*, S.I. Wiener, K. Moore, S. Kipen, and B.L. McNaughton, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

The anatomical and neurochemical organization of hippocampal circuitry exhibit a substantial septo-temporal gradient. Although it is well established that place-specific firing is a fundamental characteristic of hippocampal neurons, almost all unit recording studies in freely behaving animals have come from the septal one third, largely for technical reasons. In the present study, therefore, single units were recorded in the dorsal and ventral hippocampi of opposite hemispheres in the same rats, in many cases simultaneously. Units were first identified as the rats entered slow wave sleep. The rats then performed a food reinforced, random search task in a square chamber containing simple visual landmarks. As in dorsal hippocampus, ventral CA1 units could be classified as "complex spike" (CS-pyramidal) cells or "theta" interneurons. Both dorsal and ventral theta cells fired at relatively high rates with low spatial selectivity in the arena. As previously reported (Soc. Neurosci. Abs., 19:796), "place cells" were found among the CS cells in the ventral hippocampus; however, significantly fewer ventral units had "place fields" than dorsal units, and the spatial selectivity of ventral units was significantly lower than that of dorsal units. Thus a septotemporal gradient of spatial selectivity was found in the CA1 field of the hippocampus, complementing many other anatomical and neuropharmacological studies. A number of possible functional interpretations can be suggested from these results, including a computational advantage of representing space at different scales or a preeminence of essentially non-spatial information processing in the ventral hippocampus. Supported by NS20331 and Human Frontiers in Science.

493.9

HIPPOCAMPAL PLACE FIELDS CHANGE WITH NAVIGATIONAL CONTEXT. Y. Qin, E.J. Markus*, B.L. McNaughton and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

When general behavioral requirements are held constant, rat hippocampal complex spike cells fire selectively with respect to the animal's location. The locations of "place fields" of individual cells are generally quite robust against manipulations such as additions or deletions of visual stimuli, darkness, or allowing the rat to explore previously unexplored adjacent regions of space. Although firing rates are modulated by behavioral variables such as running velocity or direction of turning, the location of firing is relatively unaffected by the specific behavior. In the present study, the effects of the navigational requirements of the task were assessed in fixed environments. Hippocampal place cells were recorded in adult male rats (n=15) as they searched for chocolate either on a radial arm maze or a circular platform. The rats would first retrieve randomly scattered chocolate; then the task was altered by placing the food sequentially and repeatedly in 4 specific locations. This resulted in a switch from a random to a directed search pattern by the rats, typically over the course of 1-2 minutes. On the circular platform, this also involved a change in the trajectories and distribution of locations visited. The switch from random to directed search was accompanied by a pronounced change in the location of the place field in approximately 1/3 of the cells on the circular platform. There was a smaller, but significant effect when the recordings were conducted on the radial arm maze. In 2 rats the directed search was routinely followed by a second random search episode. In these cases, the place fields returned to their original location. We conclude that, if hippocampal cells encode location *per se*, the representation changes in different behavioral or navigational contexts. Supported by MH00897 & ONR.

493.10

BEHAVIORAL CORRELATES OF HIPPOCAMPAL CA1 CELLS IN A LANDMARK NAVIGATION TASK. K.M. Gothard*, W.E. Skaggs, K.M. Moore and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

To address the nature of hippocampal representation in a landmark navigation task modeled after Collett et al. (J. Comp. Physiol., 1986), three rats were trained to find food at a location predicted by two movable landmarks in a 3.5 m diameter circular arena surrounded by static background cues. On each trial the rat was released from a box to which it returned (at a different location) for reward after finding the goal. CA1 cells were recorded using a microdrive with 12 movable tetrodes (Wilson and McNaughton, Science 261:1055-1058, 1993) which enabled monitoring the simultaneous activity of up to 43 cells. Of 463 recorded cells, 199 had clear behavioral correlates during the task. Four major categories of cells were observed: 111 were place cells with single or double place fields that were stable with respect to the static background cues; 51 were box-related cells that fired either when the rat was inside the box, or as it was leaving or entering the box, regardless of the position of the box relative to the static cues; 8 were landmark/goal related cells that fired in the vicinity of the goal, but only for certain conjunctions of the location of the landmarks and the static cues; and 23 were cells related to more than one variable of the task. In some cases, the goal-related or box-related cells could be shown to fire simultaneously (± 50 msec) with place cells, suggesting that in the hippocampus multiple representations are processed simultaneously and that the hippocampus can transmit a distributed representation of specific objects and/or events within the spatial context in which they occur. Supported by NS20331 and ONR.

493.11

DYNAMICS OF VISUAL CUE CONTROL OVER HEAD DIRECTION CELLS AND PLACE CELLS. J.J. Knierim*, H.S. Kudrimoti and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Thalamic head direction (H) cells and hippocampal place cells comprise a tightly coupled system. We investigated the dynamics of visual cue control over these cells by introducing conflicting direction information between visual cues and internal cues. Rats foraged for food in a high-walled apparatus with a single salient cue card. In some experiments, we disoriented the rats before each session. This weakens visual cue control over place and H cells (Knierim et al., Soc. Nsci. Abst., 1993), although most cells have a preferred firing direction or location relative to the cue card over multiple sessions. In 30% of 119 sessions, however, firing orientation rotated 30°- 130° within the session, usually in the first few min. In 25/34 cases, rotation was from an "incorrect" orientation to the "correct" one. In 6 cases, cells rotated from one incorrect orientation to another. In 3 cases, cells started at the correct orientation, rotated away, but then returned. In no cases did cells permanently rotate away from the correct orientation. In other experiments, we abruptly rotated both apparatus and rat by 180°. Typically, cells either immediately rotated 180° with the cue, or remained at the same orientation relative to the external world. In other sessions, however, cells initially maintained their external orientations, but then rotated 180° smoothly over a few min until they returned to the original orientation relative to the cue card. Thus, visual cues can correct for large errors in spatial orientation with a slow, steady rotation of both the H cell system and the hippocampal spatial map until they realign with the cue. The progressive shift suggests that transitions between neighboring network states are preferred over transitions among less similar states. Supported by ONR and NS09052.

493.12

A DELAYED CONDITIONAL SPATIAL DISCRIMINATION TASK FOR RATS. T.M. Abdulaziz , C.A. Barnes, B.L. McNaughton, C. Duffield, L.T. Church. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

In preparation for a study of the neural correlates of route planning and short-term memory, rats were trained to perform a delayed conditional spatial discrimination task on an alternating T-maze on which the two opposite arms (reward arms) were always in the same position in space, and the orthogonal start arm alternated pseudorandomly from one side to the other. The animals were pretrained that specific food items (S1 and S2) could be found only at the associated reward arms and thus at specific locations in space. For conditional trials, animals were placed on a randomly selected start arm, and presented with a small sample of either S1 or S2, which served as a cue to guide the rat's subsequent spatial response. Only the food item presented in the start arm was made available on its appropriate reward arm. The other arm contained no reward. Thus, animals had to match the food sample given in the start arm with the correct location in space. Trials were counterbalanced to counteract response biases of individual animals or the development of a response strategy. Animals were trained to a criterion of 85%. Once animals reached this criterion, delays were imposed after presentation of the food cue, before the animal was allowed to respond. Delays ranged from 10 to 60 seconds in 10 second increments. These delays were grouped either as short (10 and 20 seconds), intermediate (30 and 40 seconds), or long (50 and 60 seconds). Percentage of correct response decreased with increasing length of delay, approaching chance by the longest delays. The effects of lesions to hippocampus and medial prefrontal cortex, as well as neuronal correlates in these areas during the delay phase are under investigation. Supported by NS20331, AG03376 and MH00897.

493.13

AGE-RELATED DECREASE IN THE NMDA-MEDIATED EPSP IN FD. G. Rao*, C.A. Barnes, B.L. McNaughton and I. Shen. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Previously we demonstrated a decline in both the non-NMDA and NMDA-mediated Schaffer collateral EPSP in senescent CA1. Here, we characterize the NMDA response in senescent fascia dentata (FD), where, although there is a smaller presynaptic fiber potential for a given stimulus intensity, indicating loss of perforant path fibers, the non-NMDA EPSP is actually larger for a given fiber volley amplitude (Barnes and McNaughton, 1980), indicating that individual perforant path synapses of old animals are more powerful (Foster et al., 1991). F344 rats (n=18, 26-35 days; n=18, 9 mo; n=18, 24-27 mo) showed an age-related deficit in spatial learning ability in the spatial version of the Morris water task, (% time in target quadrant : yng = 45.3 ± 5.2; adult = 39.8 ± 6.7; old = 24.9 ± 7.3) with no impairment in visual discrimination ability. Hippocampal slices were then prepared and incubated in low Mg^{++} ACSF. Recording and stimulating electrodes were placed in FD. Input-output curves of the relation between the presynaptic fiber volley and EPSP were constructed before and after local application of CNQX, a non-$NMDA_R$ (AMPA) antagonist. Abolition of the residual response by AP5 confirmed that it was $NMDA_R$-mediated. As found previously, prior to CNQX application the EPSP-to-fiber volley ratio was significantly larger in the old rats. After CNQX application, the $NMDA_R$ component for a given fiber volley amplitude was significantly reduced ($p<.005$) in the old rats relative to the younger age groups. This result demonstrates that components of excitatory synaptic transmission within the same hippocampal subregion are differentially affected by the aging process, even when the receptor subtypes may be colocalized. Supported by AG03376 and MH00897.

493.14

AGE-RELATED DECREASE IN CHOLINERGIC SYNAPTIC TRANSMISSION IN FD, CA3, AND CA1 OF RAT HIPPOCAMPUS. I. Shen*, G. Rao and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

The electrophysiological consequence of neurochemical cholinergic deficits in normal aging has been examined in area CA1 of hippocampus where the cholinergic slow epsp has been found to be reduced in aged rats (Potier et al., 1992; Taylor and Griffith, 1993). Whether this change occurs in all three hippocampal subregions and whether it covaries with age-related behavioral deficits has been studied here. F-344 rats (n=31, 3 wk; n=33, 9 mo; n=35, 24-27 mo) were tested in the Morris water task. There was an age-related deficit in spatial learning ability. Hippocampal slices were subsequently prepared, and the atropine-sensitive, cholinergic slow epsp was recorded intracellularly in CA1 and CA3 pyramidal cells and FD granule cells. The responses were induced by stimulation (40-50 Hz, 0.5 sec) at each of two intensities of either stratum oriens (CA1, CA3) or stratum granulosum (FD). Within each age group, the cholinergic slow epsp was larger in CA3 than in either CA1 or FD. There were no age differences in intrinsic biophysical properties of principal cells within each subregion; however, the amplitude of the cholinergic slow epsp was significantly reduced in old rats in all areas (CA1-59%; CA3-55% and FD-56%, $p<0.001$). The 3 wk and 9 mo rats did not differ. The amplitude of the slow epsp in CA1 was correlated with spatial learning on the Morris water task in old rats ($p<0.01$). In conclusion, our data suggest that cholinergic synaptic transmission in all three subregions of the hippocampus is compromised in normal aging. The possible pre- and post-synaptic loci of these changes are under investigation. Supported by AG03376 and MH00897.

493.15

ALTERED IEG INDUCTION AFTER LTP IN MEMORY-DEFICIENT AGED RATS. A. Lanahan, P.F. Worley, G. Lyford, G.S. Stevenson*, G. Rao, L. Church, and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724 and Dept. of Neuroscience and Neurology, Johns Hopkins Univ., Baltimore, MD 21205.

Aged rats show deficits in the maintenance of hippocampal LTP and in spatial memory. To identify possible molecular mechaniams of these changes, we examined the regulation of a panel of immediate early genes induced in association with LTP. Seven young (9-10 mo) and 7 old (26-28 mo) F344 rats were bilaterally implanted with electrodes for evoking and monitoring perforant path field potentials in fascia dentata (FD). Recording commenced approximately 2 weeks after surgical recovery, at which time rats were given 50 mg/kg cycloheximide (i.p.), followed by 1 hour of low-frequency test stimulation (0.1 Hz). Five young and 5 old rats received bilateral repeated 100 pulse bursts of high-frequency stimulation every 7.5 min, and 2 rats in each age group were used as implanted controls. Poly (A+) RNA (Fasttrack, Invitrogen) was prepared from FD that was microdissected from the rest of the hippocampus, and used to prepare cDNA by reverse transcription (Superscript, BRL). The cDNAs were [32P] labeled by random priming and used to screen a panel of IEGs with the "Reverse Northern" technique. Hybridization to each of the IEGs was quantified (phosphormager, Molecular Dynamics) and normalized to a non-inducible mRNA, glutaraldehyde phosphate dehydrogenase (GAPD). Most IEGs were similarly induced in the young and aged animals, however the induction of c-*fos* was much more robust in the old than the young animals and this difference was significant at p = 0.034. These observations indicate that mechanisms controlling the induction or turnover of c-*fos* are selectively altered in aged, behaviorally impaired animals. Support: AG09219, MH00897.

493.16

UNILATERAL NMDA LESIONS OF THE HIPPOCAMPUS DO NOT AFFECT WORKING MEMORY IN AGED RATS. A.H.J. Herremans, A. Van Hest*, T.H. Hijzen, J.L. Slangen. Dept. of psychopharmacology, Faculty of Pharmacy, Rudolf Magnus Institute for Neuroscience, Utrecht University, Sorbonnelaan 16, 3584 CA, Utrecht, The Netherlands.

The NMDA receptor in the hippocampal CA1 area is suggested a role in memory function. Delayed Conditional Discrimination (DCD) tasks are used to investigate working memory (WM). In the task effects on WM can be separated from other effects by examining delay dependency of an effect. Recently a DCD tasks was developed that is not sensitive to mediating behavior based on spatial cues (Herremans et al. 1994, phys & beh.). Involvement of NMDA receptors in the CA1 in the DCD task was examined in 23 aged rats. 16 trained rats were equipped with a guide canulae unilateral in the hippocampal CA1 area. Ten days after operation performance in the DCD task was impaired in the operated animals ($F(1,21)=5.56$) and not in 7 unoperated controls. No interaction with delay was found. After retraining for 12 sessions operated animals relearned the DCD task to preoperation levels. Next, eight animals were infused with NMDA (120 mMol, 0,5 μL) and eight animals received phosphate buffer. Four days after the NMDA lesion performance of lesioned animals never differed from controls during four days measured. Histological examination showed mechanical lesions of the CA1 in all operated animals. It is concluded that mechanical lesions in the CA1 temporarily impaired performance in the DCD task in old rats (recovery within 12 days) but did not affect WM. Subsequent NMDA lesions had no effect.

493.17

CEREBELLAR MECHANISMS OF EYELID CONDITIONING: MATHEMATICAL ANALYSIS OF PLASTICITY AT TWO COMPETING SITES. G.T. Kenyon* and M.D. Mauk Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Empirical evidence suggests the involvement of two sites of synaptic plasticity in simple forms of motor learning: one in the cerebellar cortex and another in the cerebellar nucleus. A mathematical model based on the synaptic organization of the cerebellar-olivary system is used to analyze the relative contributions from these two sites. Based on experimental data, we assume that plasticity at synapses from granule to Purkinje (Gr→Pkj) cells display generalized long term depression: synaptic weights decrease when active during elevated calcium induced by climbing fiber input and increase when active in the absence of climbing fiber input (during which calcium levels should be reduced). We find that incorporating standard generalized Hebbian plasticity at synapses from mossy fibers to cerebellar nucleus cells (mf→nuc) leads to runaway changes in synaptic strength. However, when a generalized Hebbian rule is modified such that mf→nuc synaptic weights only decrease when active during tonic Purkinje cell inhibition, synaptic weights become stable. Furthermore, the system is self-regulating such that neural activity is always driven to equilibrium levels at which synaptic weights remain constant. We next simulate idealized conditioning trials. An unconditioned stimulus, which increases the excitatory drive to climbing fibers, induces adaptive changes in the subsets of Gr→Pkj and mf→nuc synaptic weights encoding the conditioned stimulus (CS). We assume that Gr→Pkj synapses provide greater temporal discrimination such that a single subset of mf→nuc synapses but many different subsets of Gr→Pkj synapses are activated throughout the CS. We show that each subset of Gr→Pkj synapses active during the CS attempts to transfer plasticity to the nucleus. Thus, the relative contribution to conditioned responses made by plasticity at mf→nuc synapses is related to the weighted average across all subsets of Gr→Pkj synapses active during the CS.

493.18

CLASSICAL CONDITIONING AND VALUE-DEPENDENT LEARNING IN NOMAD, A REAL WORLD ARTIFACT. P.F.M.J. Verschure, J. Wray and G.M. Edelman*. The Neurosciences Institute, 3377 North Torrey Pines Court, La Jolla, CA 92037.

In previous work, it was demonstrated how adaptive behavior can occur in a real world artifact, NOMAD, carrying out a tracking and a block-sorting task (1). NOMAD is a mobile automaton with visual and auditory sensors and a magnetic snout that allows it to "taste" the conductivity of colored objects and pick them up. The behavior of NOMAD is controlled by a simulated neuron-based nervous system containing circuitry for the execution of reflexes and fixed or sensory-driven action patterns (avoidance, approach, exploration). States of the sensors are projected onto mapped and non-mapped neural areas. Initially, the value system is fixed and innate (e.g. "light is better than no light"). It is modeled in terms of the properties of a diffuse ascending system that can modulate synaptic plasticity. Recently, the modeling of value has been expanded to include the experience-dependent modification of value itself (acquired value) (2). In the present work, acquired value was incorporated into the nervous system of NOMAD. Our goal was to show how behavioral regularities resulting from classical conditioning can be understood in terms of value-dependent learning. A convergence of sensory and motor components takes place in a neural region that is an analog of the amygdala. Acquired value is achieved by synaptic modification of projections from the sensory areas to the diffuse ascending value system. As a result, discrimination of color preferences associated with value became more rapid and the responses became more marked. To test this anatomical and dynamical arrangement NOMAD is being subjected to classical conditioning paradigms emphasizing blocking and secondary conditioning with auditory stimuli. We analyze the conditioned behavior of NOMAD in terms of the success of avoidance and approach responses, the correlated states of its nervous system, and the effect of modifications in the stimulus environment. 1. G. M. Edelman, et al., *PNAS*, **89**, 7267-7271 (1992). 2. K. J. Friston, G. Tononi, G. N. J. Reeke, O. Sporns, G. M. Edelman, *Neuroscience*, **59**, 229-243 (1994).

493.19

NEURAL NETWORK MODEL FOR CONDITIONED AMYGDALOID NEURAL RESPONSES. K. Muramoto*, T. Ono+, and H. Nishijo+ *Dept. Elec. & Computer Eng., Fac. Tech., Kanazawa Univ., Kanazawa 920, +Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan.

The amygdaloid neuron model was constructed from single neuron activity data [1]. Significant features were the acquisition to conditioned stimuli and extinction after acquisition. A mathematical model of the amygdaloid neural network is presented to quantitatively produce the elemental physiological mechanisms of learning. General model for single neuron is proposed by McCulloch-Pitts. Main functions of the McCulloch-Pitts model are all-or-none response, potential summation and synaptic delay. Added features to the model were the acquisition and extinction to conditioned stimuli. These features were described by connection weight between neurons. When the conditioned response is followed by the unconditioned response after short delay, connection weight increases. The connection weight is always decreased by small value. Detailed temporal firing activities of the model were examined under various conditions. Results show that neurons acquired responses to conditioned stimuli associated with unconditioned ones and these responses were extinguished by extinction.

[1] Muramoto, Ono, Nishijo & Fukuda (1993) Neurosci. 52, 621-636.

493.20

MODELING BIOLOGICAL NEURONS WITH SPATIOTEMPORAL EVENT MAPPING (STEM) CELLS S.D. Murphy* and E.W. Kairiss. Interdepartmental Neuroscience Program, Department of Psychology, Center for Theoretical and Applied Neuroscience (CTAN), Yale University, New Haven CT 06511.

A biological neuron can be viewed as a match-filter that instantiates a mapping, $\mathcal{M}$, from multidimensional spatio-temporal (synaptic) events to unidimensional temporal events (action potentials). A computational abstraction of a biological neuron called a Spatio-Temporal Event Mapping (STEM) cell has been designed to perform this mapping in a general way. The three major components of the STEM architecture are (1) an input time-to-space mapping layer (2) a nonlinear spatial mapping layer (3) a recurrent time-to-space mapping layer. A STEM cell implementation of a biophysical model is developed by training on input-output mappings generated from random activation patterns applied to the biophysical model. We show that the STEM cell is capable of learning $\mathcal{M}$ for biophysical models of cortical pyramidal cells of varying complexity, and that it offers advantages over biophysical models in terms of computational efficiency, analytical tractability, and as an avenue towards comparative complexity parameterization of different biophysical models. Because STEM cells are computationally cheaper than conventional differential-equation-based biophysical models, networks of STEM cells can be used to investigate the dynamics of biological neural networks at a scale that is not practical with conventional biophysical models.

493.21

SEQUENTIAL INFORMATION PROCESSING: THE NECESSITY FOR MAPPING TIME INTO SPACE. K. Schill, C. Zetzsche and E. Pöppel *
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Problems in modeling the perception of dynamic scenes have casted doubts upon the concept of "iconic memory". Analysing current concepts of visual temporal information processing reveals that some basic inconsistencies regarding the temporal properties have not yet been resolved. We have developed a model for the representation of spatio-temporal information in early vision [1]. Its key feature is the mapping of temporal structure into *simultaneously accessible locally distributed activities*. Although the model is aimed at the description of spatio-temporal information processing, application to tachistoscopic presentations also predicts basic results obtained in this field. Furthermore, the model resolves inconsistencies of processing rates obtained with partial report and backward masking paradigms. The standard view on neural information processing, that information is represented by the spatio-temporal activity of discrete elements (neurons), is shown to *necessarily* imply a *discrete* internal representation of time. We believe that the need for the proposed mapping is so fundamental as to be expected to appear in a distributed fashion on various parallel and hierarchical levels in the nervous system. However, since on subsequent levels, sequences with larger temporal extension have to be represented, information reduction has to be applied to avoid combinatorical explosion of connections. Future theories and investigations should concentrate on the syntactical and semantical aspects of suitable integration mechanisms and how they are represented. [1] Schill K., Zetzsche C.(1994): A model of visual spatio-temporal memory: the icon revisited, Psych. Res., in press.

493.22

INFORMATION TRANSFER IN NETWORKS WITH HEBBIAN CONNECTIONS. E. Salinas, E. Marder* and L.F. Abbott. Biology Department and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

In many systems the firing rates of a population of neurons code for the value of an external variable. We investigate how information about the coded quantity can be communicated from one population (or topographic map) to another. We also explore how nervous systems might combine these representations in order to carry out coordinate tranformations. These problems are analysed using computer simulations of the responses of cells that are broadly tuned to an external quantity. We have implemented methods to decode the information in such ensembles of neuronal activities (Salinas and Abbott, *J. Comput. Neurosci.*, in press). This allows us to closely monitor changes in the coded quantity as information is transmitted between or combined in networks. We find that a value coded by one population can be faithfully reproduced in another one if the connections between them are established through a correlation based, or Hebbian type of learning mechanism. This corresponds, for example, to the generation of a motor command in the same direction as a given sensory input. Furthermore, the same algorithm can be used to couple two maps, so that the resulting set of activities codes for a linear combination of the original coded quantities. In this case a coordinate transformation is being carried out. These results demonstrate how networks can perform important computational tasks using biologically plausible learning mechanisms, such as the Hebb rule .

493.23

HIPPOCAMPUS AS A THREE-DIMENSIONAL NEURAL NETWORK FOR ASSOCIATIVE MEMORY. Y.Shigematsu and G.Matsumoto* Electrotech. Lab.,Tsukuba, 305 Japan.

A neural network model of associative memory is proposed for information processing in the hippocampus. Reccent morphological and physiological findings of three-dimensional neural network structure in the hippocanpus are considered for the modelling. We have adopted an assumption that each lamellar network accepts its respective category of information. In this model, the networks between granule cells and CA3 cells are constructed within each lamella, in which the information is processed according to their respective categories. The granule cells competitively selecte particular signals by negative self-feedback for each category. Further, the recurrent paths in the CA3 cells strengthen the associative learning within a category. Both granule and CA3 cells fire sparsely. Association among different categories of information is made by the following inter-lamellar connections of the CA1 and subiculum areas. This associative interaction has a general tendency to induce excessive correlation among the signals. Therefore, sparse firing inputs to the CA1 cells are quite reasonable to protect from the excessive interaction. The o+ther direct input paths from the entorhinal cortex to these areas are very important. These looped circuits can lead the associated output signals to the appropriate input parts of the entorhinal cortex. We can understand the three-dimensional structure and the functions of the hippocampus by this model, and the inportance of the hippocampus for associative memory as well.

493.24

NEURON EXCITABILITY AND ASSOCIATIVE MEMORY FUNCTION. E. P. Cook[1]*, M. Migliore[2], & D. Johnston[1], [1]Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030 & [2]Inst. of Interdisc. Applic. of Physics, Natl. Res. Counc., Palermo, Italy.

In previous work we showed that the ability of three different biophysical models of a hippocampal pyramidal neuron to use synaptic potentiation as a mechanism of memory storage is dependent on a match between the magnitude of the synaptic input and cell excitability (Cook et al. Soc. Neurosci. Abs., 660.4, 1993). This previous work, however, only explored memory function in model neurons that were initially at rest and in a steady state. In the present report we have compared how previous synaptic activity reduces excitability of the three different neuron models and tested whether this affects subsequent associative memory function.

Our three models were reconstructed hippocampal pyramidal neurons with the following different channel distributions and types: 1) passive dendrites with H&H-like Na^+ and K^+ channels in the soma, 2) passive dendrites with a full compliment of voltage-gated channels in the soma, and 3) a complete biophysical model that included active dendrites (Migliore et al. Soc. Neurosci. Abs., 295.13, 1993). Activation curves were computed for each model at various time points after four different stimulus paradigms: a) Somatic current injection, and b) low (subthreshold), c) medium, and d) strong synaptic inputs. The program NEURON by Michael Hines was used in all computer simulations.

Comparisons between associative networks constructed from all models show that correct recall of stored patterns is affected by previous synaptic activity in models 2 and 3 but not 1. Simulations demonstrate that the two most realistic models (2 and 3) have increased thresholds to firing after experiencing all four types of stimulus paradigms. As synaptic input is increased (c and d), however, model 3 shows even greater reductions in excitability. This is a direct result of residual $[Ca^{2+}]_i$ activating AHP K^+ channels. Model 3, which contains dendritic Ca^{2+} channels, was affected the most by all three types of synaptic activity. (Keck Center and MH10475, MH44754, MH48432, and NS11535.)

493.25

PATTERN DISCRIMINATION IN AN IMPEDANCE MATCHING MODEL OF DENDRITES K.T. Blackwell*[1], T.P. Vogl[1], D.L. Alkon[2]. [1]Environmental Research Institute of Michigan, Arlington, VA 22209; [2]Laboratory of Adaptive Systems, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD

Associative learning in Dystal (Blackwell et al., *Pattern Recognition*, 1992), an artificial neural network, is based on associative learning in *Hermissenda* (Alkon, *Memory Traces in the Brain* 1987). A crucial aspect of associative learning is the ability of a dendritic patch, consisting of a spatially contiguous group of synapses, to recognize previously learned patterns (pattern matching) (Olds et al., *Science*, 1989; Alkon, *Science*, 1984). To investigate the details of this aspect of associative learning, we developed a model of pattern matching in a group of synapses. The model is based on the finding that classical conditioning causes an increase in membrane resistance, and employs an impedance matching paradigm: when the dendritic membrane impedance equals the synaptic resistance, voltage across the dendritic membrane is maximized. The model consists of a small piece of dendritic membrane with a number of synaptic spines, each of which is modeled as an RC circuit (Koch and Zador, J. Neuroscience, 1993). The model's resistive elements have the following properties: (1) spine neck resistance increases quickly when calcium concentration exceeds its normal resting value; (2) spine neck resistance slowly adapts to the increased calcium concentration so that after repeated stimulus presentations, spine neck resistance increases only when calcium concentration exceeds the learned value. (3) During repeated stimulus presentations, dendrite membrane resistance increases slowly to match spine neck resistance. Simulations show that this model discriminates patterns that differ solely by biologically reasonable variations in signal strength across synaptic inputs.

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494.1

PARIETAL NEURAL ACTIVITY IS DISTINCT FROM HIPPOCAMPUS DURING AUDITORY DIRECTIONAL DELAYED NONMATCHING-TO-SAMPLE IN AWAKE RATS. K. Nakamura*, T. Ono, H. Nishijo, T. Hirota and R. Nagura, Dept. Electronics and Informatics, Toyama Prefectural Univ., Toyama 939-03, and Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan.

The neural network from parietal cortex (PG) to hippocampus (HF) is important for spatial recognition memory. In the present study, Sprague-Dawley rats were trained to perform a spatial working memory task, directional delayed nonmatching-to-sample (DNMS). Single unit activity was recorded from the PG and HF of an awake rat. While unit recording, the rat could be rotated in some direction or moved within a 63 cm X 63 cm horizontal field. Six speakers surrounded the field at the same height in a hexagonal arrangement. In the directional DNMS, two tones (1s in duration, identical in frequency, and separated by 2 s) were directed at the rat from either the same location (matched pair) or different locations (non-matched pair). To receive an award, the rat needed to lick the spout immediately for a non-matched pair or postpone licking for a matched pair. There were 12 of 57 neurons in the PG and 10 of 30 neurons in the HF that responded to auditory directional stimuli. Ten neurons in the PG also had significant activity during the delay period that sometimes became enhanced after the rat was rotated to an unfamiliar direction. None of the HF-neurons responded during the delay period, but occasionally activity would emerge after the rat was rotated. These results suggest that spatial working memory in the PG is persistent, whereas that in the HF is transient.

494.2

THE EFFECTS OF PARIETAL CORTEX AND HIPPOCAMPAL LESIONS ON MEMORY FOR ALLOCENTRIC DISTANCE, EGOCENTRIC DISTANCE, AND SPATIAL LOCATION IN RATS. J.M. Long*, R.P. Kesner, Dept. of Psychology, University of Utah, Salt Lake City, UT 84112.

Deficits in finding a hidden platform in the Morris swim maze (a task thought to require a "cognitive map") after lesions to the hippocampal formation or the parietal cortex have been interpreted as evidence for their role in processing spatial cues and their relationships. What has not been explored is which spatial cues or relationships are mediated by these structures. The present study explicitly assessed the effect of post-training lesions of the hippocampus or the parietal cortex on the memory for three such cues: allocentric distance, egocentric distance, and spatial location. In three separate experiments in the same apparatus, Long-Evans rats were trained on a delayed match-to-sample, go/no-go task in which the rats had to remember the distance between two identical objects (allocentric distance), the distance between the rat and an object (egocentric distance), or the spatial location of an object (spatial location). Hippocampal lesions resulted in deficits in all three tasks, whereas parietal cortex lesions did not significantly impair performance in any task. In separate experiments both hippocampal and parietal cortex lesioned rats retained the ability to discriminate allocentric and egocentric distance information, thus providing evidence against the possibility that deficits in the memory tasks could be attributed to deficits in the perception of spatial information. It is postulated that spatial information can be reduced to spatial features (i.e. distance, direction), which are processed primarily by the hippocampal formation.

494.3

Retrograde amnesia for spatial discrimination following entorhinal cortex or parietal cortex lesions in rats. Y. H. CHO* 1,2, R.P. KESNER 1 and R. JAFFARD 2, 1.Department of Psychology, University of Utah, Salt Lake City, 84112 Utah, USA; 2.Lab. Neurosciences Comportementales & cognitives, CNRS URA 339, Avenue des Facultés, 33405 Talence Cedex, France.

Our previous study has demonstrated that lesions of entorhinal cortex (EC) produced a temporally graded retrograde amnesia for spatial discrimination in mice. The present research was conducted to study further retrograde amnesia using rats as subjects as well as to extend to parietal cortex (PC), another brain region involved in processing of spatial memory.

Rats learned successively, one at a time, and every two weeks, 6 single-pair discriminations in two radial mazes (2 pairs in an 8-arm maze and 4 pairs in a 12-arm maze). Either electrolytic lesions of the EC or aspiration of the PC or sham-operation was performed on the day after subjects had attained learning criterion on the 6th discrimination. Postoperative retention performance assessed only for the last 4 discriminations indicated that the PC-lesions impaired overall performance as compared to control rats. The EC-lesions produced a more severe impairment than the PC lesions in addition to an extended temporal gradient of retrograde amnesia. The EC-lesioned rats were significantly impaired in retention of the 3 recent discriminations acquired immediately and up to 4 weeks prior to surgery, but were not different from controls for the first discrimination learned 6 weeks before surgery.

These results are consistent with previous findings obtained in mice with ibotenate lesions of the EC and suggest that the EC as well as the PC might be differentially involved in long-term storage/retrieval of spatial discrimination memory in rodents.

494.4

FUNCTIONAL DIVERSITY IN THE RAT MEDIAL PREFRONTAL CORTEX. J.K. Seamans*, S.B. Floresco, A.G. Phillips. Dept. of Psychology, University of British Columbia, Vancouver, B.C. Canada, V6T 1Z4.

The rat medial prefrontal cortex (mPFC) consists of two distinct subregions, the prelimbic cortex (PL) and the anterior cingulate cortex (AC). Most behavioral investigations have treated these subregions as homogeneous, therefore their individual contribution to the numerous cognitive functions ascribed to the mPFC remains unclear. The present study examined the effects of transient lidocaine-induced lesions of the PL or AC on delayed spatial win-shift (DSWSh) behavior on a radial-arm maze. The DSWSh task consists of a training phase where 4 of 8 arms are baited randomly and the remaining 4 arms are blocked, and a test phase where all 8 arms are open but the previously blocked arms are baited.

Lidocaine microinjections (2%, 1ul/2min) into the PL prior to training had no effect on: 1)training phase performance or 2)subsequent test phase performance as assessed when the anesthetic effects of lidocaine had dissipated. Similar injections into the AC had no effect on training phase performance, however lesioned animals made significantly more entries into previously baited arms during the test phase. Transient lidocaine lesions of the PL delivered prior to the test phase disrupted test phase performance as lesioned animals foraged randomly. Similar microinjections to the AC also impaired test phase performance, but in this case the errors were predominately revisits to previously baited arms. During the random foraging task in which 4 of 8 arms were baited transient lesions of the AC were again accompanied by significantly more revisits to baited arms. PL lesions had no effect on this task. Given that PL lesions disrupted foraging for 4 pellets on the DSWSh but not during the random foraging task, the PL may play a role in complex rule guided behaviors that utilize previously acquired information. Alternatively, the perseverative pattern of responding observed following transient lesions of the AC suggest that the AC is involved in situations which require newly acquired information to be used in a flexible manner.

494.5

CHARACTERIZATION OF A PREFRONTAL CORTEX AREA INVOLVED IN AUDITORY EVOKED ASSOCIATIVE LEARNING OF THE DEGU (OCTODON DEGUS) M. Müller and G. Poeggel*, Institute for Neurobiology, Brenneckestr.6, 39118 Magdeburg, FRG

Imprinting is one form of early filial learning. Most of the studies dealing with imprinting mechanisms were carried out on birds. However, there is a gap in our knowledge concerning imprinting behaviour in mammals. The degu (Octodon degus) provides a good experimental model for studies of auditory filial imprinting in mammals. The mothers use individual calls to attract their pups and to stimulate and probably also to reinforce suckling. Based on earlier studies, we developed a behavioural discrimination test using a Y-shaped test arena equipped with loudspeakers presenting alternatively either the mother call or various control sounds. As controls, pups of surgically muted mothers were tested. Pups from normal mothers showed a significantly higher preference for the mother call compared to pups from muted mothers. In another subset of experiments using 2-deoxyglucose (2-DG), we exposed pups from normal and muted mothers either to the mother call or control sound, respectively. In pups of normal mothers the exposure to the familiar mother call provokes an autoradiographically detectable activation of the medial prefrontal cortex (PFC) and the premotor cortex. Pups of muted mothers and pups exposed to control sounds failed to show such an activation. These areas were immunohistochemically investigated using different techniques. Antibodies against calcium-binding proteins label distinct subsets of neurons in the prefrontal cortex; particularly calretinin is abundantly present in this brain area. With the use of c-fos/ c-jun-immunohistochemistry in an identical test setup as in the 2-DG experiments we were able to identify the neurons responsible for the 2-DG staining pattern. These cells were scattered over the PFC with lack of any layering. At present, efforts are made to characterize these neurons and to look for possible alteratios at the cellular and subcellular level. (Supported by grant GSF 07 NBL 06 of the BMFT)

494.6

EFFECTS OF HIPPOCAMPAL AND MEDIAL PREFRONTAL LESIONS ON DISCRIMINATION OF DURATION IN RATS. P. Jackson-Smith*, R.P. Kesner, and K. Amann. Depts. of Psychology, Radford Univ., Radford, VA 24142* and Univ. of Utah, SLC, UT 84112.

We examined the effect of hippocampal and medial prefrontal cortex (MPF) lesions on discrimination of temporal duration and on memory for duration in rats. One short duration (2 sec) and one long duration (8 sec) was used and involved visual presentation of a 3-dimensional object. In the discrimination experiment, the door separating the rat from the stimulus was opened at the end of the time period, and latency to approach and move the stimulus was measured. For half of the rats, the stimulus covered a piece of food on the short duration presentations, but not the long durations, and the other half had the opposite contingency. In the second experiment, memory for duration was measured by presenting a study phase (short or long duration) followed one second later by a test phase. The test phase involved presentation of one of two different 3-D stimuli. One object was always correct following a short duration and the other was always correct (food underneath) following a long duration. Half of the trials involved correct pairings of duration and object and half involved incorrect pairings. Latency to approach and move the stimulus during the test phase was measured. Rats in both experiments received control, hippocampal, or MPF lesions following acquisition (significantly greater latencies on nonreinforced trials as compared to reinforced trials). Hippocampal and MPF lesioned groups showed performance deficits following surgery as compared to the control-operated groups on both tasks. Performance on the duration discrimination task returned to normal as compared to controls and pre-surgery performance, whereas both the hippocampal and MPF groups continued to perform at chance levels on the memory for duration task. Both the hippocampus and the medial prefrontal cortex appear to be involved in data-based memory for temporal information.

494.7

LESIONS OF POSTERIOR CINGULATE CORTEX AND VERTICAL LIMB OF THE DIAGONAL BAND OF BROCA IMPAIR THE LATE STAGES OF LEARNING IN A CONDITIONAL VISUAL DISCRIMINATION TASK.

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It has recently been shown that excitotoxic lesions of the anterior cingulate cortex facilitate early learning, and posterior lesions impair late learning, on a conditional visual discrimination task (Bussey et al., Soc. Neurosci. Abstr. 19: 1233, 1993). Furthermore, excitotoxic lesions the the vertical limb of the diagonal band of Broca (VDB), which result in cholinergic denervation of both these cortical areas, produce both of these effects on the same task (Muir et al., Soc. Neurosci. Abstr. 19: 1233, 1993). These results are concordant with those of others, who suggest that the anterior cingulate cortex is involved in mechanisms operative during the early stages of learning, while posterior cingulate activity predominates during later stages (Gabriel et al., Exp. Brain Res. 86: 585-600, 1991).

The aim of the present study was to determine whether the late-learning effects observed in our previous experiments were independent of the fact that animals were required to learn the task under the influence of the lesion. Accordingly, rats were trained to a criterion of 70% correct responding on two consecutive days, at which time animals received either quinolinic acid lesion of the anterior or posterior cingulate cortex, or excitotoxic AMPA lesions of the VDB. Following recovery, the animals were returned to the task and trained until they had achieved 85% correct responding on two consecutive days. The results reveal that effects on late learning observed in our previous studies were indeed reproduced: both VDB and posterior cingulate lesioned animals showed an impairment in the ability to progress through the later stages of learning the task. In addition, the effects of the lesions on a post-acquisition retention test and on extinction were examined.

494.8

INTRODUCING A NEW COMPUTER AUTOMATED TOUCHSCREEN TESTING PROCEDURE FOR THE RAT USING COMPUTER-GRAPHIC STIMULI: OBJECT DISCRIMINATION AND RECOGNITION TASKS.

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The comparative neuropsychological approach involves the comparison of brain-damaged human patients with animal models of the same disorders, using tasks designed to assess specific psychological functions. This enterprise is greatly facilitated when the tasks are as similar as possible for both species, and very difficult when they are not. Many researchers have succeeded in devising such tasks involving, for example, the use of "junk objects" in the Wisconsin General Test Apparatus or, more recently, the presentation of computer graphic stimuli on a VDU/touchscreen apparatus in primates and patient groups. Unfortunately, few tasks have been designed for the rat with this approach in mind. This is perhaps surprising given that the rat is, in psychology at least, by far the most popular and convenient laboratory animal.

Thus, we have recently developed a touchscreen procedure for the rat using computer generated visual stimuli. In addition to inter-species comparability, other advantages of this technique include the nature of the response, the rat being able to interact directly with the stimulus by nose-pokes to the VDU screen; the ease with which rats can be trained to respond in this manner; and the ability to collect data on a wide range of performance measures. These points will be discussed and data will be presented to illustrate the use of a touch-sensitive screen for rats on various tasks, including object discrimination and serial reversal learning, 8-pair concurrent visual discrimination learning, spatial and non-spatial delayed non-matching to sample and visuospatial conditional rule learning.

494.9

THE EFFECT OF CINGULATE CORTEX LESIONS ON ACQUISITION OF A SIMPLE VISUAL DISCRIMINATION AND REVERSAL, AND AN 8-PAIR CONCURRENT DISCRIMINATION TASK IN RATS: THE USE OF A NOVEL TOUCHSCREEN APPROACH.

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Cingulate cortex - the division of midline limbic cortex overlying the full rostrocaudal extent of the corpus callosum - has received little attention relative to other limbic structures such as the hippocampus. Recently it has become apparent that the cingulate cortex can be anatomically dissociated into anterior and posterior components. However, the characteristic behavioural functions of these structures remain to be elucidated. Results obtained using an active avoidance paradigm suggested that the anterior cingulate cortex mediates mechanisms operative during early learning, while posterior cingulate cortex is involved predominantly in late learning (Gabriel et al., Exp. Brain Res. 86: 585-600, 1991). More recently, it has been demonstrated that lesions of the anterior cingulate cortex facilitate early learning, and posterior cingulate cortex lesions produce a deficit in late learning, of a visual conditional discrimination task which requires the acquisition of a stimulus-response rule (Bussey et al., Soc. Neurosci. Abstr. 19: 1233, 1993.

These result have led us to investigate the role of anterior and posterior cingulate cortices in the learning of stimulus-reward associations. We have recently developed a new computer automated touchscreen testing procedure which enables the testing of rats on various tasks involving the presentation of computer graphic stimuli. The present study employed this technique to examine the effects of anterior and posterior cingulate cortex lesions on simple visual discrimination and reversal and the acquisition of an 8-pair concurrent visual discrimination task. The results show that neither anterior or posterior cingulate lesions affected acquisition of a simple discrimination. However, lesions of the anterior cingulate cortex impaired the early but not the late stages of acquisition of the 8-pair concurrent discrimination task.

494.10

RATS' VISUAL MEMORY IN A COMPUTER-CONTROLLED TESTING ENVIRONMENT.

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Rats, of the Hooded Lister and Dark Agouti strains, were trained in a fully automated setting; a Y-maze each of whose arms terminates in a pair of adjacent monochromatic VGA screens, with a food dispenser between them. The abstract stimuli include internally complex, wide-angle displays - "scenes" analogous to those whose processing is disrupted by hippocampal system lesions in primates - and more localised, homogeneous "objects". Displays can incorporate movement and brightness fluctuation to enhance their salience. We report rats' performance in tests of associative reference memory (concurrent discrimination among pairs of displays) and working memory (matching or non-matching).

494.11

INSULAR CORTEX GRAFTS RESTORE REMEMBERANCE OF PREVIOUSLY LEARNED TASTE AVERSIONS.

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Instituto de Fisiol. Celular, UNAM, México, D.F., México 04510.

Previous studies in our laboratory have shown that cortical grafts are able to induce recovery in the ability to acquire a conditioned taste aversion (CTA) in insular cortex (IC) lesioned rats. Furthermore, tissue specificity has been shown since only homotopic grafts induce recovery whereas occipital cortex (OC) tissue does not. Further studies have shown that IC lesions interfere with short and long term memory for CTA. The aim of the present study is to evaluate the effects that cortical grafts have on the recovery of memory aspects of CTA.

Twenty-nine male Wistar rats were trained for CTA by pairing a saccharin solution (0.1%) with a gastric malaise induced by an ip injection of LiCl solution (0.15M). After 4 days to allow consolidation, all animals except a control (Con) group were lesioned by microinjections of NMDA and 10 days later the lesioned animals were randomly divided in 3 groups: one recieved IC grafts from 15-day-old fetuses (TxIC), one recieved OC grafts (TxOC), and one remained lesioned. Sixty days after grafting, all animals were tested for the CTA to saccharin and then retrained for a CTA to saline taste. The animals that remained lesioned did not recall the saccharin CTA nor were able to acquire the new CTA to saline. Both grafted groups showed a recall of the CTA for saccharin similar to the control, but only the TxIC group showed values similar to the control for the acquisition of the new CTA for saline. It is noteworthy that the TxOC group was able to recall previously learned CTA but unable to learn a new one. This data suggests that the graft-induced recovery of recall is less tissue specific than recovery of acquisition of CTA, and that the IC is highly involved in the evocation aspects of CTA.

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494.12

MEMORY FOR FOOD REWARD MAGNITUDE: THE ROLE OF THE AGRANULAR INSULAR CORTEX.

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Memory for magnitude of reinforcement was assessed in rats using a go, no-go task. During the task's study phase rats were given a piece of cereal comprised of either 25% or 50% sugar. For all trials, one of the cereal types was designated positive, the other negative. On the ensuing test phase the rat was presented with an object which covered a food well. If a positive food reward was given during the study phase, a second food reward was placed beneath the object. No food reward was placed under the object if the study phase consisted of a negative food reward. Latency to object displacement was used as the measure of performance. Following the establishment of a significant difference between latency to approach the object with reward compared to latency to approach the object without reward, rats were given either medial prefrontal cortex, agranular insular cortex, infra-limbic/pre-limbic cortex or sham control lesions. Sham controls excepted, all lesion groups showed post-surgery impairment followed by recovery of performance. Furthermore, all animals transferred to a new set of cereals containing 25% and 50% sugar. Thus, independent of lesion type, all post-surgery animals were able to perceive differences between magnitudes of reward. Trials consisting of 10 and 20 second delays between the study and test phases were then introduced. Only agranular insular lesioned animals showed significant impairment at each delay. These results demonstrate that lesions to the agranular insular cortex in rats produce accelerated forgetting of reward magnitude and suggest that this structure plays a significant role in memory for affect.

494.13

NEGATIVE PATTERNING DISCRIMINATION PERFORMANCE IS IMPAIRED IN RATS WITH BILATERAL QUISQUALIC ACID LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS. **A.E. Butt* and G.K. Hodge. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.**

It was hypothesized that NBM lesions would selectively impair the ability to learn conditional, or configural, associations while sparing the ability to learn simple, or elemental, associations (see Sutherland & Rudy, 1989). To test this hypothesis, rats received bilateral quisqualic acid or sham lesions of the NBM and were subsequently tested in a negative patterning operant discrimination task. In this task, animals are food-reinforced (+) for pressing an operant chamber lever in the presence of either a light (L) or tone (T), but are not reinforced (-) in the presence of a compound stimulus comprised of the light and tone presented together (LT). Animals must learn that the reinforcement values of the elements L and T are determined by whether these stimuli occur separately (L+, T+) or simultaneously (LT-). Thus, learning to withhold responses to LT- requires the formation of a configural representation of the compound cue that is discriminable from the representations of its constituent elements. Based on evidence suggesting that simple associative learning remains intact in NBM-lesioned animals, it was hypothesized that these animals would learn to respond normally to the elements L+ and T+. It was further hypothesized, however, that NBM-lesioned animals would be unable to form the configural representation of LT- and would continue to respond in the presence of this nonreinforced compound stimulus. Data support these hypotheses. All animals were able to discriminate between the presence and absence of the reinforced elements L+ and T+, suggesting that the ability to solve simple associative discriminations is unimpaired in NBM-lesioned animals. In contrast, negative patterning discrimination performance was impaired in NBM-lesioned animals; lesioned animals made significantly more LT- responses across training sessions than controls ($p < .05$), although behavioral recovery eventually ensued. Results suggest a selective involvement of the NBM in the acquisition of configural but not simple associative tasks. (Supported by UNM RAC #93-33)

494.14

CHANGES IN ATTENTION AND NORADRENERGIC REUPTAKE SITES IN FRONTAL CORTEX OF BASAL FOREBRAIN-LESIONED RATS. C.L. Wellman* and M.A. Pelleymounter. Department of Neurobiology, Amgen, Thousand Oaks, CA 91320.

The nucleus basalis magnocellularis (NBM) is the major cholinergic projection to frontal cortex in the rat and appears to modulate cortical neuronal activity. Lesions of the NBM produce deficits in radial arm maze performance and morphological alterations in frontoparietal cortex. In the present study, we have assessed selective attention and noradrenergic receptor binding in frontal cortex of rats with bilateral lesions of the NBM. Nineteen rats received either ibotenic acid or sham lesions of the NBM. Two months after surgery, rats were trained in a blocking task. While sham-lesioned rats showed blocking, supressing significantly more to a meaningful cue than to a redundant cue, NBM-lesioned rats did not show blocking, suppressing less to both cues. The groups performed comparably on a passive avoidance task, suggesting that deficits on the blocking task were due to attentional changes rather than a learning deficit. Three months after surgery, rats were killed and their brains removed. Sections were incubated with either [3H]hemicholinium-3 (HC-3) to assess extent of cholinergic denervation or [3H]desmethylimipramine (DMI) to assess noradrenergic function and processed for autoradiography. Quantitative densitometry revealed a 28% decrease in HC-3 binding and a 24% decrease in DMI binding in frontal cortex of lesioned rats. Thus, the NBM appears to modulate noradrenergic function in frontal cortex, and alterations in NBM function may influence behavior and cortical morphology through the noradrenergic system.

494.15

EFFECTS OF MEDIAL SEPTAL LESIONS ON DELAYED GO/NO-GO RESPONSE ALTERNATION IN RATS. R. NUMAN.* Psychology Department, Santa Clara University, Santa Clara, CA 95053.

In an earlier study (Numan and Quaranta, 1990) we found that medial septal lesions impaired a two-lever (left-right) operant delayed alternation task in rats. We concluded that the impairment was due to a disruption of working memory. However, a 'spatial' interpretation of the data could not be ruled out. Subsequently (Numan and Klis, 1992), we found that medial septal lesions facilitated performance on a cued go/no-go discrimination task with a delay between the cue and the required response. This task was not spatial (only 1 lever was used), but did require working-memory. Hence, the lesion could not have produced a general working-memory impairment.

These findings, taken together, suggested to us that the medial septal lesions impaired 'response' working memory which in turn led to a compensatory enhancement of 'stimulus' working memory. If this were true, then the lesions should impair a go/no-go task based on response working-memory. The current experiment tested this hypothesis.

Rats (12 with medial septal lesions and 12 with sham operations) were tested on a discrete trial operant go/no-go response alternation task without cues. The rats were first tested for 20 days without a delay contingency, followed by 30 days of testing with a 15-sec delay between 'go' and 'no-go' trials.

The septal lesions did not impair performance at the 0-sec delay ($p>0.05$). Both groups achieved about 85% correct and acquired the basic requirements of the task. However, when the delay contingency was added, the medial septal lesions impaired performance ($p<0.025$). By the end of testing, the controls averaged about 75% correct while the septal lesioned rats averaged about 68% correct.

As the go/no-go task does not require spatial processing, the best explanation for all of these findings (impairment on L-R delayed alternation and delayed go/no-go response alternation, but facilitation on a delayed but cued go/no-go discrimination) is that the medial septal lesions produce an impairment in 'response' working memory. Such an impairment may result in a compensatory enhancement in the processing of salient environmental cues to guide behavior and improve performance on cued tasks.

494.16

RETROGRADE AND ANTEROGRADE SPATIAL MEMORY IMPAIRMENTS FOLLOWING RADIO-FREQUENCY LESIONS TO THE LATERAL INTERNAL MEDULLARY LAMINA. L.M. Savage*, A. Sweet, A. Bassett, & P.J. Langlais
VA Medical Center San Diego, CA. 92169, &
Department of Psychology, SDSU, San Diego, CA. 92182.

Rats were pre-trained on a Non-Matching-To-Position (NMTP) task in the T-maze. After initial acquisition (90 % correct for 2 sessions) subjects were randomly assigned to two treatment conditions: (A) radio-frequency lesions to the lateral Internal Medullary Lamina (IML-lesioned), or (B) probe placement in the same location (sham). Following recovery from surgery, the IML-lesioned rats took significantly more trials than shams to re-acquire the criterion. This suggests that lesions to the lateral IML region produce retrograde deficits. Once subjects re-mastered the NMTP task, delays intervals were introduced to access working memory. Although the performance of IML-lesioned rats at the shortest delay (4 s) was equivalent to shams, their accuracy at longer delays (30, 60 and 90 s) had a steeper decline. Anterograde learning and memory was subsequently assessed using the Morris water maze. Again, IML-lesioned rats demonstrated impaired spatial abilities-their latency to find the location of the hidden platform was significantly longer than shams. However, they were able to decrease their latency to find the location of a visual platform. IML-lesioned rats, relative to shams, demonstrated no deficits in learning a single trial passive avoidance task. These results suggest that lesions to the IML region produce a range of spatial deficits that parallel the pyrithiamine-induced thiamine deficiency model of Wernicke-Korsakoff syndrome.
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494.17

LATERAL DORSAL NUCLEUS OF THE THALAMUS CONTRIBUTES TO SPATIAL LEARNING. M. Palmer* & R. J. Sutherland. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

The lateral dorsal nucleus (LD) of the thalamus has reciprocal connections with retrohippocampal areas. Recent electrophysiological work implicates LD as playing an important role in the spatial navigation process, especially in encoding head direction (Mizumori & Williams, 1992). The present experiment was designed to investigate LD's role in spatial learning.

Eight rats received electrolytic lesions of LD (coordinates: -2.6, +2.6, -2.6, 5.7) of 15mA for 15s. Sixteen rats served as sham controls. All rats were naive to the Morris water task. We used a moving platform version of the water task in which the submerged platform was moved to a new position on each subsequent day, but remains in the same position over the day's trials.

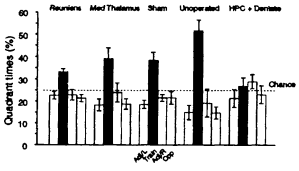
The LD rats displayed a significant deficit in learning relative to the sham controls.

We will also report on acquisition of a negative patterning discrimination and a light-tone discrimination in an operant task.

494.18

IBOTENATE LESIONS OF RAT NUCLEUS REUNIENS THALAMI FAIL TO IMPAIR SPATIAL LEARNING AND MEMORY. M.J.Dolleman-van der Weel*, R.G.M.Morris[1], and M.P.Witter. Graduate School of Neurosciences, Dept. Anatomy and Embryology, Vrije Universiteit, Amsterdam, The Netherlands, [1]Centre for Neuroscience, Univ Edinburgh, Scotland.

Monosynaptic projections from nucleus reuniens may enable it to modulate activity in the entorhinal-hippocampal circuit and, hence, play a role in learning and memory. We explored this idea using the watermaze (fixed hidden platform, 18 spatial training trials, transfer test, 4 cue trials). The reuniens (RE, n=12) was lesioned with ibotenic acid and the behavioural effects were compared to Unoperated (Unop, n=6) and sham-lesioned controls (SH, n=11), mediodorsal nucleus (MD, n=6) and hippocampal-dentate gyrus lesioned rats (HDG, n=6). ANOVAS revealed overall Groups effects in both training and the transfer test ($ps< 0.001$), but, despite a trend towards poor performance by the RE group, pairwise comparisons (Dunnett's test) revealed that only the MD and HDG groups were impaired relative to the SH group during training ($ps< 0.01$ and 0.025 respectively) and only the HDG group was impaired during the transfer test ($p< 0.025$). The cue test revealed a trend towards poorer performance in the MD group ($0.10 > p > 0.05$). Our protocol was, therefore, sufficiently sensitive to detect hippocampal dysfunction in spatial learning and memory, but showed only a nonsignificant trend towards impairment following damage to the reuniens.



In summary, the presumed modulatory input from the reuniens to the entorhinal-hippocampal circuit has only a subtle effect on spatial learning. Its function may be better revealed using other learning and memory paradigms.

494.19

ASSESSING THE PERFORMANCE OF RATS WITH LESIONS TO THE MEDIODORSAL THALAMUS ON A BATTERY OF NONSPATIAL MEMORY TASKS. T.J. Kornecook and J.P.J. Pinel*. Dept. of Psychology, University of British Columbia, Vancouver, B.C. Canada. V6T 1Z4.

Rats with bilateral electrolytic or sham lesions of the mediodorsal thalamic nucleus (MD) were tested on a battery of six object-memory tasks, which resemble those which have been used in the study of amnesia in humans and monkeys: (1) simple two-choice object discrimination, (2) discrimination reversal, (3) 8-pair concurrent object discrimination, (4) nonrecurring-items delayed nonmatching-to-sample (DNMS) with retention delays of 4, 15, 30, 60, 120 s, (5) DNMS with lists of 3, 5, and 7 samples, and (6) order discrimination. Each task made use of the same apparatus and, like the monkey versions of these tasks, each one used objects as test stimuli. All testing was conducted postsurgery.

Relative to control rats, the rats with mediodorsal thalamic lesions were unimpaired at learning the simple object discrimination, but they took significantly more trials to master both the object reversal and the concurrent object discrimination tasks. The DNMS testing revealed that the rats with MD lesions were impaired at all delays of 15s or longer, and there was an overall group impairment across the three list lengths. There was no significant difference between the controls and MD-lesioned rats on the temporal-order discrimination task. The results from this battery of memory tasks will help in fully characterizing the nature of the memory deficits associated with medial diencephalic lesions in the rat and allow for direct comparisons to similar experiments in monkeys.

494.20

MEMORY IMPAIRMENT FOLLOWING LESIONS TO THE ANTERIOR NUCLEI OF THE THALAMUS. V. Sziklas*, F. Leri, and M. Petrides. McGill University, Montreal, Quebec, Canada.

In the present study, rats with lesions to the anterior thalamic nuclei (ATN), the fornix (FX) or a control operation (OC) were trained on an eight-arm radial maze under two conditions. In the first condition, there was a 20 sec delay between choices; in the second, there was no intratrial delay. Animals with lesions to the FX improved significantly when there were no delays between choices but the performance of this group was significantly impaired in comparison with the OC animals under both conditions. The overall performance of the ATN group was significantly worse than that of the OC group and, in addition, did not improve significantly with the removal of the intratrial delay. An analysis of the distribution of errors made in the delay condition suggests that the nature of the memory deficit following lesions to the ATN and the FX might be different. Taken together, these findings suggest that restricted lesions to the ATN are sufficient to produce a working memory deficit on the radial maze under the conditions used in this study.

494.21

LESIONS OF THE TUBEROMAMMILLARY NUCLEUS FACILITATE REWARD AND LEARNING. J.P. Huston, K. Klapdor, U. Wagner, P. Zimmermann and R.U. Hasenöhrl*. Inst. Physiological Psychology, University of Düsseldorf, D-40225 Düsseldorf, Germany.

The tuberomammillary nucleus (TM) is located in the posterior part of the hypothalamus and provides the only source of neural histamine in the CNS. Our experiments performed with unilateral lesions of the TM region provide evidence for an involvement of the TM system in reinforcement mechanisms. Unilateral destruction of the TM with DC or ibotenic acid was found to increase the rate of lateral hypothalamic self-stimulation ipsilateral to the lesion site, suggesting that the TM may function as a reinforcement inhibiting neural substrate. Experiments performed with bilateral lesions of the TM region provide evidence for a role of the TM in learning and memory processes. Adult (3-month-old) and aged (31-month-old) rats with bilateral DC lesions in the TM region were tested along with sham-lesioned controls on the one-trial step-through inhibitory avoidance task and on a spatial discrimination test in a water-filled T-maze. Bilateral lesions of the TM led to significantly longer retention latencies in the step-through task and improved long-term retention in the water maze test in both adult and aged rats, indicative of superior learning. Thus, lesions of the TM may have a facilitatory effect on learning and mnemonic functioning, which is possibly related to a lesion-induced disinhibition or facilitation of reinforcement ("stamping-in") processes. *Supported by DFG.*

494.22

INVESTIGATION OF NEURAL AREAS CONTROLLING CONDITIONED TASTE AVERSIONS IN RATS: EFFECTS OF COOLING THE AREA POSTREMA. Y. Wang, D. G. Lavond, & K.C. Chambers*, Department of Psychology USC, Los Angeles, CA 90089

Four studies were designed to determine the feasibility of using cooling to investigate neural mechanisms of conditioned taste aversions (CTAs). Study 1 was designed to determine whether cooling the area postrema (AP) can induce a CTA. After 13 rats were given access to a sucrose solution, the AP of 7 of the rats was cooled for 1 hour and the AP of 6 was not cooled. Starting 2 days later, all rats were given daily extinction trials until their acquisition day consumption levels were restored. Cooling the AP induced a CTA; the rats whose AP was cooled showed a decrease in sucrose consumption but the non-cooled group did not. Study 2 was designed to determine whether preexposure to cooling of the AP could attenuate the CTA induced by cooling the AP. In 7 rats, the AP was cooled daily for 7 days and in another 7 rats the AP was not cooled. On acquisition day, the AP of all rats was cooled after consumption of sucrose solution. Preexposure to cooling attenuated the aversion induced by cooling; the non-preexposed group showed a decrease in sucrose consumption but the preexposed group did not. Study 3 was designed to determine whether cooling the AP could be used to block a CTA induced by LiCl. All rats were preexposed to cooling. After sucrose consumption, the AP of 6 rats was cooled just prior to injection of LiCl and was kept cooled for 1 hour. Another 7 rats received LiCl only. Cooling the AP attenuated the aversion induced by LiCl; rats exposed to cooling during LiCl stimulation acquired a weaker aversion than those not exposed. Study 4 was designed to determine whether cooling the AP could block a CTA induced by apomorphine (Apo). After sucrose consumption the AP of 8 rats was cooled just prior to injection of Apo and was kept cooled for 1 hour. Another 8 rats received Apo only . Cooling had no effect on the aversion induced by Apo. The results of these studies, attenuated aversion induced by LiCl but not apomorphine, replicate those of studies that have used permanent lesion techniques. Supported by USC FRIF.

494.23

DIFFERENT SUBCORTICAL AREAS SUBSERVE SPATIAL BUT NOT VISUAL DISCRIMINATION PERFORMANCE IN INBRED MICE. M. Ammassari-Teule * and C. Rossi-Arnaud °. Inst. of Psychobiology and Psychopharmacology and ° Dept of Psychology, University of Rome "La Sapienza", Rome, Italy.

C57BL/6 (C57) and DBA/2 (DBA) inbred mice differently perform spatial tasks with C57 doing better than DBA at such learning. Hippocampal lesions have been shown to impair performance in both strains while amygdaloid and frontal cortex lesions have a deleterious effect only in the "high learner" strain C57. The aim of the present study was to examine whether the genotype-dependent involvement of limbic structures remains the same across tasks which make either the spatial or the visual modality relevant for discriminating the set of baited arms. C57 and DBA mice were tested in the standard (experiment 1) or in the visually-cued (experiment 2) version of the four-baited path task in a radial eight-arm maze.

Results show that, when the spatial modality was relevant for identifying the correct arms, both hippocampal and amygdaloid lesions impaired performance in C57 while hippocampal but not amygdaloid lesions impaired performance in DBA. Conversely, when the visual modality was relevant, the two strains performed the task in the same fashion and their performance was impaired by hippocampal lesions only. Spatial but not visual discriminative learning is therefore selectively based upon the involvement of different subcortical areas in the two strains considered.

494.24

ASYMMETRICAL EFFECTS OF LESIONS IN FR2 ON LEFT-RIGHT RESPONSE DIFFERENTIATION IN THE RAT. M. Noonan*[1], D. Chmiel, Jr[1]. and S. Axelrod[2]. 1 Canisius College, Buffalo, NY 14208; 2 SUNY at Buffalo, Buffalo, NY 14214.

We compared rats with left or right lesions in supplementary motor area Fr2 (Zilles), and sham operates, on a left-right response differentiation (LRRD) water-maze task given after prior experience with a control task in the same apparatus. We found that (1) rats exhibited an overall left-turning bias during acquisition; (2) the bias was particularly pronounced on the LRRD task; and (3) right-lesioned rats performed better than left-lesioned rats.

Overall, our results provide evidence of a consistent cerebral asymmetry in the rat. The left-turning bias replicates earlier findings in our laboratory and points to an asymmetrical motor activation favoring the right hemisphere during these water escape tasks. Nonetheless, a lesion in the right Fr2 left our rats better able to acquire an LRRD task than a lesion in the left Fr2. We have previously shown that extensive right hemidecortication, compared to left decortication, improves performance on the LRRD task when it was similarly presented as a second learning experience. The present finding appears to localize the relevant asymmetry to area Fr2, and suggests that it is motor/attentional in nature.

(Supported by NSF IBN-9209551.)

495.1

DIFFERENTIAL INVOLVEMENT OF HIPPOCAMPAL SUBFIELDS CA1 AND CA3 IN ODOR DISCRIMINATION. U.S. Hess*, G. Lynch, and C.M. Gall. Dept. of Psychobiology, Univ. of CA, Irvine 92717.

Expression of the protooncogene *c-fos* is a useful marker of neuronal activity. In the present study, levels of *c-fos* mRNA were evaluated in order to define functional brain circuitries that underlie odor discrimination. Rats were trained on one odor pair in a familiar chamber with six odor alleys. Odors were emitted from any random two odor ports on a given trial and rats had to nosepoke at the positive port to receive water reward and to avoid the negative port (response to which resulted in a strobe flash). Control rats (i.e. *nosepoker controls*) performed a practiced nosepoking behavior for water reward in the same apparatus with no odor presentation. *c-fos* mRNA was quantified using in-situ hybridization of ^{35}S-cRNA probes. Hybridization to *c-fos* mRNA was substantially lower in rats performing the task (*learners*) as compared to *nosepoker controls*. Levels of *c-fos* mRNA in *learners* were lower by 30 to 40 % in i) medial glomerular and granule cell layers of main olfactory bulb, ii) medial superficial superior colliculus, iii) rostral occipital cortex, iv) dorso-lateral caudate/putamen, and v) hippocampal CA1 stratum pyramidale. Other regions measured showed no difference in *c-fos* mRNA content between *learners* and *nosepokers*. In no region were *c-fos* mRNA levels greater in *learners* than controls. These selective differences in *c-fos* mRNA content resulted in differential regional labeling suggesting primary sensory and limbic structure involvement in task performance. In olfactory bulb, the increased lateral/ medial *c-fos* ratio seen in *learners* appears functionally relevant since it is the lateral portion of the bulb which maps the positive, most frequently sampled odor (i.e., peppermint). Sustained *c-fos* expression in area CA3 of *learners*, and notably suppressed *c-fos* mRNA content in area CA1 of these rats in relation to controls, indicates a differential involvement of these two hippocampal subfields in odor discrimination. This is especially interesting in light of the postulated role of the hippocampus in memory consolidation. Supported by 1 F31 MH10510-01 and HD24236.

495.2

GLUCOSE FACILITATES ACQUISITION AND REDUCES HIPPOCAMPAL DEFICIT IN THE MORRIS WATER MAZE IN RATS. R.W. Skelton* & S.P. Ross. **Psychology, U. Victoria, Box 3050, Victoria, B.C. V8W 3P5 CANADA**

Post-trial glucose has well-known facilitatory effects on retention of inhibitory conditioning. Increases in blood glucose levels have been proposed to underlie the mnemonic facilitation produced by epinephrine, norepinephrine and nootropics. The present study examined the effects of pre- and post-trial glucose on acquisition in the Morris water maze, an aversively motivated escape-learning task which requires spatial navigation and a functioning hippocampus. The present study also examined the effects of pre-trial glucose on performance of rats recovering from bilateral transections of the perforant path (a major cortico-hippocampal-cortical pathway).

During initial acquisition, glucose (100 mg/kg) was administered for 10 days 15 min before or after 2-trial daily sessions with a submerged platform in a fixed location. Probe trials were given as the second trials of Days 5 and 10, and an undrugged probe trial was given after a 5 day undrugged retention interval. After such pretraining, rats were given bilateral knife cuts to the perforant path and allowed 5 days to recover. They were then tested for 7 days with the platform in the original location and 7 days with the platform in the opposite quadrant. Glucose (100 mg/kg) was injected 15 min prior to each daily session of 4 submerged platform trials, 1 probe trial, and (for the 1st 5 days) 1 visible platform trial.

Both pre- and post-trial glucose increased the rate of acquisition and enhanced retention. Pre-trial glucose reduced the lesion-induced deficit in navigating to the previously learned platform location, but had little effect on the smaller deficit in reversal learning.

These results extend previous reports that glucose facilitates learning and retention, and indicate that glucose can also ameliorate cognitive deficits after hippocampal damage. (Supported by grants from NSERC Canada and BC Health Research Foundation.)

495.3

FACILITATION OF PLACE LEARNING AND DEFICIT OF RELATIONAL REPRESENTATIONS INDUCED BY HIPPOCAMPAL INJECTIONS OF SOMATOSTATIN. J-L. Guillou*, L. Lamirault, J. Micheau and R. Jaffard. Lab. Neurosciences Comportementales et Cognitives URA CNRS 339 Univ. Bordeaux I Av des facultés 33405 Talence Cedex France.

Previous studies with rodents showing alterations in both spatial learning and LTP following injections of either somatostatin (SS-14) or its depletor cysteamine have generated the hypothesis that hippocampal SS-14 could play a role in spatial mapping processes. However we reported that, although intra-hippocampal SS-14 injections facilitated the acquisition (regular trials) of spatial discriminations (two overlapping pairs (A-B+) vs. (B+C-) in a 8-arm radial maze, SS-14 also impaired performance during probe trials (A-B+C) aimed at taxing relational representations, (Guillou et al., Psychobiology 21-4-,1993). In the present study the aim was to determine whether this latter impairment was due to a deficit of relational encoding during the acquisition of the task or, alternatively, to a retrieval deficit (fragment fitting) occuring only when testing was shifted on probe trials. Accordingly, SS-14 or artificial CSF was injected either before regular or probe trials, or both. As in the previous study, injections of SS-14 before both regular and probe trials facilitated regular trials and significantly impaired probe trials. Injections of SS-14 before only regular trials produced the same pattern of results. In contrast, injections of SS-14 before only probe trials did not yield any behavioral effect. Thus, injections of SS-14 into the hippocampus appear to disrupt relational representations that, in normal mice, would occur during the acquisition of the task. This SS14-induced deficit of relational representations could account for the facilitation of acquisition (by use of individual representations of each pair). However, other results along with the observation that cysteamine produces a large impairment on regular trials seem difficult to explain by the relational theory alone. Taken together, these results suggest that both spatial mapping and the formation of relational representations are two separate HPC-dependent functions.

495.4

DENTATE GYRUS DESTRUCTION AND SPATIAL LEARNING IMPAIRMENT AFTER CORTICOSTEROID REMOVAL IN YOUNG AND MIDDLE-AGED RATS. C.D. Conrad* and E.J. Roy. Neurosci., Univ. Ill., Champaign, IL 61820.

We investigated the functional and behavioral implications of chronic corticosteroid removal in young and middle-aged rats. Pre-pubertal rats were placed in these groups: adrenalectomized (**ADX**) with no hormone replacement; ADX given corticosterone chronically, **chCORT**; ADX given corticosterone acutely at time of Morris water maze testing, **acCORT**; and **SHAM**. 13 month old rats were ADX or SHAM operated only. All rats were run on the Morris water maze 12 weeks after surgery for 11 days after which, they were sacrificed and brains saved for histological analysis.

The results showed that prolonged corticosteroid absence caused major damage to the dentate gyrus and impairment on the Morris water maze. The chCORT rats had little dentate gyrus cell loss and were as efficient as the SHAMs in the Morris water maze performance whereas the acCORT rats had dentate gyrus cell loss and were impaired in the spatial acquisition task. Middle-aged ADX rats lost cells only in the dorsal blade of the dentate gyrus but they did not show a greater learning impairment in the Morris water maze relative to the middle-aged SHAMs. These results indicate that corticosteroids are trophic for the dentate gyrus and that **substantial** dentate gyrus damage impairs spatial learning.

495.5

BEHAVIORAL AND ANATOMICAL EFFECTS OF 192-SAPORIN & ANTI-DβH-SAPORIN: PASSIVE AVOIDANCE, CONDITIONED FREEZING AND OPEN FIELD ACTIVITY. R.G. Wiley*, T.G. Berbos, D.A. Lappi, M.J. Picklo & D. Robertson. VAMC & Vanderbilt University, Nashville, TN 37212 & The Whittier Institute, La Jolla, CA 92037.

Analysis of the behavioral function of the cholinergic basal forebrain (CBF) has benefitted from the development of the immunotoxin, 192-sap, that selectively destroys neurons expressing the low affinity NGF receptor (p75). Animals with high grade CBF lesions are profoundly impaired in a variety of behaviors including passive avoidance. Since the ascending noradrenergic projections innervate both the CBF and many of its targets (hippocampus, neocortex), we sought to compare rats injected intraventricularly with 192-sap (N=8) to controls (N=8) and to rats treated with another immunotoxin, anti-DβH-sap (N=8), that selectively destroys neurons (adrenergic & noradrenergic) expressing the enzyme, dopamine β-hydroxylase. Rats injected with anti-DβH-sap were slower to regain weight after surgery than rats injected with 192-saporin ($p<0.002$). Anti-DβH-sap rats also showed less of an increase in immobility in a conditioned fear paradigm than 192-sap treated rats ($p<0.003$). In a step-through passive avoidance task, anti-DβH-sap treated rats behaved similar to controls showing none of the impairment seen in the 192-sap treated rats ($p<0.001$). In the open field, there was a nonsignificant trend for increased activity among 192-sap treated rats compared to the controls and anti-DβH-sap treated rats. Immunocytochemical staining for p75 and choline acetyltransferase (ChAT) showed near complete (>90%) loss of p75+/ChAT+ neurons from the CBF in 192-sap treated rats compared to controls. Anti-DβH-sap treated rats showed about 50% decrease in p75+/ChAT+ neurons in the CBF and near complete (>90%) loss of tyrosine hydroxylase (TH)-positive neurons from the noradrenergic and adrenergic brainstem nuclei. Dopaminergic TH+ neurons were unaffected in the substantia nigra and ventral tegmental area. These results confirm that 192-sap and anti-DβH-sap produce the expected selective lesions and that animals prepared with these agents are useful in analysis of the behavioral function of CNS cholinergic and noradrenergic neural systems.

495.6

EFFECTS OF 192 IgG-SAPORIN-INDUCED LESIONS OF THE CHOLINERGIC BASAL FOREBRAIN ON BEHAVIORAL VIGILANCE. J. McGaughy*, T. Kaiser, D. Wendelin and M. Sarter. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

The available evidence supports the hypothesis that the immunotoxin 192 IgG-saporin selectively destroys the cholinergic neurons bearing p75 NGF receptors. Heckers et al. (1994) showed that infusions into the basal forebrain destroyed the cholinergic projections to the cortex and spared the cholinergic neurons projecting to the amygdala and rhinal cortex (which do not bear NGF receptors). As cortical cholinergic afferents have been assumed to mediate the subjects' ability to detect and process behaviorally significant stimuli, the effects of 192 IgG-saporin-induced lesions of the cholinergic basal forebrain on behavioral vigilance were examined. Rats received infusions of 192 IgG-saporin (0.20 μg/0.5 μl/hemisphere) after they were trained to criterion in an operant vigilance task. This task requires the animals to discriminate between signal (central panel light presented for 25, 50 or 500 msec) or non-signal events. Hits and correct rejections were rewarded, while misses and false alarms were not. Following the post-lesion retraining period, animals were treated with a benzodiazepine receptor agonist (chlordiazepoxide; 1, 3, 5 mg/kg; i.p.), and partial inverse agonist (FG 7142; 0.5, 1, 2 mg/kg; i.p.) to test the hypothesis that, in lesioned animals, the detrimental effects of the agonist are more potent than in control animals, and that the partial inverse agonist attenuates the lesion-induced impairments in vigilance performance.

495.7

192 IgG-SAPORIN LESIONS OF BASAL FOREBRAIN CHOLINERGIC CELLS: EFFECTS ON LEARNING AND MEMORY IN RATS. M. G. Baxter*[1], D.J. Bucci[1], A. A. Chiba[2], L. Thai[1], R. G. Wiley[3], and M. Gallagher[1,2]. [1]Curriculum in Neurobiology and [2]Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599; [3]DVAMC and Vanderbilt University, Nashville, TN 37232.

The development of 192 IgG-saporin, which acts as a specific cholinergic neurotoxin when infused directly into basal forebrain nuclei, permits direct assessment of the role of basal forebrain cholinergic cells in cognitive function. Male Long-Evans rats, 2-3 months old, received infusions of 192 IgG-saporin (LES) or vehicle (CON) into either the medial septal area (MSA) or substantia innominata (SI). Place discrimination in the Morris water maze assessed spatial reference memory, and a 2-trial delayed matching-to-place task in the Morris water maze assessed spatial working memory. MSA-CON and SI-CON rats did not differ in behavioral performance and were pooled for statistical analysis. MSA-LES and SI-LES rats were not impaired relative to CON rats in acquisition of the place discrimination. MSA-LES and SI-LES rats were impaired relative to CON rats in performance of the working memory task; however there was no obvious delay-dependence of the deficit at the delays tested (delays ranged from 30 seconds to 3 hours), suggesting that this deficit may not be purely mnemonic in nature. This pattern of results calls into question the central role postulated for cholinergic neurons of the MSA in learning and memory. (Supported by NIA Grant PO1-AD09973-03 to MG and an NSF Predoctoral Fellowship to MGB.)

495.8

SUBSTANTIA INNOMINATA LESIONS IMPAIR INCREMENTAL ATTENTIONAL PROCESSING IN A PAVLOVIAN CONDITIONING PARADIGM. A.A. Chiba[1]*, J.S. Han[1], P.C. Holland[2], and M. Gallagher[1], Department of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599; Department of Psychology, Duke University, Durham, NC 27706.

Holland and Gallagher (1993) proposed a role for the central nucleus of the amygdala (CN) in incrementing attention based on associations formed during appetitive conditioning. Furthermore, recent evidence indicates that regulation of attentional processes by CN may be mediated through the corticopetal system located in the substantia innominata (SI), a component of the basal forebrain system in the rat brain.

An associative learning paradigm was used to examine the role of the SI in modulating attention. Each rat was exposed to conditioned stimuli (CS) that were either consistent or inconsistent predictors of subsequent CSs. Intact control rats maintained higher levels of attention to a CS when it was an inconsistent predictor of subsequent CSs, whereas SI-lesioned rats failed to show this enhanced attention. Thus, SI-lesioned rats displayed an impairment in incrementing attention equivalent to that previously demonstrated by CN-lesioned rats. These data support the notion that CN attentional modulation is dependent on the the projections of the CN to the SI corticopetal cholinergic system. Based on this hypothesis, further experimentation using selective cholinergic lesions of the SI corticopetal system is being pursued within the identical behavioral paradigm. (Supported by an award from the Human Frontier Science Research Program.)

495.9

c-FOS EXPRESSION FOLLOWING PAIRED OF UNPAIRED CUTANEOUS NERVE STIMULI: SPECIFICITY RELATED TO LTP OF SPINAL REFLEXES. R.G. Durkovic* Dept. of Physiology, SUNY Health Sci. Ctr., Syracuse, NY 13210

In order to help define the neural circuits involved in two forms of associative reflex potentiation, spinal neurons expressing *c-fos* were examined following various sequences of cutaneous nerve stimuli. Previous work from his laboratory has shown that in the spinal cat preparation when two cutaneous nerves are stimulated in specific "paired" sequences, flexion reflexes evoked by stimulating one of the nerves exhibit LTP (Long-Term Potentiation) that lasts for hours. These reflex changes obey the general rules established for learning and memory behavior in intact animals (J. Neuroscience 6:2921, 1986). Thus, these spinal reflex changes represent a simplified model for investigating neural mechanisms of elementary forms of learning and memory behavior. The experiments reported here compare the numbers and locations of *fos* labeled neurons in the spinal cords of operative control animals (no nerve stimuli), animals given 30 "unpaired" cutaneous nerve stimuli, and animals given 30 "paired" cutaneous nerve stimulus presentations. *fos* label was differentially expressed among these groups with few neurons labeled in operative control animals and the greatest numbers of labeled neurons in "paired" animals. Furthermore, two methods of "pairing" resulted in concentrations of labeled neurons in different spinal cord regions. The paradigm that preferentially potentiates spinal circuits activated by large myelinated saphenous cutaneous nerve fibers labeled neurons principally in deeper laminae (V-VII) of spinal cord. The paradigm that preferentially potentiates spinal circuits activated by small myelinated saphenous cutaneous nerve fibers labeled neurons primarily in more dorsal laminae (I-V). The results are consistent with the hypothesis that learning and memory result from alterations in the synaptic strengths of specific sets of synapses. Supported by NSF grant IBN 9220206.

495.10

LONG TERM POTENTIATION (LTP) OF PYRAMIDAL NEURONS IN LAYER FIVE OF THE CAT MOTOR CORTEX. Akihisa Kimura*, Francesco Melis, Marcello A. Caria and Hiroshi Asanuma. The Rockefeller University, New York, N.Y. 10021.

Long term potentiation is supposed to play a crucial role in learning new skilled movements (Asanuma and Keller, 1991). A series of experiments revealed ubiquitous existence of LTP in various structures involved in execution of skilled movements. In this study, generation of LTP within the motor cortex was studied using intracellular recording and labeling (biocytin) techniques under Nembutal anesthesia. Intracortical microstimulation (ICMS) was delivered in the superficial layers and recordings were obtained from cells in layer V of the motor cortex. Of 20 labeled cells in layer V, 6 cells showed short lasting potentiation (1-4 min, 160-240% of control EPSP) immediately after tetanic ICMS (100-200 Hz, 10-30 sec) which was followed by steady potentiation (130-150% of the control) lasting until the electrode went out of the cell (7-28 min). In 12 labeled cells, only PTP (120-270% of the control) was observed. The duration of the PTP was from one to 4 minutes. All the labeled cells were pyramidal neurons. The effective stimulating sites inducing LTP were located immediately superficial to the labeled neurons. These results suggest that plasticity exists within the intracortical connections of the motor cortex that may be involved in learning of new motor skills.

495.11

INHIBITION OF NITRIC OXIDE REDUCES HIPPOCAMPAL MEDIATION OF PLACE LEARNING IN THE RAT. G. Wörtwein, B. Gustafson, P. Ermens and J. Mogensen*. Laboratory of Neuropsychiatry, University Hospital-6102, Copenhagen, Denmark.

In Experiment 1 the behavioural consequences of transection of the fimbria-fornix were investigated in animals that had acquired a place learning task after a control pretreatment or a pretreatment period during which near-total inhibition of NOS (the nitric oxide synthesizing enzyme) had been accomplished. While the lesion significantly impaired task performance in normals, the rats which had acquired the task during NOS inhibition did not reveal a lesion associated impairment. In Experiment 2 four groups of rats were studied: two groups received transection of the fimbria-fornix while the two others were subjected to sham surgery. Subsequently, one of the lesioned and one of the sham groups received a period of L-nitro-arginine (L-N-ARG) injections. During the final 5 days of injections the four groups were subjected to training on place learning. While NOS inhibition impaired task acquisition in the sham operated animals, L-N-ARG administration in fimbria-fornix transected rats failed to impair task acquisition. Conclusions: (1) Normal place learning acquisition involves hippocampus associated mechanisms that depend on NOS, and (2) Under NOS inhibition the task can be acquired by a system that differs from the one mediating task acquisition in normals by receiving reduced contributions from the hippocampus.

495.12

Stress Effects on Memory and AMPA Receptors are Abolished by Adrenalectomy. D.M. Diamond*, B.J. Branch, G.M. Rose and G. Tocco. Dept. of Pharmacology, Univ Colo Health Sci Ctr, & VA Med. Ctr., Denver, CO and Neurosciences Program, USC, Los Angeles, CA

We have reported that exposure of a rat to an unfamiliar environment (psychological stress) blocked LTP (*Psychobiology*, 18:273, 1990; *Beh. Brn. Res.*, in press) and selectively impaired hippocampal-dependent memory (*Neurosci. Abst.*, 19:366, 1993). The present work is a test of the hypothesis that the effects of stress on hippocampal function will be eliminated by adrenalectomy (ADX).

Male rats were trained on a 14-arm radial maze incorporating hippocampal-dependent (working memory) and hippocampal-independent (reference memory) components (see *Neurosci. Abst.*, 19:366, 1993 for details). Stress impaired working, but not reference, memory in intact rats. In contrast, stress had no effect on memory in ADX rats. The ADX rats did exhibit other behavioral manifestations of stress (e.g., fecal boli production). Therefore, the effects of stress on emotionality and cognition (memory) can be dissociated. We have also found that stress produces a hippocampal-specific reduction in AMPA receptor binding in intact, but not in ADX, rats. These findings indicate that stress produces adrenal-dependent effects on hippocampal plasticity, memory and glutamate receptor binding dynamics.

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495.13

INFLUENCE OF ENVIRONMENTAL STIMULATION AND BEHAVIOURAL TESTING ON SOMATOSTATINERGIC NEUROTRANSMISSION IN THE RAT BRAIN. L.Nilsson*, A.K.Mohammed, B.G.Henriksson, B.Winblad and L.Bergström, Department of Clinical Neuroscience and Family Medicine , Huddinge University Hospital, Karolinska Institutet, S-141 86 Huddinge, Sweden.

Housing rats in an enriched environment has been shown to induce morphological changes in the cerebral cortex, such as synaptogenesis and dendritic branching. In this study adult Sprague-Dawley rats (n=28) were housed for 30 days in either enriched or impoverished environments. Subsequently half of the groups were tested for spontaneous motor activity for 1 day followed by 2 days of spatial learning test. The rats housed in "enriched conditions" were characterized by superior learning ability and more rapid habituation to novel environment, when compared to their impoverished counter-parts. The relation of cortical somatostatinergic neurotransmission to learning was studied by quantification of somatostatin levels and gene expression. Somatostatin levels were significantly elevated following enriched environment, compared to impoverished conditions. Impoverished but not enriched rats also demonstrated increased cortical somatostatin levels after behavioural testing. The immmunoreactivity detected was furthermore characterized by gel filtration in two of the groups. The relative proportion of proSS (14kD) was significantly lower in the impoverished tested group compared to the non-tested group, suggesting activation of somatostatin neurotransmission occurs at the post-transcriptional level in association with learning stimulation.

495.14

BICUCULLINE ADMINISTERED POST-TRAINING INTO THE AMYGDALOID COMPLEX BLOCKS BENZODIAZEPINE-INDUCED AMNESIA. H. Dickinson-Anson* & J.L. McGaugh. *Center Neurobiology Learning & Memory, Dept. Psychobio., UC, Irvine, CA 92717.

It is well known that benzodiazepines (BZD) produce anterograde amnesia. Recent evidence suggests the amygdaloid complex (AC) GABAergic system mediates BZD-induced amnesia. Lesions of the AC block the impairing effects of a BZD on retention of inhibitory avoidance (IA) training. Additionally IA retention is impaired by pre-training intra-AC infusion of the BZD midazolam (MDZ) and enhanced by intra-AC infusion of a BZD antagonist. We recently reported that pre-training infusion of the GABAergic antagonist bicuculline methiodide (BMI) into the AC blocks the memory impairing effects of systemically administered midazolam on IA behavior. In general, BZDs impair memory when administered before, but not after, aversively motivated learning takes place, whereas GABAergic drugs affect retention when administered post-training. Consequently, it is not clear whether these drugs modulate pre- or post-training memory processes. Because BZD-induced behavioral effects are mediated by the $GABA_A$ receptor complex, it is likely that BZD-induced memory deficits are also due to post-training influences on memory processes. The present experiment examined this implication. Before training in a multiple trial IA task, male Sprague-Dawley rats (250-300g) were injected (ip) with either midazolam (MDZ, 2.0 mg/kg) or vehicle (1.0 ml/kg). Immediately following IA training BMI (2.0, 5.6, 56.0, or 197.0 pmol/ 0.5µl) or vehicle (0.5 µl) was infused bilaterally into the AC. On a 48 hr retention test the performance of the MDZ treated animals was significantly poorer than that of controls. The retention of MDZ-treated animals given intra-AC injections of the lowest dose of BMI (2.0 pmol) was comparable to that of controls, whereas higher doses of BMI impaired retention. The present results are consistent with other findings indicating that the amygdaloid complex mediates the amnestic effects of BZDs on aversive learning. Furthermore, these data suggest that BZDs impair memory by disrupting post-training processes underlying memory consolidation. Supported by PHS MH12526, NIMH & NIDA (JLM).

495.15

IMPAIRED LEARNING AND MEMORY IN MATURE AND OLD SPONTANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RATS. J.M. Wyss*, J.A. Franklin and T. van Groen, Dept. of Cell Biology, University of Alabama, Birmingham, AL 35294-0019

Recent studies in our laboratory indicate that in very old, normotensive rats, a selective breakdown occurs in the limbic cortex and that a similar disorganization occurs in spontaneously hypertensive rats (SHR), but at a much earlier age (12 compared to 30 months). The breakdown leads to a deficit in learning and memory in the 12 month old SHR. In the present study we employed a water maze task to test the hypothesis that learning and memory are disturbed in old (24 month) SHR and in old normotensive Wistar Kyoto (WKY) rats, and we examined the ability of long-term, antihypertensive therapy with captopril to attenuate the deterioration of learning and memory in old SHR and WKY. At the end of the 5 day training period, young SHR display the best performance and have the shortest escape latency, i.e., 7±2 sec, 12 month old SHR are slower to find the platform, i.e., 38±16 sec, and 24 month old SHR are still slower, i.e., 98±22 sec. In comparison, young WKY are slower than age-matched SHR, i.e., an escape latency of 25±3 sec, but 24 month old WKY are similar to 24 month old SHR and perform much worse than 3 month old SHR or WKY. SHR that were made normotensive by long-term captopril treatment were significantly better in the task than the age-matched SHR. The captopril treated WKY also showed an improved performance. It should be noted that swim speeds were similar for all groups. These data indicate that 24 month old SHR and WKY have impaired memory function, and that treatment with captopril partially improves this function in hypertensive and non-hypertensive rats.

495.16

GABAERGIC-CHOLINERGIC INTERACTION IN THE SEPTAL REGION MEDIATES BOTH ANXIETY AND SPATIAL WORKING MEMORY IN MICE. M.P. Mano, M. Belotti and D. Galey*. Lab. Neurosci. Comportementales et Cognitives, CNRS URA 339, Université de Bordeaux I, 33405 Talence France.

Convergent data suggest that the septal region may constitute an interface between anxiety and spatial working memory (SWM). Using C57BL/6 mice, we have examined the role of cholinergic and gabaergic septal neurons interaction in these two processes. The anxiety level was evaluated by comparison between exploratory activities measured in an elevated plus-maze and in a four hole-board. The SWM was assessed using a sequential alternation procedure achieved in a T-maze.

Results indicated that intraseptal infusion of scopolamine (2.5µg/0.2µl) combined with i.p. injection of diazepam (0.5 mg/kg) in same animals produced an "anxiolytic"-like effect. Furthermore, SWM was differentially affected by this treatment. Indeed, performance was unaffected when a 30 sec intertrial interval was used whereas for a 5 sec intertrial interval alternation scores were reduced below the chance level. In addition, the application of either scopolamine or diazepam alone did not induce any changes whatever the behavioral testing situation. These results suggest that a cholinergic/gabaergic septal mechanism mediates both the anxiety phenomenon and SWM. This interpretation is consistent with hypothesis that the cholinergic septo-hippocampal pathway produces anxiety (GRAY, J., Oxford University Press, Oxford, 1982).). Thus, decrease in SWM abilities observed after modulating activity of these structures seems to be the consequence of release from an anxious response, wich will induce increasing vulnerability to proactive interference rather than an accelerating forgetting.

495.17

AGE OBSCURES THE IMPAIRMENT OF LEARNING PERFORMANCE IN S100ß TRANSGENIC MICE. R. Gerlai[1], M.D. Kawaja[2] & J. Roder*[1]. 1. Mount Sinai Hospital, Samuel Lunenfeld Research Institute, and University of Toronto, CANADA; 2. Queen's University, Department of Anatomy, Kingston, CANADA

S100ß is a brain protein whose levels are elevated in Down's Syndrome (DS) and Alzheimer Disease (AD). S100ß has neurotrophic properties *in vitro* and has also been implicated in long-term potentiation (LTP), a neurophysiological correlate of memory. We have shown an LTP/synaptic depression deficiency in transgenic mice overexpressing S100ß. Here we investigate the learning abilities and neurohistology of mice from two independent transgenic lines carrying 8 and 70 copies of the human S100ß gene along with normal, non-transgenic, mice of the same background genotype (CD1). In order to reveal possible age dependent effects we analyzed mice ranging from 3 to 24 months of age. The distribution of neuronal (e.g. neurofilament, calretinin) and glial (e.g. GFAP, S100) markers among animals from each experimental group showed comparable age-dependent changes in both control and transgenic mice. The learning performance of mice was tested in various tasks of the Morris water maze. Young (3-mo-old) transgenic mice exhibited a significant spatial task dependent impairment but normal non-spatial task performance. Although a similar trend was observed in the old mice (16-mo-old), no significant difference was found between transgenic and normal mice in either of the tasks. Comparison of the age-groups showed a significant age effect: old age impaired the learning performance of mice of all genotypes in both the spatial and non-spatial tasks. Unlike the observations in DS and AD, where the neurological impairments are age dependent, our results suggest that aging in the mouse obscures the effects of overexpressing the gene for S100ß.
Supported by MRC, Ciba-Geigy, and NCE of Canada.

495.18

IMPAIRED LEARNING RESULTS FROM A PERSISTENT VIRAL INFECTION IN MICE. M.D. Brot*, L.H Gold, A. Tishon, M.B.A. Oldstone, and G.F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Lymphocytic choriomeningitis virus (LCMV) is a nonlytic murine virus that forms a model system for studying the behavioral correlates of central nervous system virus infections. Newborn mice infected with LCMV develop a persistent infection that is characterized by high viral titers in all tissues including the brain. In this model, virus persists in neurons throughout the animals' life. Previous work has demonstrated learning and locomotor deficiencies in mice infected with LCMV. The present study focuses on learning impairment in a mouse strain in which immunologic parameters of persistent viral infection in neurons, and neurons from which virus has been cleared by immunotherapy, can be directly correlated with behavioral learning procedures.

Neonatal Balb/cByJ mice were injected with LCMV or vehicle and then tested behaviorally as adults for their ability to learn a Y-maze spatial avoidance discrimination task. In this task, the mouse learns to avoid a shock by running into the non-shocked maze arm prior to the onset of the shock. The number of avoidance responses and errors were used as an index of learning. The virus-infected mice performed more poorly on this discrimination task than sham-infected controls, although they eventually reached control levels by the end of the 6 day testing period. Thus, functional deficits in Balb/c mice result from this persistent virus, which replicates previous experiments in other strains and will enable future studies to examine learning behavior of this strain following viral clearance from the brain.

495.19

HIGH DOSE MK-801 KILLS NEURONS IN A SINGLE BRAIN REGION AND CHRONICALLY IMPAIRS MEMORY. G. Brosnan-Watters, D. F. Wozniak*, A. Nardi, J. W. Olney, A. S. Fix, Washington Univ., St. Louis, MO 63110 and Eli Lilly & Co., Greenfield IN 46140.

Previously we reported that a high dose (10 mg/kg sc) of MK-801 produced chronic memory impairment in adult male mice (Brosnan-Watters et al., 1993). Here we confirm in Harlan ICR adult male mice that MK-801(10 mg/kg sc) chronically impairs acquisition performance on a spatial learning task and, in addition, produces neuronal necrosis confined to the posterior cingulate/retrosplenial (PC/RS) cortices. In Exp. I, mice were injected sc with either 10 mg/kg MK-801 (n = 10) or saline (n = 6), and perfused 4 days later. Brains were comprehensively screened histologically (H&E stained paraffin sections) for the presence of necrotic neurons. None were found in the controls nor in any brain region of the MK-801-treated mice except for the PC/RS cortices where the number of necrotic neurons ranged from 1 to 12 per section. In Exp. II, adult male mice were trained on a hole board food search task (4-corner version) using a massed trials protocol and were required to learn in a single day which 1 of the 4 holes was baited on every trial. Mice were assigned to two groups matched according to acquisition performance and then placed back on ad lib food for 4 days before being dosed with either 10 mg/kg MK-801 (n = 7) or saline (n = 8). Two weeks later they were retested on acquisition using a different baited hole. On the posttreatment test, the controls significantly improved their own acquisition scores (fewer trials to criterion), whereas the MK-801-treated mice showed no improvement. In addition, the controls performed significantly better than MK-801 treated mice on the posttreatment test. Ten weeks posttreatment, MK-801-treated mice still showed memory impairment relative to controls, this time on a newly configured hole board task. The results suggest that a high dose of MK-801 induces chronic memory acquisition impairment and raise the question of whether PC/RS neurons play a role in this deficit. Supported by AG 05681 and RSA MH 38894 (JWO).

495.20

EVIDENCE SHOWING THAT HIPPOCAMPAL PROTEIN KINASE C ACTIVITY APPEARS TO BE MORE LINKED TO LEARNING ABILITY THAN TO LEVEL OF TRAINING. X. Nogues, J. Micheau* and R. Jaffard Lab. Neurosciences Comportementales et Cognitives, URA CNRS 339, Univ. Bordeaux I, Av. des facultés, 33405 Talence Cedex, France

We have previously shown a decrease in hippocampal cytosolic PKC activity following partial learning of a reference memory task in a radial maze (Noguès et al.,.Hippocampus, in press). Conversely, membrane bound PKC activity remained unchanged, but was negatively correlated with performance level. Since this result did not fit with the translocation hypothesis, this study was designed to examine the effects of an increased level of training on PKC activity in the hippocampus.

Mice were trained in a spatial concurrent discrimination task performed in an 8-arm radial maze for 2, 5 or 12 sessions. Then, PKC activity was measured in membrane and cytosolic compartments of the hippocampus.

Behavioral results showed a slower rate of acquisition as compared to our previous studies. Biochemical results did not show any significant decrease in cytosolic PKC activity for any group. Nevertheless, membrane bound PKC activity was correlated with the number of reference memory errors at the third day of training ($r=0.74$; $p<0.001$). Firstly, these data show that the decrease in cytosolic PKC activity is not cumulative across sessions. Secondly, they confirm the correlation between membrane-bound activity and performance accuracy. Further statistical analysis on all animals tested in our previous experiments (n=77), shows that the amplitude of the PKC cytosolic decrease is correlated with performance accuracy on the second day of training ($r=0.247$; $p<0.02$; one-tail test).

Taken together, these observations support the idea that the membrane-bound PKC activity derives from a pre-existing pool that plays a significant role in the rate of acquisition. Moreover, they suggest that learning abilities at the early stages of training are linked to a decrease in cytosolic PKC activity .

495.21

WORKING AND REFERENCE MEMORY IN CXB RECOMBINANT INBRED MICE . D. F. Peeler.* Department of Neurosurgery, University of Mississippi Medical School, Jackson, MS 39216

Two types of memory which have been described - reference and working - appear able to function essentially independently. This implies not only separate CNS mechanisms, but also possibly different genetic influences. This was investigated in the CXB set of recombinant inbred (RI) strains. Association between genotype and performance in an 8-arm radial maze was assessed. Adult male mice (N=103) representing the 9 strains of the CXB set were familiarized with the maze for two consecutive days, 5 min per day, with all arms baited. For sessions 3 - 12, four arms were consistently baited. A dim lamp illuminated one corner of the room, with equipment and walls providing different shapes and textures as distant orientation cues. Each arm of the maze was dimly illuminated at its distal end. The mice were placed directly in the maze center, facing the lighted corner in sessions 1 - 7, and away from the lighted corner for sessions 8 - 12. Session duration was 5 min. Analyses utilized ANOVA and multiple range tests.

Strains show similar rates and levels of acquisition. There are significan differences among the strains for working memory (number of arm re-entries; number of baited arms entered before re-entry) and reference memory (number of non-baited arm entries), but the strain distribution patterns are different. Orientation change at the beginning of session 8 disrupted performance, presumably reflecting the contradiction of reference memory. The strain distribution pattern for reference memory indicated a possible major single gene effect. For some measures of working memory, the progenitor strains were statistically similar but the strain distribution pattern was different, implying some genetic determinants different from reference memory with no major single gene effect.

495.22

MOUSE STRAIN DIFFERENCES IN MORRIS WATER MAZE PLACE TASK PERFORMANCE

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AJ; DBA; and C57 mice were tested for acquisition and retention of place task in the Morris water maze. All animals were swum for 14 days (4 trials per day; 60 seconds maximum per trial), with the platform being moved from the NW to the SE quadrant on day 8. AJ mice were unable to reliably find the platform, while the time taken by DBA animals in escaping the pool declined gradually during the study. C57 mice reached asymptote performance in 4 days, and found the platform significantly faster than either the DBA or AJ mice, while the DBA animals were significantly faster than the AJ mice in escaping the pool. Swim paths used by the 3 strains indicated that C57 mice selected the most efficient strategy for the solution of the task, while DBA animals did not acquire the same elegance in performance. AJ mice were never able to devise an appropriate strategy for solution of the task. Neuroanatomic differences between the 3 strains are also described, and the implications discussed.

495.23

MORRIS WATER MAZE IN THE NAPLES HIGH AND LOW-EXCITABILITY RAT LINES. A.Gallo[1], H.Welzl[2]*, M.P.Pellicano[1] and A.G.Sadile[1]. [1]Lab. Neurophysiol. Behav. & Neural Networks, Dept. Human Physiol. "F. Bottazzi"; Second Univ. of Naples, 80138 Naples, I; [3]Inst. Behav. Biol., ETH, 8092 Zürich, CH.

Behavioral, histochemical, neurochemical and electrophysiological evidence supports the hypothesis that the Naples High (NHE) and Low-Excitability (NLE) rat lines may be genetic model to study hippocampal functions (Cerbone et al., Neurosci.Biobehav.Rev. 17: 295-303, 1993). To further challenge this proposal, adult male rats of the NLE, NHE and random-bred controls (NRB) were tested in a Morris water maze. A video camera system was used to monitor and record the rat behavior. In the place-learning task, one block of four trials per day was given for 5 days. Every day a rat had to find the hidden platform starting to swim from all four locations (N, S, E, W) in a random order. For each trial the escape latency and the swim distance were measured. On day 6, the platform was removed from the pool and a 30-s trial was given. The time spent in each of the four quadrants, and the total swim distance were recorded. During session 1, both NLE/NHE, but not NRB-rats, decreased the time and the distance travelled to find the platform. During the acquisition phase, both NLE/NHE spent more time to find the hidden platform, but the NHE on session 5 were not different from NRB-rats. The distance travelled was higher in the NHE than in the NLE/NRB-rats. In the platform removal test, NLE/NHE rats spent less time than NRB-rats in the quadrant where the platform had been. The results indicate that both NLE/NHE rats are coherent with a non linear model of the inverted-U type with both extremes leading to impaired spatial processing. **(Supported by CNR 92.01092.CT04 and 93.00408.CT04 grants).**

495.24

DIFFERENTIAL EXPRESSION OF NADPH-DIAPHORASE IN THE BRAIN OF THE NAPLES HIGH AND LOW-EXCITABILITY RAT LINES FOLLOWING AROUSAL AND HABITUATION TO NOVELTY. M.Papa[1]*, and A.G.Sadile[2]. [1]Inst. Anatomy, and [2]Lab. Neurophysiol. Behav. & Neural Networks, Dept. Human Physiol. "F. Bottazzi", Second Univ. Naples, 80138 Naples, Italy.

The effects of exposure to spatial novelty on the expression of nitric oxide synthase were mapped in the brain of the Naples High (NHE) and Low-Excitability (NLE) rat lines by NADPH-diaphorase (NDP-d) histochemistry. Adult male rats of the NLE, NHE and control rats (NRB) were tested for 10 min in a Làt-maze and sacrificed at different time intervals thereafter (0, 2, or 24 h). Handled unexposed rats served as controls. The brains were perfused with paraformaldehyde, cryoprotected with sucrose and processed for histochemistry for NDP-d reaction. Sections were processed for densitometric evaluation by digital image analysis. In unexposed control rats the the number of NDP-d positive cells and total optical density values were low and scattered. In contrast, following exposure to novelty, all rats showed an increased NDP-d staining in the hippocampus (granule cells, few hilar neurons and some CA1 pyramidal cells), the caudate-putamen complex, the cerebellum, and all layers of somatosensory cortex. However, NLE/NHE-rats were less positive than NRB-rats. The positivity was not due to activity per se, since immediately after exposure did not differ from baseline. In contrast, it peaked at 2h to fade out 24h later. In addition, a strong neuronal discharge induced by pentylentetrazol did not induce NDP-d 2 h afterwards. The widespread induction of NDP-d suggests that exposure to novelty activates overlapping neural networks across different organizational levels of the CNS, whereas the impaired expression in the NHE-rats provides a tool for studying the neural substrates of spatial and non spatial information processing. **(Supported by CNR 92.01092.CT04, and MURST 40% grants).**

496.1

GABAERGIC RETICULAR THALAMIC NEURONS PROJECT TO THE CONTRALATERAL MEDIODORSAL NUCLEUS OF THE THALAMUS IN THE CAT. I. Gritti*, M. Mariotti and M. Mancia. Institute of Human Physiology II, University of Milan, I-20133 Milano, Italy.

In order to investigate the topographical and chemical organization of the pathway involved in the regulation of the EEG synchronization at a thalamic level horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP) was injected into the intermediate part of the Mediodorsal nucleus (MD) of the thalamus. Retrograde transport was also combined with glutamic acid decarboxylase (GAD)-immunohistochemistry to provide additional criteria for the identification of retrograde labelled neurons. In cats under pentobarbital anaesthesia WGA-HRP was stereotaxically injected into the intermediate part of MD. After a survival time of 24-48 hrs, the animals were perfused through the ascending aorta with mixed aldehydes; 25 μm frozen sections were horizontally cut. Alternate sections were reacted for WGA-HRP visualization using tetramethylbenzidine as a chromogen with cobalt intensification, and the peroxidase anti-peroxidase technique on immunohistochemistry for GAD. In the same sections GAD-immunohistochemistry, combined with the retrograde transport of WGA-HRP, revealed that the intermediate part of MD nucleus receives an important input from GABA synthesizing neurons located in the ipsi and contralateral rostral-pole of the RE nucleus. Although the distribution of GAD-immunoreactive retrogradely labelled neurons was similar both in the ipsilateral and contralateral RE nucleus, their number was much smaller in the latter. The findings suggest that through both GABAergic ipsilateral and contralateral projections the RE contribute to a GABA mediated inhibition within the intermediate MD neurons population and to a thalamo-cortical synchronization.

496.2

NOVEL SLEEP STATE ORGANIZATION IN THE ECHIDNA: IMPLICATIONS FOR THE EVOLUTION OF REM AND NON-REM SLEEP J.M Siegel , P. Manger, R. Nienhuis, H.M Fahringer and J. Pettigrew* VAMC, Sepulveda CA 91343, UCLA, Los Angeles, CA 90024 and U. of Queensland, Brisbane, Australia

We have examined brainstem neuronal activity in the primitive monotreme mammal, the echidna (*Tachyglossus aculeatus*) across the sleep cycle. While no periods of REM sleep were seen, unit activity patterns differed from those seen in placental mammals during nonREM sleep.

Twenty-two brainstem units were recorded from the midbrain and subcoeruleus region. Unit discharge rates were significantly lower in sleep than in quiet waking ($F=3.8$, $p<.05$). However, both midbrain and pontine regions had **substantially increased discharge variability** (burst firing) throughout sleep ($p<.001$).

Therefore, neuronal activity during sleep in the echidna does not resemble that seen in nonREM sleep in placental mammals. While the discharge rate of the neuronal population decreases, as in placental nonREM sleep, discharge variability increases, as in REM sleep. We hypothesize that **both** REM and nonREM evolved from this primitive "mixed" sleep state.

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496.3

GABA RELEASE IN THE LOCUS COERULEUS AS A FUNCTION OF SLEEP/WAKE STATE. D.A. Nitz and J.M. Siegel* UCLA Dept. of Neuroscience and UCLA Dept. of Psychiatry, Los Angeles, CA 90024 and Sepulveda VAMC, Sepulveda CA 91343

Noradrenergic neurons of the nucleus locus coeruleus fire tonically throughout waking, decrease discharge in non-REM sleep, and cease discharging in REM sleep. Only serotonergic nuclei of the raphe system and histaminergic nuclei of the posterior hypothalamus display similiar firing patterns. The locus coeruleus is densely innervated by GABAergic neurons. In vitro studies have shown that application of GABA onto noradrenergic neurons potently inhibits their activity. Based on this evidence, we hypothesized that GABA mediates the inhibition of noradrenergic locus coeruleus neurons during sleep.

GABA release in the locus coeruleus was measured as a function of sleep/wake state using microdialysis and HPLC techniques. Histological analysis confirmed the placement of two dialysis probes in the locus coeruleus and peri-locus coeruleus areas. GABA release in the more caudal site was increased during both non-REM and REM sleep as compared with wake. Data from the more rostral locus coeruleus including the peri-locus coeruleus area indicated a selective increase in GABA release during REM sleep as compared to wake and non-REM sleep. We have previously reported a similiar selective increase of GABA release in the dorsal raphe nucleus during REM sleep (Soc. Neurosci. Abstr., 19:1815, 1993). These data suggest that GABA mediates the inhibition of noradrenergic locus coeruleus neurons during non-REM and REM sleep.

496.4

Simultaneous application of serotonin or carbachol facilitates NMDA-dependent bursts of nucleus basalis cholinergic neurones in guinea-pig brain slices. P. Fort, A. Khateb, B.E. Jones° and M. Mühlethaler* *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and °Montreal Neurological Inst., McGill University, Canada H3A 2B4*

Cholinergic neurones of the nucleus basalis provide the major cholinergic input towards the cerebral cortex. In a recent study we have demonstrated that these cells, which are endowed with a potent low threshold calcium spike (LTS), responded to the continuous application of NMDA by rhythmic bursts. However, in the majority of cases (85%), this effect became apparent only when the cells were artificially hyperpolarized. In parallel studies we found that both serotonin and carbachol were able to hyperpolarize cholinergic neurones. Our goal in this study was thus to identify the possible interactions in between these substances. When NMDA was applied alone, it depolarized these cells and led them to fire tonically. However, when serotonin was added concomitantly, the neurones started to slightly hyperpolarize and then discharged rhythmically in bursts (21 out of 26 experiments). This effect persisted in TTX (n=4). Interestingly the duration of the LTS was greatly increased in presence of NMDA+ serotonin, as compared to the situation of NMDA alone (tested at different levels of membrane hyperpolarization). Almost similar results were obtained for the simultaneous applications of NMDA and carbachol (9 out 19 experiments). These interactions might be the means by which cholinergic neurones could discharge rhythmically in bursts in vivo. *(Swiss NSF, Canadian MRC, Fondation Fyssen and Lyonnaise des Banques).*

496.5

Effects of muscimol on non-cholinergic neurones of the nucleus basalis in guinea-pig brain slices. A. Khateb*, P. Fort, B.E. Jones° and M. Mühlethaler *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and °Montreal Neurological Inst., McGill University, Canada H3A 2B4*

The nucleus basalis contains both cholinergic and non-cholinergic magnocellular neurones which respectively display distinct electrophysiological properties when recorded in guinea pig brain slices. Non-cholinergic cells demonstrate high frequency (30-60 Hz) subthreshold oscillations and have the ability to discharge in clusters of action potentials (recurring at 2-6 Hz). In order to assess the potential modulation of their activity by GABA, the effect of muscimol, a selective $GABA_A$ agonist, was tested on the non-cholinergic cells. Out of 31 cells tested, 23 were depolarized by high concentrations of muscimol (10^{-4} M), yet concomitantly inhibited due to a marked decrease of their membrane resistance. With smaller concentrations of muscimol (10^{-6} M), tested on 8 cells, 5 were still depolarized, but in addition their ability to fire in clusters was greatly facilitated. This effect persisted throughout the duration of the agonist's application and was perfectly reversible. Furthermore, the effect was antagonized by bicuculline, a $GABA_A$ blocker. These results suggest that locally released GABA could serve to facilitate the cluster firing pattern of non-cholinergic neurones and thus the rhythmic activity of these cells could participate in the generation and/or maintenance of theta rhythms in the forebrain *(Swiss NSF, Canadian MRC, Fondation Fyssen and Lyonnaise des Banques).*

496.6

HYPERPOLARIZING POTENTIALS IN MASSETER MOTONEURONS ARE ELICITED BY AN AUDITORY STARTLE REFLEX AFTER CARBACHOL INJECTIONS IN THE NUCLEUS PONTIS ORALIS. K.A. Kohlmeier, F. López-Rodríguez*, R.-H. Liu, F. Morales, M.H. Chase. Dept. of Physiology, Dept. of Anatomy and Cell Biology, and the Brain Research Institute. UCLA School of Medicine, Los Angeles, CA 90024.

One striking phenomenon of active sleep is the occurrence of ponto-geniculo-occipital (PGO) waves. Morrison and Bowker (Acta Neurobiol. Exp. 35:821-840, 1975) proposed that these waves are neuronal signatures of a startle response produced by internally generated signals. We have found that PGO waves are accompanied by large amplitude IPSP's recorded in lumbar and trigeminal motoneurons (Pedroarena et. al., Sleep Res., 20A:58, 1991). We therefore decided to investigate whether startle responses induced by auditory stimulation are also accompanied by the inhibition of motoneurons. For this purpose we microiontophoretically injected carbachol (200mM) into the nucleus pontis oralis of five α-chloralose anesthetized cats (40mg/kg, i.v.) because carbachol-induced muscle atonia is comparable to that which occurs during active sleep (Morales et al., J. of Neurophys., 57,4:1118-1129, 1987). Responses to an acoustic stimulus (95 dB; duration=1.5ms) were recorded intracellularly (KCitrate-filled glass electrodes) from antidromically identified masseter motoneurons both before and after the injection of carbachol. In 19 of the 20 masseter motoneurons recorded before the injection of carbachol, the auditory stimulus elicited no response. In one neuron there was a long latency depolarizing potential. After the injection of carbachol the auditory stimulus elicited a distinct hyperpolarizing potential in 20 of 21 masseter motoneurons (latency to onset=31.2±1.3ms; latency to peak=45.6±1.5ms; rise time=14.4±1.5ms; half-width=25±5.8ms; amplitude=-2.6±0.4mV). In two of the neurons the effect of hyperpolarizing and depolarizing current injection was examined; the amplitude of the stimulus-induced hyperpolarizing potentials decreased and increased, respectively, suggesting that they are inhibitory post-synaptic potentials. These findings indicate that the carbachol-activated motor inhibitory system is responsive to a startle stimulus in the α-chloralose anesthetized animal and that the processing of this sensory input includes the production of a hyperpolarizing potential in motoneurons. The physiological significance of this hyperpolarizing potential during active sleep may be to aid in insuring the maintenance of muscle atonia that otherwise might be disrupted by auditory sensory stimuli. Supported by NS 23426 and NS 09999.

497.1

CIRCADIAN MODULATION OF THE RAT ACOUSTIC STARTLE REFLEX. P. W. Frankland [*1] and M.R. Ralph[1,2], Dept. of Psychology[1] and Zoology[2], University of Toronto, Toronto, Canada M5S 1A1.

The acoustic startle reflex (ASR) in rats exhibits robust circadian modulation, with ASR amplitudes greater in subjective night. Because the ASR is mediated by a serial circuit with few synapses, it provides a simple system in which to study the circadian modulation of behavior. We sought to identify the location of modulation by recording amplitudes of startle reactions evoked either acoustically or electrically via electrodes implanted in the primary startle circuit. Responses were measured at four hour intervals over 48 hours in constant dark conditions. Startle amplitudes were greater in subjective night for acoustically evoked and electrically evoked responses from both the ventral lateral lemniscus and medial longitudinal fasciculus. These results show that circadian modulation of startle occurs at some point in the circuit after the last brainstem synapse in the caudal pontine reticular formation, at the level of spinal interneurons, motoneurons or at the neuromuscular junction.

In a second experiment, animals with electrolytic lesions of the hypothalamic suprachaismatic nucleus (SCN) exhibited no circadian variation in ASR amplitudes. Mean startle amplitudes were similar to pre-lesion basal (subjective day) levels. These results suggest that circadian rhythm of ASR is driven by the SCN, and that the SCN may enhance startle amplitudes during subjective night.

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497.2

RETICULAR THALAMIC NUCLEUS AND NEOCORTICAL PAROXYSMS IN THE RAT. G. Marini*, R. Giglio, G. Macchi °and M. Mancia (SPON: European Neuroscience Association).
Ist. Fisiologia Umana II, Univ. degli Studi; * INB-CNR, Milano; °Ist. Neurologia, Univ. Cattolica, Roma (Italy).

The reticular thalamic nucleus (NRT) has been involved in mechanisms for the induction of normal thalamocortical rhythmicity in cats. The goal of the present study was to test the hypothesis that in rats the abolition of NRT unique influence (due to absence of intrinsic GABAergic interneurons) has a more crucial role on synchronization processes than in cats.

Unilateral chemical lesions were stereotaxically placed in the anterolateral sectors of NRT of 5 ketamine-sedated rats, chronically implanted to monitor frontoparietal EEG and nuchal EMG. Ibotenic acid (20μg/μl in 1M phosphate buffer) was used. In all animals in which lesion of anterolateral NRT sectors were histologically confirmed, after 1.5-2.5h post-injection, an EEG epileptic activity (high-voltage polyspikes and spike-wave complexes) emerged abruptly from a background of delta activity primarily in the contralateral hemisphere. The number of episodes peaked at 2-3h post-injection and decreased gradually. The epileptic activity declined within 2 days. Bilateral NRT lesions failed to trigger the phenomenon and abolished EEG synchronization. The results further confirm the role of anterolateral NRT sectors in both physiological and pathological synchronization processes.

497.3

INDUCTION OF FOS PROTEIN IN THE RAT CIRCADIAN SYSTEM BY NON-PHOTIC STIMULI. S. Amir*, K. Edelstein, B. Robinson and B. Woodside. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada.

Immunohistochemistry of Fos proteins in the hypothalamic suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL) of the lateral geniculate complex is often used as a tool in studies on the neural mechanisms and cellular processes involved in photic regulation of circadian rhythms of rodents. Photic stimuli that induce phase shifts of circadian rhythms in mice, rats and hamsters also induce the expression of Fos in the SCN and IGL; photic stimuli that fail to induce phase shifts appear to be without effect on Fos expression in these structures. The effect of non-photic stimuli on Fos expression in the SCN and IGL is less well defined. Here we report that in rats, cortical injury (induced under pentobarbital anaesthesia, 50 mg/kg, by lowering a 28 gauge needle 3 mm below the surface of the skull) or immobilization stress (60 min) induce the expression of Fos in the SCN and IGL. Stimuli such as i.p. saline injection (1 ml/kg) and cage transfer induce Fos expression in the IGL. Expression of Fos in the SCN and/or IGL in response to each of these stimuli is independent of the time of treatment, and is associated with expression in other brain regions, including many cortical, subcortical and hypothalamic areas such as the paraventricular nucleus, anterior hypothalamic nucleus and preoptic nucleus. SCN cells which express Fos in response to cortical injury or immobilization are located in subregions of the nucleus that also express Fos in response to photic stimuli. Results indicate that neural components of the rat circadian system respond to diverse types of non-photic stimuli and that Fos expression in SCN and IGL should not be regarded as light-specific.

497.4

ABNORMAL EXPRESSION OF CIRCADIAN ACTIVITY RHYTHMS IN MICE LACKING THE GENE FOR THE TYROSINE KINASE *fyn*. T.S. Kilduff*, B.F. O'Hara, H.C. Heller, W.C. Dement, and D.M. Edgar. Center for Sleep and Circadian Neurobiology, Depts. of Psychiatry and Biological Sciences, Stanford University, Stanford, CA 94305.

Protein tyrosine kinases (PTKs) phosphorylate substrates involved in a variety of cellular regulatory processes and play an important role in signal transduction. We have begun to characterize the circadian system of a strain of mice in which the gene for a particular nonreceptor PTK known as Fyn has been eliminated by homologous recombination. In contrast to wildtype 129 Sv mice which entrain adequately to an LD cycle, *fyn*- mutants exhibit phase control abnormalities under LD. In mutants in which the endogenous rhythm is relative strong (2/8 animals), this abnormality was manifest as an altered phase relationship with the LD cycle: even though $\tau_{DD} > 24$ hr, activity onset preceded light offset by as much as 8 hours. The net result in these mutants is that the majority of activity occurred in the lights on period. In cases in which the endogenous rhythm was relatively weak, L/D masking of the activity rhythm occurred (5/8 animals). Under DD conditions, a progressive dampening of the activity rhythm was evident over the course of 8 months as confirmed by both chi-square and Schuster periodogram analyses; chi-square periodogram analysis was eventually unable to detect significant rhythmicity in the circadian range in 6 of 8 *fyn*- mice. One *fyn*- mutant was periodic in DD at 6 months of age, exhibited an abnormal phase relationship under LD at 10 months of age, and circadian rhythmicity was undetectable at 14-15 months of age. These results suggest that the tyrosine kinase *fyn* may be involved in the expression of the activity rhythm, if not the intrinsic timing mechanism of the circadian pacemaker and/or its processing of photic input

(Supported in part by AG06490 and AG11084).

497.5

CIRCADIAN RHYTHMS IN A GENETIC MODEL OF OBESITY. R.E. Mistlberger*, H. Lukman, and B.G. Nadeau. Department of Psychology, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

The Zucker (fa/fa) rat is a widely used model of genetic obesity characterized by a variety of metabolic and endocrine abnormalities, and by low amplitude and/or phase advanced circadian rhythms. The nature of the circadian disturbance, and its possible role in obesity, are unclear. Body temperature rhythms (Tr) were recorded by telemetry in 7 obese and 5 lean Zucker rats under a 12:12 light-dark (LD) cycle and in constant dark (DD) and light (LL). Obese rats exhibited a phase advanced Tr in LD; the acrophase of the best fitting cosine function occurred 6.7 h early by comparison with lean rats ($p<.05$). The amplitude of the cosine function did not differ between groups. The phase advance was retained in DD, indicating that it is not due to differential masking effects of light. In LL, all rats exhibited free-running Tr's with periods > 24 h (no group difference). This suggests that phase advances in LD are independent of pacemaker period and may reflect altered phase responses to light or changes downstream from the clock, in homeostatic processes or coupling to slave oscillators. Obese rats also exhibited an advanced feeding rhythm, with more intake during the light period. The role of advanced rhythms in the development of obesity was examined by measuring body weight and food intake for 60 days in 2 additional fa/fa groups (6 weeks age); one group received food ad-lib, the other only during the dark period, i.e., phase advanced feeding was prevented. Daily food intake did not differ between groups, but the nocturnal-fed rats exhibited a lower average daily weight gain ($p<.05$), suggesting that circadian abnormalities may contribute to obesity.

497.6

PROGRESSIVE CIRCADIAN RHYTHM DECLINE IN ALZHEIMER'S DISEASE. William Fishbein*, Steven Ferris and Barry Reisberg. Dept of Psychology, The City College & Graduate School, CUNY, New York 10031 and NYU Medical Center, Alzheimers Disease Center, New York 10016.

This research is concerned with developing an early behavioral marker for AD. We have accumulated circadian rest-activity data (12 days per subject) from six healthy elderly subjects and fifteen very reliably diagnosed, community residing, AD patients (mild to severe), employing a miniature ambulatory wrist monitor.

The present report describes the detection and decline of the generator(s) of the circadian process by non-linear curve fitting, an iterative process that reduces noise and detects peaks that otherwise might be missed by direct examination of circadian rhythm data. Precise measures of amplitude, area, center and width of peaks, and overall area of a single circadian cycle are determined.

In normal elderly (Global Deterioration Score (GDS) = 1, Mini-Mental State (MMS) = 30), four activity peaks are detected accounting for 95% of the variance of the 24 hour circadian rhythm. Qualitatively, the circadian rhythm looks like a square wave with small perturbations throughout the 24 hour cycle. At night, subjects' sleep periods are relatively free of waking movement activity.

In Alzheimer's Disease, advancing from mild to severe, (GDS = 4 to 6; MMS = 24 to 3) five to eight activity peaks are necessary to account for 92% to 94% of the variance of the 24 hour circadian rhythm. Most notable as dementia advances: (1) progressive increase in the number of peaks, (2) phase shift of peaks to earlier clock time hours, (3) increasing variance between peaks and troughs, and (4) progressive increase in level of activity during the nighttime. Ultimately, in subjects with severely advanced dementia the circadian rhythm disappears completely.

Although the research is in an early stage, the accumulated data indicate that with decline in cognitive and functional competence, a concurrent and profound degeneration of the circadian time keeping system occurs in Alzheimer's disease.

498.1

INVESTIGATION OF THE EFFECTS OF PHASE-SHIFTING TREATMENTS ON THE REGULATION OF PUTATIVE OSCILLATOR PROTEINS IN *APLYSIA*. C. Koumenis, M. Sloan, T. Liu, M. Nunez-Regueiro, and A. Eskin*, Dept. of Biochem. & Biophys. Sci., Univ. of Houston, TX 77204.

A circadian pacemaker located in the isolated eye of *Aplysia* is responsible for generating a rhythm of spontaneous nerve impulses. A model of this system predicts that light and 5-HT will phase shift the oscillator by modifying the synthesis of oscillator proteins at the level of translation and perhaps transcription as well. Using 2-D gel electrophoresis, we previously searched for putative oscillator proteins (POPs) in the eye whose synthesis was modified by treatments of light.

To extend these studies, proteins were labeled with ^{3}H-leucine during the last 4h of a 6h 5-HT treatment, as well as 1h and 3h after the end of the 5-HT treatment. 5-HT increased incorporation of label into POP-1, POP-2, and POP-3, while it decreased incorporation into POP-4 and POP-5. Interestingly, the effects of 5-HT on the synthesis of POP-1, POP-2, POP-4 and POP-5 are opposite to those of light. 2h pulses of DRB (a transcription inhibitor) also altered ^{3}H-leucine incorporation into all five POPs during, or shortly after, DRB exposure.

These experiments focused our attention on several POPs which we have identified using microsequencing techniques: POP-1 — lipocortin, POP-2 — porin, POP-3 — GRP78/BiP, and POP-4 — "multiple stimulus response" protein. POP-5 was identified as HSP70 by heat shock experiments.

To measure mRNA levels and other aspects of gene expression, we have selected two of these putative oscillator proteins for study. Riboprobes for BiP (a molecular chaperone in the endoplasmic reticulum) and porin (a large conductance channel protein found in the outer mitochondrial and other membranes) have been made. *Aplysia* BiP clones were obtained from the Kandel lab. *Aplysia* libraries were screened with human porin clones and a clone containing the full-length cDNA porin coding sequence has been obtained. A 199-bp sequence from the 5'-end of this clone was found to have a 60% identity with human porin. We are currently using these probes in ribonuclease protection assays to examine the effects of light and 5-HT on the mRNA levels of these two proteins.

498.2

ESTROGEN RECEPTORS ARE NOT PRESENT IN THE CIRCADIAN SYSTEM OF THE OCTODON DEGUS, A DIURNAL RODENT. [1]N. Goel*, [2]L. Smale and [1]T.M. Lee, Depts. Psychology, [1]University of Michigan, Ann Arbor, MI 48104, and [2]Michigan State University, East Lansing, MI 48824.

Steroid hormones, in particular estrogen, influence circadian rhythms in various rodents, but the site of action for estrogen's effects is not yet known. Octodon degus are hystricomorph rodents that exhibit diurnal temperature and activity rhythms (Labyak, 1993). In the degu, elevated estrogen levels during estrus lead to phase advances in the wheel-running rhythm and increased levels of activity (Labyak and Lee, in press). Because lesions of the intergeniculate leaflet (IGL) of the thalamus in hamsters, a major afferent projection to the SCN, result in changes in the phase angle of activity onset, the IGL has been suggested as a candidate for estrogen's site of action. Estrogen receptors have been located in the IGL of guinea pigs (DonCarlos, pers. comm.), but not in hamsters (Smale, unpub.). No steroid receptors have been identified in the suprachiasmatic nucleus (SCN), the primary circadian clock. The goal of this study was to identify the distribution of estrogen receptors throughout the degu brain. Three adult degu brains (one male, two female) were processed for immunohistochemical identification of estrogen receptors using the H222 antibody. ER-immunoreactive (IR) cells were found in the medial preoptic area (mPOA), bed nucleus of the stria terminalis (BNST), supraoptic nucleus (SON), arcuate nucleus, paraventricular nucleus (PVN), infundibulum, ventral posterior medial nucleus (VPM) and amygdala. No ER-IR cells were found in the SCN or IGL. Thus, it appears that estrogen cannot directly influence the circadian system via the SCN or IGL in the degu.

498.3

EXPRESSION OF GROWTH HORMONE-RELEASING HORMONE (GHRH) AND SOMATOSTATIN (SRIF) GENES DURING REM SLEEP DEPRIVATION IN THE RAT. T.Porkka-Heiskanen*, J.Toppila, M.Asikainen, F.W.Turek, D.Stenberg. University of Helsinki, Helsinki, Finland and Northwestern University, Evanston, IL 60208.

GHRH increases both SWS and REM sleep, while SRIF increases REM sleep. Antiserum to GHRH decreases sleep and inhibits sleep rebound after deprivation. We tested the hypothesis that REM sleep deprivation affects the expression of GHRH and SRIF genes. Male rats were deprived of REM sleep on small platforms for 24 or 72 h, and one group was allowed a rebound sleep of 24 h after 72 h deprivation. Large platforms and animals taken directly from their home cages served as controls to sleep deprived groups. In situ hybridization was made from 20 μm cryosections through the periventricular and arcuate nuclei using specific oligonucleotide probes for GHRH and SRIF. Number of cells expressing SRIF and GHRH was counted, as well as the area of silver grains over the labeled cells; serum GH was measured. Number of cells expressing GHRH in the arcuate nucleus was lower after 24 hours of REM sleep deprivation than in home controls, and stayed low through 72 h of deprivation. Number of cells expressing SRIF was elevated on small platforms after 24 h of deprivation but had returned to home control level at 72 h. Serum GH levels were decreased through the deprivation. We conclude that both elevated SRIF gene expression in the beginning of the deprivation and decreased GHRH gene expression through the deprivation may contribute to the low serum GH levels during the deprivation.

498.4

DIURNAL RHYTHMS OF MONOAMINES AND THEIR METABOLITES IN CORTEX, HIPPOCAMPUS AND HYPOTHALAMUS OF MALE WISTAR RATS. D.Stenberg*, M.Asikainen, J.Toppila and T.Porkka-Heiskanen. University of Helsinki, Helsinki, Finland.

Normal values for the diurnal variation of monoamine and metabolite concentrations in brain tissue of male Wistar rats were needed for studies on effects of sleep deprivation. Rats aged 3 months and adapted to LD 12:12 (lights on 0600) were decapitated (six rats with 2 h intervals for 24 h) and brain regions dissected, frozen, and subsequently analysed by HPLC-ED. Analysis of variance and cosinor analysis were used. 5-HT levels tended to decrease after onset of dark in frontal cortex (CX) and hippocampus (HC) while 5-HIAA levels showed no variation. In posterior hypothalamus (PH) both 5-HT and 5-HIAA had a 12 h rhythm with acrophase at 1210 h, while in rostral hypothalamus (MH) no diurnal variation was found. Dopamine levels in all regions tended to increase between 0600 and 1400 h, with a 24 h rhythm in PH with acrophase at 1330 h. The ratio DOPAC/DA varied greatly with 12 h rhythm in CX and HC peaking around 1500 and 0300 h. HVA had a 24 h rhythm with acrophase at 1210 h in PH, but not in CX or MH. Noradrenaline had no significant diurnal variation in any of the regions. Rat strains differ from each other in drug responsiveness and in brain chemistry. The diurnal pattern is also dependent on brain region. The measured descriptors thus form a useful basis for evaluating experimental manipulations in this rat strain.

498.5

CENTRAL THYROTROPIN-RELEASING HORMONE ADMINISTRATION PRODUCES PHASE SHIFTS IN THE CIRCADIAN RHYTHM OF HAMSTER WHEEL RUNNING BEHAVIOR. Keith A. Gary*, Patricia J. Sollars, Nedra Lexow, Andrew Winokur, and Gary E. Pickard. Department of Psychiatry, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Several lines of evidence suggest that thyrotropin-releasing hormone (TRH) plays a primary role in the regulation of arousal in mammals. Analeptic effects of this tripeptide have been established by numerous studies, and endogenous levels of TRH vary in response to increased and/or decreased CNS activity states. Modulation of arousal state would require integration and coordination of autonomic, neuroendocrine, and circadian functions. TRH and its receptors has been localized in the retina, suprachiasmatic nucleus (SCN), and pineal gland; anatomical structures associated with circadian function. This study evaluates the effects of TRH microinjection into the suprachiasmatic nucleus (SCN) of the hamster on 24 hour wheel running behavior. The precision displayed by the hamster in wheel-running activity prompted our use of this species in these studies. TRH or saline was administered both by intracerebral ventricular (icv) injection and site specifically via chronic indwelling cannulae stereotaxically placed in the SCN. Drug administration was performed at circadian times (CT) 6 and 18, times corresponding to subjective day and night, respectively. At CT 6, both phase advances and delays in wheel running activity were observed in hamsters receiving 10nM and 100nM TRH icv, whereas phase advances were observed following the same doses delivered directly in the SCN. The magnitude of the phase advance was dose dependent, with 10 nM and 100 nM TRH administration resulting in 35 and 50 minute advances in wheel running activity, respectively. No changes in wheel running activity was observed following icv TRH administration at CT 18. These data support the hypothesis that TRH influences circadian rhythmicity.

498.6

IN VIVO ANALYSIS OF ADENOSINE LEVELS IN HIPPOCAMPUS AND NEOSTRIATUM DURING REST AND ACTIVITY PERIODS. M.Pfister, J.P. Huston*, R.Schwarting, U.Decking#, J.Schrader# and H.L. Haas#. Inst. Physiol. Psychol. and Physiol.#, Univ. Düsseldorf, 40225 Düsseldorf, Germany.

Adenosine occurs in the extracellular space of the central nervous system where it exerts tonic inhibitory effects at pre- and postsynaptic levels, including reduction of excitatory transmitter release, induction of a potassium current and increase of the Ca^{++}-dependent potassium current I_{AHP}. The sedative properties of this metabolite have long been known but the question remains whether endogenous adenosine is responsible for fatigue after intense neuronal activity and/or whether it increases propensity for sleep by global or even site-specific accumulation during periods of activity. We used the in vivo microdialysis technique combined with RIA to monitor extracellular levels of adenosine in hippocampus and neostriatum of freely moving rats, kept in a normal day-night cycle (lights on from 7:00 to 19:00) for 24 hours. In some animals, adenosine increased slowly and gradually in the hippocampus but not in the neostriatum over the course of the active period. During this light-off period extracellular adenosine levels in the hippocampus were about 60% higher than the mean values of the preceeding light-on phase. The maximal increase (260%) occurred at the end of the period of activity. These results are the first obtained by in vivo microdialysis showing progressive accumulation of hippocampal adenosine during nocturnal activity in rodents, supporting its hypothe sized involvement in fatigue and/or induction of sleep propensity.

499.1

CHARACTERIZATION OF FOOD INTAKE REDUCING EFFECT OF METFORMIN IN GENETICALLY OBESE ZUCKER RATS: LACK OF EVIDENCE FOR THE INVOLVEMENT OF NEUROPEPTIDE Y. J. Rouru*, U. Pesonen*, E. Santti*, T. Rouvari*, R. Huupponen[b] M. Koulu*[c] and M. Jhanwar-Uniyal[c]. *Dept. of Pharmacology, Univ. of Turku, FIN-20520 Finland. [b]Dept. of Clinical Pharmacology, Turku Univ. Hospital, Finland and [c]Lab. of Neuroendocrinology, The Rockefeller Univ., New York, USA

Metformin is an antihyperglycaemic agent widely used for treatment of obese type 2 diabetic patients particularly because of its weight reducing effect. The hypothalamic neuropeptide Y (NPY) content was measured by radioimmunoassay and preproNPY mRNA expression was studied by quantitative *in situ* hybridization procedure in metformin treated (300 mg/kg orally for 12 days), in pair-fed and in *ad libitum* fed obese Zucker rats in order to elucidate possible mechanisms involved in the anorectic and body weight reducing effect of metformin treatment in genetically obese Zucker rats. The concentration of NPY in the hypothalamic paraventricular nucleus (PVN) was significantly higher in the metformin-treated and pair-fed rats when compared to the control animals. The expression of preproNPY mRNA in the arcuate nucleus (ARC) was similar in all three treatment groups. Both chronic metformin treatment and pair-feeding markedly lowered hyperinsulinaemia in these animals. It is concluded that the treatment with metformin and pair-feeding, which result in comparable reductions in food intake, body weight gain and hyperinsulinaemia, similarly increase NPY concentrations in the PVN while not affecting preproNPY mRNA expression in the ARC. The increase in hypothalamic NPY content may be secondary to reduction of hyperinsulinaemia during metformin treatment and pair-feeding. Thus, the anorectic effect of chronic metformin treatment cannot be explained by changes in content or expression of hypothalamic NPY.

499.2

PERIPHERAL NALTREXONE INDUCES NEUROPEPTIDE Y GENE EXPRESSION IN THE ARCUATE NUCLEUS. C. M. Kotz*, M. K. Grace, J. Briggs, C.J. Billington and A.S. Levine. University of Minnesota, St. Paul MN 55108 & VA Medical Center, Mpls MN 55417

Neuropeptide Y (NPY) induces feeding and deprivation results in increased NPY mRNA in the arcuate nucleus (ARC). Naltrexone (NTX), an opioid receptor antagonist decreases NPY-induced feeding. We thought NTX would also block deprivation-induced increases in ARC NPY mRNA. To test this, osmotic minipumps pre-filled with either 0.9% saline (SAL) or NTX (70 μg/μl/hr) were implanted subcutaneously in 32 male Sprague Dawley rats weighing 275-300 g. Half these rats were food-deprived and half were allowed food ad lib for 48 h. At study's end, NPY levels in the paraventricular nucleus (PVN) and ARC NPY mRNA levels were determined using an NPY radioimmunoassay and an NPY cDNA probe respectively. The data were analyzed by a 2 factor ANOVA (NTX level x deprivation level):

Treatment	48 h Food Intake (g)	PVN NPY levels (ng/mg PRO)	ARC NPY mRNA levels (O.D. units)
SAL ad lib	43.9[a]	36.8 ± 5.8	1.13 ± .16[a]
SAL dep	0	36.9 ± 2.4	2.18 ± .16[be]
NTX ad lib	32.1[b]	42.0 ± 5.5	2.10 ± .28[ce]
NTX dep	0	46.5 ± 6.9	3.80 ± .37[d]

Food intake was decreased 26% by NTX, PVN NPY levels were not different, and there were significant positive main effects of deprivation and NTX but no interaction among the 2 factors on ARC NPY mRNA. Thus NTX decreases normal feeding and increases deprivation-induced NPY gene expression, suggesting a complex interaction between NPY and endogenous opioids in the regulation of feeding.

499.3

NALOXONE DECREASES BOTH CARBOHYDRATE AND FAT INGESTION INDUCED BY NEUROPEPTIDE Y (NPY) A.S. Levine*, M. K. Grace, and C.J. Billington. VA Medical Center and University of Minnesota, Minneapolis, MN 55417

Central administration of NPY markedly enhances food intake in rats and appears to selectively stimulate intake of high carbohydrate diets. Naloxone has been reported to decrease intake of high fat (HF) diets much more effectively than high carbohydrate (HC) diets. In the current studies we examined whether naloxone would decrease NPY-induced intake of HC and HF diets, given individually or concurrently. In our first study we presented rats with a choice of a high carbohydrate (HC) or a high fat (HF) diet and stimulated feeding by intracerebroventricular (icv) injection of NPY. In this group of carbohydrate preferring rats (n=16), NPY induced HC intake (4.4 g/.5 hr), but failed to increase HF intake (0.3 g/.5 hr). Naloxone potently decreased intake of the HC diet at doses ranging from 0.1-3 mg/kg (.1mg/kg: 2.7 g; 0.3mg/kg: 2.5 g; 1mg/kg: 1.7 g; 3mg/kg: 1.5 g). In the second study the same rats were injected icv with NPY and presented with either the HC or the HF diet (no choice). NPY stimulated both HC (3.9 g/0.5 hr) and HF (2.9 g/0.5 hr) intake, and naloxone decreased intake of both the HC (0.3 mg/kg: 1.9 g; 3mg/kg: 1.2 g) and HF (0.3 mg/kg: 1 g; 3mg/kg: 0.4 g) diets. Thus, we found that naloxone potently decreased NPY stimulated intake of HC and HF diets. This is in contrast to the published observation that naloxone has little or no effect on HC intake in food deprived rats offered a choice between HC and HF diets. Our data lend support to the idea that an NPY-opioidergic feeding system exists in the central nervous system.

499.4

DISSOCIATIVE EFFECTS OF NEUROPEPTIDE Y ON FEEDING AND BODY TEMPERATURE AFTER INJECTION INTO DIFFERENT HYPOTHALAMIC SITES. P.J. Currie* and D.V. Coscina. Section of Biopsychology, Clarke Institute of Psychiatry, 250 College St., Toronto, ON, M5T 1R8 Canada.

Recent research has shown the perifornical hypothalamus to be a particularly sensitive brain site in eliciting feeding after local injection of neuropeptide Y (NPY). Since other work has demonstrated that NPY injected into the paraventricular nucleus of the hypothalamus (PVN) evokes both feeding-stimulatory and metabolic effects, the current study examined the simultaneous impact of this peptide on food intake and body temperature (T_{bo}) of unrestrained rats following injection into the PFH, and contrasted it with similar measures after injection into the PVN. NPY (0-235 pmol) was infused in a volume of 0.4 μl using a 28-ga. microinjection assembly. Food intake and T_{bo} were measured every 30 min for 3 h postinjection. Both PVN and PFH injections of NPY increased food intake dose-dependently in the first 30 min test interval, with the PFH showing a stronger response than the PVN. In PVN treated rats, the increased eating was associated with a significant decline in T_{bo} which was also evident 30 min after NPY injection and lasted 2 h. A mean maximal T_{bo} decline of 0.97±0.20°C occurred within 90 min following PVN treatment of 235 pmol NPY. In contrast, NPY had no apparent effect on T_{bo} when injected into the PFH. These findings support previous suggestions that while PFH NPY acts to stimulate a robust and relatively specific ingestive response, PVN NPY may participate in the integrative mechanisms responsible for the simultaneous regulation of feeding, heat conservation, and energy metabolism. (Supported by NSERC and NIN of Canada).

499.5

RAPID LOSS OF FEEDING DURING CONTINUOUS HYPOTHALAMIC INFUSION OF NPY. W.T. Chance*, X. Zhang, B. Mohapatra & A. Balasubramaniam. Dept. Surgery, Univ. Cincinnati Med. Ctr. and VA Med. Ctr., Cincinnati, OH 45267.

Anorectic tumor-bearing (TB) rats exhibit a refractory feeding response to neuropeptide Y (NPY) and decreased hypothalamic NPY concentrations. Therefore, we hypothesized that constant hypothalamic infusion of NPY would correct the anorexia exhibited by these TB rats. Following anesthetization, 24 ga. infusion cannulae were implanted into the perifornical hypothalamic area of 32 male Fischer 344 rats, 18 days after sc. MCA sarcoma or sham inoculations. These cannulae were connected to sc. Alzet minipumps (Model 2002) that infused NPY (125 ng/hr) or artificial CSF (0.5 ul/hr). Although food intake by both NPY-infused groups was increased immediately following surgery, 3 days later food intake by these groups was not different from that of the CSF-infused rats. Food intake by the TB rats continued to decline, with significant anorexia being noted 21 days after tumor inoculation. These results suggest a rapid desensitization of hypothalamic NPY receptors in both TB and control rats in the continuous presence of exogenous NPY.

499.6

CHANGES IN NEUROPEPTIDE Y GENE EXPRESSION CORRELATE WITH ALTERATIONS IN MACRONUTRIENT PREFERENCE IN THE RAT DURING ZINC DEFICIENCY-INDUCED ANOREXIA. N.F. Shay*, T.M. Rains and G. Li. Department of Food Science and Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801.

Zinc deficiency is known to cause anorexia among other physiological symptoms. The cause of this anorexia is poorly understood. We are currently investigating the role of Neuropeptide Y in the development of zinc deficiency-induced anorexia. Sprague-Dawley rats were fed modified AIN-76 diets containing either adequate or deficient levels of dietary zinc (Zn+: 30 mg/kg; Zn-: 1 mg/kg). Alterations in body zinc content were confirmed by measuring bone zinc content via atomic absorption spectrophotometry. Within 9 days of administration of a zinc deficient diet, reductions in the mRNA for preproNPY could be detected in the hypothalamus and intestine. After 28 days of diet administration, preproNPY levels were 4-fold higher in the intestine (Zn+/Zn-) and nearly 2-fold higher in the hypothalamus. In another set of studies, rats were allowed to self-select a diet from pure macronutrient diets containing fat, carbohydrate, and protein. Each diet was supplemented with complete vitamin and mineral mixes except for Zn- rats, in which Zn- mineral mix was used (Harlan). During a 28 day feeding period, the reduction in intake for Zn- was 20% (1710 ± 47 vs. 1360 ± 17 kcal). This reduction was due in total to a reduced intake of carbohydrates ($p << .01$); protein and fat intakes were statistically the same between the Zn+ and Zn- conditions. Upon repletion with zinc, normal carbohydrate intake was restored in 24-48 h, and a 50% surge in protein intake was noted for the first 5 days of repletion. This reduction in carbohydrate preference correlates with our observation of reduced NPY levels in zinc deficiency, as NPY is known to be a potent stimulator of carbohydrate intake.

499.7

EFFERENT PROJECTIONS FROM THE PERIFORNICAL AREA OF THE HYPOTHALAMUS IN THE RAT. E.M. Tomkins*, H.A. Akmal, A. Polzin, B.G. Stanley. Depts. Neurosci. & Psych., Univ. Calif., Riverside, CA 92521.

Neuropeptide Y (NPY) injected into various regions of the hypothalamus elicits robust eating responses. A recent cannula-mapping study employing nanoliter volume injections of NPY identified the medial perifornical area of the hypothalamus (mPFH), at the level of the posterior paraventricular nucleus, as the most sensitive site for NPY's eating stimulatory effect (Stanley et al., Brain Res. 1993, ***604***, 304-317).

To identify the neural projections from this area, 30 nanoliters of the anterograde tracer, biotinylated dextran amine (10%), was pressure injected into the mPFH of adult male Sprague-Dawley rats through glass micropipettes with 40-60 µm O.D. tips. Following survival times of 7-10 days, the animals were perfused, 60 µm frozen sections were collected, incubated in avidin-HRP, and visualized with a nickel-intensified diaminobenzidine reaction.

Dense projections of labelled fibers extended from the site of injection to the central gray, bed nucleus of the stria terminalis, medial and lateral preoptic areas, posterior hypothalamus, and median preoptic nucleus. Moderate projections were visible in the dorsal premammillary nucleus, parabrachial nucleus, lateral septum, paraventricular thalamic nucleus, precommissural nucleus, and locus coeruleus. Terminal labeling was also evident in the accumbens, amygdala, lateral parabrachial nucleus, and nucleus tractus solitarius. Although the roles of these projections remains to be established, by acting on PFH neurons NPY could directly influence activity in multiple intra- and extra-hypothalamic structures.

499.8

EFFECT OF FOOD DEPRIVATION ON NEUROPEPTIDE Y (NPY) MESSENGER RNA IN HYPOTHALAMUS OF SYRIAN AND DJUNGARIAN HAMSTERS. J. G. Mercer, C. B. Lawrence and P. J. Morgan*. The Rowett Research Institute, Bucksburn, Aberdeen, U.K. AB2 9SB.

Imposed manipulations of energy balance that increase food intake in rats often have no effect or elicit an attenuated response in various hamster species. Thus, Syrian, Turkish and Siberian hamsters either fail to express, or exhibit only limited post-fast hyperphagia, and are unresponsive to glucoprivation or inhibition of fatty acid oxidation (Bartness, Int.J. Obesity 14 Suppl. 3:115, 1990). Syrian hamsters are, however, sensitive to the appetite-stimulating effect of Neuropeptide Y (NPY) injected intracerebroventricularly (Kulkosky et al., Peptides 9:1389, 1989). To assess whether endogenous NPY in hamsters is sensitive to alterations in energy balance we have examined the effect of food deprivation on preproNPY mRNA in the hypothalamus of two species of hamster, the Syrian hamster, *Mesocricetus auratus*, and the Djungarian hamster, *Phodopus sungorus campbelli*. PreproNPY mRNA was studied by *in situ* hybridisation using the cloned rat gene to synthesise antisense and sense ^{35}S-riboprobes. Autoradiographic images were quantified by computerised image analysis, providing measures of integrated intensity (OD x mm^2). In the Hooded Lister rat, which was employed as a control species, 48 h food deprivation induced a 2.2-fold increase in total hybridisation in the hypothalamic arcuate nucleus (ARC), relative to fed controls. The same manipulation resulted in a 1.5-fold increase in total hybridisation in the ARC of the Djungarian hamster. In contrast, preliminary studies of the Syrian hamster indicated that NPY expression in this species may not be regulated by food deprivation.

This work was supported by SOAFD.

499.9

ALTERATIONS IN NUTRIENT METABOLISM HAVE DIFFERENTIAL EFFECTS ON GALANIN (GAL) AND NEUROPEPTIDE Y (NPY) IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI. J. Wang, A. Akabayashi, C.T.B.V. Zaia*, I. Silva, and S. F. Leibowitz. The Rockefeller University, New York, N.Y. 10021.

Hypothalamic injections of GAL and NPY have differential effects on nutrient ingestion, with NPY preferentially enhancing carbohydrate intake and GAL preferentially stimulating fat intake. The question is whether endogenous production of these peptides in hypothalamic neurons is differentially responsive to signals related to the metabolism of these nutrients. To test this, two compounds that alter carbohydrate and fat metabolism were examined in albino rats: 2-deoxy-D-glucose (2-DG, 200 mg/kg), which inhibits glucose utilization, and mercaptoacetate (MA, 600 µmoles/kg), an inhibitor of fatty acid oxidation. Intraperitoneal injection of 2-DG, compared to vehicle, caused a significant increase in NPY levels (measured via RIA), specifically in the paraventricular (PVN) and suprachiasmatic nuclei but not other areas. However, no change in GAL levels was detected. While MA, in contrast to 2-DG, produced no change in NPY levels, this metabolic inhibitor significantly reduced GAL levels, only in the lateral division of the PVN (-35%, $p<0.05$). Since cAMP production is inversely related to intracellular glucose, the impact of ventricular injections of dibutyryl cAMP (25 µg) on NPY and GAL levels was also examined. This agent caused a significant increase in NPY levels specifically in the medial PVN and arcuate nucleus, while having no impact on GAL in any area. Thus, intracellular regulatory signals for NPY and GAL production appear to differ, consistent with the differential impact of these peptides on nutrient ingestion.

499.10

THE IMMUNOLOGICAL TARGETING OF TOXINS TO NPY NEURONS OF THE ARCUATE NUCLEI DECREASES FOOD INTAKE FOR SEVERAL DAYS AND DISTURBS NPY mRNA EXPRESSION. A. Burlet*, H. Rafai, E. Grouzmann, B. Fernette, E. Angel, J.P. Nicolas and C. Burlet. INSERM U308, 38, rue Lionnois - F-54000-Nancy (France).

Cellular toxins (Tox., ricin A chain and monensin) centrally injected with a monoclonal antibody (MAb) to a neuropeptide may specifically penetrate the peptidergic neurons and induce long-lasting disturbances of neuronal functions. Presently, the toxins were injected with a MAb to the Neuropeptide Y precursor molecule (MAB to C-flanking peptide, C-PON) near the hypothalamic arcuate (ARC) or paraventricular (PVN) nuclei ; by itself, the injected C-PON-MAb was devoid of biological activity. The effects of one injection of C-PON-MAb or non specific IGg with toxins on spontaneous food intake and mRNA expression of NPY by in situ hybridization were studied.

In rats injected with the C-PON-MAb mixture into ARC, the daily food intake significantly decreased (26%, $p<0.001$) during the week after the injection. This was due to a decrease of the nocturnal food intake (30%, $p<0.001$) since the diurnal food intake was unchanged. On the day of injection, the diurnal food intake acutely increased (75%, $p<0.001$) whereas the following nocturnal food intake decreased (30%, $p<0.01$). The food intake was not modify by the injection of the non specific mixture into ARC or PVN, and by the injection of C-PON-MAb/Tox. mixture into PVN. The injection of C-PON-MAb/Tox. mixture into ARC increased the mRNA expression of arcuate NPY which decreased after the same injection performed into PVN.

The long-lasting decrease of food intake was due to an efficient immunological impairment of the arcuate NPY neurons in which the damages likely up-regulated the mRNA expression of NPY.

499.11

VAGOTOMY REDUCES FOOD INTAKE IN LEAN BUT NOT OBESE ZUCKER RATS. D. Greenberg*, D.L. Lewis, and A.J. Strohmayer. Bourne Laboratory and Departments of Psychiatry and Neurology, Cornell University Medical College, White Plains, NY, 10605.

The genetically obese Zucker rat (fa/fa) is hyperphagic compared to lean controls (Fa/?). This hyperphagia is characterized by increased meal size. The abdominal vagus has been implicated in the mediation of satiety signals and severing the abdominal vagus reverses the hyperphagia of hypothalamic obesity (Powley & Opsahl, 1974). We investigated the effects of total subdiaphragmatic vagotomy on the food intake and body weight of obese and lean male and female Zucker rats.

Male and female obese and lean Zucker rats received bilateral subdiaphragmatic vagotomies or sham operations (n=4-9 each group). Rats were placed on a low fat diet (< 1% fat) for 2 weeks and then placed on a high fat diet (30% fat) for an additional 2 weeks. Food intake and body weight was measured daily.

Vagotomy reduced the food intake (28% $p < .001$) and body weight (23% $p < .0003$) of male and female lean rats in both diet conditions. Vagotomy failed to reduce the food intake of male or female obese rats fed the low fat diet, but did reduce the food intake (22% $p < .03$) and body weight (16% $p < .02$) of obese rats fed the high fat diet.

The fact that vagotomy reduces low fat intake in lean but not obese rats indicates that intact vagal function is required for the expression of hyperphagia and obesity in obese Zucker rats under these conditions.

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499.12

ICV ADMINISTRATION OF ANTI-GALANIN ANTISENSE OLIGONUCLEOTIDE: EFFECT ON FEEDING BEHAVIOR AND BODY WEIGHT. M. G. Hulsey *, C. M. Pless and R. J. Martin. Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602

The neuropeptide Galanin has been reported to potentiate the consumption of high-fat diet in a macronutrient selection paradigm. This study investigated the effects of an ICV-administered antisense phosphorothioate oligonucleotide, directed against the Galanin mRNA, on feeding behavior and body weight in rats. Sixteen male HSD rats were cannulated in the lateral ventricle, and cannula placement was confirmed using Angiotensin II. Rats were given ad libitum access to tap water and a high-fat (60% w/w corn oil) semipurified diet. Feeding behavior was recorded by computer. Rats were given 7.5 µg of an anti-Galanin oligonucleotide (5'-cctggccatcttgagcag, N = 8) or a missense control oligo (5'-tcgccgctatacgatggc, N = 8) in the hour before the onset of the nocturnal cycle (1200-2400 h) for six consecutive days. Cumulative food intake was assessed at 12 and 24 h after each injection as well as over the six day injection period, and was subjected to one-way ANOVA. Body weight change was assessed daily, and subjected to one way ANOVA. Compared to missense controls, rats receiving the antisense oligonucleotide ate significantly more of the high fat diet ($p=.0025$) during the six day injection period. There was no effect on cumulative food intake at 12 h or 24 h after injection ($p > .05$). There was no effect on body weight change ($p > .05$). These data are incongruent with previous data from macronutrient selection paradigms where the peptide was administered, and may suggest that Galanin's role in the regulation of body energy balance may be less pivotal than previously thought.

499.13

MODULATION OF GENE EXPRESSION IN HYPOTHALAMUS OF OBESE ZUCKER (fa/fa) RATS IN RELATION TO AGE AND GENDER. M. Jhanwar-Uniyal* and A. Burlet. The Rockefeller Univ. New York, New York 10021 and INSERM U308 Nancy, France.

Obesity in genetically predispose Zucker rats is inherited as an autosomal recessive trait. Associated with this gene are various metabolic, endocrine and behavior disturbances, such as hyperinsulinemia, hypothermia, enhanced lipogenesis, low energy expenditure, increased hypothalamo-pituitary axis, and hyperphagia. These phenotypic and metabolic abnormalities are observed relatively early in life of obese rats and are gender specific. Certain peptides and hormone receptors, by virtue of their early abnormal status in fa/fa rats or their role in primary metabolic systems show response to the changes associated with the obesity. This study examines the hypothalamic gene expression (messenger RNA; mRNA) of corticotropin-releasing factor (CRF) and glucocorticoid receptor (GR) by using the Northern blot analysis in male and female, lean and obese Zucker rats of 2, 5, and 9 wk old. The results demonstrate that: 1) higher gene expression of CRF in obese rats as compared to lean at 2, 5 and 9 wk age; 2) gene expression was significantly altered with age in both lean and obese rats; 3) gene expression of GR was significantly different in female vs. male, in lean and obese rats. Thus, result of this study show that the gene expression of the CRF and GR is associated with development/maintenance of obesity.

499.14

IS THE ATP-SENSITIVE POTASSIUM CHANNEL (K_{atp}) THE NEURONAL GLUCOSENSOR? B.E. Levin* Neurology Serv. VA Med. Ctr., E. Orange, NJ 07019 and Dept. Neurosciences, NJ Med. Sch., Newark, NJ 07103.

Sulfonylureas binding sites (SBS) are associated with the K_{atp} which is responsible for glucose-induced insulin release in pancreatic ß-cells. Neurons in the ventrobasal hypothalamus and other sites are also glucosensitive, altering their firing rates when ambient glucose or sulfonylurea concentrations change. These neurons contain K_{atp} which are similar to those on pancreatic ß-cells. But the high affinity SBS (SBS_H) is not concentrated in areas with large populations of glucosensor neurons. Here, SBS_H and low affinity (SBS_L) were mapped by quantitative receptor autoradiography in rat brain using 20mM ^{3}H glyburide (GLYB). Subtraction of images in the presence (SBS_H) or absence of Gpp(NH)p to block SBS_L (SBS_H + SBS_L) were used to define the anatomical location of these binding sites. Unlabelled 1uM GLYB was used to define non-specific binding. Initial studies show SBS_H to be concentrated in areas in which sulfonylureas evoke GABA release, presumably from axon terminals. SBS_L were relatively diffuse but showed a propensity for brain sites in which glucosensor neurons are concentrated with less binding in areas containing the highest concentrations of SBS_H. These results are compatable with the hypothesis that SBS_L are linked to the K_{atp} which serves as a glucosensor on neurons. It provides a potential site for pharmacologic intervention to modulate energy intake and body weight.

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499.15

CENTRAL AND PERIPHERAL 2-DG: EFFECT ON NEUROPEPTIDE Y GENE EXPRESSION AND PEPTIDE LEVEL. S.Q. Giraudo*, M. K. Grace, A.S. Levine and C.J. Billington. University of Minnesota, St. Paul MN 55108 & VA Medical Center, Mpls MN 55417

Neuropeptide Y (NPY) induces feeding and has been associated with carbohydrate selection. 2-deoxyglucose (2-DG) blocks glucose utilization and it potentiates appetite for carbohydrates. Thus, blockade of glucose utilization could affect NPY gene expression in the arcuate nucleus (ARC) and NPY levels in the paraventricular nucleus (PVN). To test this, 2 studies were set up as follows: 1) 32 male rats were injected intraventricularly (icv) with 18.3 µmol/10µl of 2-DG and 2) 30 male rats were injected i.p 200 mg/kg of body weight. Both studies had a control (0.9% saline), a 2-DG with ad libitum food or 2-DG food deprived group. Two hours after treatment animals were sacrificed and NPY levels in the PVN and ARC NPY mRNA levels were determined using an NPY radioimmunoassay and an NPY cDNA probe respectively.

Treatment	2 h Food Intake (g)	PVN NPY levels (ng/mg PRO)	ARC NPY mRNA levels (O.D. units)
Saline icv	0.7 ± 0.2^a	20.3 ± 2.4	4.59 ± 0.18
2-DG/food icv	2.9 ± 0.7^b	19.3 ± 1.5	5.05 ± 0.34
2-DG/dep icv	0^a	19.7 ± 2.1	4.28 ± 0.25
Saline ip	1.5 ± 0.2^a	15.4 ± 1.5	5.17 ± 0.28
2-DG/food ip	5.8 ± 0.5^b	13.6 ± 2.0	4.90 ± 0.19
2-DG/dep ip	0^a	18.7 ± 1.5	5.10 ± 0.29

2-DG increased food intake 4-fold, but did not affect NPY mRNA nor peptide levels. These data suggest that 2-DG-induced feeding, through decrease glucose availability, does not involve changes in NPY activity.

INGESTIVE BEHAVIORS V

500.1

c-FOS INDUCTION IN THE PONS AND FOREBRAIN BY LITHIUM CHLORIDE AND SUCROSE AFTER CONDITIONED TASTE AVERSION FORMATION. T.A.Houpt*, J.M. Philopena, J.W. Jahng, T.H. Joh, and G.P. Smith. Bourne Lab., Dept. Psychiatry, and Lab. Molec. Neurobiol., Burke Med. Res. Inst., Dept. Neurol. Neurosci., Cornell Univ. Med. Coll., White Plains, NY 10605.

We have previously reported that intraorally infused sucrose does not induce c-Fos-like immunoreactivity (c-FLI) in the rat NTS unless a CTA has been formed to sucrose. Lesion studies indicate that brain areas rostral to the NTS are necessary for CTA acquisition and expression. Thus, we qualitatively surveyed c-FLI as a marker of neuronal activity in the pons and forebrain at the level of the hypothalamus 1 hour after 4 treatments: intraoral sucrose infusion alone (5%, 6ml/6min), LiCl alone (0.15 M, 12ml/kg i.p.), intraoral sucrose paired with LiCl for the first time, and intraoral sucrose in rats with a CTA formed by 3 previous pairings of sucrose and LiCl.

LiCl alone induced robust c-FLI in the dorsolateral PBN, dorsal tegmental gray, olfactory CTX, thalamic PVN, central and medial amygdala, hypothalamic PVN,VMH, LH, DH, SON, AH, and BNST. c-FLI in rats receiving intraoral sucrose paired with LiCl appeared qualitatively similar to the c-FLI observed after LiCl alone. Sucrose alone induced robust c-FLI in the thal. PVN, olfactory CTX, PVN, LH, and AH. Sucrose induced some but less c-FLI than LiCl in the amygdala and PBN, and little or no c-FLI in the VMH and SON. Sucrose, however, did induce more c-FLI than LiCl in LC and striatum. In rats with an established sucrose CTA, sucrose induced more c-FLI in medial PBN and VMH than unconditioned sucrose; striatal c-FLI appeared reduced. Thus c-FLI was observed after LiCl and sucrose in many areas implicated in CTA formation by lesion studies, e.g. amygdala, LH, and PBN. Extension of the survey to other brain regions and quantitation by cell-counting may reveal patterns of neuronal activity underlying CTA formation and expression.

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500.2

EFFECTS OF LITHIUM CHLORIDE PRE-EXPOSURE ON CONDITIONED PALATABILITY SHIFTS IN RATS. L. A. Eckel* and K.-P. Ossenkopp. Neuroscience Program, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Conditioned taste aversions can be produced by pairing a novel tasting substance with exposure to an emetic agent such as lithium chloride (LiCl). Using a rapid conditioning paradigm, the effects of LiCl pre-exposure on conditioned changes in taste reactivity responses were examined. Rats were pre-exposed to LiCl or NaCl (3.0 meq/kg, ip, N=12 per group) and placed in an observation box for 30 min. Conditioning occurred 2 days later when LiCl/NaCl injection was paired with intraoral sucrose infusions. Half of each pre-exposed group received LiCl injection while half were injected with NaCl. The rats were then placed in the observation box and 7 brief (30 sec) intraoral infusions of 0.3 M sucrose were administered at 5 min intervals. Both groups conditioned with LiCl produced fewer ingestive and more aversive responses than NaCl conditioned rats ($ps < .05$). Comparison between the two LiCl conditioned groups revealed that LiCl pre-exposure had no effect on ingestive responses and passive drip, but did reduce aversive responding relative to NaCl pre-exposed rats ($p < .05$). These results suggest that LiCl pre-exposure attenuates conditioning by selectively reducing aversive responses. (Supported by an NSERC to KPO).

500.3

β-ESTRADIOL-INDUCED CONDITIONED SHIFTS IN SUCROSE PALATABILITY IN MALE RATS. K.-P. Ossenkopp*, Y. J. Rabi, and L. A. Eckel. Neuroscience Program, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Conditioned taste aversions (CTA) can be produced by pairing a novel taste with exposure to a toxin. Previous studies have shown that estradiol is effective in producing a CTA. The present experiment examined the ability of estradiol to produce conditioned shifts in taste reactivity responses to sucrose in male rats. Rats were fitted with intraoral cannulae and adjusted to a 23 hr/day water deprivation schedule. On two conditioning days the animals received 1 hr access to a 0.30 M sucrose solution followed by s.c. injection of 17β-estradiol (100 μg/kg) or vehicle (polyethylene glycol). All rats were then given free access to water and 3 days later tested for their taste reactivity to 0.30 M sucrose. The rats were placed in an observation box and three 30 sec intraoral infusions of sucrose were admininstered at 5 min intervals. The orafacial and somatic responses to the sucrose taste were videotaped to assess palatability. The estradiol group exhibited significantly reduced ingestive behaviors and increased aversive behaviors ($ps<0.01$), relative to the control group. When the two groups were subsequently given a 24 hr two-bottle choice test between sucrose and water, the estradiol group displayed a significant ($p<0.01$) CTA to the sucrose. Thus, estradiol-induced CTAs seem to be based on conditioned shifts in palatability.

500.4

THE RESPONSIVENESS OF GUSTATORY NEURONS IN THE AMYGDALA OF THE MACAQUE MONKEY IS INDEPENDENT OF HUNGER. T.R.Scott* and J.Yan. Department of Psychology, University of Delaware, Newark DE 19716

Taste cells in the macaque code for quality and intensity, but may be sensitive to the motivational state of the subject as well. The dichotomy between neurons that are influenced by hunger and those that are not appears to be total: cells in OFC and hypothalamus respond to a chemical only when the monkey is motivated to consume it, while those in NTS and primary taste cortex are independent of motivational state. The amygdala receives afferents from primary taste cortex, yet has close reciprocal connections with the hypothalamus. Its functions have been associated with motivation and reward, particularly as these relate to feeding. We recorded from amygdaloid taste neurons to determine whether they were influenced by the monkey's motivational state. Subjects were two male cynomolgus macaques. Tastants were applied to the tongue, and single neuron records taken from the central nucleus of the amygdala during 15 separate experiments. Monkeys were fed to satiety using 50-cc aliquots of glucose, sucrose or fruit juice, and evoked activity was measured after each aliquot. Inhibition was nearly as common a response option as excitation. Whether inhibitory or excitatory, however, the response was unaltered as the monkey progressed from acceptance to rejection of the satiating stimulus. Thus, the decreased pleasure and acceptance of foods as satiety develops does not result from changes in gustatory activity in the amygdala.

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500.5

FOOD DEPRIVATION REDUCES STIMULUS DISCRIMINABILITY IN THE NUCLEUS TRACTUS SOLITARIUS OF THE RAT. L. J. Nolan* and T. R. Scott. Department of Psychology, University of Delaware, Newark, DE 19716

The results of human psychophysical and animal behavioral studies suggest that a state of food deprivation alters hedonic perception of taste stimuli and broadens the range of substances an animal will accept as food. We recorded from the NTS of 72-hour deprived rats to determine whether these hedonic and behavioral changes resulted from alterations in the sensitivity of the taste system. The deprivation resulted in a 10% decrease in body weight at the time of recording. The evoked activity of 45 units from food deprived rats and 40 units from calorically replete rats to an array of 13 taste stimuli was recorded and analyzed. Food deprivation significantly reduced the spontaneous firing rate of NTS gustatory neurons and attenuates the response to the sodium and lithium salts. In addition, the breadth of tuning of salt-sensitive neurons was greater in food deprived rats than in controls. We then analyzed for differences in the code for taste quality. Activity profiles evoked by the salts (NaCl, LiCl, MSG) and carbohydrates (Polycose, glucose, fructose, sucrose) across neurons were significantly more similar in deprived rats. Temporal patterns of activity in the firing rate of NTS neurons for the acids (HCl and citric) and sugars were also significantly more similar in these rats. The willingness of 72-hour food-deprived rats with a conditioned taste aversion to glucose to ingest an array of taste stimuli was tested. Results indicated a reduced ability in 72-hour food-deprived animals to discriminate between acid and sugar stimuli. The implication is that deprived rats are able to make fewer discriminations among tastants, an effect that could underlie their wider acceptance of potential foods.

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500.6

TASTE PREFERENCES FOR AMINO ACIDS AND NaCl AFTER SECTION OF CHORDA TYMPANI AND/OR GLOSSOPHARYNGEAL NERVES. E. Tabuchi[1], T. Kondoh[1], T. Voynikov[1], T. Yokawa[1], T. Ono[2], J.H. Teeter[3*], and K. Torii[1,4]. [1]ERATO, R&D Corp. of Japan, Yokohama 221, Japan; [2]Dept. Physiol., Toyama Med.&Pharmaceu. Univ., Toyama 930-01, Japan; [3]Monell Chemical Senses Center, Philadelphia, PA 19104-3308, USA; [4]Ajinomoto Co., Inc., Central Res. Lab., Yokohama 224, Japan.

Preferences for amino acids and NaCl change with the nutritional status of the animal. To learn how the gustatory nerves convey information about nutritionally dependent taste preferences, the taste preferences in rats with bilateral chorda tympani (CTX) and /or glossopharyngeal (GPX) neurotomy were determined when animals were fed a control diet or a diet deficient in L-lysine (Lys). The group receiving CTX did not select nor ingest Lys during feeding of the Lys deficient diet, and, while being fed the control diet, their intake of L-arginine was low, whereas intake of monosodium L-glutamate (MSG) was normal. The GPX group did not substantially alter its preference for Lys, L-arginine or MSG under either diet when compared with intakes of the intact group, yet it did show altered intakes of L-histidine, saline and water, along with an increase in total fluid intake. The preferences of the group receiving both CTX and GPX evidenced themselves as a combined effect of those observed when each nerve was cut. The present data suggest that primarily the chorda tympani nerve conveys taste information for some amino acids, such as Lys and arginine, that are related to a nutritionally dependent preference. The glossopharyngeal nerve may convey other tastes, such as aversive tastes, and may be involved in sensory aspects of osmotic regulation. It is concluded that preferences seen in the selection of nutrients depend on the nutritional state of the animal. This conclusion is likely true for humans as well.

500.7

SIMULTANEOUS CONTRAST EFFECTS IN CHRONIC DECEREBRATE RATS. P.S.Grigson*, M.F.Roitman[1], J.M.Kaplan[1], H.J.Grill[1], and R.Norgren. Dept. Behavioral Science, College of Medicine, Penn State Univ., Hershey, PA 17033 [1] and Dept. of Psychology, Univ. of Penn, Phila, PA 19104.

Seven rats received full supracollicular decerebrations in two hemi-transections spaced one week apart. These rats, along with 7 control subjects, were then implanted with two intraoral cannulae and one anterior digastric EMG electrode. On the first day following recovery from surgery, the rats received forty 10 sec infusions of 0.1 M sucrose. On a second day, they received identical infusions of 1.0 M sucrose. On two other days, the 2 concentrations were alternated for a total of forty infusions/day. All stimuli were delivered at a rate of 1.2 ml/min and the interstimulus interval was 30 sec. The rhythmic activity of the anterior digastric, which closely follows spout-licking, was evaluated during both the 10 sec infusion period and the 20 sec offset period. The results showed that during the offset period (1) both the intact and the decerebrate rats demonstrated a reliable concentration effect by generating more motor movements for the high than for the low concentration of sucrose. (2) While intact rats demonstrate contrast using other parameters, they failed to do so under these circumstances. (3) Regardless, chronic decerebrate rats did express simultaneous negative contrast. That is, they made fewer responses for the low concentration of sucrose when alternated with the high concentration than when the low concentration was presented alone ($p < .004$). Thus, an intact brain stem is sufficient for the occurrence of simultaneous negative contrast. Supported by DC-02216, DC-00240, MH-00653, DK-42284, and DK-21497.

500.8

EFFECTS OF HEPATIC-CELIAC BRANCH VAGOTOMY AND SITE OF POLYCOSE INFUSION ON CONDITIONED FLAVOR PREFERENCES IN RATS. E.A. Simons, F.A. Martinson, E.A. Watson, C.C. Horn, and J.C. Mitchell.* Department of Psychology, Kansas State University, Manhattan, KS 66506.

We have shown (Horn & Mitchell, *Soc. Neurosci. Abs.*, 1993) that conditioned flavor preferences using intragastric polycose infusions are not blocked by hepatic branch vagotomy. The present study extended this research using a discrete trial procedure and a hepatic-celiac branch vagotomy. A normal control (n=7) and a hepatic-celiac vagotomized group (n=8) were both implanted with gastric fistulas and a duodenal group (n=8) was implanted with duodenal fistulas. While 22 hours food deprived, rats were trained for a total of 12 days. During training, rats received a flavor (CS+) paired with intragastric or intraduodenal infusion of 5 ml of 16% w/v polycose (US) and on alternate days, received a different flavor (CS-) paired with intragastric or intraduodenal infusion of 5 ml distilled water. The flavors used were grape and cherry Kool-Aid. After training, rats were given two-bottle extinction preference testing for four days. Results showed that the combined hepatic-celiac vagotomy failed to interfere with conditioned flavor preferences. Furthermore, flavor preferences appeared to be greater in the vagotomized animals. The results showed that polycose infused into the duodenum is as effective as intragastric infusion in conditioning preferences.

500.9

HIGH FAT-MODERATE CARBOHYDRATE DIET INCREASES FLAVOR PREFERENCES CONDITIONED WITH INTRAGASTRIC FAT INFUSION IN RATS. E.A. Watson*, E.A. Simons, F.A. Martinson, C.C. Horn, and J.C. Mitchell. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

Using a discrete trial procedure, Lucas and Sclafani (*Physio. & Behav.*, 1989) found that conditioning flavor preferences using intragastric fat infusion was difficult and produced only a 60% preference. We modified their study using three groups of food deprived rats on special diets: a high fat-low carbohydrate group, a low fat-normal carbohydrate group, and a high fat-moderate carbohydrate group. Three cycles were completed each consisting of four days of 1-bottle training and two days of 2-bottle testing. During training, a CS+ flavor was paired with a 2-minute intragastric infusion of a variable amount of 7.1% corn oil emulsion and a CS- flavor with water. Rats maintained on the high fat-moderate carbohydrate diet had a mean preference as high as 80% for the flavor paired with the fat infusion (individual preferences reached as high as 98%). Rats in the high fat-low carbohydrate, and the low fat-normal carbohydrate groups showed no preference.

500.10

OPIOID SUBTYPE ANTAGONISTS IN MEDIAL HYPOTHALAMUS ALTER DEPRIVATION, GLUCOPRIVIC AND PALATABLE FOOD INTAKE IN RATS. J.E. Koch*, M.J. Glass, M.L. Cooper and R.J. Bodnar. Dept. of Psychology & Neuropsychology Doctoral Program, Queens Coll., CUNY, Flushing, NY 11367.

Intraventricular injections of selective μ and κ opioid subtype antagonists decrease intake after food deprivation (FD), 2-deoxy-D-glucose (2DG) hyperphagia and sucrose intake in rats. Opioid agonist injections into the medial hypothalamus (MH), particularly the paraventricular nucleus, stimulate food intake. The present study compared the abilities of naltrexone (NTX), the μ-selective antagonist beta-funaltrexamine (βFNA) and the κ-selective antagonist norbinaltorphamine (NBNI) microinjected into MH to alter deprivation (24h) intake, 2DG (500 mg/kg i.p.) hyperphagia, or sucrose (10%) intake.

Deprivation intake was significantly reduced by NTX (10 μg: 26%), βFNA (5 μg: 26%) or NBNI (5 μg: 31%) in MH. 2DG hyperphagia was significantly reduced by NTX (10 μg: 69%), βFNA (20 μg: 83%) or NBNI (20 μg: 69%) in MH only after 2h. Neither NTX, βFNA nor NBNI in MH altered sucrose intake. The MH effects are compared with ventricular effects to infer MH mediation of opioid antagonist actions on different forms of intake. Supported by NIDA DA 04194,

500.11

ANALYSIS OF CENTRAL OPIOID RECEPTOR SUBTYPE ANTAGONISM OF HYPOTONIC AND HYPERTONIC SALINE INTAKE IN WATER-DEPRIVED RATS. R.J. Bodnar*, M.J. Glass and J.E. Koch. Dept. of Psychology and Neuropsychology Doctoral Program, Queens Coll., CUNY, Flushing, NY 11367.

Intake of either hypotonic or hypertonic saline solutions is modulated in part by the endogenous opioid system. Morphine and selective mu and delta opioid agonists increase saline intake, while general opioid antagonists reduce saline intake in rats. The present study evaluated whether intracerebroventricular administration of general (naltrexone) and selective mu (beta-funaltrexamine, 5-20 ug), mu_1 (naloxonazine, 50 ug), kappa (nor-binaltorphamine, 5-20 ug), delta (naltrindole, 20 ug) or $delta_1$ (DALCE, 40 ug) opioid receptor subtype antagonists altered water intake and either hypotonic (0.6%) or hypertonic (1.7%) saline intake in water-deprived (24 h) rats over a 3 h time course in a two-bottle choice test. Water intake was significantly reduced by both peripheral naltrexone (0.5-2.5 mg/kg) in the hypotonic (-56%) and hypertonic (-57%) saline paradigms and central naltrexone (1-50 ug) in the hypotonic (-56%) and hypertonic (-31%) saline paradigms. Peripheral naltrexone significantly reduced hypertonic saline intake (-48%), but not hypotonic saline intake, while central naltrexone significantly reduced hypotonic saline (-44%) intake, but not hypertonic saline intake. Water intake was significantly reduced following mu (-53%) and kappa (-51%) receptor antagonism, but not following mu_1, delta or $delta_1$ receptor antagonism. In contrast, neither hypotonic nor hypertonic saline intake was significantly altered by any selective antagonist. These data are discussed in terms of opioid receptor subtype control over saline intake relative to the animal's hydrational state and the roles of palatability and/or salt appetite. Supported by NIDA DA 04194.

500.12

BLOCKADE OF DIETARY OBESITY DEVELOPMENT BY CENTRAL SELECTIVE OPIOID ANTAGONISTS IN RATS. J.L. Cole*, L. Leventhal, G.W. Pasternak, W.D. Bowen and R.J. Bodnar. Neuropsychology Doctoral Prog., Queens Coll., CUNY, Flushing, NY 11367; Dept. of Neurology, Memorial Sloan-Kettering Cancer Ctr., New York, NY 10021; Div. of Medicinal Chemistry, NIH, Bethesda, MD 20892.

Chronic injections of naloxone or naltrexone transiently reduce food intake and body weight, probably due to a limited duration of action. The irreversible mu_1 antagonist, naloxonazine, reduced body weight and food intake over 2 weeks in normal rats, but failed to affect these variables in dietary-obese rats. Since acute administration of opioid antagonists reduce palatable intake, the present study examined whether body weight and intake of a high-fat, sweetened condensed milk and chow diet would be altered by daily (11 days) central microinjections of long-acting mu (beta-funaltrexamine: BFNA, 20 ug), mu_1 (naloxonazine: NAZ, 50 ug), kappa (nor-binaltorphamine: NBNI, 20 ug), $delta_1$ (DALCE, 40 ug) or $delta_2$ (naltrindole 5'-isothiocyanate: NTII, 20 ug) opioid subtype antagonists. Rats exposed to the palatable diet gained significantly more weight (+9% vs. +5%) than chow-fed controls. Significant reductions in body weight occurred immediately and progressed thereafter for each antagonist: BFNA (-9%), NAZ (-11%), NBNI (-6%), DALCE (-7%) and NTII (-7%). Corresponding decreases in milk, fat and chow intakes were also observed following antagonist treatments. These data implicate each opioid receptor subtype in maintainance of body weight under palatable conditions. Supported by NIDA DA 04194.

500.13

CENTRAL KAPPA AND MU OPIOID ANTAGONISTS DECREASE SUCROSE INTAKE IN SHAM-FEEDING RATS. L. Leventhal*, T.C. Kirkham, J.L. Cole and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367 and Bourne Labs, Cornell Univ. Med. Ctr., White Plains, NY 10605.

Opioid antagonists decrease food intake in rats, especially palatable intake. While sucrose intake is significantly increased by mu, kappa and delta agonists, it is decreased by general, mu and kappa antagonists, but not by mu_1, delta or $delta_1$ antagonists. Since sucrose intake acts postingestively, studies with intact rats cannot distinguish between opioid modulation of hedonic orosensory or postingestive factors. In sham-feeding rats in which postingestive factors are eliminated, peripheral naloxone stereospecifically decreased sucrose intake by shifting its concentration-dependent effects. To assess whether central kappa and mu opioid receptor subtypes alter sucrose intake through orosensory mechanisms, the present study evaluated whether selective kappa (nor-binaltorphamine: NBNI) or mu (beta-funaltrexamine: BFNA) opioid antagonists reduced sucrose intake in sham-feeding rats in a manner similar to peripheral naltrexone. Naltrexone (0.01-1 mg/kg, sc) dose-dependently reduced sucrose (20%) intake in sham-feeding rats with its peak inhibitory effects occurring after 15-30 min. This corresponds with the premise of interference with the maintainance and not initiation of intake. NBNI (20 ug) and BFNA (20 ug) significantly decreased sucrose intake in sham-feeding rats in a manner both similar to their respective effects in intact rats, and to peripheral naltrexone's effects in sham-feeding rats. Thus, kappa and mu receptors appear to reduce sucrose intake by acting on the hedonic orosensory charactistics of the ingestate. Supported by NIDA DA 04194.

500.14

EEG RESPONSES TO ICE CREAM AND PAIN: OPIOID AND PREFERENCE EFFECTS. **D.D. Krahn*, B.A. Gosnell, R.J. Davidson, J. Williams, L. Redmond, L. Vitse. University of WI Dept of Psychiatry, Madison, WI 53792.**

Research by our group and others has demonstrated that (1) treatment with opioid agonists increases and treatment with opioid antagonists decreases intake of and preferences for highly preferred foods and fluids in animals and humans; (2) intake of highly palatable foods affects the endogenous opioid system and alters responses to painful stimuli; and (3) states associated with positive affect and approach behaviors are associated with relative activation of the left frontal cerebral hemisphere (RAL) as measured by EEG in humans. In order to determine whether the eating of a highly preferred food resulted in altered response to pain, changes in mood, and RAL due to effects on the endogenous opioid system, we are conducting an experiment in which the hedonic, EEG, and analgesic responses of young female subjects (classified as ice cream likers or non-likers on the basis of their answers to a questionnaire) to ingesting ice cream (750 cal in 10 minutes) followed by either naloxone or saline are measured. The same responses are also measured in likers and nonlikers during a no eating, saline trial. Initial results in three ice cream likers and two nonlikers are available; additional data are being collected. After ice cream ingestion, naloxone treatment resulted in a large shift to relative right activation on the EEG in likers; this effect was not seen in nonlikers. Also, the three likers showed a relative increase in right-sided EEG activation on the no eating day in response to repeated painful stimuli, but on days on which they received ice cream and saline, they demonstrated relative left-sided activation. These preliminary data support the hypothesis that subjects who highly prefer ice cream differ from those subjects who do not prefer ice cream in their affective and analgesic responses to palatable food ingestion. This research was supported by NIDA grant DA05471.

500.15

NITROUS OXIDE STIMULATES FOOD INTAKE IN NON-DEPRIVED RATS. D.A. Czech* and C.T. Kazel Department of Psychology, Marquette University, Milwaukee, WI 53233.

Previous research suggests that nitrous oxide (N_2O) exerts one or more of its effects through a benzodiazepine (BZP) receptor system. Further, BZP's are known to influence ingestive behaviors in several animal species. This study examines a possible N_2O effect on food intake in the non-deprived rat.

In expt. 1, male Long-Evans hooded rats were exposed to one of four concentrations of a N_2O and O_2 mixture (10-40% N_2O) or to room air circulated through an enclosed environment. Following an initial acclimation to gases, ground chow and tap water were provided *ad lib* in the same environment for a 60 min test. Intakes at 30 and 60 min were corrected for body weight and evaluated (here and in other expts.) with ANOVA procedures and, as appropriate, Dunnett's or adjusted Student's-*t* tests; alpha level was set at $P<0.05$. Food intake increased in a dose-related manner with increasing concentration of N_2O, differing significantly from room air controls at 20% N_2O.

In expt. 2, pretreatment with 10 or 20 mg/kg (ip) of the BZP receptor blocker, flumazenil, failed to attenuate the hyperphagia induced by a challenge concentration (30%) of N_2O.

In expt. 3, pretreatment with the opioid antagonist, naltrexone (0.1-10.0 mg/kg, sc), attenuated 30% N_2O-induced hyperphagia in a dose-related manner; all doses were significant relative to vehicle control. These data fail to support an hypothesis that N_2O-induced hyperphagia in the rat is mediated by a BZP receptor. The data do, however, implicate an opioid mechanism.

500.16

EVIDENCE FOR INNERVATION OF THE HEPATIC PARENCHYMA IN THE RAT. M.I. Friedman, M. Ketchum, P. Ulrich, P.A.S. Breslin* and C. DellaCorte. Monell Chemical Senses Center, Philadelphia, PA 19104.

Although innervation of the hepatic parenchyma has been demonstrated in many species, its existence in the rat has been controversial. The intralobular innervation of human liver has been characterized using immunocytochemical staining for a variety of neural markers. To determine whether the hepatic parenchyma of rats is innervated, we examined liver tissue immunohistochemically for the presence of neurofilament protein 200 (NF 200) using a monoclonal antibody to NF 200. Reaction product indicative of fine neuronal processes was observed in close association with hepatocytes in certain areas of the liver. The processes were seen transversing several hepatocytes and often had a beaded appearance indicative of varicosities. NF 200-like immunoreactivity was also found in association with the hepatic vasculature suggesting a vascular innervation as well. Western blot analysis of liver tissue confirmed the presence of NF 200. These observations provide evidence for intralobular innervation of the rat liver.

500.17

HEPATIC PORTAL AND VENA CAVA GLUCOSE AND AMINO ACID INFUSIONS DECREASE DAILY FOOD INTAKE IN RATS. A. E. Willing & H. S. Koopmans*. Dept. of Medical Physiology, Univ. of Calgary. Calgary, Canada, T2N 4N1.

Intravenous infusions of glucose and/or amino acids into the vena cava cause daily food intake to decrease by 50% to 100% of the kilocalories (kcal) infused (Walls & Koopmans, 1992). Since glucose and amino acids are normally absorbed from the gut into portal circulation before they are taken up or metabolized in the liver or passed on to the rest of the body, we examined whether glucose and amino acids infusions into the hepatic portal vein (HP) of male Lewis rats would lead to better caloric compensation than vena cava (VC) administration. A 10 kcal glucose infusion decreased daily food intake to a similar degree in the HP (3.3 ± 1.5 kcal) and VC groups (2.5 ± 4.9 kcal, $p > .90$). Daily food intake was significantly decreased ($p < .01$) during the 20 kcal infusion by 12.8 ± 4.8 (HP) and 14.7 ± 3.1 kcal (VC). There was no significant difference between the two routes of infusion ($p > .75$). With the 20 kcal amino acid infusion food intake was significantly reduced ($p < .005$) by 17.4 ± 4.9 kcal in the HP group and 17.7 ± 2.7 kcal in the VC group. Again there was no difference in route of infusion ($p > .90$). There was no significant effect of treatment or route of infusion on the rate of body weight gain. These results show once again that infused nutrients can provide part of a signal that controls daily food intake. Furthermore, the liver is not likely to be the site where this signal is generated even though it plays a central role in nutrient metabolism. The infusion of glucose or amino acids into the portal vein would generate high hepatic plasma values of these nutrients but produced reductions in daily food intake that were similar to those found after vena cava infusion.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: MATING

501.1

DIFFERENTIAL MATING STIMULATION EFFECTS FOS EXPRESSION IN BRAIN AND SPINAL CORD OF THE FEMALE RAT. E. Komberg, J.-W. Lee and M.S. Erskine*. Department of Biology, Boston University, Boston, MA 02215 USA

Threshold amounts of cervical stimulation (CS) received during mating are essential for the initiation of the prolactin (PRL) surges of early pregnancy in the rat. The expression of FOS in brain and spinal cord in response to amounts of CS which are sufficient or insufficient to induce PRL surges was examined. Ovariectomized rats primed with estrogen (EB, 40µg/kg) and progesterone (2mg/kg) received subthreshold (mounts-without-intromission; MO), perithreshold (5 or 10 intromissions, I), suprathreshold (15 I) mating stimulation from males or were taken directly from the home cage (HC). Animals were sacrificed 1 hr post mating, and brain and spinal cord sections were stained for FOS protein using standard techniques. Numbers of FOS-immunoreactive (FOS-IR) cells increased in proportion to the number of I in the medial amygdala (mAMYG) and bed nucleus of the stria terminalis (BNST) and in response to I compared to MO and HC in the preoptic area (POA) and ventromedial nucleus of the hypothalamus (VMN). The L6 segment of the cord showed graded numbers of FOS-IR cells following 5 or 15 I; this effect was restricted to laminae II and X. At thoracic (T13), lumbar (L1-L3) and sacral (S1-S2) levels, I stimulation did not increase FOS above HC levels. MO animals at all spinal levels examined had significantly higher FOS-IR than HC and I animals, suggesting an analgesic effect of CS on neural activity in the cord. Since the mAMYG, BNST and L6 showed graded responses to CS, these areas may be involved in acquisition of information relevant to initiation of PRL surges. Supported by HD21802 to MSE.

501.2

ALTERNATIVE MALE TYPES IN TREE LIZARDS HAVE DIFFERENT DELAYED STEROID HORMONE RESPONSES TO AGONISTIC ENCOUNTERS. R. Knapp* and M.C. Moore. Dept. of Zoology, Arizona State Univ., Tempe, AZ 85287-1501.

Steroid hormones are known to mediate agonistic behavior in a variety of species. We are examining the role of testosterone (T) and corticosterone (B) in agonistic behavior in a species where a male can be one of two permanent types with respect to aggressiveness. Previously, we reported finding a delayed (1-day) increase in plasma B levels, but no changes in T, in aggressive-morph male tree lizards (*Urosaurus ornatus)* who won short encounters in the laboratory (*Amer. Zool.*, 32(5):24A, 1992). We have now repeated the experiment to determine whether this delayed effect of winning occurs under more natural conditions, and if it also occurs in the less aggressive morph. Staged encounters were run using a tethered intruder male presented to marked lizards in the field. The two types of males differed in their hormonal response to the encounter. Winning staged encounters had no significant effect on plasma T or B levels in the territorial, more aggressive morph one day following the encounter. However, males of the less aggressive, satellite/nomadic morph exhibited decreased T (ANCOVA, $p = 0.02$) and increased B levels ($p = 0.003$) relative to controls. A contributor to morph-specific levels of aggression documented earlier (*Horm. Behav.*, 26:568, 1992) may be a morph-specific response of T and B levels following male-male encounters. However, morph differences in aggressiveness may not be mediated by differential sensitivity of plasma T to increases in B, as i.p. injections of 0-600 ng B revealed no difference in T levels for the two types of males. The results do, however, suggest a hormonal mechanism whereby frequency of male-male encounters may influence whether a male of the less aggressive morph is a satellite or nomad.

501.3

EFFECTS OF TESTOSTERONE (T) AND PROGESTERONE (P) ON CCK-INDUCED LORDOSIS IN THE ESTRADIOL BENZOATE (EB)-PRIMED MALE RAT. P. Butler, J.G. Kohlert and G.J. Bloch*. Dept. of Psychology, BYU, Provo, UT 84602.

Sulfated CCK octapeptide (sCCK-8) increases lordosis behavior when microinjected into the medial preoptic nucleus (MPN) of the EB-primed male rat (Bloch et al, Physiol Behav 46,'89.) The male does not normally express high levels of lordosis, however, even though MPN levels of CCK and CCK mRNA are high. To test whether T inhibits the lordosis response to CCK, saline (SAL) or 50ng sCCK-8 were microinjected into the MPN of adult-gonadectomized EB-primed male rats implanted with blank (Bk) or T-filled Silastic capsules. 3 behavioral tests were spaced 1 week apart. Males received a P-filled capsule immediately after the 2nd test. sCCK-8 increased lordosis in BK- and T-treated males. (Bk: LQ=25.6±4.8(SAL) vs 52.2±7.6(CCK) p<0.02, paired t test; T:LQ=35.5±7.8 (SAL) vs 67.5±7.4(CCK), p<0.01.) Interestingly, with P treatment, lordosis was very low in T- vs Bk-treated males (LQ= 21.7±16 vs 71.1±10.2, p<0.025); sCCK-8 abolished this effect: LQ's=72.5,78.0 for Bk- and T-treated males. Although T is reported to inhibit lordosis in EB-primed females, T was not inhibitory in males. Thus, CCK injected into the MPN stimulated lordosis in the EB-primed male with or without T, administered alone or in combination with P. Low levels of lordosis in the male do not therefore result from a suppression by gonadal steroids of neural mechanisms mediating the lordosis-stimulating effects of CCK. (NIH HD27334)

501.4

PREPUBERTAL TESTOSTERONE (T)) DEFEMINIZES OR MASCULINIZES MEDIAL PREOPTIC AREA (MPOA) STRUCTURES IN THE RAT. R. Mills*, J.K. Graham and G.J. Bloch. Dept. of Psychology, BYU, Provo, UT 84602.

Gonadotropin secretion and sexual behaviors are defeminized or masculinized in adulthood by prepubertal T treatment of female and NeoGx male rats (Bloch et al, Neurosci Biobehav Rev, in press). The periventricular preoptic (PvPO), anteroventral periventricular (AVPv), and medial preoptic nucleus (MPN) and its central and medial divisions (MPNc,MPNm) were measured in adult-sacrificed females and NeoGx males that received blank (Bk-) or 7mm T-filled Silastic capsules during days 15-30 of age. The width of the PvPO in T-treated, constant-estrus females and NeoGx males decreased 19% and 18%, respectively, vs Bk controls (P<0.06,<0.03); the volume of the AVPv decreased by 35% and 29% (p's<0.03). Cell number and density decreased significantly within the PvPO of T-treated NeoGx males but not T-treated females. Similarly, the T treatment increased the size of the MPNm by 26% in NeoGx males but not females. There were no effects on the size of the MPNc and MPN, although the MPNc of gonadally intact males was 45% larger than in NeoGx males. Interestingly, the morphology of the MPNm and PvPO was affected more by T in NeoGx males than in females, similar to results with sexual behavior and gonadotropin secretion. Thus, a prepubertal T treatment that defeminizes or masculinizes reproductive function in females and NeoGx males has analogous effects on sexually dimorphic structures of the MPOA.

501.5

LHRH MICROINJECTED INTO THE MEDIAL PREOPTIC NUCLEUS (MPN) INCREASES SEXUAL BEHAVIOR IN THE MALE RAT. G.J. Bloch, J.G. Kohlert, P. Butler, J. Stowell, S. Huber, W. Johnson, J. Sovereign, N. Kuemmerle, M. Walden, & D. Fleming*. Psychology Dept., BYU, Provo, UT 84602.

Galanin (GAL) microinjected into the MPN facilitates male-typical sexual behavior in the rat (Bloch et al, Physiol Behav 54,'93). GAL stimulates LHRH and is found within LHRH cells located within the medial preoptic area (MPOA). Others have reported that subcutaneous LHRH facilitates male sexual behavior. I.c.v. LHRH also is stimulatory, but this result is not site-specific. No effect was noted with LHRH in the MPOA, but gonadally intact males behave maximally and thus may have been insensitive. We determined the behavioral response of .3ul saline (SAL) or 20,50,100 200,500ng LHRH microinjected into the MPN of gonadectomized male rats implanted with 2mm testosterone-filled Silastic capsules; these produce low levels of behavior, allowing for measurement of facilitatory effects. The percentage of males that mounted (M), intromitted (I), or ejaculated (Ejac) increased with higher doses of LHRH. M and I latencies (ML,IL) decreased dose-responsively. ML and IL scores in 500ng LHRH males, for example, were 144±50 and 211±61 sec, respectively, vs 372±96 and 593±109 sec for SAL controls. M and I frequencies, Ejac latencies, and post-ejaculatory intervals were not affected. Thus, as with GAL, LHRH microinjected into the MPN appears to increase sexual arousal. GAL and LHRH may interact to regulate sexual behavior. (Supported by NIH HD 27334)

501.6

INHIBITION OF TESTOSTERONE AROMATIZATION IN THE INTACT MALE RAT PREOPTIC AREA ENHANCES ESTROGEN RECEPTOR-LIKE IMMUNOREACTIVITY AND REDUCES MATING. A. N. Clancy, D. Zumpe and R. P. Michael*. Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, GMHI, 1256 Briarcliff Rd., Atlanta, GA. 30306.

Copulatory behavior was studied in 5 groups of sexually experienced, intact male rats in which: (i) the nonsteroidal aromatase inhibitor Fadrozole (CIBA-Geigy CGS 16949A) was delivered bilaterally into the medial preoptic area (POM) together with normal saline given s.c. via osmotic minipumps, (ii) normal saline was delivered bilaterally into POM together with the same Fadrozole dose given s.c., (iii) Fadrozole was delivered bilaterally into the cerebral cortex together with saline given s.c., (iv) Fadrozole was delivered bilaterally into the lateral preoptic area together with saline given s.c., and (v) unoperated controls. Mounting and ejaculation were significantly decreased in rats receiving Fadrozole in POM compared with the behavior of rats in the other 4 groups. Few differences occurred between rats in the latter 4 groups, all of which continued to mate. The H222 (Abbott Laboratories) and ER-715 anti-estrogen receptor (ER) antibodies were used to examine the distributions of ER immunoreactive (ERir) neurons in gonadectomized controls and some of the rats in groups i, ii and v. Since labeling of ERir neurons with the H222 antibody, but not the ER-715 antibody, is inhibited by estrogen, areas labeled by both antibodies presumably were sites where the conversion of testosterone (T) into estradiol (E_2) was blocked. Intense H222 ERir neuronal labeling was confined to the POM of males in group i, and significantly more labeled neurons were found in the POM of males in group i than were found in the POM of males in group ii. The ER-715 antibody labeled neurons in the brains of all rats. Results show that conversion of T into E_2 in POM is important for copulatory behavior in male rats and that H222 ERir can be used to identify the neurons in POM affected by Fadrozole. (Funded by USPHS grant MH 19506 and the Emory University Research Committee).

501.7

SPECIES DIFFERENCES IN ESTROGEN SENSITIVITY IN WHIPTAIL LIZARDS L. J. Young* and D. Crews, Dept. of Zoology, Univ. of Texas, Austin TX 78712

C. uniparens is a unisexual species of whiptail lizard whereas *C. inornatus* is a sexual species and the maternal ancestor of *C. uniparens*. Together they represent an excellent model for investigating the evolution of hormone-brain-behavior relationships. Plasma levels of estradiol in *C. uniparens* are approximately 5-fold lower than those of female *C. inornatus* in a similar reproductive state. This translates into species differences in dose-response curves for estradiol benzoate (EB)-induced female sexual behavior (receptivity) (p<0.01). Ten-fold smaller dosages of EB are capable of stimulating receptivity in*C. uniparens* compared to female *C. inornatus*. *In situ* hybridization analysis indicates that estrogen induction of progesterone receptor (PR) expression in the ventromedial nucleus of the hypothalamus (VMH) also differs between the species. EB was significantly more potent in ovariectomized C. *uniparens* than in ovariectomized female *C. inornatus* at inducing PR gene expression within the VMH (p<0.01). Preliminary results indicate that species difference in estrogen receptor expression in the VMH may account for the differences in behavioral and molecular sensitivities to EB. These results indicate that the differences in behavioral sensitivity to estrogen lies in the estrogen target neurons in the brain area (VMH) controlling receptive behavior. Furthermore, these differences in sensitivity to estrogen may provide insight into the evolution of species differences in circulating hormone levels and of hormone sensitivity of steroid dependent behaviors in general. Supported by NSF Pre-doctoral Fellowship (LJY) and NIMH 41770 (DC).

501.8

FOS EXPRESSION FOLLOWING ELECTRICAL STIMULATION OF THE VOMERONASAL ORGAN. M. Meredith* and G. Fernandez-Fewell. Program in Neuroscience, Florida State University, Tallahassee FL 32306.

c-fos expression in the forebrain following electrical stimulation of the vomeronasal organ (VNO) was compared with Fos patterns obtained previously after mating and pheromonal stimulation in male golden hamsters, . Electrodes were implanted unilaterally via a midline incision in the palate. Two 125 um teflon coated wires were bared 0.5 mm at the tip and inserted one near the anterior end, one midway along the vomeronasal capsule. The wires were sealed in place with cyanoacrylate tissue adhesive and their free ends passed under the skin around the nose then along the dorsal skull surface to a connector mounted on the parietal bone. The palate was sutured and the animals allowed 3 days to recover. Via a flexible cable connected with a constant current stimulator, animals were stimulated for 45 min intermittently with low current in their home cages, or were connected but not stimulated for 45 min. Typical stimulation currents were 120-175 uA (0.5-1 ms pulses) and produced no stimulus driven movements in the awake animal. Low level Fos activation occurred in accessory olfactory bulb, medial preoptic area and medial amygdala {both anterior (MeA) and posterior (MeP)}. The activation in the MeA on the side ipsilateral to the stimulation was similar to activation previously seen in mated and pheromonally stimulated animals. Fos expression was also seen in the anterior medial preoptic/diagonal band region, including a few LHRH immunoreactive (ir) cells; a colocalization never seen in male hamsters following mating behavior or stimulation with female chemical stimuli. This may reflect activation of the LHRH system by vomeronasal input, but could also reflect activation of the Nervus terminalis system. Supported by NIH grant DC 00406.

501.9

FOS PATTERNS IN MALE HAMSTER VOMERONASAL PATHWAYS: PHEROMONE STIMULATION AND EFFECT OF EXPERIENCE Gwen. Fernandez-Fewell* and Michael Meredith Neuroscience Program, Florida State University, Tallahassee, FL 32306

Removal of vomeronasal organs (VNX) from sexually naive, but not from sexually experienced male hamsters results in severe deficits in mating behavior. Previous studies of Fos patterns in VNX and intact inexperienced males indicate a preferential activation of VN pathways (Accessory olfactory bulb, Medial amygdala; Me) during mating behavior and pheromonal stimulation. Activation in the medial preoptic area (MPOA) mainly reflected copulatory performance. Here, c-fos expression was used to analyze patterns of neural activity in sexually experienced intact and VNX males exposed to pheromonal stimulation either alone or during mating. Males from each group were placed in clean boxes with fresh bedding and each exposed to female hamster vaginal fluid (HVF) or a sexually receptive female for 45 mins. After an additional 45 mins they were perfused with 4% paraformaldehyde. Control animals were put into clean boxes with fresh bedding and perfused 90 mins later. Fifty um vibratome sections were processed for immunocytochemistry using a polyclonal Fos antibody. (Cambridge Research). When exposed to HVF, experienced males had higher levels of Fos expression in the MPOA than did inexperienced males, whether intact or VNX. Mated animals all had extensive Fos expression in MPOA but here there appeared to be a denser expression in intact than VNX animals. Fos expression in Me, among HVF exposed males, was highest in intact experienced animals with lower levels in inexperienced animals and in VNX animals. Mated animals had a higher overall level but similar relative levels of expression. Supported by NIH Grant DC00906.

501.10

STEROID-INDUCED REPRODUCTIVE BEHAVIOR IN OBESE ZUCKER FEMALE RATS. C.L.M. Bivens* and D.H. Olster. Department of Psychology & Neuroscience Research Institute, University of California, Santa Barbara, CA 93106.

Obese Zucker female rats are infertile. Anecdotal evidence suggests ovary-intact obese Zucker females display less reproductive behavior than their lean counterparts. The present study was designed to compare reproductive behavior induced by exogenous steroid hormones in ovariectomized (OVX) lean and obese Zucker rats. OVX adults were given estradiol benzoate (EB, 15-100 μg/kg) or EB plus progesterone (P, 2-10 mg/kg), and tested for sexual receptivity. At the highest EB dose obese Zucker females displayed lordosis less frequently than lean rats (Lordosis Quotient, LQ = 8 ± 6%, vs. 32 ± 13% respectively, $p<0.01$). At the lowest doses of EB plus P lean females were maximally responsive (LQ = 93 ± 4%); Zucker obese females, in contrast, were only slightly receptive (LQ = 26 ± 11%, $p<0.00003$). Increasing the dose of either EB or P, administered in combination with the lowest dose of the other hormone, produced responses in obese Zucker females that were comparable to those observed in lean rats. These data suggest that considerably higher doses of EB and/or P are required to elicit robust lordosis responses in OVX obese Zucker, as compared to lean rats. This behavioral hyporesponsiveness may contribute to infertility in the obese Zucker female rat. (This work was supported by NIH HD 23483 and the American Psychological Association.)

501.11

ESTROUS FEMALE ELICITS DOPAMINE RELEASE IN MPOA OF INTACT, BUT NOT CASTRATE, MALE RATS. J. Du, D. S. Lorrain, L. Matuszewich, and E. M. Hull*. Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

Extracellular levels of dopamine (DA) metabolites DOPAC and HVA increased in the medial preoptic area (MPOA) of male rats during copulation; DA was below the sensitivity of the assay (Hull et al., 1993). The presence of an estrous female increased the catecholamine amperometric signal in MPOA, but the contribution of DA could not be determined (Blackburn & Pfaus, 1992). This experiment tested whether extracellular DA, serotonin (5-HT) and their metabolites in the MPOA of male rats were affected by exposure to an estrous female behind a barrier or by copulation. We also tested if testosterone (T) facilitates the DA response to the female. Microdialysis samples were assayed using an LC Packings capillary column and Antec electrochemical detector. In intact males, DA, DOPAC and HVA increased in the presence of the female and during copulation; 5-HT and 5-HIAA were not affected. In castrates with T replacement DOPAC and HVA increased significantly in the presence of the female and during copulation; DA increased slightly. In castrates without T, DA fell throughout the experiment, and DOPAC and HVA did not rise in the presence of the female; these animals did not copulate. Since DA in MPOA enhances male sex behavior (Hull et al., 1986), T may influence copulation in part by increasing DA release in MPOA in response to an estrous female. (NIMH grant #40826 to EMH)

501.12

SEXUAL EXPERIENCE INCREASES COPULATION INDUCED FOS-LIKE IMMUNOREACTIVITY IN THE MPOA. L.A. Lumley* and E.M. Hull. Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

Male sexual behavior increases the induction of the immediate early gene c-fos in the medial preoptic area (MPOA) (Robertson et al., 1991), a brain area important in the regulation of male sex behavior. We recently reported that a D1 antagonist lowered the number of copulation induced Fos-like immunoreactive cells in the MPOA (SN, 1993). Male rats improve their copulatory efficiency with sexual experience. We hypothesized that sexual experience may affect copulation induced Fos in the MPOA. In the current study, we compared copulation induced Fos-like immunoreactivity in sexually experienced relative to sexually inexperienced male rats. One group of male rats received sexual experience every five days for seven weeks, at which time they were able to achieve three ejaculations in a 30 minute interval. Another group of male rats of equal age received no sexual experience prior to the test day. On the test day, all males were allowed to achieve one ejaculation, and brains were subsequently assayed for Fos-like immunoreactivity. A noncopulatory control group was also included. After one ejaculation, an increase in Fos-like immunoreactivity in the MPOA was observed in sexually experienced relative to sexually inexperienced males. Since dopamine in the MPOA is released during copulation and is important in the regulation of male sexual behavior, increased Fos-like immunoreactivity may reflect a more sensitive dopamine system in sexually experienced male rats. Supported by NIMH grant #MH40826 to EMH.

501.13

AXOTOMY AND TESTOSTERONE SENSITIVITY OF THE RAT BULBOSPONGIOSUS MOTOR SYSTEM. W.F. Collins, III,* W. Hu and M. Gonzalez. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Rat bulbospongiosus (BS) motoneurons exhibit androgen dependence in adulthood. However, it is not clear whether androgens act directly on BS motoneurons or indirectly via the BS muscle. To address this question, we have examined the effect of axotomy on the androgen sensitivity of the rat BS motor system.

The BS muscle nerve was sectioned unilaterally in 180 gm male rats anesthetized with ketamine and xylazine. The rats were then castrated (with or without testosterone pellet implants) or sham-castrated (sham). After 28 days survival, the rats were sacrificed, and the left and right BS muscles were removed and weighed. Transverse sections from L_5-L_6 spinal cords were counterstained with cresyl violet, and the somal cross sectional areas of 20 randomly selected motoneurons in both the left and right dorsomedial nuclei from each rat were measured. The data were analyzed using ANOVAs.

As expected, castration produced decreases in mean BS muscle weight and motoneuron somal cross sectional area (73% and 17% of sham controls; respectively) on the axonally intact control side. Chronic denervation of the BS muscle resulted in a 53% decrease in weight (compared to contralateral control), and the BS muscle exhibited a further loss in weight (59% decrease compared to denervated sham) following castration. Chronic axotomy produced a 23% decrease in BS motoneuron somal cross sectional area (compared to contralateral control). Furthermore, somal cross sectional area of axotomized BS motoneurons was 12% smaller in castrated rats compared to axotomized sham controls. All castration effects were reversed by testosterone administration and are significant to $p<0.01$.

Thus, both denervated BS muscles and axotomized BS motoneurons are sensitive to androgen. However, the effect of castration on axotomized BS motoneurons is attenuated compared to that observed in axonally intact BS motoneurons.

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502.1

DISCRIMINATION LEARNING WITH INJECTIONS OF MORPHINE INTO THE PARABRACHIAL NUCLEUS, BUT NOT INTO THE VENTRAL TEGMENTAL AREA (VTA) T.V. Jaeger* and D. van der Kooy, Dept. of Anatomy, Univ. of Toronto, Toronto, Canada M5S 1A8.

In a previous study, we demonstrated a dissociation between the neuroanatomical sites which mediate the motivational effects of morphine and its discriminative effects. Morphine was found to have both aversive and rewarding motivational effects in the VTA, but not in the parabrachial nucleus (PBN). Conversely, morphine produced discriminative effects in the PBN, but not in the VTA. To extend these studies, we use a taste aversion model of discrimination to show that 1) the discriminative effects of morphine within the PBN are specific to the activation of opiate receptors and 2) injections of morphine into the PBN, but not into the VTA, may serve as a stimulus for discrimination learning. Rats were injected with morphine (5 mg/kg, i.p.) 15 min prior to the presentation of a 0.1% saccharin solution. Lithium chloride (130 mg/kg) was injected immediately after 20 min of exposure to the flavour. On alternate days, an injection of 0.9% saline both preceded and followed the presentation of saccharin. Unilateral guide cannulae were then implanted into the PBN and generalization to central routes of administration was evaluated following the microinjection of 2.5 and 5 µg of morphine and 2.5 and 5.0 µg of (+)-morphine. Stimulus generalization was observed with 2.5 and 5.0 µg of morphine, while the inactive (+)-isomer of morphine failed to produce responses reliably different from the saline training condition.

Separate groups of rats received bilateral cannulae implants into either the PBN or the VTA and were trained in the discrimination task using either 2.5 or 5.0 µg of intracerebral morphine as the training stimulus. Rats with implants into the VTA did not demonstrate differential responding under either dose of morphine tested. Rats with implants in the PBN, however, quickly learned the task, consuming significantly less saccharin after morphine than after saline injections. The data demonstrate that injections of morphine into the PBN are *sufficient* to support discrimination learning.

502.2

AVERSIVE EFFECTS OF THE SYNTHETIC CANNABINOID CP 55,940 IN RATS I. S. McGregor* and C. Issakidis, Dept. Psychology, University of Sydney, NSW, 2006, Australia.

Despite the widespread use of cannabis, questions relating to the addictive potential of this drug are a matter of considerable controversy both scientifically and politically. The recent development of highly selective and potent synthetic cannabinoids, such as CP 55,940, have allowed new avenues of research into cannabinoid effects. The present study investigated the hedonic properties of CP 55,940 in rats across a variety of paradigms. An initial experiment showed that a low dose of CP 55,940 (10 µg/kg) increased locomotor activity, indicating behavioural excitement, but that a higher dose (100 µg/kg) caused a sedative effect. A second experiment using the conditioned place preference paradigm showed that rats developed a powerful aversion to an environment in which they received CP 55,940 (100 µg/kg). A lower dose (10 µg/kg) produced a weaker aversion. In a third experiment using the conditioned taste aversion paradigm it was shown that rats developed a strong aversion to a fluid (saccharin) that was ingested just prior to administration of 100 µg/kg CP 55,940. In a final experiment it was shown that rats under the influence of CP 55,940 (10 or 100 µg/kg) displayed a higher rate of anxiety-related ultrasonic vocalisation in an environment in which they had previously received mild footshock. This suggests that the aversive effects of CP 55,940 summated with the aversive effects of the environment to magnify anxiety. It is concluded that CP 55,940 is aversive to rats, probably due to an anxiogenic effect. The utility of animal models for increasing understanding of human cannabis use is therefore open to question.

502.3

FUNCTIONAL MAGNETIC RESONANCE IMAGING OF NICOTINE EFFECTS ON BRAIN ACTIVATION BY A VISUAL SPATIAL WORKING MEMORY TASK IN HUMANS. E.A. Stein*, A.S. Bloom, J. Pankiewicz, J-K. Cho, S.A. Fuller, D.C. Osmon+, Y. Suchy+, and J.R. Zigun. Medical College of Wisconsin and University of Wisconsin+, Milwaukee, WI 53226.

Nicotine facilitates human attention, memory and sensorimotor function. A visual spatial working memory (VSWM) task that utilizes these functions activates several frontal and parietal cortical regions as measured by functional magnetic resonance imaging (FMRI). Since evidence exists for the involvement of forebrain dopamine (DA) systems in VSWM as well as the effects of nicotine on DA systems, we examined the effects of nicotine on task-induced FMRI activation. Single shot echo planar imaging was obtained on a 1.5 Tesla GE Signa scanner using an insertable, balanced torque, 3-axis head gradient coil designed for rapid gradient switching. To obtain images throughout the entire brain volume, a shielded quadrature elliptical endcapped transmit/receive birdcage radio frequency coil was used. Four experienced smokers performed the VSWM task while FMR data were acquired (TR=5 sec; TE=40 msec; 8 mm slice thickness). A trial consisted of 7 cycles of task repetition (30 sec rest, 45 sec task). Nicotine (0.75, 1.5 or 3.0 mg) or saline was injected iv over a 15 sec period after the third task cycle. The most common nicotine effect observed (defined as appearing in 4 contiguous pixels) was an increase in the magnitude of the task-induced activation response in both frontal and parietal regions with doses as low as 0.75 mg. In each case, an increase in activation magnitude was seen within 1 minute of drug (but not saline) administration. The average injection-induced increase in signal was 5.3% after saline, 53.5% after the low (0.75 mg) nicotine dose, and 86.3% after the medium (1.5 mg) nicotine injection. The present data suggest that nicotine enhances regional neuronal activation induced when performing a VSWM task.

502.4

MICRODIALYSIS ASSESSEMENT OF NUCLEUS ACCUMBENS GLUTAMATE DURING IV HEROIN SELF-ADMINISTRATION K. Bonter,*[1,2] K. Leeb,[1,3] D. Pocock,[1,2] R. A. Wise,[1,3] Ctr Stud Behav Neurobiol[1] and Depts Chem Biochem[2] and Psychol,[3] Concordia Univ., Montréal, Que, CANADA H3G 1M8

Nucleus accumbens septi (NAS) is identified as a critical structure for the habit-forming actions of opiates, and glutamatergic afferents from the frontal cortex have been suggested to regulate NAS activity. Accordingly, the present study was designed to determine fluctuations in NAS glutamate levels during intravenous heroin self-administration. Rats with NAS dialysis probes were allowed to self-administer heroin. Extracellular fluid from NAS was sampled at 4min intervals by micro-dialysis and glutamate was assayed using HPLC with electrochemical detection of OPA amino acid derivatives. Glutamate concentrations in the dialysate increased during heroin self-administration stabilizing at 200% to 400% above baseline. Glutamate concentrations appeared to increase further during extinction conditions that followed the self-administration period. The glutamate elevation during self-administration coincides with reported elevations in dopamine; the elevation in glutamate during extinction occurs at a time when dopamine levels are falling. Thus behavioral states appear capable of dissociating fluctuations in extracellular levels of the two transmitters, suggesting a more complex glutamate-dopamine interaction than has been recently hypothesized.

502.5

IBOGAINE ANTAGONISM OF MORPHINE-INDUCED HYPERACTIVITY: ENHANCEMENT BY PRIOR MORPHINE EXPOSURE AND ROLE OF KAPPA OPIOID RECEPTORS. S.M. Keefner* and S.D. Glick, Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208

Ibogaine, an indole alkaloid, is currently being investigated as a potential anti-addictive therapy. It has been shown to decrease morphine self-administration and to inhibit both morphine-induced locomotor stimulation and morphine-induced dopamine release in the nucleus accumbens and striatum of rats. However, in both rats and humans, the effects of ibogaine appear to be quite variable; the present study sought to determine if prior morphine history might account for individual differences in sensitivity to ibogaine. Female Sprague-Dawley rats, pretreated for 2 days with morphine (30 mg/kg, i.p.) before receiving ibogaine (40 mg/kg, i.p.), showed significantly less locomotor activity in response to morphine (2.5-30 mg/kg, i.p.) than controls pretreated with saline prior to ibogaine. Thus, prior morphine exposure enhanced ibogaine antagonism of morphine-induced locomotor activity. To study whether ibogaine's effects may be related to activation of the kappa opioid receptor, for which ibogaine has affinity, two kappa agonists were administered using the same treatment parameters. The selective kappa agonists, U50488 (10 mg/kg, i.p.) and U62066 (1 mg/kg, i.p.), when administered in place of ibogaine, mimicked ibogaine's effects. Furthermore, in preliminary studies, the selective kappa antagonist nor-binaltorphimine (10 mg/kg, s.c.) appeared to reverse the ibogaine-induced antagonism of morphine. These results suggest that the long-lasting effects of ibogaine may be due to persisting levels of ibogaine and/or an ibogaine metabolite interacting at kappa opioid receptors to yield anti-addictive effects. (Supported by DA03817)

502.6

HEROIN SELF-ADMINISTRATION PRODUCES BIOCHEMICAL ADAPTATIONS SIMILAR TO CHRONIC MORPHINE TREATMENT IN BRAIN REWARD REGIONS. A.W. McClenahan, D.W. Self, D. Beitner-Johnson, R.Z. Terwilliger, and E.J. Nestler*. Dept. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508

The mesolimbic dopamine system, specifically the ventral tegmental area (VTA) and its projection to target neurons in the nucleus accumbens (NAc), mediates some of the reinforcing effects of drugs of abuse. Previous results from rats forcibly treated with chronic morphine found several biochemical adaptations in the VTA and NAc (see Neuron 11:995, 1993). This experiment validates the previous findings by using the animal model of drug self-administration (SA), which better approximates human drug addiction. In these studies, rats self-administered heroin in limited, daily 6-hr test sessions for three weeks. A yoked-heroin and a yoked-saline control group were used for comparison. The findings show that opiate SA produces similar biochemical adaptations, when compared to animals receiving yoked injections of saline, as produced by chronic, forced opiate treatments. These include an increase in tyrosine hydroxylase (TH) and glial fibrillary acidic protein, and a trend toward a decrease in neurofilaments, in the VTA, and an increase in cAMP-dependent protein kinase activity and a trend toward a decrease in Gi proteins, in the NAc. Interestingly, heroin SA produces one important adaptation in the NAc, a decrease in TH levels, not seen in animals treated chronically with morphine. There were no differences between heroin SA rats and rats receiving yoked heroin injections.

Although the heroin self-administering rats failed to show signs of physical dependence, biochemical adaptations in the mesolimbic dopamine system of these rats suggest that these changes could contribute to the motivational aspects of drug addiction.

502.7

REINSTATEMENT OF HEROIN SELF-ADMINISTRATION BEHAVIOR BY EXPOSURE TO STRESS AFTER PROLONGED EXTINCTION

Y. Shaham*, J. Stewart. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

We have shown previously that non-contingent IV priming injections of heroin, or intracranial injections of morphine into VTA reinstate heroin self-administration behavior in rats following a period of extinction. Naltrexone blocks these effects, and given alone reduces baseline responding. Here we report that first-time exposure to 10 min of intermittent footshock reinstates previously extinguished heroin self-administration behavior.

Male and female rats were trained to self-administer heroin (6.125-50 µg/kg/infusion, IV) during four 3-h sessions/day over a 2-week period. The drug-reinforced behavior was extinguished by at least 16 sessions (lever presses resulted in saline infusions). Reinstatement was studied following non-contingent infusions of saline, 50 µg/kg heroin, naltrexone-precipitated opioid withdrawal (5 mg/kg naltrexone, SC, 40 min after 10 mg/kg morphine, SC) and by 10-min of footshock (1 mA; 0.5 sec on, mean off period of 40 sec) .

Reinstatement was observed following footshock stress and priming injections of heroin, but not following saline or naltrexone-precipitated withdrawal. Most importantly, footshock stress reinstated self-administration behavior upon first exposure, and again more than one month after the termination of heroin self-administration. These findings suggest that exposure to some stressors can precipitate relapse, possibly by mimicking the neurochemical effects of a heroin priming injection and not by inducing opioid-like withdrawal symptoms.

502.8

OVARIAN HORMONES DO NOT ALTER SELF-ADMINISTRATION OF HEROIN IN OVARIECTOMIZED FEMALE RATS

J. Stewart,* B. C. Woodside and Y. Shaham. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

We studied the effect of ovarian hormones on the initiation and maintenance of heroin self-administration in ovariectomized female rats. In Experiment 1, the effects of 10 µg estradiol benzoate (EB) and 0.5 mg progesterone (P) on the reinforcing efficacy of heroin were studied. Animals were trained to self-administer heroin (50 µg/kg per infusion, IV) on a progressive ratio schedule, 4 sessions per day, 4-h each. Animals were tested with descending doses of heroin (50, 25, and 12.5 µg/kg) in 5-day blocks. Neither EB, given on the third, nor P, given on the fifth day of each block, affected number of heroin infusions taken.

In Experiment 2, we studied the initiation of IV self-administration of heroin on an FR1 schedule using an ascending series of doses (6.125 - 50 µg/kg per infusion). Following ovariectomy, 8 of 16 animals were injected with 10 µg EB on 3 occasions every 3 days before self-administration training and every 3 days throughout training. Twelve 3-h sessions per dose, 4 session per day, were given. No differences in rate of responding between groups were observed at any of the heroin doses; most animals achieved stable responding at the 25 µg/kg dose. Finally, no group differences were found when animals were tested again using a descending series of doses.

These results suggest that circulating ovarian hormones do not affect the sensitivity of female rats to the reinforcing effects of heroin.

502.9

THE DEVELOPMENT OF A CONDITIONED PLACE PREFERENCE (CPP) TO MORPHINE. I. EFFECTS OF LESIONS OF VARIOUS CNS SITES.

M.C. Olmstead* and K.B.J. Franklin. Department of Psychology, McGill University, Montreal, Quebec, Canada.

The CPP paradigm is used to assess the reinforcing effects of incentive stimuli in that animals spend more time in the presence of cues previously associated with rewarding stimuli than those associated with neutral stimuli. In order to determine the neural substrates mediating the reinforcing effects of morphine, separate groups of male Long-Evans rats received neurotoxic lesions of various CNS sites and were tested for the development of a CPP to morphine (2 mg/kg X 3 pairings). Lesions of the hippocampus, periaqueductal gray, or pendunculopontine nucleus disrupted the CPP. Lesions of the entire ventral striatum reduced the CPP whereas selective lesions of anterior or medial portions were ineffective. Lesions of the dorsal striatum, lateral amygdala, or mesolimbic dopamine system changed the pattern of CPP behaviour but did not reduce the CPP. It appears that the development of a CPP to morphine is a complex phenomenon which depends on interactions between a number of brain structures.

502.10

INTRAVENOUS MORPHINE SELF-ADMINISTRATION BY RATS WITH LOW VS. HIGH SACCHARIN PREFERENCES.

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An experiment was performed to determine the relationship between saccharin preference and morphine self-administration. On the basis of voluntary intake of a 0.1% saccharin solution in 1 hr daily sessions, low and high preference groups (n=8/group) of male Sprague-Dawley rats were selected from a larger group. Oral morphine consumption (0.5 mg/ml) was then measured in the selected rats in 1 hr daily sessions. The groups did not differ in either water-deprived or non-deprived conditions. Jugular catheters were then implanted in all rats. Five days after surgery, daily 1 hr sessions in operant chambers began, during which rats were allowed to self-administer morphine sulfate (i.v.) at unit doses beginning at 0.04 mg/kg/infusion; over the course of the experiment, the dose was raised to 0.08 and then to 0.16 mg/kg/infusion. Fourteen rats completed the study through the end of the sessions at 0.08 mg/kg/infusion; 10 completed the entire study. The groups did not differ in the number of infusions obtained at 0.04 mg/kg/infusion. Over the course of the 0.08 mg/kg sessions, saccharin-preferring rats began to self-administer significantly more morphine than rats with a low saccharin preference. For example, averaged over sessions 16-20 at this dose, the high saccharin rats obtained 10.5 ± 2.3 infusions per session, whereas the low saccharin rats obtained 4.1 ± 0.8 ($p<0.05$). Increasing the unit dose from 0.08 to 0.16 mg/kg/infusion caused an increase in self-administration in the low saccharin group and a decrease in self-administration in the high saccharin group. The positive relationship between saccharin intake and morphine self-administration may be due to their mediation by a common reward mechanism. Supported by NIDA DA05471 and DA06827.

502.11

EFFECT OF IBOGAINE ON MORPHINE AND NALOXONE-INDUCED PLACE CONDITIONING.

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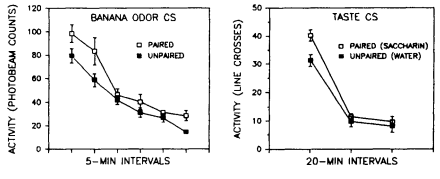
Ibogaine has been proposed to serve as an agent to reduce craving for addictive agents. A series of experiments were conducted to determine the effects of ibogaine (40 mg/kg, ip) on place preference conditioning produced by morphine (5 mg/kg, ip) and on place aversion conditioning produced by naloxone (1-2 mg/kg, ip). Ibogaine alone was found to be ineffective in producing either a place preference or a place aversion; however, it did interfere with the establishment of a morphine-induced place preference after 1, but not after 4 conditioning trials. These results are contrasted with the effects of ibogaine on naloxone-induced place aversion conditioning. In addition, the ability of ibogaine to attenuate the expression of a previously learned morphine-induced place preference was assessed.

502.12

TASTE- AND ODOR-SPECIFIC LOCOMOTOR SENSITIZATION USING MORPHINE.

R. A. Bevins*, K. Shanmugham, & M. T. Bardo. Dept of Psychology, Univ of Kentucky, Lexington, KY 40506.

Context-specific locomotor sensitization occurs when a distinct environment (context) previously paired with morphine elicits an increase in activity relative to a context that was explicitly unpaired. Given that this work has typically used a context conditioned stimulus (CS), it is unclear whether a context CS is necessary for locomotor sensitization, or whether other CS types would work. A context can be viewed as a polymodal stimulus composed of many elements (eg, auditory, olfactory, and tactile). The present work examined the ability of these more unimodal cues (odor and taste) to serve as CSs in a conditioned sensitization paradigm. Adult male Sprague-Dawley rats were used in both the taste and the odor studies. Rats received 8 conditioning trials in which a morphine injection (2 mg/kg, SC) was immediately followed by 15-min exposure to the CS (eg, saccharine taste or banana odor) in a chamber. Using a CS-alone test to assess locomotor sensitization, we found greater activity in morphine-paired rats than in unpaired control rats (see Fig below). Thus, olfactory and gustatory elements of a context are capable of controlling Pavlovian conditioned increases in locomotor activity.



502.13

ORAL DISCRIMINATIVE AND REINFORCING EFFECTS OF ETONITAZENE IN RHESUS MONKEYS. E.D. Pakarinen and J.H. Woods*. Dept. of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0626.

To induce drinking of drug, rhesus monkeys were provided with prandial etonitazene (ETZ, 2.5 ug/ml) drinking opportunities (e.g., Carroll and Meisch, 1978, *Psychopharmacol.*, **59**: 225-229). In the Carroll and Meisch study, it was reported that all subjects orally self-administered more ETZ relative to water. The purposes of the present study were to replicate the oral reinforcing effect of ETZ and to extend the observations with ETZ (0.019 - 5 ug/ml) to a situation in which ETZ was provided concurrently with water in rhesus monkeys. Fluid deliveries were provided following contact responses on solenoid-operated drinking spouts. After the induction procedure, less than the majority of a set of monkeys reliably self-administered more ETZ than water when presented concurrently. The intake of ETZ increased in a concentration-related manner across monkeys. The oral discriminative effects of ETZ were also studied in monkeys trained to discriminate fentanyl (0.0032 mg/kg, s.c.) from vehicle. As expected, ETZ generalized in the fentanyl-trained monkeys, however, ETZ had a slow onset of discriminative effect when delivered orally. ETZ may not be as efficacious as an oral reinforcer among rhesus monkeys as originally reported; large individual differences across monkeys were evident. Significant individual differences have not been noted in studies of intravenous etonitazene reinforcement in primates. (Supported by USPHS Grants DA 00254, DA 05325, DA 07268, and DA 08568).

502.14

THE DEVELOPMENT OF A CONDITIONED PLACE PREFERENCE TO MORPHINE. II. EFFECTS OF MICROINJECTIONS INTO VARIOUS CNS SITES. K.B.J. Franklin* and M.C. Olmstead. Dept. Psychology, McGill University, Montreal, Quebec, Canada.

In the CPP paradigm, animals will approach and maintain contact with an environment previously paired with morphine administration. The present study investigated the neural sites where morphine may produce its reinforcing effect. Bilateral infusions of morphine (1 ug in 0.5 ul over 1 min X 2 pairings) into the lateral ventricles, periaqueductal gray (PAG), or ventral tegmental area (VTA) produced a CPP. Injections into the dorsal striatum, frontal cortex, hippocampus, lateral amygdala, lateral hypothalamus, pedunculopontine nucleus, posterior hypothalamus, ventral pallidum, or ventral striatum did not produce a CPP. Injections 1 mm dorsal to the PAG or VTA were also ineffective. Naloxone methiodide injections (5 nmole in 0.5 ul over 1 min) into the PAG or VTA blocked the CPP induced by systemic morphine (4 mg/kg X 1 pairing). Activation of either PAG or VTA opioid receptors is sufficient to produce morphine reinforcement but the effect may involve an interaction between the two sites.

502.15

EFFECTS OF ADENOSINE AGONISTS AND ANTAGONISTS ON MORPHINE WITHDRAWAL SYNDROME. G.B. Kaplan*, M.T. Sears. Dept. of Psychiatry and Human Behavior, Brown University and Veterans Affairs Medical Center, Providence, RI 02908.

Central opiate actions depend, in part, on the involvement of adenosine. We examined the effects of selective adenosine agonists and antagonists in opiate withdrawal. Male CD-1 mice were implanted with morphine (75 mg) or placebo pellets and a withdrawal syndrome was precipitated by injecting opiate antagonist, naloxone (5 mg/kg, i.p.) 72 hours later. Animals (N=3-8 per dose) were injected i.p. with either vehicle, A_1 agonist R-N^6-(2-phenylisopropyl)adenosine (0.05 mg/kg), A_{2a} agonist, CGS 21680 (0.05 mg/kg) or A_1 antagonist, 1,3-dipropyl-8-cyclopentylxanthine (1 mg/kg) or A_2 antagonist, 3,7-dimethyl-1-propargylxanthine (1 mg/kg) and placed in a plexiglass cylinder. The following behaviors were counted over a 20 minute period: jumps, wet dog shakes, weight loss and diarrhea while teeth chattering, forepaw treads and tremors were checked for their presence during each 1 min interval. In naloxone-precipitated withdrawal groups, A_1 agonist R-PIA decreased jumps and diarrhea by 49 and 58%, respectively, while A_2 agonist decreased jumps by 68% but increased wet dog shakes by 81%. A_1 antagonist increased jumps and tremors by 67 and 30%, respectively, and A_2 antagonist increased wet dog shakes by 68%. In conclusion, adenosine A_1 agonist inhibited, and both adenosine antagonists enhanced withdrawal behaviors, providing support for the role of adenosine receptors in opiate dependence. Follow-up studies will examine acute and chronic agonist and antagonist effects on opiate withdrawal at other doses.

DRUGS OF ABUSE: BEHAVIOR II

503.1

MK-801 Non-differentially Attenuates Both Associative and Non-Associative Morphine Tolerance

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Morphine tolerance is mediated by both associative and non-associative factors. Animals tested in a context which has been repeatedly paired with drug are more tolerant in that context than in others, indicating situationally-specific or associative tolerance. Non-associative tolerance simply results from physiological changes following drug exposure, and does not depend upon contextual cues. Previous experiments have shown that spinal neurotensin mediates associative morphine tolerance. The present experiments examined the role of NMDA receptors in both situationally-specific and non-situationally specific morphine tolerance. Two groups of rats were administered morphine (5mg/kg) once a day for 4 consecutive days and then tested for morphine analgesia (tailflick): one group was tested in the same context in which they had received all prior morphine and the second group was tested in an equally familiar context which had been associated with prior saline injections. A third group of drug naive but similarly handled rats were also tested for morphine analgesia.. Before testing, subjects were intrathecally injected with either MK-801 (2.5, 5.0 or 10 nmol in 5 µl) or vehicle. Both 2.5 and 5 nmol of MK-801 attenuate morphine tolerance. This reversal of tolerance occurred independently of the rats' prior experience in the testing context indicating that unlike neurotensin, NMDA receptors in the spinal cord play a general role in the expression of morphine analgesic tolerance.

503.2

UNIQUE PATTERNS OF MORPHINE AND NICOTINE EFFECTS ON EVOKED RESPONSES IN THE NUCLEUS ACCUMBENS (NAS). C.Eyl. and R. Hakan* Dept. of Psychology, Univ. of North Carolina at Wilmington, Wilmington, N.C. 28403

Behavioral studies have indicated that the nucleus accumbens septi (NAS) is critically involved in drug reward. The purpose of studies in our lab is to elucidate the neuropharmacological nature of NAS related brain circuits an d to determine their role in the mediation of psychoactive drug effects. The present study examined the effects of morphine and nicotine on the amygdala-NAS afferent pathway. Electrical stimulation of the amygdala was used to evoke single-unit responses in the NAS of anesthetized rats. Baseline parameters of extracellularly recorded evoked responses were established and then the effects of systemic morphine and nicotine were examined. Both morphine and nicotine consistently inhibited these evoked responses (15/15 and 7/8 respectively). Also, morphine inhibited spontaneously active single-units in the NAS while nicotine did not. These results are meaningful when compared to the previously established effects of morphine and nicotine on NAS unit responses evoked by fimbria and ventral pallidal (VP) stimulation (Hakan and Eyl, 1993; Hakan et al., 1994). Fimbria evoked responses are inhibited by nicotine but not by morphine whereas both morphine and nicotine have mixed effects on NAS unit responses evoked by electrical stimulation of the VP. Thus, the pattern of effects of these drugs on different NAS circuits are unique. Further analysis of drug effects on NAS circuitry should help explain both the common and distinct reinforcing properties of different psychoactive compounds.

503.3

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON MORPHINE TOLERANCE, PHYSICAL DEPENDENCE AND ABSTINENCE SYNDROME IN THE RAT. George A. Matwyshyn, N.P. Plotnikoff* and Hemendra N. Bhargava, Dept. Pharmaceutics and Pharmacodynamics, Univ. Ill. at Chicago, IL 60612.

The effects of L-N^G-monomethyl-arginine (NMMA) on the development of tolerance to and dependence on morphine as well as on the naltrexone-induced abstinence symptoms were determined. Male Sprague-Dawley rats were made tolerant to and dependent on morphine by s.c. implantation of 4 morphine pellets during a 3-day period with 2 pellets on am of day 1 and 2 pellets on pm of day 2. NMMA (2, 4 or 8 mg/kg, s.c.) was administered twice a day for 3 days. The pellets were removed on day 4 and tolerance and dependence were assessed 6 hr after the pellet removal. The development of tolerance to the analgesic but not to the hyperthermic action of morphine was inhibited by 4 and 8 mg/kg doses of NMMA. Physical dependence development was also inhibited as evidenced by the decrease in jumping behavior induced by naltrexone (5 mg/kg, i.p.) but the other symptoms were not affected. The doses of NMMA which inhibited the tolerance and dependence development were unable to inhibit the naltrexone-induced withdrawal symptoms in morphine-dependent rats. It is possible that much higher doses of NMMA are required to inhibit antagonist-induced withdrawal in morphine-dependent rats (Supported by a Research Scientist Development Award K02-DA-00130 from the National Institute on Drug Abuse).

503.4

NEUROCHEMICAL SUBSTRATES IN THE EXPRESSION OF THE OPIATE WITHDRAWAL SYNDROME: ROLE OF SPINAL NITRIC OXIDE. J.J. Buccafusco, A.V. Terry*. Dept. Pharmacology and Toxicology Medical College of Georgia and Dept. VA Med. Ctr., Augusta, GA 30912.

In the morphine-dependent rat, the expression of autonomic symptoms of naloxone-precipitated withdrawal are mediated to a large extent by an ascending spinal cholinergic muscarinic pathway. The purpose of this study was to determine which neurotransmitter systems participated in the efferent, bulbo-spinal component of this pathway. Our recent studies in non-dependent rats indicated that the pressor response to intrathecal (I.t.) injection of the muscarinic agonist carbachol was mediated by a spinal NMDA-glutamatergic pathway linked to a NO generating system. Rats were made dependent on morphine by a continuous yolked i.a. infusion of increasing doses over 5 days. Withdrawal was precipitated by the i.a. injection of 0.5 mg/kg of naloxone. Mean arterial pressure (MAP), heart rate and several behavioral symptoms of withdrawal were measured over a 60 min observation period. In control dependent rats, i.a. injection of naloxone produced a rapid increase in MAP up to about 30 mmHg over the first 5 min after injection; thereafter, MAP slowly decreased to pre-withdrawal levels. Signs of opiate withdrawal, including body shakes, escape behavior, etc. accompanied the MAP changes. I.t. pretreatment with 100 or 200 nmol of the NMDA antagonist MK801 produced a dose-dependent inhibition of the opiate withdrawal response up to about 60%. I.t. injection of 100 or 200 nmol of the NO synthase inhibitor, L-NAME also significantly inhibited the expression of the withdrawal response. This inhibition was reversed in the presence of 100 nmol L-arginine. Also, L-NAME demonstrated essentially no affinity for spinal muscarinic receptors as assessed using a competition assay with [^{3}H]methylscopolamine. These results are consistent with a role for an NMDA-NO system in the expression of spinal opiate withdrawal. Supported by the Div. Res Svc. Dept. Vet. Affairs.

503.5

ETHOANALYSIS OF THE OPIATE WITHDRAWAL SYNDROME IN RATS E. F. Espejo[1], L. Stinus[2]* and M. Cador[2]. (1) Esc. Ciencias de la Salud, Univ. de Sevilla, Sevilla, Spain, (2) INSERM U.259, Univ. de Bordeaux II, Bordeaux, France

Physical signs during the rat's opiate withdrawal syndrome are usually evaluated by the Gellert-Holtzman score (J. Pharmacol. Exp. Ther. 205: 536-546, 1978). However, this score was not elaborated according to a modern behavioral criterion, and it appears to be somewhat arbitrary. The objectives of this study were thus: i) to analyse the overall rat's behavior during the opiate withdrawal syndrome, and ii) to evaluate the validity of the classic score. Rats were implanted with morphine pellets (75 mg x 2, s.c.), and assigned to six groups (n = 10), receiving naloxone (0, 0.01, 0.05, 0.1, 0.5, 1 mg/kg i.p.) to precipitate the withdrawal syndrome. Behavior was videotaped and analysed by an ethogram and ethological techniques. Score value as well as frequency, duration and latency of each pattern were quantified. A cluster analysis allowed to discern the behavior structure. Ethogram was composed of: sniff, walk-sniff, rearing, leaning posture, wet dog shake, autogroom, face-washing, genital-groom, abnormal posture, swallowing, intermittent walking, jumping, teeth-chattering, freezing, elongation, yawn, and lying. Number of ejaculations, defecations and micturitions, as well as other physical signs (diarrhea, irritability, weight loss, etc.) were also evaluated. Results revealed that abnormal posture and intermittent walking, core patterns, changed in a dose-related fashion. Wet dog shakes and jumping, main behavioral signs included in the classic score, were only enhanced in a dose-related manner with intermediate doses. Significant changes in weight loss and irritability were found to be dose-dependent. Score values were gradually enhanced after naloxone injection, except for the highest dose (1 mg/kg). In conclusion: i) the Gellert-Holtzman score is useful, but it appears not to be very accurate for high naloxone doses, and ii) abnormal reactions, weight loss and irritability are the best indicators of the opiate withdrawal syndrome "level", and they might be the basis of a simpler "ethological" score.

503.6

OPIOID RECEPTOR SUBTYPES MODULATE PONTINE EXCITATORY AMINO ACID (EAA) LEVELS DIFFERENTLY IN BUTORPHANOL AND MORPHINE DEPENDENCE. Yangzheng Feng, Tianshu Zhang, Robin W. Rockhold* and Ing K. Ho, Dept. of Pharmacol. & Toxicol., Univ. of Miss. Med. Ctr., Jackson, MS 39216-4505

To clarify the involvement of different opioid receptor subtypes in regulation of brain glutamate (Glu) /aspartate (Asp) levels during opioid withdrawal, extracellular fluid (ECF) levels of Glu/Asp were examined with *in vivo* microdialysis of the locus coeruleus (LC) following precipitation of withdrawal using antagonists selective for μ (CTOP; D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2), κ (Nor-BNI; norbinaltorphimine) or δ (NTI, naltrindole) opioid receptors in conscious morphine or butorphanol dependent SD rats. Dependence was induced by intracerebroventricular (i.c.v.) infusion of morphine (26 nmol/1 μl/hr), butorphanol (26 nmol/1 μl/hr), or saline (1 μl/hr) for 3 days. Microdialysis probes (2 mm tip) were inserted into the LC 24 hr before precipitation of withdrawal by i.c.v. injection of 48 nmol/5 μl of CTOP, Nor-BNI, or NTI. Behavioral evidence of withdrawal was detected following CTOP challenge in morphine and butorphanol infused rats, and following Nor-BNI challenge in butorphanol infused rats, but not in other groups. Levels of Glu in the LC increased to 409% and 258%, respectively, above basal values in the first 15 min sample following CTOP injection in morphine (n=5) and butorphanol dependent (n=5) rats, while Asp increased to 258% (n=5) and 183% (n=5) of basal levels in the same groups. Withdrawal precipitated by Nor-BNI produced significant increases in LC levels of Glu (200% of basal; n=6) and Asp (132% of basal; n=6) only in butorphanol dependent rats. No changes in EAA levels were noted following NTI injection, in any group. Opioid withdrawal is associated with increased levels of EAAs, within the LC. These increases are mediated primarily through μ opioid receptors in morphine dependent rats. In contrast, both κ and μ receptor subtypes regulate EAA levels during withdrawal from butorphanol. (Supported by DA 05828).

503.7

SUBUCTANEOUS INJECTION OF AN ANALOG OF NEUROPEPTIDE FF PREVENTS NALOXONE-PRECIPITATED MORPHINE ABSTINENCE SYNDROME. J.R. Lake*, D.A. Smith, J.A. Jones, J. Morel, A.E. Claunch, P.A. Stevens, K.K. Ho[1], J. Liu[1], K. Burgess[1] and D.H. Malin Univ. of Houston-Clear Lake, Houston, TX 77058, and [1]Texas A&M Univ., Dept. of Chemistry, College Station, TX 77843.

Neuropeptide FF (NPFF) has antiopiate activity and may play a role in opiate dependence and abstinence syndrome. Previously, the C-terminal tetrapeptide of NPFF was dansylated to increase lipophilicity and penetration of the blood-brain barrier. This compound, dansyl-PQRFamide, precipitated morphine abstinence following s.c. administration. In the present study, the C-terminal -Phe was removed from dansyl-PQRFamide in an effort to convert it to a NPFF-antagonist. In Expt. 1, 12 opiate-naive rats were injected in the 3rd ventricle with either 1 μg dansyl-PQRamide (n=6) or 20% ETOH:water vehicle alone (n=6) 6 mins prior to receiving 10 μg NPFF. Rats pretreated with dansyl-PQRamide had a significant, p<.001, 67% decrease in morphine abstinence-like signs compared with rats pretreated with vehicle alone. In Expt. 2, 12 morphine-dependent rats were injected subcutaneously with 13 mg/kg dansyl-PQRamide (n=6) or 20% ETOH:water vehicle alone (n=6) 5.5 mins prior to receiving 0.125 mg/kg naloxone. Rats pretreated with dansyl-PQRamide exhibited a significant, p<.001, 76% reduction in overall abstinence signs as compared with rats receiving vehicle alone. (NIDA DA06554 and Texas Advanced Technology Program.)

503.8

INHIBITION OF MORPHINE TOLERANCE AND DEPENDENCE BY DIAZEPAM AND ITS RELATION TO CYCLIC AMP LEVELS IN THE RAT BRAIN. M.-J. Sheu, P. Sribanditmongkol, D. Santosa and G.A. Tejwani*. Dept of Pharmacology, College of Medicine, Ohio State University, Columbus, OH 43210

We have recently observed that met-enkephalin may be involved in the inhibition of morphine tolerance and dependence by diazepam (Sribanditmongkol et al. Brain Res. in press). We now propose that cyclic AMP may also play an important role in this phenomenon. Male Sprague-Dawley rats were made tolerant-dependent by s.c. implantation of six morphine pellets. Diazepam (0.25 mg/kg body wt.) was injected i.p. in some rats. Control rats were implanted with placebo pellets and injected daily with saline or diazepam. Morphine tolerant rats were sacrificed 1 h. after injections. Abstinence was induced by injecting s.c. naloxone (10 mg/kg) and withdrawal effects were observed for 30 min. There was no change in cyclic AMP levels in hypothalamus, striatum or cortex of morphine-tolerant animals compared to rats given placebo. Diazepam had no effect on cyclic AMP levels in these animals. Morphine abstinent rats showed a significant increase in cyclic AMP levels in striatum (71%), cortex (126%) compared to placebo treated rats. This increase in cyclic AMP levels was attenuated in morphine abstinent rats injected with diazepam. It is concluded that cyclic AMP may be involved in the inhibition of morphine dependence by diazepam.

503.9

CENTRAL ADMINISTRATION OF NITRIC OXIDE SYNTHASE INHIBITOR ATTENUATES NALTREXONE-INDUCED WITHDRAWAL IN MORPHINE-DEPENDENT MICE. Sanjay N. Thorat* and Hemendra N. Bhargava, Dept. Pharmaceutics and Pharmacodynamics, Univ. Ill. at Chicago, Chicago, IL 60612.

The effects of N^G-monomethyl-L-arginine (L-NMMA) an inhibitor of nitric oxide synthase (NOS) on naltrexone-induced abstinence symptoms in morphine-dependent mice were determined. Male Swiss-Webster mice were rendered dependent on morphine by subcutaneous implantation of a morphine pellet for 3 days. Mice which served as controls were implanted with placebo pellets. Six h after pellet removal, mice were injected intracerebroventricularly (i.c.v.) with either vehicle or L-NMMA (0.1, 1.0 and 10 μg/mouse). Five minutes later, the animals were injected with 50 μg/kg, s.c. of naltrexone and the intensity of withdrawal symptoms was determined. Naltrexone-induced stereotyped jumping response was used as an index. Mice were put individually on a platform following the naltrexone injection and the number of mice jumping off the platform or the total number of jumps within a 15- min observation period were noted. I.c.v. administration of L-NMMA (0.1, 1.0 and 10 μg/mouse) 5 min prior to the injection of naltrexone attenuated the stereotyped jumping response in a dose-dependent manner. These results confirm our earlier report (Brain Res., 642: 153-159, 1994) and strongly suggest a role for CNS nitric oxide in opioid withdrawal phenomenon. In addition, these studies suggest that NOS inhibitors may be clinically beneficial in managing the symptoms of morphine withdrawal (Supported by a Research Scientist Development Award K02-DA-00130 from the National Institute on Drug Abuse).

503.10

PRECIPITATED MORPHINE WITHDRAWAL FROM THE NEONATE THROUGH THE ADOLESCENT RAT. K. Jones* and G. A. Barr. Dept. of Psychology, Hunter College, NY, NY 10021 and New York State Psychiatric Institute, 722 W. 168th Street, NY, NY 10032.

There are few reports on the morphine withdrawal syndrome in the developing animal. In this study, we examine the behavioral effects of precipitated withdrawal in morphine dependent 7, 14, 21, and 42 day old rats. On the first treatment day (which differs for each age group), the litter was removed from the dam (except for postweaning rats which were housed separately) and individual rats were injected with morphine sulfate (10 mg/kg, i.p., b.i.d., 7 day-old pups also received a dose of 3 mg/kg, i.p.) for 6.5 days. Controls included saline injected groups. The last injection was on the morning of the 7th day. For preweaning rats, two hours after the last morphine treatment a single pup was injected with one of several doses of naltrexone (0, 0.3, 1.0, 3.0, 10.0, mg/kg) and observed in a blind manner with the remainder of the litter for a total of 20 minutes. Ongoing behaviors were recorded every 15 seconds. When the observation period ended, the pup was anesthetized and placed back into the observation chamber with the remainder of the litter. Postweaning rats were tested individually on fresh bedding in the same manner as were preweaning pups. Naltrexone injected pups that had been chronically treated with morphine displayed greatly increased specific behaviors and were activated. The results also show that morphine abstinent animals demonstrate withdrawal behaviors that were appropriate to the age group examined. For example, morphine abstinent 7 day-old pups show increased head movements, rolling, wall climbing, and paw movements. Morphine treated 42 day-old animals displayed the classic adult-like behaviors. These results clearly show that a morphine withdrawal-like syndrome can be described in the very young rat and that this syndrome is reflective of the specific behavioral repertoire appropriate to the age of the animal. (Supported in part by DA-06600)

503.11

ABSTINENCE PRECIPITATED BY NALOXONE FOLLOWING A SINGLE DOSE OF MORPHINE: POTENTIATION FOLLOWING A SECOND EXPOSURE. C.J. Heyser*, G.F. Koob, and G. Schulteis. Dept of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Recent studies in humans with no prior history of opiate abuse reported naloxone-precipitated withdrawal signs following a single exposure to morphine, and this effect was markedly enhanced following a second morphine exposure (Azorlosa et al., Psychopharmocology, 114:71, 1994). In a recent report using an established animal model of this acute dependence-like phenomenon, the response disruptive effects of naltrexone on operant responding in rats were enhanced 4 hrs after a single injection of morphine, with a further potentiation of this effect following weekly exposures to morphine and naltrexone for 2 months (Adams & Holtzman, J Pharmacol Exp Ther, 253:483, 1990). Using a similar operant measure, the present study sought to determine if this increase in sensitivity to an opiate anagonist could be observed following only 2 morphine exposures, as was seen with humans. Rats were trained to lever press for food on an FR-15 schedule of reinforcement. Rats were pretreated with morphine (2, 5, or 10 mg/kg s.c.) followed 4 hours later by an injection of naloxone (0 - 3.0 mg/kg s.c.). Prior morphine administration dose-dependently increased naloxone's response disruptive effects relative to morphine-naive baseline, and this effect was markedly potentiated following a second morphine/naloxone exposure 7 days later. The current results confirm previous reports in humans and rats that withdrawal-like signs may be elicited following acute exposure to opiates, and demonstrate a potentiation of this effect with a second exposure. These findings suggest that the development of dependence on opiates as defined by the manifestation of precipitated withdrawal is a progressive phenomenon that may begin with a single dosing. Supported by grant DA04043.

503.12

CLOCINNAMOX STUDIES IN MORPHINE-TOLERANT AND NONTOLERANT RATS TRAINED TO DISCRIMINATE MORPHINE. E.A. Walker*, T.M. Richardson, Y. Wu and A.M. Young. Dept. of Psychology, Wayne State Univ., Detroit, MI 48202.

The effects of irreversible opioid antagonist clocinnamox (C-CAM) on morphine (MS) and fentanyl (FT) were compared in rats before and after repeated injections of MS to determine the role of agonist efficacy in tolerance. Male, Sprague-Dawley rats (n=20) were trained to discriminate 3.2 mg/kg MS from saline under a FR 15 schedule of food reinforcement. Cumulative doses of MS (0.32-10 mg/kg) or FT (0.0032-0.056 mg/kg) produced MS-appropriate responding and decreased response rates. In nontolerant rats, 10 mg/kg C-CAM decreased MS potency by 7-fold, but failed to significantly alter FT potency to produce MS-like stimulus effects. Repeated treatment with 20 mg/kg/day MS for 7-14 days decreased MS and FT potency to produce MS-like stimulus effects by approximately 4-fold. In these MS-tolerant rats, 10 mg/kg C-CAM further decreased MS and FT potency to produce MS-like stimulus effects by an additional 8-fold. No withdrawal signs were observed after C-CAM administration. Quantitative analysis of these data suggest that chronic opioid treatment may increase the apparent efficacy required for an agonist to exert MS-like discriminative stimulus effects. (Supported by DA03796 and KO2 DA00132).

503.13

Clonidine Antagonizes The Rewarding Effects Of Morphine Only In Opiate Withdrawn Rats As Well As The Aversive Effects Of Opiate Withdrawal Itself, K. Nader* and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, M5S 1A8.

Lesions of the brainstem tegmental pedunculopontine nucleus block morphine's rewarding effects only in drug naive animals. Conversely, dopamine antagonists block the rewarding effects of morphine only in opiate deprived rats, as well as the aversive effects of morphine withdrawal itself. As clonidine, an α_2-noradrenergic receptor agonist, has been reported to antagonize the somatic signs of opiate withdrawal, we asked if clonidine would block the motivational effects of opiate withdrawal as well as respect the boundary between nondeprived and deprived motivational states. In a place conditioning paradigm, clonidine (0.05 mg/kg ip) blocked the rewarding effects of morphine in opiate withdrawn rats (as well as the aversive properties of withdrawal itself), but did not affect morphine place preferences (2 and 20 mg/kg) in previously drug naive rats. These results suggest that the motivational system activated in opiate deprived animals includes dopaminergic and noradrenergic components that are in series with each other. Furthermore, clonidine blocked the acquisition of morphine (15 mg/kg), but not LiCl (15 mg/kg), conditioned taste aversions. Given that dopamine antagonists also block morphine, but not LiCl, conditioned taste aversions, we suggest that the aversive effects of both opiate withdrawal and morphine conditioned taste aversions are mediated by the same neurobiological substrates.

503.14

SHORT-TERM TOLERANCE DEVELOPS BEFORE LONG-TERM TOLERANCE IN RATS. Heather L. Burton and Catherine P. Cramer*. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

The development of tolerance to morphine analgesia was investigated in three separate experiments with the purpose of comparing "short-term" and "long-term" mechanisms. "Short-term" tolerance occurs with closely spaced injections of high doses, whereas, "long-term" tolerance is characterized by low to moderate doses at widely-spaced intervals (Tiffany & Baker, 1985).

In the first two experiments Long-Evans hooded rats received i.p. injections beginning on Day 1 and continuing through Day 6. Twenty minutes following the injection, pups were tested for latency to retract a hindpaw from a 52°C hotplate. On Day 7 the litters were each divided into 6 test conditions. In four of the conditions pups received either 0, 1.0, 3.0, or 10.0 mg/kg morphine and were tested for analgesia 20 min. following injection. The two other conditions were 10 mg/kg naloxone and a saline control. These groups were then tested for hyperalgesia, weight changes (1 and 4 hours postinjection) and locomotor behavior.

In Experiment 1, pups received 0, 1.0, 2.0, 5.0 or 10.0 mg/kg of morphine sulfate once a day on Days 1-6. Experiment 2 followed the same format, with the exception that dosages were 0, 0.6, 3.0, 15.0 mg/kg , twice daily, 8 hours apart. In Experiment 3, the pups also received two daily injections of either 0, 0.6, 3.0, or 15.0 mg/kg of morphine but were tested on Day 3.

In Experiment 1, the results of Fanselow & Cramer (1988) were confirmed. Under long-term parameters tolerance was not observed at Day 7. The results of Experiment 2 show shifts in the dose-response curve at Day 7 indicative of tolerance. Withdrawal was not observed in either of the first two experiments. In Experiment 3, a shift in the dose-response curve was observed on Day 3 and withdrawal was indicated by both hyperalgesia and behavioral effects.

In sum, neonatal rats seem capable of short-term, but not long-term, tolerance during the first week, perhaps due to the nonassociative properties of the short-term paradigm.

503.15

MORPHINE WITHDRAWAL IMPAIRS ACQUISITION OF A MORRIS WATER MAZE TASK: POTENTIAL INVOLVEMENT OF PROCEDURAL MEMORY.
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Behavioral and neurochemical changes in rats during early and longterm morphine withdrawal were investigated. Experimental subjects (M) were implanted with osmotic pumps (8.75 mg/240 µl/day over 7 days). Controls (C) received sham implants of similar size and weight. One or 21 days following pump removal, subjects were tested in for 8 days in a Morris water maze (MWM) task. Rats were sacrificed and high affinity choline transport was assessed in the hippocampus (HPC) and striatum (STR). M subjects exhibited a significant decrease in bodyweight during withdrawal that recovered by 21 days after pump removal. M subjects trained during early withdrawal exhibited significantly longer escape latencies due to a possible failure of procedural memory. However, during sequential probe trials these subjects exhibited a significant preference for the target area and accurate search strategies suggesting intact declarative memory. Nine days after removal of the osmotic pumps, HAChT in M subjects was elevated in the STR but not in the HPC. M rats trained during longterm withdrawal exhibited no deficits in any measure of MWM performance. HAChT in these rats was comparable to C levels in both the HPC and STR. Early morphine withdrawal was accompanied by increased striatal HAChT and impaired MWM acquisition.

503.16

EFFECT OF MORPHINE TOLERANCE AND ABSTINENCE ON NITRIC OXIDE SYNTHASE ACTIVITY IN BRAIN REGIONS AND SPINAL CORD OF THE MOUSE.
Marc J. Barjavel and Hemendra N. Bhargava*, Dept. Pharmaceutics and Pharmacodynamics., Univ. Ill. at Chicago, Chicago, IL 60612.

Male Swiss Webster mice were rendered tolerant to and dependent on morphine by subcutaneous implantation of a pellet containing 75 mg of morphine base, for 3 days. Nitric oxide synthase (NOS) activity was determined in the brain regions (cortex, striatum, pons-medulla and hypothalamus) and spinal cord of morphine tolerant and abstinent mice. NOS activity was measured by the conversion of [^{3}H]arginine to [^{3}H]citrulline. In tolerant mice, the pellets were left intact, whereas in abstinent mice the pellets were removed 6-8 hr prior to sacrificing. NOS activity was found to be significantly increased (16%) in cortex of morphine-tolerant mice, but in striatum, hypothalamus, pons-medulla and spinal cord it was unchanged. In morphine-abstinent mice, the activity of NOS was not altered in spinal cord, hypothalamus, pons-medulla cortex and spinal cord but was increased by 32% in the striatum. In vitro, morphine did not modify the NOS activity. It is concluded morphine tolerance and abstinence increases the activity of NOS in certain brain regions (Supported by a Research Scientist Development Award K02-DA-00130 from the National Institute on Drug Abuse).

503.17

TOLERANCE AND CROSS-TOLERANCE TO CANNABINOID LIGANDS AFTER CHRONIC ADMINISTRATION OF ANANDAMIDES.
E. Fride* and R. Mechoulam. Dept. of Natural Products, Hebrew University of Jerusalem, Israel

The development of tolerance to cannabinoid receptor ligands has been well established. However, it is not known whether repeated injections of the recently discovered "anandamides", a family of endogenous cannabinoid ligands, also induce tolerance to subsequent administration of anandamides or cannabinoid drugs. Ten daily injections (*i.p.*) of anandamide (20:4, n-6 [ANA] or 20:6, n-6, 20 mg/kg) were administered to adult female C57/BL mice. Twenty four h after the last injection, animals were challenged with 20 mg/kg of ANA or Δ^9-tetrahydrocannabinol (THC) and tested for a tetrad of responses, typically seen after administration of cannabinoid drugs.

After chronic injection of the anandamides, full tolerance to ANA was seen for catalepsy in the "ring test", while partial but significant tolerance was seen in the open field test for motor activity, for hypothermia and for analgesia on a hot plate. Under these conditions, cross-tolerance was also displayed to a challenge dose (10 mg/kg) of Δ^9-THC. Full responsivity to ANA was restored 1 week after cessation of the chronic injections. Ten daily injections of a lower dose (1 mg/kg) of ANA did not induce tolerance. In contrast, 10 daily injections of a very low dose (0.001 mg/kg) of ANA, which has been shown to attenuate the response to Δ^9-THC (Fride et al., *submitted*), induced a slight increase in the response to ANA for some parameters.

These results indicate that repeated administration of anandamides induces tolerance to cannabinoid receptor ligands. Further, the tendency for increased sensitivity to ANA after chronic administration of a very low dose of ANA, support our previous suggestion that a Gs protein mediated signalling pathway may be involved in the receptor activation by the anandamides (Fride et al., *submitted*).

503.18

DIPOLE SOURCE LOCALIZATION OF THE P300 DURING OPIATE/COCAINE WITHDRAWAL AND BUPRENORPHINE TREATMENT IN HEROIN AND COCAINE ABUSERS.
E. M. Kouri *, S. E. Lukas and J. H. Mendelson. Clinical Neuropsychopharmacology Laboratory, Alcohol and Drug Abuse Research Center, McLean Hospital, Belmont, MA 02178.

The present study was conducted to determine the utility of using novel dipole localization algorithms to identify the origin of the auditory P300 ERP during a cycle of withdrawal from opiate/cocaine use and buprenorphine or placebo treatment. Fifteen male volunteers between the ages of 25-40 who met DSM-IIIR criteria for concurrent opiate and cocaine dependence provided informed consent to participate in this study. Subjects were admitted to an inpatient treatment unit and were tested before, after 12 days of detoxification and then on the 15th day of either buprenorphine (12 mg/day, s.l.) or placebo treatment. P300 ERPs were elicited with an auditory oddball task. To estimate the dipole solution, we assumed a 3 shell model (brain, skull and scalp) and a single source model. Treatment with placebo resulted in an apparent P300 source that was ventral but of larger magnitude than during buprenorphine treatment. These results suggest that P300 source localization may be a sensitive indicator of CNS function during drug withdrawal. (Supported by NIDA Grants DA03994, 06116, 00115, and 00064)

503.19

ELEVATIONS AND PHASIC FLUCTUATIONS IN NUCLEUS ACCUMBENS DOPAMINE (DA) DURING IV HEROIN SELF-ADMINISTRATION.
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Fluctuations in extracellular nucleus accumbens DA and DOPAC levels were monitored in 4-min microdialysis samples from rats engaged in IV heroin self-administration. DA levels were elevated to 200-400% of baseline during self-administration, fluctuating phasically between responses. Each injection caused a short-latency increase in DA levels; a subsequent decrease preceded the next lever-press. Drug requests (lever-presses) normally occurred well before DA levels fell to near-normal levels. DOPAC levels increased in the first minutes and remained elevated through the period of drug availability, showing no significant fluctuations that were time-locked to the response cycle. These observations confirm that self-administered IV doses of heroin are sufficient to activate the mesolimbic DA system; independent evidence implicates ventral tegmental mu and delta receptors in the mechanisms of this opiate action. The patterns of elevation and phasic fluctuation were similar to those seen in cocaine and amphetamine self-administration; this is consistent with the suggestion of a common mechanism for the habit-forming actions of these otherwise disparate agents. These and other microdialysis data taken during self-administration studies remain difficult to reconcile with earlier interpretations of *in vivo* voltammetry data.

503.20

SELF-ADMINISTRATION OF HEROIN, COCAINE, AND HEROIN PLUS COCAINE IS REDUCED BY THE CO-ADMINISTRATION OF THE D3 SELECTIVE COMPOUND 7-OHDPAT.
C. Duvauchelle*, J. Francher, T. Sapoznik and C. Kornetsky. Lab. Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Because of the recently postulated role of the D3 dopamine receptor in mediating the rewarding effects of cocaine (COC) (Caine & Koob, 1993), the D3 selective compound 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OHDPAT) was co-administered i.v. to male Wistar rats self-administering heroin (H), COC, or a combination of H+COC (speedball) on a progressive ratio schedule (PR). 7-OHDPAT (1.0, 2.0, or 4.0 µg/kg/infusion) reliably decreased the PR breakpoint of H (.009, .018, or .036 mg/kg/infusion), COC (.15 mg/kg/infusion), and low doses of H+COC (.009+ .15 mg/kg, respectively). The decrease in PR was characterized by periods of complete cessation of lever pressing accompanied by an increased incidence of catalepsy, oral stereotypy and hypomotility in most subjects. These results suggest that there are multiple effects of 7-OHDPAT that interfere with the self-administration of H, COC and H+COC. (Supported by Grants DA02326 and DA00099 to CK).

503.21

DIFFERENTIAL EFFECTS OF DOI ON COCAINE (COC) AND MORPHINE (MOR)-INDUCED EXTRACELLULAR DOPAMINE (DA) RELEASE IN THE NUCLEUS ACCUMBENS (NA)
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There is previous evidence that blockade of 5-HT3 receptors can inhibit the effect of COC and MOR to increase extracellular DA in the NA. The purpose of this study was to determine if DOI, a direct acting 5-HT2A/2C agonist, can influence the effect of COC and MOR on extracellular DA in the NA in awake rats using microdialysis. The systemic administration of DOI (2.5 mg/kg, sc) alone had no effect on basal extracellular NA DA levels. COC (10 mg/kg, i.p.) produced an increase in extracellular DA in the NA of 323% over baseline. Pretreatment of rats with DOI 30 min. prior to the administration of COC did not significantly alter the increase in extracellular DA produced by COC. MOR (5mg/kg, s.c.) produced an increase in extracellular DA in the NA of 206% over baseline. DOI pretreatment 30 min. prior to MOR inhibited the MOR-induced increase in extracellular DA. These findings suggest that MOR and COC-induced increases in extracellular DA in the NA are regulated differently by the activation of 5HT2A/2C receptors. The ability of DOI to suppress MOR-induced DA release in the NA suggests that non-hallucinogenic 5-HT2 agonists may have a role in the treatment of addiction.

503.22

NICOTINE INCREASES MET-ENKEPHALIN CONTENT AND PREPROENKEPHALIN mRNA IN MOUSE STRIATUM.
N.H. Neff*, R.K. Dhatt, T.A. Wemlinger, G.A. Tejwani and M. Hadjiconstantinou. Depts. of Pharmacology, Psychiatry and The Neuroscience Program. Ohio State University College of Medicine. Columbus, Ohio 43210

Behavioral evidence suggests that opioids play a role in nicotine addiction. Indeed, naloxone reportedly can precipitate withdrawal behavior in animals treated with nicotine chronically. We now present evidence that acute or chronic administration of nicotine increases met-enkephalin immunoreactivity (Met-Enkl) in the mouse striatum. The response to a single dose of nicotine is time-dependent, with Met-Enkl content reaching a maximum 30-60 min post-injection and returning to near normal by 6 hr. The nicotine-induced increase of Met-Enkl is blocked by pretreatment with the nicotinic antagonist mecamylamine, but not by the muscarinic antagonist atropine. Daily administration of nicotine for 2 weeks also increases striatal Met-Enkl. In addition to increasing Met-Enkl content, chronic nicotine increases preproenkephalin mRNA in the striatum when assayed after 7 or 14 days of treatment. We postulate that nicotine addiction is associated with enhanced brain content of opioids which could explain withdrawal behavior when nicotine addicted animals are treated with naloxone.

503.23

EVIDENCE FOR A WITHDRAWAL SYNDROME FOLLOWING CHRONIC ADMINISTRATION OF AN ANABOLIC STEROID TO RATS.
Katherine R. Bonson, Nancy A. Garrick* and Dennis L. Murphy. Laboratory of Clinical Science, National Institute of Mental Health, Building 10, Room 3D41, 9000 Rockville Pike, Bethesda, MD 20892.

In the past decade, anabolic steroids have been recognized as drugs of abuse, especially among athletes and body builders. Chronic administration of testosterone and other androgens has been shown to induce behavioral changes in rats and in humans which includes increases in aggression and alterations in mood. A putative withdrawal syndrome following chronic self-administration of anabolic steroids has been described in humans but has not been demonstrated experimentally in either humans or in rats. The present study shows that distinct behavioral effects emerge in two strains of rats following withdrawal from long-term but not short term administration of testosterone propionate (TP) (30 mg/kg/day). In both Wistar and Fawn-Hooded rats, withdrawal from TP following ten weeks of daily administration induced a behavioral syndrome characterized by facial tremor, head-weaving, full-body lurches and ptosis. This syndrome appeared to be of a greater intensity in the Fawn-Hooded strain. These behaviors subsided within two weeks of withdrawal from the androgen. There were no significant changes in temperature, food intake or body weight over the same period. In comparison, three weeks of daily TP administration did not produce a consistent behavioral syndrome following withdrawal in either Wistar or Fawn-Hooded rats, although there was a significant increase in yawning behavior. These results show evidence for the first time of a withdrawal syndrome in rats following chronic administration of high-dose anabolic steroids.

DRUGS OF ABUSE: METABOLISM—PHARMACOLOGY

504.1

THE LOCAL CEREBRAL METABOLIC EFFECTS OF MORPHINE SENSITIZATION IN THE RAT.
M.A. Kraus*, J.M. Piper, R.T. Livezey and C. Kornetsky. Lab. Behavioral Pharmacology, Boston University School of Medicine, Boston, MA, 02118.

Repeated administration of morphine sulfate (MS) will sensitize a rat to oral stereotypic effects. The effects of this sensitization on local cerebral metabolic rate of glucose ($LCMR_{glu}$) was determined in male F-344 rats. Rats were sensitized by four 10 mg/kg MS (sc) injections at 12 hr intervals. Two control groups were administered saline. One week later, the sensitized animals and one control group, morphine-control, were administered a 0.5 mg/kg challenge of MS 10 min prior to the 2-[C^{14}]deoxyglucose bolus. The second control group received saline in place of MS. $LCMR_{glu}$ in 49 brain sites was significantly greater in the sensitized rats than the morphine-control rats after the MS challenge. There also were increases in $LCMR_{glu}$ in the sensitized animals compared to the saline-controls in a number of structures. These results suggest that MS sensitization involves increased metabolic activity in multiple brain systems including mesolimbic and motor structures. (Supported by Grants DA02326 and DA00099 to CK)

504.2

THE EFFECT OF NALOXONE, MORPHINE, AND THEIR COMBINATION ON LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE IN THE RAT.
C. Kornetsky*, M.A. Kraus and J.M. Piper. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

The present experiment used 2-deoxy-D-[^{14}C]glucose (2-DG) to determine the effects of naloxone (NX), morphine sulfate (MS), and the combined treatment of NX+MS on local cerebral metabolic rates for glucose ($LCMR_{glu}$) in rats. Four groups were studied (N = 18): saline, NX, MS, and NX+MS. On test day, NX (1 mg/kg s.c.) was administered 20 minutes prior to and MS (4 mg/kg s.c.) 10 minutes prior to the 2-DG bolus. NX, by itself, significantly decreased $LCMR_{glu}$ in the locus coeruleus, a structure believed to be involved in the expression of opiate withdrawal, suggesting a role of endogenous opioids in the regulation of central noradrenergic activity. Also, NX significantly increased $LCMR_{glu}$ in the mediodorsal thalamic nucleus which has extensive reciprocal connections with the limbic system and prefrontal cortex, suggesting an impact on the integration of limbic and cortical activity. Finally, NX blocked the inhibitory effects of MS on $LCMR_{glu}$. (Supported by Grants DA02326 and DA00099 to CK).

504.3

MOLECULAR MODELING OF ANANDAMIDE V.M. Showalter and M.E. Abood*. Dept. of Pharmacology/Toxicology, Virginia Commonwealth University, Richmond, VA 23298.

Other investigators have utilized molecular modeling to gain more insight into how cannabinoid ligands bind to their receptor. Anandamide (arachidonylethanolamide) is thought to be the endogenous ligand at cannabinoid receptors. The purpose of this study was to extend the cannabinoid pharmacophore to include anandamide. The structures of cannabinoid ligands were modeled using SYBYL. The structure of Δ9-tetrahydrocannabinol (Δ9–THC) was constructed based on x-ray crystal data for Δ9-THC. The structure of anandamide was generated by adding ethanolamine to the crystal structure of arachidonic acid; a systematic search was performed about the rotatable bonds and each conformer was extensively energy minimized. The minimum energy conformation of Δ9-THC then served as a template for the alignment of anandamide conformers. Preliminary superimpositions suggest that anandamide conformers can mimic the portions of Δ9-THC thought to be involved in receptor recognition and activation. (Supported by DA-07027 and DA-05274)

504.4

DOSE DEPENDENCE OF IBOGAINE NEUROTOXICITY. H.H. Molinari*, I.M. Maisonneuve, and S.D. Glick. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208

Ibogaine is claimed to be an effective treatment for opiate and stimulant addiction. O'Hearn and Molliver (1993), however, showed that in rats ibogaine can cause degeneration of cerebellar Purkinje cells. The present study extended their observations using female Sprague Dawley (Charles River) rats given IP injections of ibogaine and allowed to survive seven days after the last injection. Purkinje cell degeneration was evaluated with a Fink Heimer II stain; enhanced glial cell activity, with a GFAP antibody stain.

One set of animals received an ibogaine dose tested by O'Hearn and Molliver: 100 mg/kg per day for three consecutive days. All of these animals displayed bilaterally symmetric, parasagittal strips of Purkinje cell degeneration. The degeneration was more extensive than suggested by O'Hearn and Molliver, consistently occurring in the medial simple lobule and Crus 1 as well as the vermis and intermediate regions of lobules 5, 6, and 7.

A second set of animals received a dose of ibogaine capable of reducing morphine self administration (Glick et al., 1991): a single injection of 40 mg/kg. No evidence of Purkinje cell degeneration was found in these animals. Thus, ibogaine in sufficient quantities can cause neurotoxicity but the toxicity may not occur with doses that have anti-addictive effects. (Supported by NS29771 and DA03817.)

504.5

CHARACTERIZATION OF A CANNABINOID RECEPTOR IN THE ROUGH-SKIN NEWT. K.Soderstrom[1], F.L.Moore[2], P.H.Franklin[1]* and T.F.Murray[1]. [1]College of Pharmacy, and [2]Department of Zoology, Oregon State University, Corvallis OR 97331.

Binding of the synthetic cannabinoid [^{3}H]CP-55940 to membranes prepared from brains of rough-skin newts (*Taricha granulosa*) was investigated using a rapid filtration assay. Binding equilibrium was reached by 480 min at 15°. Specific binding was saturable whereas non-specific binding (determined in presence of 10 μM levonantradol) was linearly related to [^{3}H]CP-55940 concentration. Saturation isotherms provided a Kd value of 10.5 nM and Bmax of 1.64 pmol/mg protein. Estimates of Kd obtained by kinetic analyses agreed with equilibrium saturation results. Competition experiments using unlabeled CP-55940 and levonantradol yielded Ki values of 8.6 nM and 5.9 nM respectively. Affinity differences between the amphibian receptor and values previously reported for the rat (0.2 - 4.0 nM) may be explained by differences in incubation temperatures; 15° for the newt vs. 30° for the rat. Using a rat brain membrane preparation we found that Kd varied inversely with temperature: Kd = 12.5, 6.5, and 3.9 nM at 5°, 15°, and 30°C. Behavioral experiments suggest that the cannabinoid binding site has physiological relevance. Newts treated with 5 μg of levonantradol IP displayed significantly decreased locomotor activity (measured in crossings of lines painted on a testing-arena floor during a three minute period): Treated animals averaged 4.1 line crossings vs. 11.4 in vehicle controls ($p = 0.01$, 2-tailed t-test). The same dosage significantly decreased the incidence of male clasping behavior (a characteristic mating behavior): clasping was seen in 20% of treated animals vs. 68% of vehicle controls ($p < 0.001$, Rank Sum Test). These behavioral correlates render the rough-skinned newt a useful system for the study of cannabinoid pharmacology.

504.6

ANANDAMIDE AND Δ^9-THC DILATION OF IN VIVO CEREBRAL ARTERIOLES IS BLOCKED BY INDOMETHACIN. S. F. Moore, K.A. Willoughby and E.F. Ellis*. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

An endogenous ligand of the Δ^9-THC receptor, arachidonyl ethanolamide (anandamide, AN) has been shown to cause some behavioral and physiological alterations similar to those induced by Δ^9-THC. We investigated the effect of increasing, topically applied doses (10^{-12} - 10^{-3} M) of AN and Δ^9-THC on in vivo cerebral arteriolar diameter using the acute cranial window technique in anesthetized rabbits. Both AN and Δ^9-THC induced a dose-dependent dilation, with a maximal dilation of 22%. AN and THC were active at doses as low as 10^{-11} M. Dilation by both agents was completely blocked by topical co-application of the cyclooxygenase inhibitor indomethacin. Superoxide dismutase plus catalase, which block cyclooxygenase-dependent dilation induced by bradykinin and arachidonic acid, were inactive on AN- and Δ^9-THC-induced dilation, implying dilation is not via vasoactive, dilator oxygen radicals. HPLC analysis of ^{3}H-AN passed under the cranial window showed approximately 20% metabolism to ^{3}H-arachidonic acid (AA). Following IV injection of ^{3}H-AN in mice both ^{3}H-AN and ^{3}H-AA were found in brain. Reported increases in cerebral blood flow caused by marijuana may be related to formation of brain eicosanoids. Endogenous AN may modulate cerebral blood flow. Supported by DA 07027 and a Javits Neuroscience Investigator Award.

504.7

BINDING OF [^{3}H]MK-801 TO BRAIN REGIONS AND SPINAL CORD OF MICE AND RATS TREATED CHRONICALLY WITH MORPHINE. **Krishnamurthy P. Gudehithlu*, Poluru L. Reddy and Hemendra N. Bhargava, Dept. Pharmaceutics and Pharmacodynamics., Univ. Ill at Chicago, Chicago, IL 60612.**

The effect of morphine tolerance-dependence and abstinence on the binding of [^{3}H]MK-801 to brain regions and spinal cord of mice and rats was determined. Male Swiss-Webster mice and Sprague-Dawley rats were rendered tolerant-dependent by implanting a pellet (mice) containing 75 mg of morphine base for 3 days and 6 pellets during a 7-day period (rats), respectively. In tolerant-dependent animals, the pellets were left intact where in abstinent animals pellets were removed 6 hr (mice) and 16 hr (rats), respectively before sacrificing. In the absence of glutamate and glycine, the binding (B_{max}) of [^{3}H]MK-801 was decreased by 10% in the cerebral cortex of morphine tolerant but not in the abstinent rats. In the presence of glycine and glutamate, the binding was decreased in midbrain and spinal cord of tolerant rats, and in hypothalamus, midbrain and spinal cord of morphine abstinent rats. In morphine tolerant mice, the binding of [^{3}H]MK-801 was decreased in pons-medulla and hypothalamus but was increased in spinal cord. In morphine abstinent mice, the binding of [^{3}H]MK-801 was increased in hippocampus. It is concluded that chronic treatment of mice and rats with morphine produces differential effects on central NMDA receptors. Additionally, different changes are seen when the binding is done in the absence or presence of glycine and glutamate **(Supported by a Research Scientist Development Award K02-DA-00130 from the National Institute on Drug Abuse).**

504.8

PRENATAL EXPOSURE TO MORPHINE ALTERS BRAIN μ OPIOID RECEPTOR CHARACTERISTICS IN ADULT FEMALE RATS. Agnes Rimanóczy[1] and Ilona Vathy*. Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461. [1]Dept. Psychiatry, A. Sz-Gyorgyi Medical School, Szeged, Hungary.

The present study investigated the opioid binding characteristics of membranes of different brain regions of adult female rats exposed to morphine in utero (10 mg/kg/twice daily on days 11-18 of gestation). Females were ovariectomized (OVX) at least one week prior to sacrifice, and some females were injected with 3 μg of estradiol benzoate (EB) 48 hours before sacrifice. Saturation binding assays were carried out using the ^{3}H labeled, highly selective ligand D-Ala-Gly-N-Methyl-Phe-Gly-ol (DAMGO) to label μ opioid receptors. The maximal binding capacity (B_{max}) and affinity (K_d) were estimated in the hypothalamus (HYP), preoptic area (POA), frontal cortex (CX), striatum (STR), ventral tegmental area (VTA) and cerebellum (CER). Prenatal exposure to morphine reduced the B_{max} and the K_d about 25% in the HYP of OVX females when compared to OVX, saline-treated females. A 3 μg EB injection increased the B_{max} and the K_d about 25% in the HYP of morphine-exposed females without affecting saline-treated females. These effects of prenatal morphine were not apparent in either the B_{max} or the K_d in the POA, CX, STR, VTA or CER. The increase in hypothalamic μ receptor binding capacity in prenatally morphine-exposed females in response to estrogen treatment may be related to the deficit seen in adult sexual behavior of prenatally morphine-exposed females in a previous study (Vathy and Katay, Dev. Brain Res., 68:125-131, 1992). Supported by DA 05833 awarded to IV.

504.9

INTERACTION OF TOLUENE WITH GABA AND GLUTAMATE (GLU) IN THE HIPPOCAMPAL SLICE PREPARATION. N. Shinsky* and G. T. Pryor. Neuroscience Department, SRI International, Menlo Park, CA 94025.

Toluene (T) is inhaled for its euphoric effects. We reported that T markedly enhanced electrically evoked responses elicited by single pulses in the hippocampal slice preparation (*Neurosci. Abst.* 19(2), 1461, 1993). This effect was antagonized by GABA and muscimol. To explore further its neuropharmacology, we also examined the effect of T on responses elicited by paired-pulses and its interaction with compounds affecting the GABA and GLU neurotransmitter systems. Responses evoked by single-pulse stimulation (0.1 Hz) of Schaffer collaterals in the stratum radiatum were recorded from pyramidal cells of area CA1 in transverse hippocampal slices from mice. Paired stimuli, separated by 20 msec, were periodically interspersed with the single-pulse train. In this paradigm, there was an increase in the response to the second relative to the first pulse (facilitation). T enhanced paired-pulse facilitation. This enhancement by T was antagonized by GABA and muscimol, but not by baclofen. The competitive NMDA antagonist CGS-19755 was without effect on T-enhanced facilitation; however, after T was washed out, it markedly enhanced paired-pulse facilitation, suggesting a residual effect of T. CNQX, an antagonist at the quisqualate site, blocked the response to single- and paired-pulses, alone and with T. After washout of CNQX and T, the response to single pulses only partially recovered, whereas paired-pulse facilitation was markedly enhanced. These results further characterize the interactions of T with specific neurotransmitter systems in the brain, possibly related to its psychoactive effects.

DEVELOPMENTAL DISORDERS: CORTICAL INJURY MODELS

505.1

CATECHOLAMINE METABOLITES IN THE CEREBROSPINAL FLUID OF HYDROCEPHALIC RABBITS. Ch.E.Olmstead, M.C. Wehby-Grant, W.C.Peacock, J.A.Lazareff, R. S. Fisher, A. Fluharty K.F.Faull and E.N.Orlino Division of Neurosurgery and Dept of Anatomy. U.C.L.A. Sch. of Med., Los Angeles,CA 90024

We have been using the rabbit with chronic kaolin-induced hydrocephalus to assess the factors determining the success of ventriculoperitoneal shunting for infantile hydrocephalus. To that end we have been developing a battery of neurological, behavioral, radiological and anatomical tools to describe the progress of the untreated disease and to evaluate the efficacy of treatment.

In the current study the effects of hydrocephalus on the levels of tyrosine, tryptophan and 5HIAA were determined by HPLC with fluorimetric detection in 6 day old normal (N=14) and in 26 to 69 day old hydrocephalic (N=11) rabbits. Samples of cerebrospinal fluid (50 - 100 µl) were obtained either by puncture of the *cisterna magna* or directly from the lateral ventricle at the time of shunt insertion. Samples were stored at -80°C until processed.

The hydrocephalic rabbits showed significant decreases in both tyrosine and tryptophan levels and a highly significant increase in the serotonin metabolite 5HIAA. There was a significant positive correlation between the intracranial pressure(ICP) and the increase in 5HIAA, but not with the two precursor amino acids.

These data suggest that the increased ICP adversely affects the mechanism of removal of the serotonin metabolite from the cerebrospinal fluid.

505.2

STRUCTURAL AND FUNCTIONAL ASSESSMENT OF CHRONIC KAOLIN-INDUCED HYDROCEPHALUS IN THE RABBIT. M.C.Wehby-Grant, Ch.E.Olmstead, W.J.Peacock, T.H.Hsu, G.Olavarria, D.Hovda, R.S.Fisher. Depts Neurosurgery and Anat., U.C.L.A. Sch. of Med.,Los Angeles, CA 90024

Rabbits with chronic kaolin-induced hydrocephalus have few neurological deficits despite profound ventriculo-megaly. We report here both structural and metabolic sparing in the somatosensory cortex in chronic untreated animals. Hydrocephalus was induced at 4-6 days of age. Control (N=15), untreated hydrocephalic (N=18) and shunted (N=5) animals were sacrificed at survival periods between 10 and 366 days. The brains were either processed for quantitative 2-deoxy-D-glucose auto-radiography and cytochrome oxidase(N=19), stained with cresyl violet for somatosensory cortex barrel fields (N=16),or silver impregnated for quantitative neuronal golgi (N=6).

Glucose utilization and oxidative metabolism were significantly higher in the chronic hydrocephalics than in normal, with the highest metabolic activity in the outer cortical layers in both groups, while the lowest activity was seen in the white matter. The organization of the barrel fields in the facial portion of cortex was preserved. The areas of the individual fields were significantly larger in the hydrocephalic than in the normal littermates. Shunted animals showed intermediate sized fields. In the hydrocephalics the apical dendrites of the layer IV neurons remained perpendicular to the cortical surface, but the dendritic shafts are thickened and the spines much more prominent.

These data show that even with profound ventriculomegaly, the structural organization of the cortex is maintained, and there is considerable functional activity in the cortical mantle of the chronic hydrocephalic rabbit.

505.3

ELECTRON MICROSCOPY OF THE CEREBRAL CORTEX IN YOUNG HYDROCEPHALIC AND SHUNT-TREATED H-TX RATS. H.C.Jones[2*], C.A.Boillat[1], N.G.Harris[2], G.L.Kaiser[1]. 1.Dept of Pediatric Surgery, Univ. of Berne, Switzerland. 2.Dept of Pharmacology, Univ. of Florida, Gainesville, FL 32610.

Shunt-treatment in hydrocephalic infants results in marked clinical improvement but little is known about the cellular effects of hydrocephalus and the response to shunting. The H-Tx rat has inherited hydrocephalus with onset in late gestation and is a useful model in which to study these effects. Rats were shunt-treated at two ages: 4-5 days after birth (before the CSF pressure increases) or at 10 days (after the pressure starts to increase). Cortical tissue was prepared from these rats (n=7) at 21 days of age and from age-matched control (n=6) and hydrocephalic rats (n=8). The ultrastructure of the pyramidal cells and surrounding neuropil from layers V and VI was examined in a Phillips 200 transmission electron microscope. In hydrocephalic rats the cortex was frequently edematous with swollen irregularly-shaped neurites. The neurones had watery cytoplasm, swollen mitochondria, multivesicular bodies, expanded endoplasmic reticulum and dispersed nuclear chromatin. Synaptic contacts were few. Tissue from rats shunted at both ages showed a dramatic recovery of neuronal organization and contained neurones with increased metabolic activity, with frequent Golgi complexes, centrioles, mitochondria and ribosomes. Some neurites showed remaining injury and synaptic contacts were only partly restored. The abnormalities seen in hydrocephalic rats were prevented more effectively in the rats shunted soon after birth at 4-5 days. It is concluded that shunt treatment, if carried out early enough, has the potential for normalizing cortical ultrastructure.

505.4

HIGH RESOLUTION ^{1}H-NMR ANALYSIS OF CEREBRAL CORTEX FROM CONTROL AND HYDROCEPHALIC H-Tx RATS. N.G. Harris[*Φ], B.A. Inglis[Y], H.C. Jones[Φ], R.W. Briggs[6]. Depts. of [Φ]Pharmacology, [Y]Biochemistry and [6]Radiology, University of Florida, Gainesville, FL 32610 USA.

The H-Tx rat develops hydrocephalus in late gestation due to an obstruction of the cerebral aqueduct and if left untreated, affected animals die within 4-6 weeks after birth. By 21 days there is gross lateral ventricular expansion and severe cortical thinning with abnormal cell morphology. Cerebral glucose metabolism and blood flow (CBF) are greatly reduced so that in some areas CBF is below the ischemic threshold. Although many of these gross pathophysiological changes are reversible by the placement of a ventriculosubcutaneous shunt soon after birth, it is not known to what extent the shunted brain is able to function normally. Therefore in an attempt to provide baseline information on the more discrete, biochemical changes that occur in the unshunted hydrocephalic rat cortex, we have used high resolution 1-dimensional ^{1}H-NMR spectroscopy to analyse perchloric acid extracts of cortex prepared by the freeze-funnel technique using spontaneously-breathing control and hydrocephalic rats at 21 days after birth (n=4&4). Many cerebral metabolite concentrations in the hydrocephalic cortex were significantly reduced from control. N-acetyl aspartate (NAA) at 2.02ppm was 3.1±0.54µmol/g wet weight (means±sem) in the hydrocephalic cortex compared to 5.9±0.51 in the control ($P<0.01$), creatine/phosphocreatine at 3.04ppm was 2.0±0.33 and 3.9 ±0.32 ($P<0.01$) in hydrocephalic and control cortex respectively and taurine at 3.44ppm was 3.6±0.51 and 7.6±0.79 ($P<0.01$). The following metabolites were also significantly reduced: alanine, glutamate, aspartate, glycerophosphocholine /phosphocholine, inositol. These results confirm our previous measurements of a disrupted cortical metabolism and further indicate that the biochemistry of the hydrocephalic cortex is severely disturbed. This data establishes a good baseline from which subtle differences in the biochemistry of the shunted hydrocephalic cortex can be investigated.

505.5

QUANITITATIVE ULTRASTRUCTURAL BASIS OF GERMINAL MATRIX MICROVASCULAR MATURATION. L.R.Ment*, W.B.Stewart, C.C.Duncan, and J.A.Madri. Yale Univ. School of Med., New Haven, CT 06510

The risk period for intraventricular hemorrhage(IVH) of the preterm neonate is the first 3 - 4 days and is independent of gestational age. We hypothesized that the risk period is attributable to perinatal induction of maturation of the germinal matrix (GM) microvessels.

Newborn beagle pups (N = 6) were anesthetized and systemically perfused and the brains were prepared for electron microscopic examination. Examination of electron micrographs from the GM of animals on the first, fourth and tenth postnatal days demonstrated no difference in perimeter lengths and capillary and endothelial cell areas; in contrast, luminal areas decreased across postnatal age ($p = 0.05$). Increases were found in basement membrane area between days 1 & 4 ($p = 0.01$) and tight junction length ($p = 0.02$). No such changes were noted in the periventricular white matter. On day 1, 19% of GM capillary perimeter was not covered by glial endfeet. By day 10, only 5% was bare. The microvessels of the white matter had approximately 5% bare perimeter regions at all three ages examined. These data suggest that the risk period for IVH in the preterm infant represents the interval of development of the blood brain barrier in the GM microvasculature. (supported by NS 32578)

505.6

CONJUNCTIVE EYE MOVEMENTS IN CEREBRAL PALSY. M.LeGare* S.Lee and H.Zhang. Dept. of Psychology and Biomedical Engineering Program, California State University, Sacramento, CA 95819.

Do the conjunctive movements of the eyes in cerebral palsy (CP) differ from the norm? If so, this deficit would affect spatial vision in CP. The right and left eye positions of 6 CP and 6 normal (N) adults were measured by the binocular 7000S (Micromeasurements, Inc., Farmington CT). Square, sine and triangle waveforms in the time domain were stimuli for 24, 10sec tests of horizontal and vertical saccadic and pursuit movements (0.3,0.5 Hz;$\pm$4,$\pm$8 degrees). The pooled mean coefficients of determination (r^2) for right and left eye positions across all tests for N ranged from 0.96$\pm$0.03 to 0.98$\pm$0.006 and for CP from 0.80$\pm$0.24 to 0.90$\pm$0.08. The mean r^2 was significantly lower for CP than N on all tests (Mann-Whitney U;$p<.02$) except for horizontal saccades. The degree of binocularity across CP subjects did not coincide with the severity of the disorder. While the final common path for coupling the two eyes' positions may be in the tegmentum and pons, these results indicate that this visuomotor feature is not necessarily conserved in CP and its contribution to poor aimed movement control in CP may not be trivial. (Supported by NSF-BCS9107276 and a CSU-S/CA Award to ML.)

505.7

ABSENCE OF BRAIN ELASTIN IMMUNOSTAINING IN WILLIAMS SYNDROME. A.M. Galaburda,* P.P. Wang, M. Rossen, and U. Bellugi. Beth Israel Hospital and Harvard Medical School, Boston, MA, 02215 and The Salk Institute for Biological Studies, La Jolla, CA 92037.

Williams syndrome (WS) is a rare genetic disorder resulting in characteristic facies, heart defect, and a unique neurobehavioral profile. Cytoarchitectonic neocortical anomalies include exaggerated horizontal laminar organization, increased cell packing density, abnormally clustered and oriented neurons. Posterior forebrain areas are markedly diminished in volume. We have suggested that the increased cell packing density is in part the result of infantile hypercalcemia. (Galaburda, A.M. *et al., NeuroReport.* 5:753-757, 1994).

The recent report that WS is associated with hemizygous deletion including the elastin locus (at chromosome 7q11.23) and contiguous genes (Ewart, A.K. *et al., Nature Genetics* 5:11-16, 1993) suggests that the brain symptomatology of WS may be due to abnormal expression of elastin, laminin B1, and/or acetylcholinesterase.

In one brain from a WS patient aged 8 months we were able to immunostain normally for laminin B1 but not for elastin compared to controls. Another WS brain stained normally for acetylcholinesterase.

Although little is known about elastin's brain distribution and function, there is ample evidence that other extracellular matrix proteins are neurodevelopmentally active. Abnormal elastin activity may contribute to the developmental malformation in WS. This work was supported by NIH grants HD20806, HD26022, NS22343, DC01289, and a grant from the Oak Tree Philanthropic Foundation.

505.8

SELECTIVE INCREASE IN NEURON SIZE IN AGING MICRENCEPHALIC RATS WITH NEOCORTICAL TRANSPLANTS. M. H. Lee,*A. Heaney, A. Disenhouse and A. Rabe. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

The rat with prenatally induced micrencephaly, a model of congenital brain defects, displays life-long cognitive deficits, and a premature aging-related deterioration of spatial learning competence. We recently demonstrated that a neocortical transplant grafted in the hypoplastic cerebrum during the second postnatal week ameliorated the premature aging-associated decline in learning.

To determine whether a life-long presence of a neural transplant would protect the micrencephalic brain from aging-related deterioration, the cerebral cortex and hippocampus were examined in old (19-26 months) rats with methylazoxymethanol-induced micrencephaly that had received a solid transplant of normal E18 neocortex at 10 days of age. The cortical pyramidal neurons were larger in transplant-bearing rats (T) than in micrencephalic rats without transplants (M), while there was no significant difference between the T group and normal controls (C). No group difference was observed in the size of cortical granular cells. In the hippocampus, neuron size in subfields CA2 and CA3 did not differ between T and C rats, while in the M rats neurons were smaller than in T and C rats. On the other hand, no group difference in size was seen in CA1 and CA4. It needs to be determined whether these selective transplant-induced differences in neuron size are responsible for cognitive improvement in the old micrencephalic rat.

505.9

THE DISTRIBUTION OF DYSTROPHIC AXONS PREDICTS CLINICAL FEATURES OF NEUROAXONAL DYSTROPHY: A CASE STUDY. L.A. Goodman*, I. Rapin, and D.W. Dickson. Departments of Pathology and Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.

Neuroaxonal dystrophies are neurodegenerative disorders characterized clinically by dementia and motor abnormalities, and pathologically by dystrophic axons that appear as spherical eosinophilic granular structures called spheroids. In immunocytochemical and electron microscopic studies of a case of late infantile neuroaxonal dystrophy, the distribution of spheroids correlated with the patient's clinical signs. This 11 year old girl had markedly delayed motor and language development, ataxia, severe behavioral disturbances, sleep abnormalities and autonomic dysfunction. At autopsy, the brain was grossly normal. Routine sections revealed numerous spheroids in many gray matter regions. Immunocytochemical studies showed them to be positive for ubiquitin and amyloid precursor protein (APP), weakly positive for parvalbumin and synaptophysin, and negative for neurofilament, Aβ, tau and PHF-tau. Additional markers demonstrated an intense microglial and astrocytic reaction associated with the spheroids. Ultrastructural studies revealed the spheroids to be filled with membrane-bound organelles containing electron-dense granular material and membranous lamellae. Adjacent neuronal perikarya were normal. Spheroids occurred at axon terminals as shown by their localization to target regions of major fiber tracts, positive staining for synaptophysin and APP, and juxtaposition to neuronal perikarya. They were most numerous in the hippocampal endplate, septal nuclei, nucleus accumbens, hypothalamus, certain cranial nerve nuclei, dorsal column nuclei, and dorsal horns of spinal cord, but virtually absent from cerebral cortex and white matter. The selective distribution of spheroids within limbic, sensory, autonomic and reticular activating systems thus corresponded closely to the patient's clinical manifestations. (Supported in part by NIA AG06803.)

505.10

HEATED WATER-INDUCED CONVULSIONS: A MODEL FOR STUDYING THE EFFECTS OF EARLY FEBRILE CONVULSIONS AND PERINATAL EXPOSURE OR WITHDRAWAL FROM DRUGS UPON THE SENSITIVITY OF THE CNS. S. B. Sparber,* B. J. Klauenberg and L. W. Thomas. Department of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Exposure to heated water for not more than 4 minutes caused convulsions in rats during postnatal development. Following exposure to water of 45° C, rats in each age group (24, 32, 36 and 56 days) had more convulsions compared to rats exposed to water of 44° C (N=12-26/group). The occurrence of a convulsion was inversely related to age. The younger rats experienced more convulsions, reached greater maximum colonic temperatures, higher convulsion temperatures and had more rapid increases in temperature. Overall, the results suggest that the rate of temperature change (i.e. heat transfer in the case of an external source) may be the most salient factor. The rates at which temperature increased in rats that convulsed were significantly greater than those that didn't convulse at each age. The maximum body temperature reached at each age was generally not different for convulsing and nonconvulsing rats following exposure to the heated water bath. The younger rats were able to withstand greater temperature changes (e.g.slope of rise; peak temperature, etc.) than older rats without experiencing a convulsion. Although fewer of the older rats convulsed, their convulsion temperatures were lower, their slopes were shallower and, in the case of the oldest rats, these parameters were significantly different from the other age groups, suggesting that older rats are actually more susceptible to hyperthermia-induced convulsions if their thermoregulatory capacity is exceeded. This procedure may be a useful developmental model for inducing febrile seizures, since convulsions were induced during the first exposure (i.e. no kindling; (1)) without causing mortality. It may also be a sensitive procedure for unmasking altered sensitivity of the CNS of subjects exposed to or withdrawn from drugs during development. Supported by USPHS grants DA 01880, DA 04979 and HD 07279.

(1) A kindling-like effect induced by repeated exposure to heated water in rats. Klauenberg, B.J. and Sparber, S.B. Epilepsia, 25:292-301, 1984.

505.11

BEHAVIORAL CONSEQUENCES OF NEONATAL INJURY OF THE NEOCORTEX. G.D. Rosen,* N.S. Waters, A.M. Galaburda, and V.H. Denenberg. Beth Israel Hospital and Harvard Med. School, Boston, MA, 02215 and Univ. of Connecticut, Storrs, CT 06268.

Focal developmental neocortical anomalies, including molecular layer ectopias and microgyria, are associated with developmental dyslexia. Some autoimmune mice spontaneously develop ectopias that are similar in appearance to those seen in dyslexic brains. In addition, these mice have behavioral anomalies, some of which are associated with ectopias, others with the immunological disorder. In an attempt to further test the dissociation of the behavioral effects of autoimmunity and of cortical malformation we induced ectopias (with puncture wounds) and microgyria (with freezing lesions) in the neocortex of one-day old mice without immune disorders.

DBA mice received either a puncture wound or freezing lesion of either the left or right hemisphere. Some mice received sham surgery. Compared to sham-operated animals, mice with either puncture wounds or freezing lesions performed poorly in discrimination learning, in a spatial Match-to-Sample task, and in a Lashley Type III maze. In shuttlebox avoidance conditioning, in which immunological disorder diminishes performance, there was no difference between lesioned and sham animals. These results (1) support the dissociation between the effects on behavior of developmental neocortical anomalies and autoimmune disease, (2) point out similarities between spontaneous and induced neocortical malformations, and (3) fail to support behavioral differences between ectopias and microgyria.

This work was supported, in part, by NIH grant HD20806.

505.12

DIFFERENCES IN NEURONAL AND GLIAL MORPHOLOGY IN HUMAN SUBSTANTIA NIGRA IN INTRA-UTERINE GROWTH RETARDATION (IUGR) AND COT DEATH CASES. [1]E.Markova, [2]C.V.Howard* & [2]D. van Velzen. [1]Moscow Brain Research Institute, 5 Obukha, 103064 Moscow, Russia & [2]Dept. of Fetal and Infant Pathology, University of Liverpool, L69 3BX. UK

Free-floating sections of the mid-brains of 4 control, IUGR and cot death cases for each of three postnatal ages, i.e. 1 week, 1 and 4 months, were prepared for Golgi staining, Braitenberg modification. In addition the 1 week group was immuno-histochemically stained for GFAP and TH .

Qualitatively abnormal changes in Golgi stained dendrites, including varicosities, enlarged shafts of dendrites and aneurysm-like changes, were noted in the IUGR and cot death groups, which were similarly affected. Many neurons showed growth-cone like dendritic endings and also filopodium-like very thin dendrites arising from the soma or proximal dendrites.

60 neurons from each case were measured, using a Leitz Orthoplan 3-D system. In both pathological groups a significant reduction in lengths of total dendritic tree, unbranched dendrites and terminal dendritic segments was revealed. In addition there was a reduction in the maximal radius of the dendritic trees in the IUGR and cot death groups compared to control.

A significant reduction in soma size of TH positive neurons with a concomitant increase in soma size of GFAP positive astrocytes was measured in IUGR and cot death groups compared to control.

The data suggests the presence of delayed maturation and pathological changes in SN neurons and a hypertrophic astrocytic reaction, possibly associated with chronic hypoxic conditions during prenatal development. These results support the known clinical association between IUGR and cot death. It also may be relevant to findings of behavioural changes and impairment of cognitive function in groups of low birth weight infants.

EPILEPSY: ANTICONVULSANT DRUGS—AMPA AND NMDA RECEPTOR

506.1

THE EFFECTS OF VALPROIC ACID ON PKC ACTIVITY IN THE RAT HIPPOCAMPUS.
G.Y.-P. Lee*, T.J. Teyler and L. M. Brown.
Depts. Neuro. and Pharm., NE Ohio Uni. Coll. of Med., Rootstown, OH 44272-0095

Valproic acid can suppress the NMDA induced [3H]-NE efflux in rat cortical slices (Eup. J. Pharm. 254 (1994) 307-309), the NMDA receptor mediated synaptic responses (Brain Res. Bull. 33 (1994) 333-336), and PKC activity in cultured cells (Soc. Neurosci. Abstr. 19 (1993) 750). Enhanced PKC activity may underlie the central role of the NMDA receptor in neuronal plasticity (TINS 15 (1992) 333-339). Valproic acid induced alterations in PKC may serve as a mechanism underlying the ability of valproic acid to suppress NMDA activity.

Brain slices (350 μm) from the hippocampus of Long-Evans hooded rats were used. The control slices were incubated in ACSF, and experimental slices were incubated in ACSF containing 100 μg/ml valproic acid for 20 mins. After the treatment, slices were washed in Tris-HCl buffer, and prepared for PKC phosphorylation assay (Brain Res. 524 (1990) 144-148). Under the treatment of valproic acid, the cytosol PKC activity was 279 /μg/pmole/min (decreased about 35%); and the membrane PKC activity was 44 /μg/pmole/min (decreased about 27%). Valproic acid had a tendency to suppress PKC activity in both membrane and cytosol components.

506.2

SUPPRESSION OF REFRACTORY STATUS EPILEPTICUS BY U54494A AND DIAZEPAM IN THE LITHIUM-PILOCARPINE MODEL IN RAT.
A. Handforth*, VAMC West Los Angeles, Los Angeles, California 90073.

Treatment of clinical status epilepticus (SE) is frequently unsatisfactory; many cases are refractory to treatment or develop serious adverse events. We have utilized the lithium-pilocarpine SE model to investigate potential new therapies. In this model rats pretreated with LiCl (3 mM/kg ip) develop SE after pilocarpine is injected (30 mgs/kg ip). Once EEG-recorded continuous fast spiking is attained the SE is highly refractory to all conventional SE therapies.

U54494A has been shown to antagonize seizures in several models, but does not possess the sedative properties of the related kappa opioid agonist U50488H. We found that U54494A, 24-30 mgs/kg, infused iv over 1 to 15 minutes (n=12) converted fast continuous spiking to periodic epileptiform discharges (PEDs). Higher doses that stopped all epileptiform activity caused subsequent mortality. Diazepam 20-30 mgs/kg iv as monotherapy did not slow or stop continuous fast spiking (n=6). Diazepam 2-10 mgs/kg iv given 10 min after U54494A-induced PEDs stopped all epileptiform activity with subsequent recovery of the rat (n=7). Diazepam administration after lower doses of U54494A that did not cause PEDs did not stop SE (n=6). The combination of U54494A and diazepam thus is effective in stopping SE in this model of refractory SE. Supported by Upjohn.

506.3

THE BROAD SPECTRUM ANTICONVULSANT ADCI BLOCKS TTX SENSITIVE VOLTAGE-ACTIVATED Na CHANNEL CURRENTS IN NE1-115 MOUSE NEUROBLASTOMA CELLS. G. White*, Neurogen Corp. Branford, CT 06405.

ADCI is a novel anticonvulsant drug that is efficacious in a wide variety of seizure models. ADCI is a hybrid analog of both carbamazepine (a Na channel blocker) and MK-801(an NMDA receptor un-competitive antagonist) and appears to have anticonvulsant activity consistent with both antagonist activities. While the NMDA antagonist activites of this compound have been characterized electrophysiologically (Rogawski et al, J. Pharm. Exp. Ther., 1991) little direct evidence is available concerning ADCI's activity as a Na channel blocker. In the present series of experiments, the sodium channel activity of ±ADCI and its enantiomers were evaluated using the whole-cell patch-clamp technique in a cell line (NE1-115) in which TTX sensitive Na currents were isolated using the following recording solutions: external (in mM): 100 NaCl, 25 TEA, 1.8 $CaCl_2$, 5 $CsCl_2$, 1 $MgCl_2$, 1 $CoCl_2$, 25 glucose, 5 HEPES, pH 7.35; internal (in mM): 115 $CsCl_2$, 4 MgATP, 0.6 GTP, 5 EGTA, 10 HEPES, pH 7.35. ADCI was found to block Na currents in a manner similar to that of carbamazepine. Both drugs were ~5 fold more potent as blockers at -65 mV than at -85 mV. Similar to carbamazepine, ADCI shifted the h_∞ curve of the Na channels in a hyperpolarizing direction and showed a use dependent block of current amplitude. No differences were observed in block of Na channel activity among racemic (±)ADCI and its two (+ and -) enantiomers. 100 nM of the NMDA antagonist, +MK-801, had no detectable effect on Na currents. These findings suggest that the spectrum of anticonvulsant activity of ADCI results from its blocking action at TTX-sensitive Na channels in addition to its NMDA antagonistic activity.

506.4

BEHAVIORAL CHARACTERIZATION OF A NOVEL COMPETITIVE AMPA RECEPTOR ANTAGONIST (LY293558).
D.R. Helton*, P.L. Ornstein, M.J. Kallman and J.P. Tizzano. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 4613

Competitive AMPA receptor antagonists protect against convulsions and neuronal injury in animal models. LY293558 is a parenterally active, selective, competitive AMPA (2-amino-3(3-hydroxy-5 methyllisoxazol-4-yl) propionic acid) receptor antagonist. In this study, the anticonvulsant and behavioral effects of LY293558 were evaluated following intravenous administration (0,1,3, or 10 mg/kg) in CD-1 mice. Convulsive thresholds for intracerebral administration of S-AMPA, electroshock and pentylenetetrazol were dose-dependently increased. Clinical observations at higher doses included labored breathing (3 and 10 mg/kg), ataxia, hyperreactivity or hypoactivity (10 mg/kg). No overt clinical observations were noted at 1 mg/kg. LY293558 produced quantitative reductions in spontaneous ambulatory and nonambulatory activity levels at 10 mg/kg. In addition, sensorimotor reactivity was decreased at the high dose of 10 mg/kg. Muscle tone and pain thresholds (writhing and tail flick) were not affected by LY293558 treatment. These data suggest that LY293558 has anticonvulsant activity in several animal models.

506.5

EFFECTS OF DEXTROMETHORPHAN (DM) ON KAINIC ACID (KA)- INDUCED SEIZURE IN RAT. H. Kim*, K. R. Pennypacker, G. Bing and J. S. Hong. LMIN-NIEHS, Research Triangle Park, NC 27709.

Several studies showed that DM exerts both anticonvulsant and proconvulsant effects depending on the experimental conditions. In this study, we examined the effects of DM on KA- induced cell loss in the hippocampus and changes in AP-1 DNA binding activity which are associated with seizure activities. KA (8mg/kg, i.p) administration produced robust behavioral convulsions lasting 4- 6 hours. Pretreatment with DM (12.5- 75 mg/kg, p. o) 15 minutes before KA injections reduced the seizure activities as well as lethalities in a dose dependent manner. Histochemical studies revealed a severe loss of cells in CA1 and CA3 fields of the hippocampus in KA- treated rats. DM pretreatment also reduced the degree of cell loss in a dose depend fashion. Biochemical studies also showed that DM pretreatment attenuated the KA-induced increase of AP-1 DNA binding activity in hippocampus. These results indicate that DM is an effective anticonvulsant to antagonize KA- induced seizures and its related changes.

506.6

VALPROIC ACID, PHENOBARBITAL AND PHENYTOIN DO NOT DISPLACE [^{3}H]MK-801 BINDING L.M. Brown*, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

NMDA antagonists have anticonvulsant properties and are capable of enhancing the effects of other anticonvulsant agents (Urbanska et al., Eur. J. Pcol. 200; 1991; 277-282). Previous studies have demonstrated that the antiepileptics; valproic acid, phenobarbital and phenytoin inhibit NMDA-stimulated [^{3}H] norepinephrine efflux in rat cortical slices (Brown et al., Eur. J. Pcol. 254; 1994; 307-309). The ability of the antiepileptics to inhibit [^{3}H]MK-801 binding in rat cortical homogenates was examined to determine if these drugs have the ability to interact with the NMDA channel site.

[^{3}H]MK-801 binding was performed using the method of Javitt and Zukin (PNAS. 86; 1989; 740-744). Well washed rat cortical homogenates were incubated in the presence of [^{3}H]MK-801 (5 nM), glutamate (10 μM), D-serine (10 μM) and varying concentrations of antiepileptic drug in a 5 mM tris acetate buffer. The binding reaction was carried out for 4 hrs at 37°C. The binding reaction was terminated by vacuum filtration and radioactivity determined using liquid scintillation counting.

PCP competed for [^{3}H]MK-801 binding with an IC_{50} of 6.2 μM ± 3.2 μM. Valproic acid (1 μM to 10 mM) did not displace [^{3}H]MK-801 binding. Phenobarbital and phenytoin in concentrations of 0.1 μM to 3 mM also failed to displace [^{3}H]MK-801 binding. These results indicate that the ability of antiepileptics to inhibit NMDA stimulated efflux of [^{3}H]norepinephrine does not involve an interaction at the NMDA channel site.

506.7

MK-801 DISRUPTS THE NETWORK OF BRAIN REGIONS AFFECTED BY KAINIC ACID AND PREVENTS THE MANIFESTATION OF LIMBIC CONVULSIONS. A.M. Planas [1*], M.A. Soriano [1,2], I. Ferrer[2] and E. Rodríguez-Farré[1]. [1]Dep. Pharmacol. & Toxicol., CID, CSIC, 08034 Barcelona, and [2]Unitat de Neuropatologia, Hospital Princeps d'Espanya, Universitat de Barcelona, Spain.

MK-801 reduces the convulsant and excitotoxic effects of kainic acid administration. This study examined the effect of MK-801 on the regional expression of heat-shock protein-70 (HSP70), HSP70 mRNA and neuropathological outcome following systemic kainic acid. HSP70 is a marker of cellular stress that does not invariably precede cell death (Sloviter and Lowenstein., 1992). Rats received either kainic acid (9 mg/kg), MK-801 (1 mg/kg), or MK-801 one hour prior to kainic acid. Kainic acid alone caused convulsions in 74% of treated rats. Convulsing rats expressed HSP70 mRNA and HSP70, predominantly in limbic regions. Necrosis was also seen in select limbic areas. Pre-treatment with MK-801 reduced the percentage of convulsing rats to 4% and inhibited HSP70 mRNA and HSP70 expression mainly in thalamus and cortex. HSP70 mRNA and protein was maintained in CA3, subiculum and the amygdala. Rats pre-treated with MK-801 and not convulsing showed no histopathological changes. These data identify brain regions putatively involved in the manifestation of limbic convulsions and through which the anticonvulsant effect of MK-801 might be mediated.

Sloviter and Lowenstein (1992) J. Neurosci., 12: 3004-3009.

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506.8

THE ANTICONVULSANT REMACEMIDE ALLOSTERICALLY BLOCKS THE NMDA RECEPTOR: INTERACTION WITH THE POLYAMINE SITE. Swaminathan Subramaniam, Sean D. Donevan and Michael A. Rogawski*, Epilepsy Research Branch, NINDS, Bethesda, MD 20892.

Remacemide [2-amino-*N*-(1-methyl-1,2-diphenylethy)acetamide] is a novel anticonvulsant drug that is metabolized to an open channel blocker of the NMDA receptor. Remacemide itself is also a weak NMDA receptor antagonist, but it appears to act, at least in part, by a mechanism distinct from that of the metabolite. We used voltage-clamp recordings from cultured rat hippocampal neurons and [^{3}H]dizocilpine binding to rat forebrain membranes to investigate the mechanism of the remacemide block. Remacemide inhibited NMDA induced currents and displaced [^{3}H]dizocilpine binding to rat forebrain membranes at a concentration of ~50 μM. The block of NMDA induced currents at −60 mV was weakly voltage-dependent and was reduced by ~15% at +60 mV. To eliminate the voltage-dependent component of block, subsequent experiments were carried out at +60 mV. Concentration-response curves for remacemide were obtained in the presence and absence of the polyamine spermine. Spermine (1 mM) shifted the remacemide isotherm to the right (IC_{50} increased from 95 to 198 μM), whereas the purported polyamine site antagonist diethylenetriamine (1 mM) produced a comparatively smaller shift (IC_{50} increased to 139 μM). Using the rate of dissociation of [^{3}H]dizocilpine as a measure channel activity, spermine (0.3–100 μM) was observed to markedly increase the rate of unbinding, whereas remacemide (10–100 μM) slowed unbinding. The concentration-response relationship for remacemide in the presence of different concentrations of spermine indicated a competitive interaction between the anticonvulsant and the polyamine. We conclude that remacemide allosterically inhibits NMDA receptor channel opening via an action at the polyamine site.

506.9

THE ANTI-CONVULSANT AND ANTINOCICEPTIVE EFFECT OF 5-NITRO-6-METHYL-7-CHLORO-2,3-QUINOXALINEDIONE (NMCQX) AND 5-NITRO-6,7-DIMETHYL-2,3-QUINOXALINEDIONE (NDMQX), TWO NOVEL NMDA RECEPTOR/GLYCINE SITE ANTAGONISTS IN THE MES AND FORMALIN TESTS. M. Tran[1], K. Lutfy[2], L. Kwon[2], S.X. Cai[1], J.F.W. Keana[3] and E. Weber*[2]. [1]**Acea Pharmaceuticals, Inc., 1003 Health Sciences Road West, Irvine, CA 92715;** [2]**Dept. of Pharmacology, UC Irvine, Irvine, CA 92717;** [3]**Dept. of Chemistry, University of Oregon, Eugene, OR 97403.**

Recent studies have suggested a modulatory role for the N-methyl-D-aspartate (NMDA) receptor in several physiological as well as pathological states, e.g., LTP, epilepsy, ischemia, pain etc. In the present study we, respectively, evaluated the anti-convulsant and antinociceptive effect of NMCQX and NDMQX in the maximal electroshock (MES) model and in the formalin test in Swiss Webster mice. In the MES test, mice were injected i.p. with NMCQX (1.25-7.50 mg/kg; N=15 mice/dose) or NDMQX (5.0-15.0 mg/kg; N=15 mice/dose). Control mice were injected with Tris (0.05 M) or Arginine (0.1 M), respectively. Seizures were then induced by applying a rectangular pulse, 50mA, 60 pulses/sec, 0.8msec pulse width and 0.2 sec train length at the time of drugs' peak effect. Both compounds dose-dependently protected mice from generalized clonic-tonic seizures induced by electroshock. In the formalin test, following a 1 hr accomodation period mice were injected i.p. with NMCQX (1.0-10.0 mg/kg, N=7-13 mice/dose) or NDMQX (0.5-20.0 mg/kg, N=8-9 mice/dose). Control mice were injected with Tris (0.05 M) or Bis-Tris (0.2 M), respectively. Mice were then injected with formalin and the amount of time that each mouse spent licking and/or biting the injected paw was recorded for 1 hr. A s.c. injection of formalin produced a biphasic nociceptive response, i.e., a transient early phase (0-5 min) followed by a tonic late phase (15-50 min). Both compounds produced a dose-dependent antinociceptive effect in both phases of the formalin test. These data suggest a modulatory role for the NMDA receptor in MES-induced seizures and in formalin-induced nociception. (Supported by Acea Pharmaceuticals, Inc. and NIDA Grant DA 06726)

506.10

FELBAMATE, A NOVEL ANTIEPILEPTIC, REVERSES NMDA/GLYCINE STIMULATED CALCIUM ION INFLUX IN CULTURED RAT HIPPOCAMPAL NEURONS. L.A. TAYLOR*, R.D. McQUADE AND M.A.B. TICE, **Schering Plough Research Institute, Kenilworth, NJ 07033.**

Felbamate, 2-phenyl-1,3-propanediol dicarbamate, is a novel, orally active anticonvulsant that is reported to exhibit neuroprotectant properties. Felbamate has recently been approved for the treatment of Lennox-Gastaut syndrome and partial onset seizures in the United States and Europe. Although its mechanism of action has yet to be fully elucidated, felbamate appears to inhibit the spread of seizures and to elevate seizure threshold in animal models.

A proposed mechanism of action for felbamate is via the N-methyl-D-aspartate (NMDA) receptor complex. Neurotransmission mediated by the NMDA receptor complex has been shown to be associated with seizures, ischemic neuronal injury, and other neurological disorders. The neutral amino acid glycine, which acts as a co-agonist for the activation of the NMDA receptor channel, is postulated to be involved with seizure disorders and neuroprotection. Previous studies demonstrate the ability of felbamate to inhibit binding at the glycine site on the NMDA receptor.

The present study examined the effects of felbamate on NMDA/glycine-stimulated calcium ion influx in cultured rat hippocampal neurons.The results of these experiments demonstrate that felbamate inhibits NMDA / glycine-stimulated calcium ion influx with a minimal effective concentration of 100 μM. Dose response studies suggest a concentration-dependent relationship between felbamate and the reversal of calcium ion influx. However, due to limited solubility, an extensive range of concentrations of felbamate could not be studied.

506.11

SELECTIVE ANTAGONISM OF THE ANTICONVULSANT EFFECTS OF FELBAMATE BY GLYCINE. M. Cohen-Williams, D. T. McHugh, A. Barnett and V. L. Coffin*, Schering - Plough Research Institute, Kenilworth, NJ 07033.

Felbamate (2-phenyl-1,3-propanediol dicarbamate) is a novel antiepileptic with a unique structure; however, its mechanism of action is unknown. Felbamate has been shown to bind to strychnine-insensitive glycine sites (McCabe et al., 1993) but thus far, this action has not been linked to its anticonvulsant effects. In the present study the effects of glycine on felbamate's ability to block convulsions was studied in two rodent models: N-methyl-D-aspartate (NMDA)-induced and electroshock-induced convulsions. Briefly, NMDA (0.5nmol/mouse) was injected i.c.v. and mice (CF-1) were observed for 0-30 min for clonic and myoclonic seizures. Electroshock-induced seizures (50 mA. 60 HZ, 0.2 s through corneal electrodes) produced tonic seizures in at least 95% of the mice. The ED50 for felbamate was shifted from 59 mg/kg to 705 mg/kg by pretreatment with glycine (800 mg/kg i.p.), with a potency ratio of 0.08 ($p < 0.05$). An even greater shift was observed for blocking NMDA-induced convulsions. This interaction appears to be unique to felbamate since the ED50's for phenytoin, valproate, carbamazepine and phenobarbital were not increased by glycine. Plans to study newer anticonvulsants are under way. These findings suggest that felbamate blocks the actions of glycine and thus is the first anticonvulsant clinically available that acts by this novel mechanism.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID VII

507.1

FUNCTIONAL CHARACTERIZATION OF A TRANSCRIPTIONAL REGULATOR OF AMYLOID PRECURSOR PROTEIN GENE

M. Grilli*, M. Ribola, B. Bossini, M. Memo and P.F. Spano. Pharmacology Sec., Dept. of Biomed. Sci. & Biotech., University of Brescia, Medical School, Brescia, Italy.

Since several observations underscore the potential contribution of APP gene overexpression or dysregulated expression to Alzheimer's Disease pathogenesis we judged important to elucidate molecular mechanisms of APP gene regulation at the transcriptional level and, as reported previously (Grilli et al. Society for Neuroscience, 1993, 421.8), we have identified a nucleotide sequence we refer to as APPκB in the APP gene regulatory region which specifically binds a protein complex belonging to the NFκB/Rel family of transcription factors. We now report that in transfection studies with constructs in which the APPκB sequence has been linked to a heterologous eukaryotic promoter (herpes simplex virus tk promoter) governing expression of the bacterial CAT reporter gene, this nucleotide region behaves as a transcriptional repressor. The complex able to bind the APPκB sequence, unlike most of the NFκB/Rel family members, is constitutively present in nuclear extracts from various rat brain areas and cell lines we examined. It is feasible that this repressor protein may contribute to maintain low levels of APP gene expression also in vivo and that an alteration at any step in this regulatory pathway may result in deregulated expression of the APP gene. Future experiments will be directed to investigate alterations of this pathway in patients affected by Alzheimer's Disease.

507.2

TWO NUCLEAR FACTOR BINDING DOMAINS ACTIVATE TRANSCRIPTION FROM THE HUMAN AMYLOID ß-PROTEIN PRECURSOR (APP) PROMOTER. Wolfgang W. Quitschke* and James P. Matthews Department of Psychiatry and Behavioral Science, State University of New York at Stony Brook, Stony Brook, NY 11794-8101.

The promoter of the APP gene was analyzed for its ability to activate transcription in selected cell lines that showed variant levels of endogenous APP transcripts. Transient transfection assays showed that 94 base pairs 5' to the main transcriptional start site are sufficient for high levels of cell-type specific promoter activity.

Two nuclear factors that bind to this region in a sequence specific manner were identified by mobility shift electrophoresis, DNase foot printing, and methylation interference. One of the factors binds to the recognition sequence GGATCAGCTGAC, here designated as APBα. The other binding domain APBß contains the recognition sequence GCCGCTAGGGGT

The contributions of the binding domains APBα and APBβ to the activity of the APP promoter were assessed by introducing block mutations into the respective recognition sequences, which were analyzed by transient transfection. The results suggested that APBβ contributes approximately 70-90% and APBα 10-30% to the transcriptional activity from the APP promoter in all cell lines analyzed.

507.3

AMYLOID PRECURSOR PROTEIN: LOCALIZATION OF mRNA AND PROTEIN ISOFORMS IN RAT OLFACTORY EPITHELIUM AND ALTERATIONS IN EXPRESSION DURING NEURONAL DEGENERATION AND REGENERATION. N. Zaidi and B. Talamo*, Tufts Medical School, Boston, MA 02111.

The major component of the senile plaques in Alzheimer's disease (AD) is βA4 or amyloid protein, which is derived from a larger integral membrane protein, Amyloid Precursor Protein (APP). βA4 is toxic to neurons in culture and may contribute to neurodegeneration in AD. APP is widely distributed in neurons and other cell types, but the role of APP under normal physiological conditions and in AD is not understood. A single APP gene generates various transcripts by alternative splicing in both humans and rodents. APP 695 is the predominant form in rat central nervous system, while APP 751 and 770 forms (containing a Kunitz protease inhibitor (KPI) domain) are abundant in peripheral tissues. Recent studies suggest a role for APP 695 in neuronal differentiation; KPI-containing isoforms have been implicated in neuronal repair, regeneration and synaptogenesis. Olfactory neurons in nasal mucosa turn over throughout life at a slow rate, but accelerated turnover can be achieved by olfactory bulbectomy, thus providing an excellent model for examining the role of APP in neuronal differentiation and repair in adult animals *in vivo*. We have previously used RT-PCR to show that three major APP isoforms are expressed in nasal olfactory tissue at ratios different from those in brain. Western blots confirm that these APP protein isoforms are present in nasal olfactory tissue. *In situ* hybridization with digoxigenin-labeled riboprobes shows that APP mRNA is localized primarily in the neuronal layer of the olfactory epithelium. Immunocytochemical studies using a C-terminally directed APP antibody that recognizes all full length isoforms show that APP is localized both in the neuronal layer of olfactory epithelium, and in nerve bundles. Experiments are in progress to localize APP isoforms by using isoform specific oligonucleotide probes in control olfactory epithelium and during neuronal degeneration and recovery following bulbectomy.

507.4

CHANGES IN AMYLOID PRECURSOR PROTEIN AND RNA IN PRIMARY CULTURES OF EMBRYONIC CORTICAL NEURONS UNDERGOING GLUTAMATE-INDUCED NEURODEGENERATION. D. Willoughby*, I. Rozovsky, and C. E. Finch. Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

Extracellular deposits of ß-amyloid (Aß) in disease-affected regions of the brain are a diagnostic feature of Alzheimer's disease (AD). Aß is a 39-43 amino acid peptide derived from the amyloid precursor protein (APP). A Kunitz protease inhibitor (KPI) domain is encoded by an alternatively spliced exon of the APP gene. KPI-mRNA may be related to Aß deposition or neuronal cell death in the AD brain.

To examine possible relationships between APP RNA alternative splicing and neuronal cell death, we measured alternatively spliced APP-mRNAs in primary cultures of rat embryonic cortical neurons treated with 250 uM glutamate. By LDH release assay, glutamate-induced cell death was ≤ 20% after 4 hours of glutamate treatment, with variable cell death after 9 hours. Cell death was 45%, after 24 hours of glutamate treatment. KPI-mRNA was increased 2-fold after 6 hours of glutamate treatment, while APP-mRNA lacking KPI was unchanged. Aminophosphovalerate, a NMDA receptor antagonist blocked glutamate-induced increases in KPI-mRNA. Thus, induction of KPI-mRNA was dependent on NMDA-receptor activation.

Recently, it was shown by M. P. Mattson and co-workers that APP protected cultured rat hippocampal neurons from excitotoxin-induced cell death. We measured APP in cell lysates and conditioned media of neurons treated with 250 uM glutamate. APP was decreased in cell lysates of glutamate treated neurons within 1 hour of glutamate treatment. Cellular APP remained depleted for at least 20 hours following glutamate addition to the cultures. APP was also reduced in conditioned media 4 hours after glutamate addition. These results suggest that sustained glutamate receptor activation results in rapid degradation of APP. Induction of KPI-mRNA may be a compensatory response to glutamate-induced APP depletion in the neuronal cell. supported by AG00093 and AG7909.

507.5

ACIDOSIS, NOT HYPOGLYCEMIA, STIMULATES PROCESSING OF BETA-AMYLOID IN CULTURED HIPPOCAMPAL NEURONS, G.J. Brewer* Southern Illinois Univ. Sch. Med., Springfield, IL 62794.

Reductions in cerebral glucose or increases in lactic acid in Alzheimer disease (AD) may signal pathogenic metabolism of the β-amyloid precursor protein (APP). We tested these stressors of cell metabolism on cultured rat embryonic hippocampal neurons. After 4 days of growth in serum-free medium, either lactic acid was added to the medium or the medium was exchanged with a medium containing lower glucose. After 24 hr., whole cells and cell extracts were tested for immunoreactivity with antibodies specific for residues 17-24 of βA4 (4G8) or residues 645-694 near the C-terminus of APP_{695} (369W). Hypoglycemia was found to have no effect on APP synthesis or processing. In contrast, lactic acid stimulated processing of APP. Less APP was secreted into the medium and more was found in the cytoplasm, processed to forms of apparent 21 and 25 kD. These forms were reactive with the antibodies directed against sequences for βA4 and the C-terminus of APP. Acidosis in the brains of the elderly may contribute to processing of APP that is potentially amyloidogenic. Supported by the Illinois Dept. Public Health.

507.6

TIME COURSE OF AMYLOID PROTEIN PRECURSOR, APOLIPOPROTEIN E, GLIAL FIBRILLARY ACIDIC PROTEIN, B-AMYLOID ACCUMULATION, AND MICROGLIAL ACTIVATION FOLLOWING A BILATERAL CAROTID OCCLUSION IN THE GERBIL. J.A. Oostveen*, D.B.Carter, E.Dunn and E.D.Hall, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The post-ischemic time course of the expression of amyloid protein precursor (APP), apolipoprotein E (APO-E), glial fibrillary acidic protein (GFAP), and β-amyloid (β-AP), as well as the activation of microglia were examined immunocytochemically in the selectively vulnerable CA1 region of the hippocampus of gerbils subjected to 10 min of bilateral carotid occlusion-induced forebrain ischemia. These markers were compared to the time course of CA1 neuronal necrosis. Loss of 90% of the CA1 neurons occurred between 24 and 72 hrs after ischemia, after which no further neuronal necrosis was observed. At 24 hrs postischemia, there was a decrease in APP, β-AP, APO-E, and GFAP in the CA1 region. Although there was no microglia activation at 24 hrs, by 2 days, activated microglia were observed throughout the hippocampus. This coincided with a dramatic increase in the expression of APP, APO-E and β-amyloid between days 2 and 7. GFAP expression paralleled this increase, which is indicative of an activation of astrocytic protein synthesis. Reactive microglia were also observed in the CA1 region from days 4 to 7. It has been shown that the E_4 isoform of APO-E, when oxidized, avidly binds to β-AP and, thus, increases the likelihood of β-AP deposition. Therefore, it is postulated that the increased expression of amyloid proteins coincident with an increased production of APO-E in response to ischemic neuronal necrosis provides conditions that are favorable for the post-ischemic formation of amyloid deposits. Production of oxygen radicals by activated microglia may enhance the binding of β-amyloid to APO-E.

507.7

MICROGLIAL PHAGOCYTOSIS OF PRE-AGGREGATED Aβ 1-42 AFTER INTRA-HIPPOCAMPAL INJECTION IN RATS. S.A. Johnson*, R.L. Bethel and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Mature plaques in Alzheimer's disease brain are intimately associated with amoeboid microglia that express surface markers consistent with antigen presentation and phagocytosis. EM studies showed intracellular amyloid fibrils in plaque-associated microglia, yet it is unclear if such intracellular amyloid was phagocytosed or synthesized. Microglia in culture contain detectable APP mRNA, but in situ hybridization of AD cortex or hippocampus did not detect APP mRNA in plaque-associated amoeboid microglia.

Since cultured microglia and fibroblasts accumulate exogenous Aβ 1-42, we examined the fate of Aβ 1-42 after intra-hippocampal injection in rats. Synthetic Aβ 1-42 was aggregated in PBS (1 mg/ml, pH 7.0, 48 h, 25° C) with N-terminal biotinylated Aβ 1-42 (bio-Aβ; gift of Dr. C. Glabe) prior to stereotaxic injection (1ul). In pilot experiments, rats were sacrificed at 1 and 5d post-injection. Injected amyloid was visualized by avidin-biotin complex (ABC-HRP/DAB) histochemistry without antibodies, or by double-label histochemistry using nickel/DAB to detect cell-specific antibodies.

We detected intra-cellular bio-Aβ by ABC only at 5d post-injection. Double-label histochemistry showed bio-Aβ in Griffonia simplicifolia isolectin1B4 positive macrophage/microglia. Combination ICC with anti-GFAP showed no intracellular accumulation of Aβ in astrocytes, but showed direct contact of astrocyte endfeet on extracellular Aβ. Numerous bio-Aβ-containing microglia (macrophage) surrounded the injection site. Some bio-Aβ-microglia were distant from the main Aβ 1-42 depot, occasionally surrounding, or even within, blood vessels, suggesting a route for clearance from the brain. This demonstrates microglial phagocytosis of extracellular, aggregated Aβ 1-42 in rat brain and suggests that microglia also phagocytose extracellular amyloid in the AD brain. Supported by AG10673 (SAJ) and AG07909 (CEF).

507.8

MICROGLIAL REMOVAL OF AMYLOID ß PEPTIDE (Aß) IS PREVENTED BY PROTEOGLYCANS. L. M. Shaffer*, M.D. Dority, S.G. Younkin and K.R. Brunden. Gliatech, Inc., Cleveland, OH 44122, and Case Western Reserve Univ., Dept. of Pathology, Cleveland, OH 44106.

In the Alzheimer's Disease (AD) brain, activated microglia are found closely associated with the cores of mature senile plaques. A major constituent of the plaque is Aß, a 40-43 amino acid peptide which aggregates to form fibrillar extracellular deposits. We have previously demonstrated that primary cultures of rat microglia and a human monocyte cell line are capable of removing Aß under conditions where the fibrillar peptide was in solution or immobilized on a substrate, mimicking a plaque-like structure. There is thus a question of why plaques in the AD brain persist in close proximity to activated microglia. This persistence could result from the association of Aß with other plaque components, resulting in inhibition of the microglial removal mechanism. Since proteoglycans are an invariable feature of all amyloidoses and are known to bind Aß, they may contribute to plaque formation and persistence by altering microglial processing of Aß. We therefore examined whether proteoglycans could prevent removal of Aß by rat microglia and the human monocyte line, THP-1. Using thioflavin-T staining to detect immobilized Aß before and after treatment, it was found that both cell types showed good adhesion to the peptide substrate and removed the Aß in approximately one week. Because astrocytes are a likely source of brain proteoglycans, primary rat astrocytes were grown in culture dishes containing immobilized Aß to allow secreted molecules to associate with the peptide. Upon hypo-osmotic removal of the astrocytes and subsequent seeding of microglia or THP-1 cells, the conditioned Aß was found to be resistant to breakdown. Direct application of proteoglycan purified from astrocytes onto the Aß substrate also resulted in inhibition of microglial processing of the peptide. These data suggest that astrocyte-derived proteoglycans may play a role in the persistence of Aß.

507.9

AN IN VIVO STUDY ON FACTORS AFFECTING AMINO ACID AND APP RELEASE FROM CORTICOSTRIATAL NEURONES, USING PUSH-PULL AND MICRODIALYSIS TECHNOLOGY.
S. Dijk, P.T. Francis, G.C. Stratmann, M.A. Smith and D.M. Bowen. (SPON: Brain Research Association). Institute of Neurology, Dept. Neurochemistry, 1 Wakefield street, London WC1N 1PJ, U.K.

A maintained inhibition on cortical pyramidal neurones through the 5-HT_{1A}-receptor may contribute to the cognitive symptoms of Alzheimer's disease. In this study in the rat it was investigated whether a selective 5-HT_{1A}-receptor antagonist would enhance the activity of cortical pyramidal neurones, and whether an increased neuronal activity would stimulate secretion of APP. Striatal microdialysis and HPLC was used to assess aspartate and glutamate release as a marker of activity. APP in striatal push pull perfusate was measured with Western blotting, using the N-terminal antibody 22C11. Topical application of the selective 5-HT_{1A}-antagonist (+) WAY 100135 (50 μM) on the surface of the frontal cortex increased significantly basal glutamate release in the striatum, and potentiated NMDA induced aspartate and glutamate release. Depolarisation of striatal neurones with a potassium challenge through the push pull probe induced an increase in 22C11 immunoreactivity. These results indicate that a selective 5-HT_{1A}-antagonist may be useful in attenuating the cognitive symptoms of Alzheimer's disease. In addition, as an increased secretion of APP may result in less amyloidogenic fragments being produced, an increased neuronal activity may also slow down the progression of the disease. Supported by the Brain Research Trust.

507.10

MUSCARINIC CONTROL OF AMYLOID PRECURSOR PROTEIN (APP) PROCESSING IN CULTURED HUMAN NEURONS DERIVED FROM A TERATOCARCINOMA CELL LINE (NT2N). A. M. Wertkin[1], B. A. Wolf[1], B. B. Wolfe[2], V. M.-Y. Lee[1]*. [1]Dept. of Path. and Lab. Med., Univ. of Pennsylvania Med. School, Philadelphia, PA 19104. [2]Dept. of Pharmacology, Georgetown Univ. School Med., Washington, D.C. 20007.

It is established in the literature that carbachol, a muscarinic agonist stimulates increased APP secretion in a variety of neural and non-neuronal cell lines transfected to express the M1 and M3 muscarinic receptors. Occupancy of M1 and M3 receptors results in phospholipase C activation via a G-protein. These data have led to the theory that APP metabolism is controlled, at least in part by neurotransmitters, i.e., acetylcholine. We aim to provide evidence for the muscarinic control of APP processing in NT2N cells, which may provide a unique model system for understanding the contributions of neurons to amyloidogenesis. Previously, we have provided evidence that the NT2N cells solely express the 695 amino acid APP isoform and secrete intracellularly produced Aβ peptides. We now demonstrate that: NT2N cells express similar levels of endogenous M2 and M3 receptors as well as the GTP-binding protein, Gq. Carbachol, an M1 and M3 agonist, induces the activation of phospholipase C in NT2N cells as evidenced by a time-dependent increase in the levels of the second messenger end-products diacylglycerol and inositol triphosphate (IP_3) which peaked at two min. Subsequently, as would be expected from the increased IP_3 levels, carbachol increased intracellular Ca^{2+} levels measured fluorometrically in single NT2N neurons. Carbachol treatment of 30 min causes at least a two-fold increase in the release of APP_S into the conditioned media. Furthermore, preliminary experiments suggest that the level of Aβ peptides secreted into the conditioned media may be reduced with extended carbachol treatment. We conclude that APP processing in the NT2N human neurons is regulated by the muscarinic receptor / phospholipase C signal transduction pathway.

507.11

PKC STIMULATION BUT NOT CHOLINERGIC RECEPTOR STIMULATION INCREASES SECRETION OF THE ß-AMYLOID PRECURSOR PROTEIN IN CULTURED RAT CORTICAL NEURONS J. Mills* and P.B. Reiner
Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

The progressive deposition of the amyloid ß-peptide (Aß) is an early and invariant feature of Alzheimer's disease pathology. The Aß sequence is contained within a much larger membrane-spanning glycoprotein, the ß-amyloid precuror protein (ßAPP) which can be processed by multiple proteolytic pathways. Conventional secretory processing of ßAPP is thought to be nonamyloidogenic, since cleavage occurs within the Aß sequence, releasing the soluble ectodomain of ßAPP (APP_S). Alternative processing routes are potentially amyloidogenic yielding the Aß segment intact.

PKC signal transduction pathways have been implicated in the regulation of APP_S secretion. These data are derived from studies in continuous cell lines. It is not known whether or not this same mechanism of control occurs in central neurons. In the present investigation, we examined whether direct or indirect stimulation of PKC increases the secretion of APP_S in primary cultures of rat cortical neurons. Activation of PKC by the phorbol ester phorbol 12,13-dibutyrate (PDBu) or phorbol 12-myristate 13-acetate (PMA) significantly increased the release of APP_S. In contrast, activation of the PLC/PKC-coupled muscarinic m_1 receptor by oxotremorine-M did not significantly increase basal release of APP_S. Similarily, chemically induced depolarization using 35 mM KCl did not alter APP_S secretion. Our data indicate that although PKC stimulation can alter ß-APP processing in central neurons, neuronal activity and cholinergic neurotransmission does not.

507.12

INVOLVEMENT OF PROTEIN TYROSINE PHOSPHORYLATION IN SECRETORY PROCESSING OF THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE. B.E. Slack*, M. A. Petryniak, J. Breu, K. Srivastava, and R.J. Wurtman, Department of Brain and Cognitive Sciences, MIT, Cambridge MA 02139.

Secretory cleavage of the amyloid precursor protein (APP) of Alzheimer's disease (AD) is stimulated by the muscarinic agonist carbachol in human embryonic kidney (HEK) cells expressing human muscarinic m1 or m3 receptor subtypes, but not in cells expressing m2 or m4 receptors (Nitsch et al., Science 258:304,1992). The m1 and m3 subtypes are coupled to phosphatidylinositol (PI) hydrolysis; the m2 and m4 subtypes are not. The effect of carbachol was blocked by the protein kinase inhibitor staurosporine, and mimicked by phorbol esters, which directly activate protein kinase C (PKC), suggesting that PKC regulates APP processing. However, a more selective PKC inhibitor, GF 109203X, completely blocked the response to phorbol esters, but only partially inhibited (by 30%) the increase in the release of soluble derivatives of APP (APPs) elicited by carbachol. Since staurosporine also inhibits protein tyrosine kinases, we investigated the involvement of tyrosine phosphorylation in the regulation of APPs release. Carbachol increased tyrosine phosphorylation of a number of proteins in HEK cells, and this effect, like APPs release and PI turnover, was preferentially coupled to m1 and m3 receptors. Moreover, an inhibitor of protein tyrosine phosphatase (vanadyl hydroperoxide) stimulated APPs release, while tyrosine kinase inhibitors reduced the effects of carbachol and vanadyl hydroperoxide on APPs release. The results suggest that both PKC and protein tyrosine phosphorylation contribute to the stimulation of APP processing by muscarinic receptor activation. (Grant #MH-28783 of NIMH)

507.13

PROCESSING OF THE AMYLOID PRECURSOR PROTEIN WITH THE CODON 670/671 MUTATION IS ALTERED IN THE SECRETORY PATHWAY. R.G. Perez* and E.H. Koo, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

A mutation at codon 670/671 of ßPP (KM → NL) from one form of familial Alzheimer's disease results in significantly elevated Aß release by an undefined mechanism. Because we have recently demonstrated that Aß production and release involves the internalization and recycling of cell surface ßPP, we asked whether the codon 670/671 mutation alters ßPP processing in this pathway. As expected, Aß release was significantly elevated in CHO cells transfected with KM → NL mutation (δNL) as compared to ßPP751 (WT) cells. However, the kinetics of Aß release was different in δNL as compared to WT cells such that Aß release occurred earlier in δNL cells. The enhanced release of Aß in δNL cells was correlated with an increase in an intracellular 12 kDa C-terminal ßPP fragment. In addition, a lower molecular weight ßPPS species was present intracellularly and released into medium of δNL cells. By epitope mapping, this shorter ßPPS from δNL cells is consistent with a "ß-secretase" cleaved product. The premature intracellular cleavage of ßPP by ß-secretase resulted in a 50% reduction of full length ßPP sorted to the cell surface. Moreover, Aß release was not significantly elevated in δNL cells after cell surface iodination, demonstrating that although Aß generation also occurred after internalization in δNL cells, processing of ßPP was not altered in this pathway. In sum, our studies demonstrated that the KM → NL mutation resulted in a selective alteration of ßPP processing by ß-secretase. More importantly, our results suggest that the early cleavage of ßPP at the ß-secretase site occurs in a different compartment within the secretory pathway in δNL cells.

507.14

CATHEPSIN S: AN Aβ GENERATING/ DEGRADING ENZYME ? S. Petanceska and L. A. Devi * Dept. of Pharmacology , NYU Medical Center, NY 10016

Cathepsin S is a cysteine lysosomal protease resistant to neutral pH. Our studies in rat brain as well as peripheral tissues have shown that it is preferentially expressed in cells from the mononuclear phagocytic lineage. Of all cell lines tested so far cathepsin S transcript could be detected only in monocytic leukemia cell lines.

Studies of entorhinal cortex lesions in rat brain have shown an upregulated expression of cathepsin S in activated versus resting microglia, suggestive of an important role for this protease in processes of neuronal degeneration and regeneration.

We have studied the changes in the level of cathepsin S transcripts that occur upon differentiation of the HL 60 leukemia cell line. The possibility of cathepsin S to be secreted in the media of normal leukemia cells, differentiated leukemia cells as well as macrophages challenged with inflammatory agents was also assessed.

Cysteine lysosomal proteases have been implicated to take part in the metabolism of the Amyloid Precursor Protein (APP). The fact that cathepsin S is expressed in microglia in brain, a cell type pertinent to the pathology of Alzheimer's and other neurodegenerative diseases, its resistance to neutral pH and its substrate specificity (preference for small neutral amino acids N-terminal to the cleavage bond), make cathepsin S a strong candidate for a protease involved in the generation of the Aβ peptide from the APP. On the other hand, other research groups have shown that secreted media from microglia are able to cleave soluble and aggregated Aβ peptide.

Here we present the processing pattern obtained by in vitro proteolysis of the wild type and mutant variants (Swedish and Dutch) of the holo APP by cathepsin S. The ability of cathepsin S to degrade soluble and/or aggregated Aβ peptide is also addressed. Supported by NS26880 (L. D.)

507.15

EXPRESSION AND PURIFICATION OF RECOMBINANT SUBSTRATES FOR APP SECRETASES. J.K. Kawooya, T.L. Emmons, D. Zitkus, S. Trusko, D. Lang, R. Scott, R. Siman* and B.D. Greenberg. Cephalon, Inc. 145 Brandywine Pkwy. West Chester, PA 19380

The growing interest in the identification, isolation and inhibition of secretases which generate Aβ peptides from Alzheimer's amyloid precursor protein (APP) calls for the production of highly purified enzyme substrates. The purified substrates must be soluble, and free of proteolytic contaminants. In this report we describe the recombinant expression and purification of soluble full length APP from the baculovirus insect cell system, and the COOH-terminal 100 amino acid APP segment (C100) from *E. coli* inclusion bodies. Full length APP was extracted from the insect cell membranes with 6M urea, purified by a combination of anion exchange and immobilized nickel affinity chromatography, then refolded in physiological buffer by dialysis. Cells seeded in one liter of conditioned medium yielded 2 mg product at ~98% purity. C100 was extracted from *E. coli* cells that were pre-washed with a mixture of formic acid/acetic acid, solubilized in 8M Guanidine-HCl, and purified by size exclusion chromatography using Guanidine-HCl as the mobile phase. The protein was refolded by dialysis against physiological buffer in the presence of polyethylene glycol. Fifteen mg purified soluble C100 was recovered from one liter of fermentation beer at ~95% purity. Both purified substrates were shown to be devoid of contaminating proteolytic activity, as neither was degraded during extended incubation (4 hours to 7 days) under conditions optimal for activation of the four common classes of mammalian proteases. These purified soluble proteins will be useful tools for studying physiological and pathological functions of APP, and for the identification and characterization of proteases involved in Aβ peptide formation.

507.16

THE LOCALIZATION AND INTERNALIZATION OF CELL SURFACE ßAPP IN CULTURED NEURONS. T.Yamazaki, D.J. Selkoe and E.H. Koo*. Brigham and Women's Hospital and Harvard Medical School, Boston MA 02115.

At the cell surface, the ß-amyloid precursor protein (ßAPP) may be released after cleavage by α-secretase or internalized without cleavage. Recent evidence suggests that the latter pathway may be important in the constitutive production and release of amyloid ß-protein. Using monoclonal antibodies that recognize the extracellular domain of ßAPP, we previously demonstrated that cell surface ßAPP in CHO cells is internalized via coated pits and targeted to lysosomes. In this study, we demonstrate that in primary rat hippocampal neurons, a population of full length ßAPP is similarly located on the cell surface and subsequently endocytosed. ßAPP was present on the cell surface of neurons cultured at low density shortly after plating (Banker Stage 1). Immature neurons (Stage 3) showed punctate ßAPP immunostaining on the soma. On axons and minor neurites, ßAPP staining was distributed in a distinctly segmental pattern. In mature neurons (Stage 5), the pattern remained discontinuous and was located principally on axons. ßAPP immunoreactivity was colocalized with α1, α5, and ß1 integrins, but not with NCAM, synaptophysin, transferrin receptor or clathrin. In immature neurons, internalized ßAPP was located in a compartment which contained fluid-phase endocytic markers. This staining pattern was coarsely granular and was located both in the perikaryon and neuritic processes. In mature neurons, internalized ßAPP was located principally in axons and soma. To demonstrate endocytosis of ßAPP from axonal surface, dissociated sympathetic neurons were grown in compartment (Campenot) cultures. Using this system, ßAPP was clearly internalized from the cell surface of distal axonal sites and retrogradely transported to the neuronal soma. In summary, these studies in primary neuronal cultures show that 1) in early stages, cell surface ßAPP was not distributed in a polarized manner, 2) in mature cultures, surface ßAPP was localized predominantly in the axonal rather than somatodendritic compartment (Haass,1994), 3) ßAPP showed a tight association with integrins, a result consistent with its putative role in adhesion, and 4) full-length ßAPP is retrogradely transported from the surface of distal axonal sites to the cell body.

507.17

PROCESSING OF ALZHEIMER'S ß-AMYLOID PRECURSOR PROTEIN IN C6 GLIOMA CELLS. E. Morato[2], L. López-Mascaraque[1*] and F. Mayor[2], jr. [1]Instituto Cajal, 28002 Madrid and [2]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM). Universidad Autónoma, 28049 Madrid, Spain.

The major neuropathological characteristic of Alzheimer's disease is the deposition in the brain of insoluble aggregates of ß-amyloid peptide (ß/A4), a small (39-43 residues) fragment contained within a family of large glycoproteins, the ß-amyloid precursor proteins (ßAPP). The understanding of the intracellular processing of ßAPP in nervous cells and of the pathways leading to ß/A4 generation are essential issues to be addressed. In this context, we have investigated the subcellular location and processing of ßAPP in an experimental model of glial cells in culture (C6 rat glioma cells), by using several affinity-purified antipeptide antibodies raised in our laboratory against different domains of both the ß/A4 peptide and ßAPP. Antibodies raised against the N and C-terminal regions of the ß/A4 peptide can detect the presence of a soluble 4 KDa peptide in the conditioned medium of C6 glioma cells (Morato & Mayor, (1993) FEBS Letters 336, 275-278), in line with recent reports in other cell types. Consistent with this observation, normal α-secretase activity appears to be low in C6 glioma cells, whereas large soluble ßAPP derivatives containing the C-terminal portion of the ß/A4 molecule are detected in the conditioned medium. Immunofluorescence studies show a preferential intracellular location of ßAPP antigens, and a rapid reinternalization of plasma membrane ßAPP, which accumulates in endosomes/lysosomes in the presence of inhibitors of lysosomal proteases. Our results confirm a distinct processing of ßAPP in glial cells, and further suggest that glial cells may prove a major source of ß-amyloid production in the nervous tissue. Cultured glial cells may be an important experimental tool in investigating the modulation of the cellular pathways involved in ß/A4 generation and its posible alteration in pathological circumstances. Supported by CAM C105/91,Boehringer Ingelheim and Fundación Ramon Areces.

507.18

NEURONS DERIVED FROM A HUMAN TERATOCARCINOMA CELL LINE GENERATE INTRACELLULAR Aβ PRIOR TO ITS SECRETION INTO CONDITIONED MEDIUM R.S. Turner*[1] and V.M.-Y. Lee[2], Depts. of [1]Neurology and [2]Pathology and Laboratory Medicine, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104-4283.

Alzheimer's disease (AD) brain is characterized pathologically by neuronal and synaptic loss, neurofibrillary tangles which consist primarily of the microtubule-associated protein tau, and amyloid plaques which consist primarily of a 4-kDa peptide (Aβ) derived from one or more of at least three alternatively spliced amyloid precursor proteins (APP). We examined the kinetics and intracellular localization of APP metabolism in cultured neurons derived from a human teratocarcinoma cell line (NT2). These neurons constitutively secrete a 95-kDa secretase fragment of APP (APPs) and Aβ. Metabolic labeling of NT2 neurons with [^{35}S]methionine followed by immunoprecipitation of APP, APPs and Aβ from cell lysates and conditioned medium revealed that Aβ was first recovered from cell lysates and, after an approximate 2 hour delay, from conditioned medium. In addition, cellular Aβ plateaued after 8 hours while Aβ in conditioned medium continued to increase. This suggests that Aβ is first generated intracellularly soon after APP synthesis and then secreted into medium. In the presence of 10 μg/ml Brefeldin A, cellular APP accumulated with no evidence of its metabolism, thus precluding the secretion of APP fragments into medium. Ammonium chloride (10 mM) decreased the recovery of Aβ in medium, suggesting that an acidic intracellular compartment is required for its generation and secretion. Collectively, these data suggest that Aβ is synthesized within the distal Golgi or endosomal/lysosomal compartment of NT2 neurons. Since a considerable fraction of APP is metabolized to Aβ and secreted, NT2 neurons may serve as a unique model system of APP metabolism and Aβ synthesis, thus providing insights into the molecular basis of amyloid formation in AD brain.

507.19

BACTERIAL EXPRESSION, PURIFICATION OF FULL LENGTH AND CARBOXYL TERMINAL FRAGMENT OF ALZHEIMER AMYLOID PRECURSOR PROTEIN AND THEIR PROTEOLYTIC PROCESSING BY THROMBIN. Y.H. Chong, W.Choi, S.H. Kim, K.S.Min, Lyhna Hong, C.W. Park, and Y.H. Suh*, Dept. of Pharmacology, Coll. of Med. and Neurosci. Res. Center, Seoul National Univ., Seoul 110-799, Korea.

Human amyloid protein precursor(APP770) and its carboxyl terminal portion(CT105) including β/A4 domain were highly expressed using strong expression systems in E. coli. These recombinant APP peptides were purified with a combination of urea solubilization and ion-exchange chromatography and used for proteolytic processing by thrombin. Three thrombin cleavage sites were predicted by the decrease of APP770 and the appearance of Mr 56, 27 and 18 KDa fragments containing β/A4 domain on SDS-PAGE gel and on the immunoblot. A similar but limited proteolysis of platelet APPs exposed to thrombin resulted in the stimulated production of 60 and 27 KDa carboxyl terminal peptides containing the intact β/A4. This thrombin mediated proteolysis was completely blocked by hirudin, the specific thrombin inhibitor. These results suggest that thrombin may play a role in altered processing of APP to generate potentially amyloidogenic intermediates in vivo leading to amyloid deposition.

507.20

EXPERIMENTAL CHLOROQUINE MYOPATHY IN RATS -MODEL FOR APP PROCESSING-

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Deposition of β/A4 protein is most characteristic pathological change in Alzheimer's disease. There have been no good models for studying β/A4 production. Chloroquine, a potent lysosomotropic agent, induces muscle pathology in experimental animals similar to human rimmed vacuole (RV) myopathy, in which β/A4 deposition was observed. In this study, we present morphological, immunopathological, and biochemical data in regard to APP processing in this experimental model.

Innerved and denerved soleus muscle from chloroquine treated and untreated WKA rats were studied. Atrophy of muscle fibers, numerous dense granule bodies, and vacuoles were seen in denerved soleus muscle of chloroquine treated rats. Progressive destruction of muscle, and proliferation of connective tissue were present. Dense granular bodies, or cytoplasm were stained with anti cathepsin D, and several anti APP antibodies. Western blot analysis showed that 49, 50 kDa bands and 20 kDa band were detected in buffer soluble, and detergent soluble fractions with anti β9-25, and anti C terminus antibodies respectively in chloroquine treated rats.

This experimental model seems to be useful for study of APP processing And present data suggests lysosomal pathway is involved in β protein production.

507.21

APP EXPRESSION IN THE CELL LINE NTERA2. R.E.B. Watson, P.R. Heath, P.W. Andrews and R.C.A. Pearson*. Department of Biomedical Science, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK.

The cell line NTera 2 is a useful tool for studying the mechanisms of protein processing with relevance to human neurodegenerative disease (Andrews, 1984; Wertkin *et al*, 1993) particularly the differential expression of the mRNAs encoding for Amyloid Precursor Protein (APP) isoforms. Previous work (Ackerman *et al*, Mol. Brain Res., in press) has shown using cDNA libraries that APP_{695} and APP_{770} are present in undifferentiated and differentiated cell libraries. APP_{751} was observed in libraries from differentiated, neuron-like cells only.

The study used *in situ* hybridization histochemistry to examine the occurrence of APP mRNA transcripts and Western blotting to examine the protein isoforms produced. Oligonucleotide probes were produced corresponding to regions encoding the βA4 sequence (βA4; 30mer), to a sequence specific to APP_{695} (APP_{695}; 30mer), to exon 8 (APP_{770}; 57mer) and to 30 bases on each side of this region (APP_{751}; 60mer). Cell lysates from undifferentiated (NT2D1), 14 day treatment with Retinoic Acid (RA; 14dRA), 28 day treatment with RA (28dRA) and mature neurons were separated on a 7.5% acrylamide gel, transferred to nitrocellulose and probed with the antibody 22C11 (Boehringer Mannheim).

APP_{695} mRNA was shown to increase through differentiation with RA. Expression of APP_{770} decreased with time in RA whilst expression of APP_{751} increased to be maximal in the differentiated neuron-like cells. βA4-containing mRNA was observable throughout differentiation. On Western analysis, one band of molecular weight (mw) 117kDa was present at all times, although the density of immunostaining increased with neuronal differentiation; this band probably represents APP_{695}. A second band of mw 132kDa was present in protein extracted from mature neurons and also visible from extracts of 28dRA. It was not present in NT2D1. Stem cells and 14dRA showed an immunostained band at 112kDa. This was present but faint in 28dRA and was not seen in extracts from mature neurons. Both the 112 and 132kDa band probably represent KPI-containing isoforms of APP. By correlation with the *in situ* data we propose that the 112kDa product represents APP_{770} and the 132kDa product APP_{751}. This study confirms the value of this culture system in the understanding of the processing of APP.

507.22

IRON LEVELS MODULATE α-SECRETASE CLEAVAGE OF AMYLOID PRECURSOR PROTEIN. S. Bodovitz*, M. Falduto, D.E. Frail, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, Il. 60208

The amyloid precursor protein (APP) is a membrane-spanning glycoprotein that is the source of βA4 peptides which aggregate in Alzheimer's disease to form senile plaques. APP is cleaved within the βA4 sequence to release a soluble N-terminal derivative (APP_{sol}) which has a wide range of trophic and protective functions. In the current paper, we have examined the hypothesis that iron availability may modulate expression or processing of APP, whose mRNA contains, based on sequence homology, a consensus Iron Response Element (IRE). Radiolabeled APP and its catabolites were precipitated from lysates and conditioned medium of stably transfected HEK 293 cells using antibodies selective for C-terminal, βA4, and N-terminal domains. Relative abundance of the different APP catabolites under different conditions of iron availability was determined by quantitative densitometry after separation by SDS-PAGE. Data show a specific effect on the production of APP_{sol}. Using standard conditions previously established for IRE studies, it was found that iron chelation reduces APP_{sol} production, while iron elevation augments it. No changes were observed in levels of immature and mature APP holoprotein, nor in C83, βA4, and p3 peptides. The specificity for modulatory changes in APP_{sol} suggests that iron acts at the level of secretase activity. In addition to its modulatory effects, iron at very high levels was found to inhibit maturation of APP and production of its down-stream catabolites without blocking formation of immature APP. The data establish a potential physiological role for iron in controlling the processing of APP. If APP_{sol} were to function trophically, as suggested by other studies, the current conclusion would suggest that changes in iron and iron-regulating proteins in Alzheimer's disease could contribute to neuronal degeneration by decreasing the production of APP_{sol}.

507.23

IRON AND ALUMINUM EFFECT AMYLOID EXPRESSION. J. Hu and J. R. Connor* Dept. of Neuroscience & Anatomy, Penn State University, College of Medicine Hershey PA 17033

Iron is consistently found in abundance in cells surrounding neuritic plaques. The robust expression of transferrin receptors on these cells suggests that iron uptake is high and on-going in the plaque vicinity raising the possibility that iron is involved in plaque formation. Aluminum is also a consistent component of neuritic plaques. This study was undertaken to test the hypothesis that iron and aluminum exposure will affect amyloid accumulation by cells. Two human cell lines were studied: SK-N-SH (neuroblastoma) and SW-1088 (astrocytoma). The cells were grown to subconfluence in DMEM medium supplemented with 10% FCS. For experimental manipulation, the cells were exposed to serum free DMEM containing 100uM $FeCl_3$ or 100uM $AlCl_3$ for 2,3 or 4d. The experimental medium was changed after 2 days. Both iron and aluminum increase intracellular and secreted APP. In addition, following iron exposure, an increase in a truncated form of APP was seen using Western blot analysis. These observations were specific to the astrocytoma cell line. No change was observed in the neuroblastoma. The metals had minimal somewhat transient effects on expression of APP mRNA in the astrocytoma. A modest decrease in expression within the first two days of exposure was followed by a return to normal. In the neuroblastoma, APP mRNA levels are initially very low and these increase in response to metal exposure after 3 days. These data demonstrate that APP levels in cells are affected following exposure to metals but that cell response is specific. The data further suggest that the effect of metals on APP is at the level of posttranslation.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID VIII

508.1

CEREBRAL AMYLOID ANGIOPATHY IS ASSOCIATED WITH CORTICAL NEURONAL LOSS. C. Zarow*, S.A. Lyness, H. Chui. Department of Neurology, University of Southern California, Rancho Los Amigos Medical Center, Downey, CA 90242. The deposition of ß-amyloid in cerebral blood vessels (so-called cerebral amyloid angiopathy (CAA)) is a frequent finding in Alzheimer disease (AD). An association between CAA and cerebral hemorrhage and infarction is well-recognized, but potential ongoing effects of CAA on neuronal survival are unknown. We examined the relationship between the severity of CAA and numbers of neurons in five neocortical areas and entorhinal cortex in 103 neuropathologically-confirmed cases of AD. CAA was rated on a semi-quantitative scale (0 to 3) in thioflavin S-stained leptomeningeal and cortical vessels. Neurons, neurofibrillary tangles, and senile plaques were counted in complete cortical columns in semi-adjacent sections stained with cresyl-violet or Bielschowsky silver method. Some degree of CAA was found in at least one region in 82% (84/103) of cases. Severe CAA (grade 3 in at least one region) was found in 29% (30/103) of cases. Severity ratings for leptomeningeal and cortical CAA were highly correlated. Furthermore, inverse correlations were found between the severity of cortical CAA and neuronal number in area 22 (r=-0.22, p=.045) and the severity of leptomeningeal CAA and neurons in area 9 (r=-0.21, p=0.06). The relationship between CAA, neurofibrillary tangles, senile plaques, and age at symptom onset and neuronal number was further examined using multiple regression analyses in each anatomical region. Again, cortical CAA in area 22 (p=0.007) and leptomeningeal CAA in area 9 (p=0.02) were signifiant predictors of neuronal loss. We hypothesize that CAA may compromise blood flow and the delivery of essential nutrients to neurons; alternatively, ß-amyloid may exert a direct neurotoxic effect. (NIH 2P50AG05142 and AG07624)

508.2

IN VIVO EFFECTS OF THE AMYLOID ß PROTEIN IN THE RAT BRAIN. T. Duong*, P.J. Acton and B. Ghetti. Indiana Univ. Sch. of Med., Terre Haute Center, Terre Haute, IN 47809 and Indiana Alzheimer Disease Center, Indianapolis, IN 46202.

The specific aim of this study was to compare the long-term *in vivo* effects of senile plaque cores (SPC) or synthetic amyloid ß peptide (Aß) in the rodent brain at 2 different ages. SPC-enriched pellets were isolated from the temporal cortex of Alzheimer brains. Synthetic Aß (1-40) was purchased from Bachem, Philadelphia, PA., dissolved in phosphate buffered saline at a concentration of 50 µM and incubated at 37°C for 48 hrs prior to use. Pressure injections were performed in the cortex of young (6 months) and old (20 months) male Fischer 344 rats and the animals were allowed to survive 1 or 3 months. At the end of the survival periods, the rats were euthanized and perfused. The brains were removed and processed for histology and immunohistochemistry. Hyperplasia and hypertrophy of astrocytes surrounding the injection site were observed at up to 3 months in all cases. The injection sites were clearly seen at 1 and 3 months due to cavitation and deposition of the injected SPC or Aß. These deposits were strongly immunoreactive to Aß antiserum at 1 month but were less so at 3 months. ALZ50 immunoreactivity, which is indicative of abnormal cytoskeletal changes, was seen at and around the injection site at 1 month but was substantially decreased at 3 months. No major difference was seen between the use of SPC or Aß, or between young or old animals. **Thus, over a long survival period, acute *in vivo* injection of SPC or Aß in the rat brain results in clearance of the injected deposits and attenuation of abnormal cytoskeletal changes.** Supported by grant #P30AG10133.

508.3

HISTOLOGICAL AND BEHAVIORAL EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF Aß(1-42) IN THE MALE RAT. T.Giordano[2]*, W.A. Dornan[1], A. W. Bannon[2], N.W. Kowall[3], A.R.V. McCampbell[1], A.R. Peterson[1], G.P. Tinkler[1]. [1]Dept. of Psychology, Illinois Wesleyan Univ, Bloomington, IL 61701, [2]Neuroscience Dept.,Abbott Laboratories, Abbott Park, IL 60064, [3]Geriatric Res Educ Clin Ctr, Veterans Admin Med Ctr, Bedford MA 01730.

Alzheimer's disease is characterized by the extracellular deposition in the brain of insoluble aggregates of the amyloid ß peptide (Aß). Evidence from several laboratories have demonstrated the neurotoxicity of Aß *in vitro*, and in vivo. Very few studies, however, have examined the behavioral effects of Aß when injected into the rat brain. In a series of experiments, a comparative histological and behavioral assessment were conducted following injections of Aß(1-42) into the hippocampus and medial septum of male rats. Experiment 1: animals received bilateral injections into the hippocampus of 3.5 nmol/.5µL of either Aß(1-42), Aß(1-42) in a scrambled form, or DMSO. In experiment 2, animals were injected bilaterally into the medial septum with 3.5 nmol with Aß(1-42), the scrambled sequence or DMSO. In experiment 1, animals were tested on a radial arm maze. In experiment 2 animals were tested following intra septal injections of Aß(1-42) using the Morris Water Maze. Following the behavioral tests, animals were sacrificed and analyzed for ChAT activity in the hippocampus either 7 or 14 days following injections. In experiment 3, another group of animals were injected with 3.5 nmol Aß(1-42) into the medial septum or hippocampus. Following a two week survival period, animals were scarified and their brains were processed for immunocytochemistry using the ALZ-50 antibody. Our preliminary results indicate that bilateral injections of Aß(1-42) into the hippocampus or medial septum had no significant effect on spatial learning in the male rat. Additionally, no significant differences were found in ChAT activity activity in the hippocampus following injections of Aß into the septum at 7d (controls = 15.877 $\pm$ 0.371 pmol/min/µg ptn; Aß = 15.65 $\pm$ 0.78 pmol/min/µg ptn) or at 14 d. The histological study is in progress, and the results of this study will be also presented at this conference.

508.4

COMPARISON OF THE EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF Aß(1-42) IN COMBINATION WITH GLUCOCORTICOIDS AND IBOTENIC ACID ON THE ACQUISITION AND RETENTION OF A SPATIAL TASK IN MALE RATS. W.A. Dornan[1]*, T. Giordano[2], A.R.V. McCampbell[1], H.K. Wijeweera[1], J.S. Pequette[1], L.L. Chapman[1], S.M. Bond[1], A.R. Peterson[1], L.J. Hickman[1], [1]Dept. of Psychology, Illinois Wesleyan Univ, Bloomington, IL 61701, [2] Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064.

Alzheimer's disease is characterized by the extracellular deposition in the brain of insoluble aggregates of the amyloid ß peptide (ßA), a 39-43 amino acid peptide. Although the cause of neuronal degeneration in AD remains unclear, one major focus is on the role of ßA. Evidence from several laboratories have repeatedly demonstrated the neurotoxicity of ßA *in vitro*, however, other investigators have reported little direct neurotoxicity, but rather an increase in the vulnerability of neurons to EAAs. Furthermore, very few studies have examined the behavioral effects of Aß when injected into the rat brain. In a series of experiments, we assessed the effects of bilateral injections of ßA(1-42) 3.5nmol/.5µL into the hippocampus on both acquisition and retention of a spatial task in the male rat. In experiment 1, intact or adrenalectomized animals were injected with either ßA(1-42) or a scrambled sequence of ßA with either daily injections of 7mg/1mL corticosterone at a concentration previously reported to induce artificial stress levels or sesame oil (controls). In experiment 2, animals were injected with either ibotenic acid (3nmol/µL), ibotenic acid and ßA(1-42) in a scrambled sequence; or IBO in combination with ßA(1-42). Animals in experiments 1 and 2 all animals were pre-trained on a radial arm maze task with 5 of 8 arms baited. In group 1, half of the animals were pre-trained, the other half were tested for acquisition. After surgery the animals were tested for two weeks with the configuration conserved. Animals were tested for another week with the new configuration. A variety of behavioral parameters were measured using the radial arm maze. Our preliminary results indicate that there is a clear disruption of learning performance in animals that received ßA(1-42) in combination with corticosterone.

508.5

INTRAHIPPOCAMPAL BETA-AMYLOID 25-35 ATTENUATES RETENTION OF ONE-TRIAL/DAY REWARD LEARNING 4 WEEKS LATER. D.H. Malin*, P.G. Negrete, B.J. Tomsic, R.E. Plotner, M.K. Crothers, S.A. Garcia, R.M. Greenwood, S. Jordan and J.R. Lake. University of Houston-Clear Lake, Houston, TX 77058.

It has been suggested that the complete neurotoxic activity of BAP 1-40 may be contained in the fragment BAP 25-35. In a previous study from this laboratory, multiple intrahippocampal injections of BAP 25-35 impaired retention of one-trial reward learning 2 weeks later. The present study investigated whether similar injections would cause such severe, long-lasting degenerative changes that the task could not be retained, even if training began 4 weeks afterward. The task employed is sensitive to aging and to modulation of cholinergic mechanisms. The subjects were 39 male rats, 3-4 months old. They received 7 hippocampal injections of 6 nmol BAP 25-35 (n=21) or distilled water alone (n=18), bilaterally. After recovery and deprivation to 82% of initial weight, they received an initial training trial 28 days post-injection, and 3 subsequent daily retention trials. The apparatus was a sunburst maze with 4 level alleys and 1 baited ascending alley with a grid floor. Rats were scored for errors (entrances into unbaited alleys). Each rat's retention score was its decrease in errors relative to the baseline trial. The control rats steadily reduced their errors from day to day, while the BAP-treated rats showed little improvement. Tukey's HSD procedure revealed significant, $p<.01$, differences between treatment groups on the last 2 retention trials.

508.6

EFFECTS OF INCUBATED AND NON-INCUBATED INTRAHIPPOCAMPAL BETA-AMYLOID 25-35 ON RETENTION OF ONE-TRIAL/DAY REWARD LEARNING. P.G. Negrete, M.K. Crothers, J.R. Lake, R.E. Plotner*, B.J. Tomsic, S.A. Garcia, S.H. Spell, K.K. Roberts and D.H. Malin. Univ. of Houston-Clear Lake, Houston TX 77058.

It has been suggested that the complete neurotoxic activity of BAP 1-40 may be contained in the fragment BAP 25-35 and that pre-incubation of amyloid peptides may increase their aggregation and neurotoxicity. Previously, multiple intrahippocampal injection of BAP 1-40 impaired retention of learning that occurred 2 weeks later. In this study, 3-4 month-old rats received 7 hippocampal injections per side of 6 nmol BAP 25-35 (n=14) incubated for 24 hrs at 37^0C or non-incubated BAP 25-35 (n=14) or distilled water alone (n=33). After deprivation to 82% of initial weight, they received an initial training trial 14 days post-injection, and 3 subsequent daily retention trials. The apparatus was a sunburst maze with 4 level alleys and 1 baited ascending alley with a grid floor. Each rat's retention score was its decrease in errors (unbaited alleys entered) from the baseline trial. Retention scores, averaged across 3 trials were 5.4 ± 1.4 (S±SEM) for the control group, 1.7 ± 0.6 for the incubated BAP group and -1.4 ± 1.8 for the non-incubated BAP group. ANOVA indicated a significant, $p<.05$, drug effect and post-hoc analysis (Tukey's HSD) indicated that only the non-incubated BAP group was significantly different, $p<.01$, from controls. One-sample t-tests indicated significant retention in all groups except non-incubated BAP.

508.7

EVALUATION OF SPATIAL LEARNING IN RATS RECEIVING β/AMYLOID INFUSION. B.A.Tate*, M.Rojas, R.E.Majocha and C.A. Marotta Dept. of Psychiatry and Human Behavior, Miriam Hospital and Dept. of Neuroscience, Brown University, Providence, RI 02906.

A chronic intraventricular β/amyloid infusion paradigm was used to induce cognitive and memory deficits. During infusions of either β-amyloid peptide (1-40) or glucose vehicle, adult male rats were tested in a spatial memory task. Animals were required to locate a hidden platform to escape from a pool of water. Early in testing, the amyloid infused rats had significantly longer latencies to escape than glucose infused rats, but eventually reached latencies equal to control animals. After four days of testing, the platform was removed and the amount of time spent in each quadrant of the pool was measured. Only the glucose-infused animals spent significantly more time in the quadrant that previously contained the platform. Therefore, amyloid-infused rats showed subtle memory deficits. When animals were sacrificed, immunostaining of the brains revealed β/amyloid-positive deposits and significant gliosis in the brains of amyloid-infused rats.

508.8

INFLUENCE OF AMYLOID PRECURSOR PROTEIN ON TROPHIC RESPONSE OF PC12 CELLS TO NERVE GROWTH FACTOR. R.E. Majocha, S. Agrawal#, E. Humke, J. Tang# and C.A. Marotta*+. Depts. of Psychiatry & Human Behavior and Neuroscience*, Brown U. and Miriam Hospital, Providence, RI, 02912; and, Hybridon, Inc.#, Worcester, MA. 01605.

The relationship between NGF and the amyloid precursor protein (APP) is of interest with respect to the therapeutic potential of growth factors applied to Alzheimer's Disease. Changes in APP levels and distribution in PC12 cells treated with NGF have been reported. To explore the relationship between APP and NGF we applied antisense oligonucleotides (AO), containing a phosphorothioate backbone, complementary to the 5' end region of APP mRNA, to stimulated and unstimulated PC12 cells. APP AO significantly reduced the level of APP compared to an unrelated AO. Maintaining APP AO in cultures (2μM, 48hr.) both prior to and during NGF addition inhibited stimulation of cell size and neurite extension, as compared with controls. Since reduction of APP levels by AO reduced the response of PC12 cells to NGF, APP may be a modulating element of the trophic factor cascade.

508.9

CHARACTERIZATION OF IMMEDIATE EARLY GENE INDUCTION BY Aβ IN CULTURED NEURONS. A.J. Anderson*, C.J. Pike and C.W. Cotman. IRU in Brain Aging, Department of Psychobiology, University of California Irvine, Irvine, CA 92717-4550.

The protein products of the Jun and Fos immediate early gene (IEG) families are cooperative transcriptional regulatory factors which act as cellular 'third messengers' and have been implicated in regulating the expression of many genes, including the amyloid precursor protein (APP). We have previously demonstrated that immunoreactivity for Jun- and Fos-related proteins is increased in AD tissue and associated with neuronal pathology (Exp. Neurol. 125: 286-295), that these proteins are induced by Aβ in cultured hippocampal neurons, and that Jun proteins are not induced in cells that are resistant to Aβ-mediated cell death (submitted). Several of these genes have been implicated in the control of apoptosis in other systems. As a result, we have suggested that the expression of some IEGs (e.g. one or more of the Jun proteins) may be related to or participate in Aβ-induced apoptosis, and that the expression of these genes may be an early event in AD pathology. Immunostaining with an antibody that recognizes a variety of Jun proteins indicates that Aβ induces a sustained increase in Jun proteins in culture, whereas other agents which induce IEGs such as phorbol esters produce a more typical and transient elevation in Jun protein expression. More specific antibodies reveal a similar pattern of Aβ-induced c-Jun immunoreactivity, but do not provide evidence for the induction of JunB in response to Aβ. These data suggest some specificity in the cellular response to Aβ, which should be apparent in the regulation of these genes at the mRNA level.

508.10

EFFECTS OF α_1-ANTICHYMOTRYPSIN ON THE TOXICITY OF β-AMYLOID FRAGMENT 25-40 IN RAT PRIMARY CULTURED NEURONS. N. NISHIYAMA*, T. KOBAYASHI AND H. SAITO. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, JAPAN.

β-Amyloid is the main component of senile plaques. Neurotoxic effects of β-amyloid peptide has been suggested to be involved in the pathogenesis of Alzheimer's disease (AD). We used a novel synthetic peptide $\beta_{25\text{-}40}$, corresponding to β-amyloid residues 25-40 which contained presumable toxic domain, to test its effect on neuronal cells. α_1-Antichymotrypsin (ACT), a serine protease inhibitor, colocalizes with amyloid deposits. ACT might contribute to the development of amyloid deposits and pathogenesis of AD through its protease inhibitory activity. We investigated the combination effects of ACT and a novel $\beta_{25\text{-}40}$ on rat primary cultured neurons. Single cell suspensions of hippocampus and cerebral cortex were prepared from fetal rat brain, and plated at either high ($1x10^5$ cells/cm^2) or low ($5x10^3$ cells/cm^2) cell density. Desired concentrations of drugs were applied in chemically defined medium. The cells were visualized by cresyl violet staining or MAP2 immunohistochemistry. In some cases, cell viability was evaluated by MTT assay. Although ACT (0.1-10 μg/ml) did not affect the neuronal survival in high density culture, it decreased the number of surviving neurons concentration dependently. $\beta_{25\text{-}40}$ (0.3-30 μM) significantly reduced cell viability in both high and low density cultures. Combination of ACT (10 μg/ml) with $\beta_{25\text{-}40}$ did not alter the toxicity of $\beta_{25\text{-}40}$ in high and low density culture (F-value = 2.22 and 0.61, respectively). These results suggested that both ACT and $\beta_{25\text{-}40}$ were neurotoxic but that there was no synergistic interaction between them.

508.11

HEPARAN SULFATE GLYCOSAMINOGLYCAN ATTENUATES NEURODEGENERATION INDUCED BY β-AMYLOID 25-35 BUT NOT β-AMYLOID 1-42 A.G. Woods*, D.H. Cribbs, E.R. Whittemore & C.W. Cotman. IRU in Brain Aging, University of California, Irvine, Irvine, CA 92717.

β-amyloid and heparan sulfate are found in neuritic senile plaques in Alzheimer's disease (AD). The co-localization of heparan sulfate and amyloidogenic proteins is a common feature of amyloidoses. β-amyloid 1-42 has been previously reported to be toxic to neurons in vitro, and the aggregation and toxic activities have been associated with amino acids 25-35. The role of heparan sulfate in the pathenogenesis of amyloidoses however, is unknown. To investigate the interaction of β-amyloid and heparan sulfate in a cell culture model, rat hippocampal neurons were exposed simultaneously to heparan sulfate glycosaminoglycan and either 25μM β25-35 or β1-42. We observed that heparan sulfate attenuated β25-35-induced process degeneration and neuronal death in a dose-dependent manner with an optimal effective dose at 15μg/ml. Concentrations as low as 0.5μg/ml were observed to have some protective influence. Heparan sulfate alone at these concentrations did not significantly increase cell survival. Heparan sulfate had no significant influence on β1-42 induced toxicity, even at concentrations as high as 50μg/ml. These data suggest that the neurotoxic effects of β25-35 may not be identical to those of the full-length peptide. It is further suggested that β1-42 retains its biological effect even when associated with heparan sulfate, however the activity of smaller fragments of β1-42 may be modified by heparan sulfate.

508.12

CLUSTERIN (APO J) FROM A STABLY TRANSFECTED MAMMALIAN CELL LINE PROTECTS AGAINST AMYLOID ß (Aß) NEUROTOXICITY. K. S. Fuson*, L. N. Boggs, M. Baez, D. McClure, J. Corvalan, D. Hepburn, G. Becker, L. Churgay and P. C. May. Lilly Research Laboratories, CNS and Biotechnology Divisions, Eli Lilly and Company, Indianapolis, IN 46285.

Clusterin (Apo J, SGP-2, ADHC-9, SP-40,40) was recently found bound to Aß in cerebrospinal fluid and proposed to be a chaperone for Aß [Ghiso *et al* (1993) Biochem J. 293:27]. Since the aggregation/conformation state of Aß is important for its neurotoxicity, interactions with clusterin may affect Aß toxicity. To test the hypothesis that clusterin may modulate Aß neurotoxicity, we expressed human clusterin (80 kD) by cloning the pADHC-9 (~1.6 kb) cDNA into the expression vector pGT-d and stably transfecting the hamster cell line AV-12. Clusterin was purified from conditioned medium to ~95% homogeneity by anion exchange and size exclusion chromatography. To study the putative relationship between clusterin and Aß, we evaluated Aß neurotoxicity in the presence and absence of clusterin using primary hippocampal cultures from fetal (E18) rats. Clusterin activity was assessed against 50 μM Aß (Bachem lot ZK840), a well-characterized lot of Aß that shows prominent neurotoxicity over a four-day timecourse. Co-treatment for four days with 50 μM Aß and 31.25 nM to 500 nM clusterin, but not BSA or PBS controls, resulted in Aß neuroprotection in a dose-dependent manner. These results suggest that clusterin may interfere with the adoption of a neurotoxic conformation/aggregation state of Aß *in vitro* and may have a similar function *in vivo*.

508.13

PATHOGENIC INTERACTIONS BETWEEN C1Q AND AMYLOID ß PEPTIDE IN ALZHEIMER'S DISEASE. Scott Webster* and Joseph Rogers. Sun Health Research Institute, Sun City, AZ 85372.

Soluble amyloid ß peptide (Aß) is present in CSF of normal individuals. This finding (and others) has made it increasingly important to identify mechanisms by which Aß becomes aggregated into its pathogenic fibrillar, cross ß-pleated form in the Alzheimer's disease (AD) brain. We have previously shown that C1q, the first component of the classical complement pathway, binds to synthetic Aß peptides. Such binding activates the classical complement cascade and potentiates Aß aggregation in vitro.

The present studies had two objectives. First, we modified a previously reported fluorimetric assay so as to demonstrate dramatic enhancement of Aß aggregation by C1q at physiologically relevant, nanomolar concentrations. Second, we employed enzymatically generated C1q fragments to characterize the C1q binding site for Aß. The latter appears to be within the globular head region (GHR) of C1q, a finding that may clarify not only the mechanism by which C1q augments Aß aggregation and ß structure, but also the mechanism by which Aß activates C1q. The GHR provides multiple binding sites for the Fc region of Ig. By analogy, it must also provide multiple binding sites for Aß, an optimal quality for nucleating Aß aggregation. Likewise, Aß and Ig share a similar target for binding and activating C1q, the GHR. Thus, complement activation in the AD brain is interactive with and may even be a consequence of Aß aggregation into its pathogenic form.

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508.14

AGGREGATED β-AMYLOID INDUCES REACTIVE ASTROCYTOSIS BOTH *IN VITRO* AND IN ALZHEIMER'S BRAIN. C.J. Pike*, B.J. Cummings, and C.W. Cotman. Irvine Research Unit in Brain Aging, *University of California, Irvine, CA 92717 USA.*

The senile plaques characteristic of Alzheimer's disease (AD) are often associated with astrocytes expressing a reactive phenotype. Since β-amyloid (Aβ) is the primary component of plaques, reactive astrocytosis in AD may result in part from direct effects of Aβ on astrocytes. To investigate this possibility, we first examined the effects of Aβ peptides on cultured astrocytes. Aβ did not induce significant cell loss in these cultures. However, upon exposure to aggregated but not soluble Aβ, astrocytes rapidly and stably adopted a stellate, process-bearing morphology consistent with the reactive phenotype. This altered morphology was accompanied by increased immunoreactivities for two markers of reactivity, basic fibroblast growth factor (bFGF) and glial fibrillary acidic protein (GFAP). In addition, similar to their interaction with senile plaques, astrocytic processes *in vitro* were observed to envelop Aβ aggregates. To investigate whether Aβ may induce similar effects on astrocytes in the AD brain, we triple-labeled sections of hippocampal formation from six AD subjects with thioflavine-S and antibodies raised against GFAP and either Aβ or bFGF. Upon scoring the intensity of each label within individual plaques, we found that reactive astrocytes were co-localized specifically with plaques exhibiting positive but not negative thioflavine staining, a marker of β-sheet structure characteristic of aggregated Aβ. Similar to the *in vitro* findings, we observed increased immunoreactivity for bFGF in reactive, plaque-associated astrocytes. These data suggest that Aβ, in an aggregation-dependent manner, can induce a reactive astrocyte phenotype. Thus, in addition to its hypothesized direct neurodegenerative effects, Aβ may indirectly affect the progression of AD via reactive astrocytosis.

508.15

β-AMYLOID STEREOISOMERS EXHIBIT SIMILAR STRUCTURAL AND BIOLOGICAL PROPERTIES: IMPLICATIONS FOR MECHANISM OF TOXICITY. S.L. Weinstein*, D.H. Cribbs, C.J. Pike, and C.W. Cotman. Dept. of Psychobiology, I.R.U. in Brain Aging, *Univ. of California, Irvine, CA 92717 USA*

Neurodegenerative changes in Alzheimer's disease (AD) have been hypothesized to be mediated by ß-amyloid protein (Aß), an amphipathic peptide composed of a 42-residue fragment of the ß-amyloid precursor protein. Previous studies have shown that the assembled form of Aß is toxic to cultured neurons, and that the Aß(25-35) fragment may be the active region mediating this toxicity. To determine if Aß binds to neurons via a stereospecific ligand-receptor interaction, we synthesized (D) and (L) stereoisomers of Aß(25-35). Purified peptides were shown to comigrate on HPLC. Phase-contrast microscopy, electron microscopy and sedimentation analysis revealed that the two peptides exhibit nearly identical structural characteristics and aggregation levels. In addition, both isomers induced similar levels of toxicity in cultured hippocampal neurons. These data suggest that the neurotoxic actions of Aß do not result from classic stereoisomer-specific ligand-receptor interactions. Rather, Aß neurotoxicity may result from adsorption to surface proteins, perturbation of cellular membranes, or other processes in which fibril features of the amyloidogenic peptide are important. This possibility is consistent with recent reports of similar neurotoxic actions by amylin and prion, amyloidogenic proteins which share structural similarities with Aß but lack amino-acid homology. Further investigation of the mechanism of Aß-induced toxicity will likely benefit attempts to understand the neurodegeneration that occurs in AD and perhaps other amyloid-related disorders.

508.16

AN ANALYSIS OF THE SEQUENCE REQUIREMENTS OF THE AMYLOID B-PEPTIDE FRAGMENT (25-35) FOR ITS FIBRILLISATION AND CYTOTOXIC PROPERTIES. J.B. Davis*, S. Wood[$], M.S. Clark, G.W. Roberts, R.B. Wetzel[$]. Dept. Molecular Neuropathology, SmithKline Beecham Pharmaceuticals, Harlow, CM19 5AD, UK; [$]Dept. Biopharmaceuticals, SmithKline Beecham Pharmaceuticals, King of Prussia, PA19406, USA.

The fibrillisation and cytotoxicity of peptide analogues of the amyloid β-peptide (25-35) fragment are being studied in order to determine the sequence and conformational requirements for these properties. Previous reports have demonstrated the neurotoxicity of the β-peptide (1-40) and the (25-35) fragment (Behl et al. 1992 BBRC 186, 944) a correlation between fibrillisation and cytotoxicity (Pike et al. 1993 J. Neurosci. 13, 1676) and also a cell surface binding requirement (JBD unpublished observation) but have not related them to sequence.

The fibrillisation and cytotoxic properties of analogues of the amyloid β-peptide (25-35) fragment, containing proline, alanine or D-amino acid substitutions, were tested. Peptides which did not fibrillise were found to be non-toxic, confirming a requirement for peptide aggregation. Loss of activity might be attributed to (i) loss of non-specific peptide aggregation, (ii) loss of quaternary structure, (iii) an additional loss of sequence specificity, due to the substitution. Chemical aggregation of the peptide to BSA failed to rescue activity, suggesting that the tertiary structure of the aggregate is important for toxicity. Future substitutions will further define β-peptide's determinants for cell surface binding.

508.17

AGGREGATION OF ALZHEIMER'S AMYLOID ß-PROTEIN AND ITS NEUROTOXICITY: ENHANCEMENT BY ALUMINUM. M. KAWAHARA*, M. KATO, K. MURAMOTO, K. KOBAYASHI, H. MORI#, and Y. KURODA, *Department of Molecular & Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu-shi, Tokyo 183, #Department of Molecular Biology, Tokyo Institute for Psychiatry, Setagaya-ku, Tokyo 156, Japan.*

It has been suggested that the aggregation of amyloid ß-protein enhances its neurotoxicity, and may play a key role in the deposition of senile plaques. We revealed that aluminum, an epidemiologic risk factor for Alzheimer's disease, promoted the aggregation of synthetic amyloid ß-protein (ß1-40) using immunoblotting and centrifugation (KAWAHARA *et al., B.B.R.C.*,198:531-535(1994)). Here, we report that such significantly aggregated ß1-40 has neurotoxic effects on cultured rat hippocampal neurons.

After incubation at 37°C for 24 h in 25 mM HEPES buffer (pH 7.0), ß1-40 (obtained from BACHEM) was separated by SDS-PAGE and immunostained by antibody to amyloid ß-protein (αß42). In the presence of 500 µM of $AlCl_3$, ß1-40 formed aggregates from higher molecular weight up to the gel top. The aggregation was inhibited by the presence of deferoxamine, a chelator of aluminum. Other metals including Zn^{2+}, Cu^{2+}, Fe^{3+} caused only a small degree of aggregation compared to Al. Pre-treated solutions of ß1-40 (aged-ß), pre-treated solutions with Al (aged (+Al)-ß), and ß1-40 without pre-treatment (new-ß) were applied to cultured hippocampal neurons obtained from 18-day embryo after 8-10 days in culture. After 7 days, cells were fixed and stained by antibodies to MAP2 and αß42. Neurons treated with aged (+Al)-ß showed a remarkable degeneration compared to aged-ß or new-ß. The neurites of the neurons were also damaged. Widespread,fibrous deposits of ß1-40 appeared on cells treated with aged-ß and aged (+Al)-ß, but not with new-ß. More condensed and characteristic deposits were observed in aged (+Al)-ß. These results suggested that the degeneration of neurons and the deposition of amyloid ß-protein are enhanced by Al.

508.18

THE ROLE OF FIBRIL FORMATION IN THE MECHANISM OF BETA AMYLOID TOXICITY. **A. Lorenzo and B.A. Yankner*. Department of Neurology, Harvard Medical School and the Children's Hospital, Boston, MA 02115.**

Recent studies suggest that the physical state of beta amyloid (βA) is an important determinant of its neurotoxic property. To determine whether a specific aggregated form of βA is required for toxicity, we have established defined conditions where βA predominantly forms either fibrillar or amorphous aggregates, as determined by Congo red staining and electron microscopy. Rat hippocampal cultures were treated with 20 µM of the different preparations for 4 days. After treatment there was significant cellular βA deposition in both preparations as determined by βA immunocytochemistry. Cultures treated with the fibrillar preparation showed a large proportion of Congo red-positive aggregates, accompanied by typical morphological changes of βA toxicity (shrinkage and clumping of neuronal cell bodies, dendritic retraction and axonal tortuosity), and by significant neuronal loss (viable neurons: 54±3%). Cultures treated with the amorphous preparation showed only occasional Congo red-positive aggregates, some neuronal clumping, few morphological abnormalities and slight neuronal loss (viable neurons: 84±5%). These results suggest that fibril formation may be necessary for βA neurotoxicity. We have recently reported that pancreatic islet cell toxicity of amylin is mediated by the fibrillar form of the peptide. Taken together, these findings suggest that amyloid fibril formation may be the pathogenic mechanism underlying cellular degeneration in different amyloidoses.

508.19

Aβ-EVOKED DEGENERATION OF DIFFERENTIATED SH-SY5Y HUMAN NEUROBLASTOMA CELLS. G.Stevens*,M.P.Lambert, S.Sabo, K. Barber, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanson, IL 60208.

Aβ peptides are known to induce neurodegeneration in cultures of rat brain cells and rat neural cell lines (Yankner, et al., 1990, *Science*, 250: 279-282; Behl, et al., 1992, *Biochem Biophys. Res. Comm.* 186: 944-950). The current data show that these peptides induce similar neurodegeneration in SH-SY5Y neuroblastoma cells, extending characterization of Aβ toxicity to a human nerve cell line. Human SH-SY5Y cells respond to aggregated Aβ with changes in cell shape, membrane blebbing, antigenic modification, loss of attachment to the substrate, and cell death. Aβ peptides require aggregation for maximum toxic effects, as cellular degeneration is evoked by aggregated Aβ 1-42 and aggregated Aβ 4-41 (Ile to Cys) but not by monomeric Aβ 1-40. Aged (pre-aggregated) Aβ 1-40 also evoked neurodegeneration.

Antigenic changes comprise upregulation of Alzheimer's-type tau epitopes, recognized by the PHF-1 and Alz-50 monoclonals. These particular changes in tau support the connectivity between this *in vitro* model and mechanisms leading to neurodegeneration in Alzheimer's disease. A significant feature of the SH-SY5Y response is that cells must be differentiated before they become sensitive to the degeneration evoked by Aβ. Signaling pathways leading to Aβ-evoked neurodegeneration thus are under experimental control, becoming complete only when proliferating cells withdraw from the cell cycle and develop a postmitotic phenotype.

508.20

POTENTIAL ROLE OF TGF-ß1 IN ALZHEIMER'S DISEASE: REGULATION OF APP EXPRESSION AND INHIBITION OF ßAP NEUROTOXICITY. **M. F. Galindo, J. H. M. Prehn*, V. P. Bindokas and R. J. Miller.** Dept. Pharmacol. and Physiol. Sciences, Univ. of Chicago, Chicago, IL. 60637.

Transforming growth factor ß1 (TGF-ß1) is found in plaques of patients with Alzheimer's disease. The ß-amyloid peptide (ßAP), the major component of these plaques, has been shown to be directly toxic to neurons and has thus been linked to the pathogenesis of Alzheimer's disease. In the present study we determined whether TGF-ß1 is able to modulate ßAP neurotoxicity and the synthesis of its precursor protein (APP).

Cultured rat hippocampal neurons were exposed to 0.1 - 10 µM of the ßAP fragment 25-35 (ßAP25-35) for a period of 5 days. Exposure to ßAP25-35 led to a significant decrease in neuronal viability, which was maximal at 1 µM. Terminal deoxynucleotidyl transferase-based immunodetection demonstrated that neuronal injury was accompanied by extensive DNA fragmentation, an indication of "apoptotic" cell death. A single addition of TGF-ß1 (10 ng/ml) inhibited both ßAP25-35 neurotoxicity and reduced the extent of DNA fragmentation. Repeated treatments with TGF-ß1 (4 x 10 ng/ml) completely prevented the death of the hippocampal neurons. In contrast to ßAP, its precursor protein (APP) is thought to actually have neurotrophic and neuroprotective properties. We next determined the effects of TGF-ß1 on APP expression both in cultured rat hippocampal neurons and in secondary cultures of astrocytes. Treatment with TGF-ß1 (0.1 - 10 ng/ml) led to an increased neuronal and glial production of APP, as determined by Western blotting and immunocytochemistry with a monoclonal antibody specific for APP.

We conclude that, in addition to its effect on Ca^{2+} homeostasis and bcl-2 expression, an increase in APP expression could underlie the protective effects of TGF-ß1 against ßAP neurotoxicity.

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DEGENERATIVE DISEASE: ALZHEIMER'S: NEUROPATHOLOGY AND NEUROTRANSMITTERS—OTHER SYSTEMS

509.1

PHOSPHOINOSITIDE HYDROLYSIS IN HUMAN POSTMORTEM BRAIN MEMBRANES: ALTERATIONS IN ALZHEIMER'S DISEASE. A.F. Greenwood*, L. Song, R. Powers and R.S. Jope, Dept. of Psychiatry , Univ. of Alabama, Birmingham, AL 35294.

Membranes prepared from postmortem human prefrontal cortex hydrolyzed exogenous [^{3}H]phosphatidylinositol PI) when (i) phospholipase C (PLC) was stimulated with Ca^{2+}; (ii) GTPγS or NaF was added to stimulate G-proteins coupled to PLC, or (iii) carbachol, ACPD, histamine, ATP, or serotonin plus GTPγS were added with GTPγS. Only the response to ATP was influenced significantly by the duration of the postmortem interval (measured between 5 and 21 hr, n = 16). There was a decline in responses to several stimulants correlated with increased age (measured between 18 and 100 yr, n = 23). Utilization of antibodies to block responses indicated that Gq and PLC-β were the active subtypes mediating human membrane PI hydrolysis. ATP induced the greatest responses of the agonists tested and results indicate that it acts through P_2-purinergic receptors. Comparisons of membranes from Alzheimer's disease and age-matched controls indicated that Ca^{++}-stimulated PLC activity was unaltered but the responses to GTPγS and carbachol were reduced. Further comparisons between AD and controls with other agonists and comparisons of G-protein levels are also measured.

509.2

PERIPHERAL BENZODIAZEPINE RECEPTORS IN LYMPHOCYTES OF PATIENTS WITH ALZHEIMER'S DISEASE AND MULTI-INFARCTUAL DEMENTIA. C. Ferrarese*, A.Scuratti[1], G.Bianchi, R.Cavarretta, R.Riva, G.Bianchetti[1] and L.Frattola. Dept. of Neurology, University of Milan, Monza and (1) Alzheimer's Unit, Sacro Cuore Institute, Brescia, Italy.

Peripheral benzodiazepine receptors (PBR) are modified in brain areas in animal models of stress and in Alzheimer's disease (AD) patients. Since PBR are present also in circulating lymphocytes, where appear to modulate immunologic functions, we presently investigated possible modifications of PBR in lymphocytes of patients with dementia, to investigate their role as putative peripheral markers of neurochemical alterations in degenerative disorders. 15 AD, 11 multi-infarctual dementia (MID) patients (NINDS-ADRDA and DSM-IIIR criteria) and 10 age and sex matched controls were selected for peripheral blood withdrawal and lymphocyte preparation. Frozen lymphocyte pellets were resuspended and incubated with various concentrations of [H^3]PK-11195, specific ligand of PBR, to analyze receptor density (Bmax) and affinity (Kd). PBR Bmax in aged controls was similar to Bmax of young volunteers; a high variability of Bmax was observed in AD, although the mean was unchanged respect to controls. On the contrary, MID patients presented PBR densities significantly reduced respect to controls. No change of Kd was observed in any patient population. These findings may indicate a biochemical eterogeneity in different AD patients and a possible role of PBR in neurochemical modifications observed in degenerative disorders.

509.3

AMELIORATIVE EFFECTS OF MCI-225, A NOVEL PSYCHOACTIVE COMPOUND, ON AMNESIA AND ATTENTION DEFICIT IN RODENTS. J. Eguchi, T. Yuasa, A. Tobe#, M. Egawa# and K.-I. Saito*. Pharmaceuticals Lab. I, Research Center, Mitsubishi Kasei Corporation, 1000 Kamoshida-cho, Midori-ku, Yokohama 227, JAPAN. #Clinical Research Center, MKC.

Effects of a new compound MCI-225, [4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate hydrochloride], on memory and attention were examined. In water maze task in rats, MCI-225 (1-10 mg/kg, p.o.) ameliorated scopolamine-induced amnesia. Consecutive administration of MCI-225 (0.3 and 1 mg/kg, p.o.) reversed the passive avoidance failure in the basal forebrain-lesioned rats. MCI-225 at 1-10 or 10 and 30 mg/kg, p.o. reversed the attention deficits in mongolian gerbils or rats, whose cortical noradrenergic neurons were destroyed by N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine or 6-hydroxydopamine, respectively. In the range of doses used, MCI-225 showed no abnormal behavior. Neurochemical studies showed that MCI-225 potentiated noradrenergic neuron pre- and post-synaptically, by inhibition of noradrenaline uptake (IC50 = 69nM) and by increasing the c-AMP formation via modification of α receptor-mediated potentiation to β receptor stimulation.

509.4

STATUS OF SEROTONIN TRANSPORTER SITES (5-HTT) IN THE AGING HIPPOCAMPUS AND DORSAL RAPHE NUCLEUS (DRN) IN ALZHEIMER'S DISEASE. S. M. Tejani-Butt, J. Yang, I. Halasz* & G. A. Ordway[1]. Dept. of Psychiatry, Univ. of Penn. School of Medicine, Phila., PA 19104 and [1]Dept. of Psychiatry & Human Behavior, Univ. of Mississippi Medical Center, Jackson, MI 39216.

There is increasing evidence that the functional integrity of the 5-HT system is compromised in age-related cognitive disorders. Several reports have indicated neuropathological changes in the DRN, including modest to severe losses of DRN neurons in Alzhemier's Disease (AD). Since ~40% of DRN neurons are considered to be 5-HT immunoreactive, this study measured 5-HTT binding sites to obtain a quantitative assessment of the status of 5-HT innervation in the DRN in AD and in the aging hippocampus. Sections were taken from the mid-region of the DRN from 8 subjects who had died of AD and 5 controls, and from the mid-region of the hippocampus from 18 subjects who had died of natural causes, ranging in ages from 19-80 years. A significant decrease in ^{3}H-cyanoimipramine binding to 5-HTT sites was seen in the DRN in AD cases versus controls, with the most significant reductions (~30%) occuring in the lateral wings ($p<0.01$) of the DRN complex. In contrast, regression analysis revealed no significant effect of age on 5-HTT binding sites in the hippocampus (dentate gyrus, CA_1, CA_2, $CA_{3,4}$) and entorhinal cortex. The results indicate that the integrity of 5-HT neurons in the DRN, as measured by 5-HTT binding, may be compromised in AD, whereas the integrity of 5-HT terminals in the hippocampus may be unaffected by age. (Research funds from USPHS grant NS 31699).

509.5

EXPRESSION OF D2 RECEPTOR "COLUMNS" IN TEMPORAL CORTEX OF ALZHEIMER'S IS REDUCED

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We have previously described the autoradiographic mapping of D2-like receptors with [^{125}I]epidepride in human brain (Joyce et al., JPET, 253:1253,1991), showing high expression in limbic regions. Reductions in the number of D2-like receptors in the DG, CA3, subiculum and perirhinal regions are observed in Alzheimer's disease (AD) (Joyce et al, 1993; Neurosci. Lett; Ryoo and Joyce, J. comp.Neurol., 1994). Multimodal regions of the temporal cortex that provide afferents to parahippocampus show a very different pattern of D2 receptor expression than other cortical regions. Dense columns of D2 receptors are observed in temporal association cortex that are bracketed by regions of very low numbers of receptors. The bands either cross all cortical layers, or show a paucity of binding in the mid-layers (Goldsmith et al., 1991 Soc Neurosci. Abstr). They have a mean width of 2.75mm (± 0.62), and are continuous for an anterior-posterior distance of at least 1500 µm. The majority of bands are observed in the lateral and inferior aspects of the superior temporal gyrus, with occasional bands observed on the lateral surface of the middle temporal gyrus and on the parahippocampal cortices. No bands of D2 binding are observed in anterior Heschl's gyrus or in the posterior primary auditory cortex. Hence, these columns form modular bands that travel in the rostral-caudal axis of these multimodal association areas of the temporal lobe. We compared AD cases (n = 11; 79 ± 10 yrs; PMI = 7.8 ± 6 hrs) with controls (n = 8; 64 ± 14 yrs; PMI = 10 ± 7 hrs) for frequency of these columns. Whether expressed as an absolute number of columns or relative frequency for each of four rostral-caudal levels of the temporal cortex, there was a significant difference (ANCOVA, p = 0.0001) between groups (Control = 2.5 ± 2 columns/level vs AD = 0.2 ± 1) **Funded by MH 43880, AG 09215.**

509.6

Differential regulation of RNA editing for an AMPA receptor subunit gene in the prefrontal cortex of Alzheimer's patients, schizophrenics and controls. S. Akbarian*, M.A. Smith and E.G. Jones. *Dept of Anatomy and Neurobiology, University of California Irvine, CA 92717.*

Studies in rat brain have demonstrated that the RNA for the GluR-B (2) subunit of the AMPA receptor is subject to RNA editing, by which a selected glutamine codon (CAG) is changed to an arginine codon (CGG). AMPA receptors containing GluR-B (2) polypeptides derived from edited RNA show upon stimulation much less Ca^{2+} permeability in comparison to AMPA receptors containing GluR-B (2) polypeptides derived from unedited RNA (*Sommer et al. ,1991, Cell 67:11).* In the rat, the ratio of unedited vs. edited GluR-B (2) RNA changes from 1:99 during early brain development to 0.01:99.99 in the adult brain (*Burnashev et al. ,1992, Neuron 8:189)*

Dysregulation of GluR-B (2) editing could potentially cause AMPA and Ca^{2+} mediated neurotoxicity. In the present study, the ratio and percentages of unedited and edited GluR-B (2) mRNA was analysed in the prefrontal cortex of Alzheimer's patients, schizophrenics and controls, using polymerase chain reaction with radiolabeled oligonucleotides, restriction enzyme analysis and scintillation radiography. In the Alzheimer's brains the relative abundance of unedited RNA was tenfold higher in comparison to age-matched controls (1.60% and 0.06%, $p < 0.05$) while in age-matched schizophrenics it was 0.39%. The abnormally high proportion of unedited GluR-B(2) mRNA in the Alzheimer's brains indicates that RNA editing for GluR-B(2) subunit is compromised and could potentially be involved in mechanisms that underly chronic neurodegeneration. It will be of interest to study possible dysfunction of AMPA/KA receptor RNA editing in other neurodegenerative diseases such as Amyotrophic lateral sclerosis or Huntington's disease. Supported by NIMH grant MH44188 and by NARSAD and Stanley Grants.

509.7

THE C-TERMINAL AMYLOID PRECURSOR PROTEIN FRAGMENT His 657-Lys 676 REVERSES NORADRENALINE- AND ENKEPHALIN-INDUCED INHIBITION OF VOLTAGE SENSITIVE CALCIUM CURRENTS IN NG108-15 HYBRID CELLS. H.W.G.M. Boddeke*, I. Meigel, P. Boeijinga and R. Swoboda, Sandoz Pharma Ltd., Preclinical Research, CH 4002 Basle, Switzerland.

Recently it has been reported that the cytoplasmic C-terminal amyloid precursor protein (APP) sequence, His 657-Lys 676, forms a complex with isolated G_o, a major GTP-binding protein in the brain (Nishimoto et al., 1993). In this study we have investigated the effect of the APP fragment His 657-Lys 676 (APP 657-676), its reverse sequence and the smaller APP fragment Gly 659-Lys 676 upon calcium currents in NG108-15 neuroblastoma x glioma hybrid cells. In addition, we have investigated the interaction of these peptides with calcium current suppression mediated by adrenergic α_{2B} and opioid δ receptors.

APP 657-676 (10^{-6}-10^{-4}M) did not affect calcium currents per se but clearly blocked calcium current suppression mediated by both adrenergic α_{2B}- and opioid δ receptors in a concentration-dependent manner. The reverse APP fragment 676-657 and the shorter APP fragment Gly 659-Lys 676 did not affect calcium current suppression by adrenergic α_{2B}- and opioid δ receptors.

Our results show an interaction of C-terminal APP with adrenergic α_{2B}- and opioid δ receptor-mediated calcium current suppression downstream of the receptor, possibly via the GTP binding protein G_o.

Nishimoto I., Okamoto T., Matsuura Y., Takahashi S., Okamoto T., Murayama Y., Ogata E., Nature, 362, 75-79, 1993.

509.8

REGULATION OF THE OLFACTORY BULB ALZHEIMER'S PRECURSOR PROTEIN (APP) BY LESIONING OF THE OLFACTORY BULB. M.C. Wilson, V. Ramkumar, R. Wang, D.N. Dhanraj, R. Struble*. Dept. of Pharmacology, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794-9230 USA

Recent evidence suggest that APP modulates the activity of the guanine nucleotide regulatory protein, G_o, a major growth cone protein. Growth cone associated protein (GAP-43) stimulates the binding of GTP to G_o and thereby promotes its activation. Olfactory nerve deafferentation of the olfactory nerve led to an approximately 2-fold increase in GAP-43 in 3 to 4 weeks which is associated with regeneration of the olfactory nerve (Verhaagen et al., J. Neurosci. Res. 26, 31-44). This study determined whether other components of this signalling pathway were regulated by olfactory nerve lesioning. Lesioning was performed by administering a single injection of diethyldithiocarbamate (DDTC) to Wistar rats (200-250 g). Vehicle-injected animals were used as controls. The levels of APP, G_o and GAP-43 were determined by Western blotting. Olfactory nerve lesioning produced a transient increase in expression of APP (2-3 fold) by 6 weeks that recovered to control levels by 12 weeks. The levels of GAP-43 were also transiently increased (~ 2 fold) by 6 weeks and recovered by 12 weeks. In contrast, G_o was decreased (~ 3 fold) over this time period and showed partial recovery (70-80% of control level) by 12 weeks. This upregulation of APP and GAP-43 might represent an attempt by the cell to compensate for the decrease in G_o. Olfactory nerve lesioning might provide a useful in vivo model to study.

509.9

Regulation of the β-APP promoter activity through a Ras-dependent pathway. T. Massamiri*,T.L. Deng and J.H.Brown. Dept. of Pharmacology, Univ. of Calif. San Diego, CA 92093-0636.

We have previously shown that the β-amyloid precusor protein (β-APP) gene promoter is activated by the transcription factor c-Jun via a functional distal AP-1 site. We propose that stimuli that lead to activation of Ras-dependent pathways would also regulate AP-1 activity to induce β-APP expression. Human astroglial cells (1321N1) were transiently co-transfected with a β-APP promoter luciferase reporter gene (a 593bp *HindIII/BamHI* proximal promoter fragment of a human β-APP genomic clone) along with an expression vector for constitutively activated Ras. Our results show that expression of activated Ras increases β-APP-luc promoter activity approximately 6 fold, suggesting that stimulation of the Ras pathway can activate the β-APP promoter. Further experiments were carried out with a mutated form of the β-APP promoter containing a dysfunctional distal AP-1 site (Mut-β-APP-luc) which did not respond to co-expression of c-Jun or c-Fos. The mut-β-APP-luc promoter was not inducible by activated Ras. We conclude that stimulation of the ras pathway leads to transactivation of β-APP expression via its distal AP-1 site. Studies are currently under way using a dominant negative Ras expression constuct to establish a requirement for Ras in the regulation of the β-APP promoter activity by cellular mediators.

509.10

IMPAIRMENTS OF Gsα FUNCTION IN HUMAN BRAIN CORTEX OF ALZHEIMER'S DISEASE : COMPARISON WITH NORMAL AGING. H. Ozawa*, E. Hashimoto,S. Hatta[1] T. Saito, L. Frölich[2], H. Ohshika[1], N.Takahata, and P. Riederer[2]. Dept. of Neuropsychiatry and [1]Pharmacology, School of Medicine, Sapporo Medical University, Sapporo 060, Japan. [2]Dept. of Psychiatry, Würzburg University, D-97080 Würzburg, Germany.

GTP binding (G) proteins are believed to be key elements on the downstream of intracellular signal transduction from many receptors. In this study, we examined the quantity and quality of G proteins in membrane preparations from normal adult human cerebral cortex (17-89 years old) and subjects with dementia of the Alzheimer type (DAT). The immunoreactivities by immunoblotting with polyclonal antibody of Giα and Gqα subunits were correlated inversely with normal age in parietal, temporal and occipital cortex regions. The Gβ subunit immunoreactivities showed a negative correlation with age in temporal and occipital cortex areas. The function of G proteins was examined by photoaffinity GTP analog [azidoanilido GTP (AAGTP)] binding. AAGTP labeling to Gsα and Gi/oα and the ratio of Gsα to Gi/oα AAGTP binding showed no age-dependent changes. There are no significant differences in the amount level of GsH,GsL, Gi, Go, Gq, Gβ subunits by immunoblotting between DAT and matched controls in these cerebral cortex regions. In contrast, photoaffinity GTP labeling of Gsα but not Gi/oα was decreased in DAT compared to controls in parietal and temporal cortex but not occipital cortex regions. These results suggest that disturbances of post-receptor trans-signaling in DAT are attribute to functional changes of stimulatory G proteins (Gs) independent of alterations in the gross level and normal aging process on those proteins.

509.11

IDENTIFICATION OF AN 8-HYDROXYGUANOSINE NUCLEOTIDE IN CSF THAT BLOCKS GTP BINDING TO β-TUBULIN. B.E. Haley*, E. Duhr and S. S. M. David, College of Pharmacy and Center on Aging, Univ. of Kentucky, Lexington, KY 40536-0082.

In comparison to control brain samples it has previously been shown that approximately 80% of Alzheimer's Diseased (AD) brains have the GTP binding site on β-tubulin substantially blocked and unable to be photolabeled with [^{32}P]8N$_3$GTP. Also, addition of the cytosolic fraction of AD brain homogentates was able to decrease this photoinsertion in control brain tubulin (Khatoon et al., Ann. Neurol. **26**, 210, 1989). Now we report that CSF from AD patients contains a very selective inhibitor of [^{32}P]8N$_3$GTP photoinsertion into β-tubulin of control brain. This inhibitor is in the flow through of centricon filtration and is stable to heat treatments of 90°C for 10 min. Treatment of CSF with cation binding resins enhanced inhibitory activity whereas treatment with anion binding resins or with alkaline phosphatase totally abolished inhibitory activity. Ion exchange HPLC of centricon filtered CSF and testing of the fractions for ability to reduce [^{32}P]8N$_3$GTP photoinsertion into β-tubulin identified one UV absorbing compound with an absorbance max of 291nm that blocked β-tubulin photolabeling. This compound was tenatively identified as a phosphorylated form of 8-hydroxyguanosine or 8-hydroxydeoxyguanosine. It is proposed that levels of this compound in the CSF may be a measure of the levels of hydroxy radical (OH·) or singlet oxygen ($^{1}O_2$) in the brain. Also, these results imply that the radicals or endoperoxides formed by OH· or $^{1}O_2$, leading to 8-hydroxyguanosine nucleotides, may interact with various GTP binding proteins permanently modifying their biochemical properties. Supported by NIH grant GM-35766 and the Lexington Clinic Foundation.

509.12

ALTERATIONS IN THE NEURONAL CYTOSKELETON FOLLOWING METABOLIC INHIBITION AND EXCITOTOXIC INSULT. Z. Pang and J.W. Geddes*. Sanders-Brown Center on Aging and Dept. Anatomy & Neurobiology, Univ. Kentucky, Lexington, KY 40536.

Impaired energy metabolism can induce 'excitotoxic' neuronal death and may contribute to the pathogenesis of late-onset neurodegenerative disorders such as Alzheimer's disease. To determine if impaired energy metabolism could play a causal role in the formation of neurofibrillary tangles, we administered the mitochondrial inhibitor malonate (a reversible inhibitor of succinate dehydrogenase) to rats via intra-hippocampal injection. Immunocytochemistry was used to examine cytoskeletal changes at different postinjection time points. Within six hours after injection, malonate (2 μmoles) resulted in neuronal cell death, loss of MAP2 and MAP1B, and relative sparing of tau in the hippocampus. At the drug injection site, damage was widespread and involved most cell types in the hippocampal formation. Distal to the injection site CA1 neurons were most vulnerable, although CA3 neurons were also affected. Intrahippocampal injection of the excitotoxin quinolinate resulted in a similar pattern of cell loss and cytoskeletal disruption. These results support the hypothesis that metabolic inhibition and excitotoxic insult may contribute to the sparing of tau and loss of other cytoskeletal proteins in tangle-bearing neurons in Alzheimer's disease. Supported by AG10678.

509.13

STAGE-SPECIFIC INCREASES IN CSF EXCITATORY AMINO ACIDS IN ALZHEIMER'S DISEASE. J.G. Csernansky*, M.E. Bardgett, Y.I. Sheline, and J. Olney. Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

Increased release of excitatory amino acids (EAA) has been proposed to be involved in the pathogenesis of degenerative brain diseases, such as Alzheimer's disease (AD). However, inconsistent changes in cerebrospinal fluid (CSF) concentrations of EAA have been reported in patients with AD compared to controls, perhaps because the patients studied had illnesses of mixed severity. To determine whether there are stage-specific changes in CSF concentrations of EAA in patients with AD, we measured the CSF concentrations of glutamate, aspartate, and taurine (a comparison neutral amino acid) using high pressure liquid chromatography in 32 AD patients and 11 controls, recruited from the Alzheimer's Disease Research Center. Twenty patients had early AD (CDR stage 0.5 - 1.0) and 12 patients had advanced AD (CDR stage 2.0 - 3.0).

ANOVA revealed a significant increase in aspartate concentrations in advanced patients compared to early patients and controls ($F=5.15$, $p=.010$, post-hoc Fisher's PLSD - normals = early AD < advanced AD). A similar trend was observed for CSF glutamate concentrations ($F=2.49$, $p=.095$). No significant differences in CSF taurine concentrations were observed among the groups. CSF aspartate concentrations were highly correlated with glutamate concentrations ($r=.84$, $p<.0001$), but not with taurine concentrations. These results indicate that CSF EAA concentrations are abnormally increased in patients with advanced but not early AD, and suggest that increased EAA release may play a pathophysiological role late in the disease. This project was supported by the Alzheimer's Disease Research Center Grant P50 AG05681.

509.14

REDUCTION IN NON-NMDA GLUTAMATE RECEPTOR SUBUNIT IMMUNOREACTIVITY IN THE ENTORHINAL CORTEX OF PATIENTS WITH ALZHEIMER'S DISEASE PATHOLOGY. R. Sheffield, M.D. Ikonomovic, R.P. Yasuda[1] B.B. Wolfe[1] and D.M. Armstrong*. Allegheny-Singer Research Institute, Medical College of Pennsylvania, Pittsburgh, PA 15212; [1]Dept. Pharmacology, Georgetown University.

In a previous study (Brain Research 639 (1994) 207-216) we employed immunohistochemical techniques and demonstrated a reduction in GluR2/3 and GluR1 immunoreactivity in the entorhinal cortex (EC) of patients with Alzheimer's disease. In the current study, Western blot analysis is employed in order to determine the extent to which selected GluR subunits are reduced in the EC. Moreover, we seek to determine whether the decrease in GluR2/3 immunoreactivity within layer II pyramidal neurons in the EC parallels alterations in the expression of various cytoskeletal markers. Throughout these studies we examined normal control subjects and subjects displaying variable degrees of AD pathology. In patients with clinical and pathological verification of AD, GluR2/3 protein was reduced by nearly 50% of controls while GluR1 was decreased by 40%. Cell counts of GluR2/3 immunoreactive neurons demonstrated that the reduction of GluR2/3 could be attributed largely to a loss of layer II-labeled cells. In contrast, labeled cells were abundant in layers V and VI even in those cases with severe AD pathology. Examination of subjects with relatively mild AD pathology in the EC revealed a number of findings applicable to layer II neurons: (1) the decrease in GluR2/3 appears to precede the actual loss of these neurons as determined in Nissl-stained tissue sections; (2) the reduction in GluR2/3 immunolabeling is observed prior to a decrease in MAP2 or neurofilament protein; (3) in some but not all cases, GluR2/3 immunolabeling is reduced prior to the appearance of PHFs. These data suggest that the reduction in GluR2/3 within the EC occurs early in the disease process and may be contributing to cytoskeletal pathology. This work is supported by NIH grant AG08206 and The American Health Assistance Foundation.

509.15

NON-NMDA GLUTAMATE RECEPTOR SUBUNIT IMMUNOREACTIVITY IN THE HIPPOCAMPUS OF NORMAL CONTROLS AND PATIENTS WITH ALZHEIMER'S DISEASE PATHOLOGY. M.D. Ikonomovic*, R. Sheffield and D.M. Armstrong. Allegheny-Singer Research Institute, Medical College of Pennsylvania, Pittsburgh, PA 15212.

In a companion work, we report a decrease in GluR2/3 immunoreactivity in the entorhinal cortex of patients with AD. That study prompted us to investigate the immunolabeling of this receptor subunit within the projection fields of the perforant pathway (i.e., the hippocampus). Throughout our studies anterior and posterior aspects of the hippocampus were examined. Within the anterior hippocampus of normal control patients, we observed numerous labeled neurons within the stratum pyramidale. In addition, intense peroxidase reaction product was observed within the stratum moleculare. Examination of more posterior sections revealed a distinctive and highly consistent pattern of GluR2/3 immunolabeling. Within the dentate gyrus, granule cells were labeled as was the molecular layer. Throughout the CA subfields, numerous pyramidal neurons were GluR2/3 positive. These labeled neurons were particularly abundant in the CA3/CA2 subfields. In many instances, labeled cells within the stratum pyramidale extended their dendrites through the stratum radiatum and into the stratum moleculare. In AD subjects, the anterior hippocampus was characterized by a marked reduction of GluR2/3-labeled cells within the stratum pyramidale and nearly a complete absence of labeling within the stratum moleculare. In most instances, within those regions where we observed a marked reduction in GluR2/3 labeling we also observed numerous neurofibrillary tangles. In contrast to the anterior hippocampus, studies of the posterior hippocampus of subjects with AD demonstrated that the extent and intensity of immunolabeling was comparable if not greater than that observed in controls. Moreover, in regions of intense GluR2/3 labeling, neurofibrillary tangles were relatively sparse. At present, studies are underway which seek to determine the precise relationship between GluR2/3 expression and neurofibrillary pathology. This work is supported by NIH grant AG08206 and the American Health Assistance Foundation.

DEGENERATIVE DISEASE: ALZHEIMER'S—CELLULAR MECHANISMS OF DEGENERATION

510.1

SYNAPTOPHYSIN AND ELECTRON MICROSCOPIC ASSESSMENT OF REACTIVE SYNAPTOGENSIS. S.W.Scheff*, J. Tewes, S.A. Baldwin, and J.W. Geddes. Center On Aging, Dept. Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536-0230

The entorhinal cortex provides a major input to the ipsilateral hippocampal dentate gyrus. A unilateral entorhinal cortex lesion denervates the outer molecular layer triggering synaptogenesis and the replacement of lost synaptic connections. This reactive replacement of synaptic connections was originally studied with quantitative electron microscopy (EM). More recent experiments have employed synaptophysin immunoreactivity (SI) to quantitatively assess synapse numbers utilizing light microscopy. No one has directly compared actual numbers of synapses observed with EM to values obtained with SI.

Adult Fisher 344 rats were subjected to a unilateral entorhinal cortex lesion and killed at 2,4,10,15,30 and 60 days after the injury. Tissue was processed for both immunoreactivity utilizing an antibody directed against synaptophysin, and for EM employing standard techniques. For the EM prepared tissue, the number of synapses per $100\mu^2$ was ascertained. An image analysis device was used to obtain the corrected optical density for the SI prepared tissue.

Both techniques demonstrated a loss of synaptic contacts immediately following the lesion, although the magnitude was significantly less for the SI material. A time dependent change in staining could be observed in the SI material which, mimicked but did not parallel the rate and magnitude of synapse replacement observed with EM. The present results call into question the use of SI for the accurate quantitative assessment of synaptic number.

Supported by AG05144.

510.2

SYNAPTIC DENSITY IN THE HIPPOCAMPAL DENTATE GYRUS INNER MOLECULAR LAYER IN PATIENTS WITH ALZHEIMER'S DISEASE. D.A. Price, D.L. Sparks* and S.W. Scheff Center On Aging, University of Kentucky, Lexington, KY 40536-0230

Substantial synapse loss occurs in many cortical areas associated with Alzheimer's disease (AD). These brain regions are primary association cortex which normally receive significant inputs from many other association regions known to have AD pathology. The hippocampal formation is one of the primary areas affected in AD, and responsible in part for some of the impaired memory and dementia characteristic of the disease. We previously reported a substantial loss of synaptic numbers in the outer molecular layer of the hippocampal dentate gyrus. This area normally receives a direct input from the ipsilateral entorhinal cortex which has significant neuronal loss in AD. The inner molecular layer of the dentate gyrus receives most of its input from within the ipsilateral and contralateral hippocampus, and may not be affected by the disease process. The present study determined whether or not this area of the hippocampus is capable of maintaining synaptic numbers in AD patients as compared to neurologically normal controls.

Human brains were obtained at postmortem examination from patients who met the NINCDS-NIA criteria for AD and from age-matched controls. All tissues were obtained within 10 hours postmortem. The tissue was coded and prepared for ultrastructural examination. The density of synapses and the synaptic apposition length was determined for each subject. Because of ongoing research with these tissues the group designation remains coded at this time but will be discussed in full at the meetings. Correlations with synaptic density in the outer molecular layer will be presented. Supported by AG05144.

510.3

SYNAPTIC ANTIGEN REACTIVITY WITH A SERIES OF MONOCLONAL ANTIBODIES. A. Pruchnicki*, R. Bowser, W.G. Honer [1] and P. Davies. Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY, and (1) Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC (Canada).

In a previous report, a series of 16 monoclonal antibodies (the SP series) to presumed synaptic antigens were produced. (W.G. Honer, et al, Brain Research 609:9-20 (1993). This report is to verify that these antibodies do, in fact, react with synaptic antigens. The antibodies were produced by injecting mice with protein from whole brain homogenates which was partially purified by eluting from an affinity column of the monoclonal antibody EP10, which reacts with a synaptophysin-like antigen, attached to agarose. Antigens presumably associated with synaptophysin in synaptic vesicles or membrane fractions would also be enriched by this method.

Normal brain homogenates were prepared and fractionated to produce a synaptic vesicle rich fraction. Electrophoresis on a 12% acrylamide gel was performed and immunoblotting was done with each of the 16 SP series antibodies, along with EP10 and a commercial anti-synaptophysin antibody. When equal amounts of protein from each of the fractions was examined this way, there was a pattern of enrichment of the antigens in the synaptic vesicle fraction. These antibodies, therefore, are reacting with antigens associated with a synaptic vesicle fraction, as predicted. We have confirmed that the SP monoclonals are reactive with five different proteins of this fraction. Further work with synaptic vesicles fractions isolated from Alzheimer's disease brains is ongoing.

510.4

TWO-DIMENSIONAL GEL ELECTROPHORETIC ANALYSIS OF CORTICAL PROTEINS FROM ALZHEIMER AND AGE-MATCHED CONTROL: INITIAL CHARACTERIZATION OF AN UNIDENTIFIED PROTEIN IN ALZHEIMER BRAIN. M.L. CHOUINARD*, H. MARUSAWA, M. McKINNEY AND K. SUGAYA *MAYO CLINIC, JACKSONVILLE, FLORIDA, 32224, AND FUJISAWA PHARMACEUTICALS, TOKYO, JAPAN*

Fibrillary tangles and amyloid plaques, the traditional markers of Alzheimer's disease (AD), are identified only post-mortem, and are present to a varying degree in nondemented aged brain as well. In addition, a definitive causative role in AD, for either of these complexes and their associated protein components, has not yet been shown. Here, we have attempted to identify proteins in cortex of AD brain as potential markers of the disease using high resolution two-dimensional electrophoresis in an effort to provide immuno-reactive targets more specific for AD and perhaps more indicative of AD etiology.

Protein from prefrontal cortex of AD and age-matched control brain was extracted, solubilized and separated first by isoelectric point, followed by molecular weight using the Millipore 2D-gel electrophoresis system. The region between 25 and 70 KD and pH 6-8 contained at least one or more spots as identified by silver and coomassie stain in AD samples that were not present in controls. One of these spots (30 KD) was targeted for further analysis by electrophoretically blotting onto PVDF membrane. The target spot was cut out of the membrane after being revealed by coomassie stain and sequenced using an automated amino acid sequencer. Two sequences were obtained and used to synthesize peptide decamers, both beginning five residues from the N-terminus. These peptides were identical, except for a single amino acid residue (A/W) at position nine in the peptide or position 14 from the N-terminus of the protein. These peptides were injected into rabbits and antisera for each peptide was recovered and used to identify the target spot. However, immunoblots using the A-peptide revealed three or more spots in control and five or more spots in AD samples within the same pH region but with a much larger molecular weight and possibly greater abundance than the target spot. Furthermore, the A to W amino acid substitution completely changed the specificity of the antibody, revealing a single spot within the same molecular weight region detectable in AD as well as normal brain. Results obtained in this study suggest, assuming the accessibility of antibody to the antigenic site of the proteins, the possibility of an alternative processing of proteins observed in AD relative to controls.

510.5

CHARACTERIZATION OF *apl-1*, AN AMYLOID PRECURSOR-LIKE GENE IN THE NEMATODE *CAENORHABDITIS ELEGANS*. J. Daigle*, and C. Li, Dept. of Biology, Boston University, Boston, MA 02215

Alzheimer's disease is a neurodegenerative disease for which the cause is still unknown. Accumulation of a β-amyloid peptide in the brain of patients has been associated with the disease. This β-peptide is derived from a larger amyloid protein precursor (APP), for which the normal function and processing remain to be understood. Recently, a family of APP-related proteins have been identified in different organisms. We are interested in elucidating the function of APP by looking at *APP*-related genes in the nematode *Caenorhabditis elegans*.

We have previously reported the isolation and characterization of an *APP*-related cDNA from *C. elegans*, *apl-1*, and have shown that the predicted APL-1 protein is a member of the APP family of proteins. The genomic region corresponding to *apl-1* has been identified and characterized; *apl-1* spans 4 kbp and contains 12 exons and 11 introns. It has been positioned to the left arm of the X chromosome of *C. elegans*.

We are interested in studying the function of *apl-1* by generating "knockout" worms, using a transposon insertional inactivation strategy. A mutant stain containing a transposon inserted in *apl-1* has been isolated by Ron Plasterk and kindly provided to us. The transposon insertion has been located to exon 12, prior to the transmembrane domain coding sequence. Further characterization of the mutant phenotype is underway. To determine the expression pattern of APL-1, antibodies against different parts of APL-1 are being generated and will be used to perform immunocytochemistry.

510.6

STRUCTURE OF APP PROTEOGLYCAN AND DETECTION OF A NOVEL APP PROTEIN. MN Pangalos, J Shioi, S Efthimiopoulos, J Ripellino, LM Refolo*, NK Robakis. Dept. Psychiatry and Fishberg Ctr. Neurobiol., Mt. Sinai. Med. Ctr., NY, NY 10029.

We have previously reported a novel chondroitin sulphate proteoglycan form of the Amyloid Precursor Protein (CSPG-APP) produced in C6 cells (Shioi et al, J. Biol. Chem. 1992; 267:13819). Further work has shown that other cell types produce CSPG-APP including human kidney 293 cells transfected with APP_{751}.

In an effort to characterise the glycosaminoglycan chain attachment sites on the APP core protein, CSPG-APP was purified from [^{35}S]- sulphate labelled media collected from transfected 293 cells. Media was passed through a dextran sulphate Sepharose column and APP and CSPG-APP containing fractions were then passed through an FPLC ion-exchange column. The resulting CSPG-APP fractions were digested overnight with Endoproteinase lysine-C and the peptide products separated by ion-exchange chromatography. CSPG containing peptide fractions, as determined by sulphate label, DMMB dye binding assay and chondroitinase ABC digestion, were immobilized onto membranes and subjected to protein sequence analysis.

Sequencing data indicated one predominant peptide. The first 10 amino acids corresponded to known APP sequences, however these were followed by 10 amino acids which have not been previously identified with any published APP sequence. Molecular biology techniques are now being used to identify and clone this unique APP cDNA.

510.7

CHARACTERISTICS OF SECRETASE CLEAVAGE OF APP FROM PC12 CELL MEMBRANES J.A. Ripellino*[1], Y. Chen[1], S.B. Roberts[2], K.M. Felsenstein[2], and N.K. Robakis[1], [1]Dept. of Psychiatry and Fishberg Center for Neurobiology, Mount Sinai Medical Center, New York, NY 10029, and [2]Bristol-Myers Squibb, Wallingford, CT 06492

The primary component of amyloid plaques, the hallmark of pathology in Alzheimer's Disease, is a 39-43 amino acid long peptide known as the amyloid-β-peptide. β-peptide is derived by proteolytic processing of a larger precursor protein known as Amyloid Precursor Protein (APP). One pathway for APP processing which results in cleavage within the β-peptide domain prevents the production of amyloidogenic products and occurs via an enzyme known as "α-secretase". In this report we show that a subcellular fraction of PC12 cells is enriched in "α-secretase" activity. After incubation at 37°C of membranes devoid of peripherally-associated molecules, a soluble 120kD fragment of APP is produced which on 7% SDS-PAGE has the same mobility as purified, secreted APP. This APP species is recognized by N-terminal specific antibodies but is not recognized by C-terminal specific antibodies. The enzymatic activity resulting in the release of the Nexin II form of APP has a pH range of 7.5 to 10.0, is differentially regulated by divalent cations, and is inhibited by two serine protease inhibitors. Moreover, the activity appears to be tightly membrane associated since extraction with 0.5M NaCl or sodium carbonate did not reduce the production of Nexin II.

510.8

THE PRION PROTEIN FRAGMENT PrP106-126 INDUCES CORTICAL TYPE I ASTROCYTES PROLIFERATION *IN VITRO*: CHARACTERIZATION OF THE MOLECULAR MECHANISMS INVOLVED. T.Florio*, M.Grimaldi, M. Salmona°, O. Bugiani#, F. Tagliavini#, G.Forloni° and G.Schettini. Dept. of Neuroscience, Sect. of Pharmacology, Univ. of Naples Federico II, Naples;°Inst. of Pharmacological Research Mario Negri and #Neurological Inst. Carlo Besta, Milan Italy.

The cellular prion protein (PrP^{C}) is a sialoglycoprotein of unknown function, highly expressed in neurons. In transmissible and genetic neurodegenerative diseases such as scrapie of the sheep and Creutzfeld-Jacobs encephalopathy of the humans, PrP^{C} is converted in an altered form (PrP^{SC}), identical in its primary structure but distinguishable from the normal protein for its resistance to protease digestion. In fact PrP^{SC} accumulates in the brain of affected individuals in protease-resistant, amyloid-like aggregates. Recently, it was reported that a fragment of PrP (PrP106-126) was able to induce neuronal apoptotic death *in vitro*, and that this peptide can polymerize in amyloid fibrils. Since all the PrP-related neurodegenerative diseases are characterized by both neuronal death and astrogliosis, our study was aimed to identify a direct proliferative effect on primary cultures of cortical astrocytes of the PrP106-126 fragment and which intracellular mechanisms were involved.

PrP106-126 induced a remarkable increase in quiescent (growth in absence of serum) rat cortical astrocytes assayed as both tritiated thymidine incorporation and exosaminidase proliferation assay. Specific antagonists for different intracellular pathways were used to identify the molecular mechanism of action of PrP106-126. (MURST 40% 1992,1993 to G.S.)

510.9

EFFECTS OF ETHANOLAMINE (Etn) ADMINISTRATION ON THE LEVELS OF Etn AND CHOLINE IN PLASMA, BRAIN EXTRACELLULAR FLUID (ECF) AND BRAIN TISSUE, AND ON THE LEVELS OF BRAIN PHOSPHOLIPIDS IN RATS: AN *IN VIVO* AND *IN VITRO* STUDY. E. De Micheli* and R. J. Wurtman Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

The sources and fates of brain ethanolamine (Etn) are poorly known and the effects of its administration have not been investigated, even though the cortical levels of this base are shown to be reduced in neurodegenerative pathologies with hypocholinergic function like Alzheimer's disease and Huntington's disease. We studied the effect of i. p. different Etn doses (10^{-3}, 5 x 10^{-3} and 10^{-2} mol/L/kg) on its and choline's (Ch) levels in arterial plasma and brain extracellular fluid (ECF) of awake rats. We also studied its effects on brain levels of Etn, Ch, and their respective major phospholipids. Etn administration caused dose dependent increases in Etn levels within plasma ($p<0.05$) and brain ECF ($p<0.05$), and also in brain Etn ($p<0.05$) and phosphatidylethanolamine ($p<0.05$). Systemic Etn also increased Ch levels in plasma (not completely dose dependently), brain ECF (dose dependently) ($p<0.05$), and in brain tissue ($p<0.05$); brain levels of phosphatidylcholine also increased. Possible metabolic pathways relating these increased Etn are discussed, as well as possible mechanisms of the decrease brain Etn in Alzheimer's disease.

510.10

DISTRIBUTION AND SEMI-QUANTITATIVE ANALYSIS OF TYROSINE HYDROXYLASE mRNA IN THE NORMAL HUMAN AND ALZHEIMER'S DISEASE LOCUS CERULEUS. J. Leverenz*, M.A. Miller, P.E. Kolb, E.R. Peskind and M.A. Raskind. Departments of Medicine (Neurology) and Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, 98195.

Locus ceruleus neuronal loss is well documented in Alzheimer's disease, though concomitant decline of noradrenergic function has not been convincingly demonstrated. We have, therefore, become interested in the regulation and function of locus ceruleus neurons in both the normal state and in Alzheimer's disease. Brainstems with the complete locus ceruleus from both normal elderly patients and those with Alzheimer's disease were collected and snap frozen. The entire locus ceruleus was serially sectioned from rostral to caudal extent and prepared for anatomic, immunohistochemical, receptor binding and in situ hybridization studies. We have developed in situ techniques for use in human brain tissue to examine for the presence and level of expression of tyrosine hydroxylase message using a 153 bp riboprobe (cDNA generously provided by Karen O'Malley, Washington Univ.). Our initial studies have demonstrated message for tyrosine hydroxylase in the locus ceruleus in both the normal and Alzheimer's disease brain. The findings are consistent with previous reports in other primate and rat brain. We will describe the distribution and level of expression of tyrosine hydroxylase message. We believe these studies will help elucidate the influence of Alzheimer's disease on locus ceruleus function and explain some of the paradoxes seen in the noradrenergic system in this disease.

510.11

LOCALIZATION OF THE DEFICIT IN CYTOCHROME OXIDASE (COX) ACTIVITY AND COX SUBUNIT mRNA WITHIN THE CEREBRAL CORTEX IN ALZHEIMER'S DISEASE (AD). K. Hatanpää, D.R. Brady, J. Stoll, S.I. Rapoport, K. Chandrasekaran*. Laboratory of Neurosciences, NIA, Bethesda, MD 20892.

Quantitative COX histochemistry was used to measure COX activity in tissue sections from motor and temporal cortices of 12 AD and 7 control brains. The method was calibrated with tissue sections from homogenates of known COX activity. We also used *in situ* hybridization to localize mRNA of COX subunit III (COX III) and 16S rRNA, a marker of total mitochondrial RNA levels. To ensure identical hybridization conditions, slides containing sections from the temporal and motor cortex of the same patient were paired and hybridized with the sections facing each other, with the probe between them.

Within the AD brain, COX activity in temporal cortex, a region typically affected in AD, was 29% lower than in motor cortex ($p<0.01$), a region typically spared in AD. The levels of COX III mRNA in temporal cortex relative to motor cortex were 35% lower ($p<0.01$), while 16S rRNA showed no difference. Control cases showed no differences between the two brain regions in COX activity or COX III mRNA levels. There were no significant correlations between the decreases in levels of COX activity or COX III mRNA and the numbers of neurofibrillary tangles or senile plaques in the AD brains.

The levels of COX activity and mRNA were plotted as a function of distance from the pial surface. The AD cases showed the most pronounced decrease in layers I-III. Studies are under way to relate these deficits to the *pre mortem* clinical state of the disease, to loss of neurons and synapses, and to altered regulation of mitochondrial gene expression.

510.12

PROPERTIES OF A POTENT AND CELL PERMEABLE INHIBITOR OF THE CHYMOTRYPSIN-LIKE ACTIVITY OF THE MULTICATALYTIC PROTEINASE COMPLEX. S. Wilk* and M.E. Figueiredo-Pereira
Dept. of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, New York 10029, USA.

The multicatalytic proteinase complex (proteasome; MPC) is a high molecular weight enzyme found in all eukaryotic cells. There is mounting evidence that MPC participates in several fundamental cellular processes including the degradation of ubiquitin-protein conjugates. To explore its physiological role, N-benzyloxycarbonyl-Ile-Glu(OtBu)-Ala-Leucinal [Z-IE(OtBu)AL-CHO], a peptide aldehyde inhibitor of the chymotrypsin-like activity of MPC, was synthesized. This compound potently inhibits hydrolysis of succinyl-LLVY-methylcoumarinylamide (IC_{50}=0.25 μM) by purified bovine pituitary MPC. Other catalytic activities of MPC were much less affected. Furthermore, this hydrophobic aldehyde is cell permeable. Due to its discriminating inhibitory effects Z-IE(OtBu)AL-CHO may be a powerful tool to investigate the mechanisms leading to accumulation of ubiquitin-protein conjugates in neurodegenerative diseases. (Supported by NIH grants NS-29936, MH-00350).

510.13

ACCUMULATION OF UBIQUITIN-PROTEIN CONJUGATES IN A NEURONAL CELL LINE FOLLOWING TREATMENT WITH AN INHIBITOR OF THE MULTICATALYTIC PROTEINASE COMPLEX (20S PROTEASOME). M.E. Figueiredo-Pereira*, K.A. Bergt & S. Wilk
Depts of Pharmacology and Anesthesiology†, Mount Sinai School of Medicine, CUNY, New York, New York 10029, USA.

Accumulation of ubiquitin-protein conjugates is common in abnormal brain inclusions of patients with idiopathic chronic neurodegenerative diseases. Covalent binding of ubiquitin to proteins is viewed as a means by which proteins are marked for subsequent degradation. We report that exposure of HT4 cells (a mouse neuronal cell line) to a potent permeable inhibitor of the multicatalytic proteinase complex (MPC) causes accumulation of ubiquitinylated proteins. In contrast, incubations with a calpain inhibitor or with a lysosomotropic agent failed to produce detectable ubiquitin-protein conjugates. These results suggest that a malfunction in the ubiquitin/ATP-dependent protein degrading system, of which MPC is the "catalytic core", could be a factor in the accumulation of ubiquitin protein conjugates identified in neurodegenerating brains. The MPC-inhibitor may therefore be a useful tool for developing a model for the study of neurodegenerative diseases. (Supported by NIH grants NS-29936, MH-00350, GM-34852 and MH-48125).

510.14

IMMUNOREACTIVE CERULOPLASMIN IN NORMAL AND ALZHEIMER'S DISEASE BRAIN. D. Loeffler*[1], L. Vaysman[1], H. Nguyen[1], K. Leone[1], P. LeWitt[1], P. Juneau[2], L. Kanaley[3], and A. Sima[4]. [1]Sinai Hospital, Detroit, MI 48235, [2]Warner Lambert Co., Ann Arbor, MI 48106, [3]Brain Tissue Resource Center, McLean Hospital, Belmont, MA 02178, and [4]University of Michigan, Ann Arbor, MI 48109

Ceruloplasmin (CP) provides the majority of anti-oxidant activity in plasma due to its ferroxidase activity. This study examined cellular distribution of CP in normal and AD brain (n = 5/group). Immunoperoxidase staining of formalin-fixed normal brain showed faint neuronal staining in frontal cortex, parietal cortex, and caudate, with variable staining in hippocampus, putamen, substantia nigra (SN), and cerebellum. Neuronal staining was more widespread and of greater intensity in AD (vs. normal) brain in frontal and parietal cortex, caudate, and hippocampus, and immunoreactive astrocytes were present. Differences between AD and normal brain were not seen for putamen, SN, and cerebellum. These results suggest that neuronal production of CP may be increased in some regions of AD brain. (Tissue provided by Dr. E. Bird, Brain Tissue Resource Center.)

510.15

AN INFORMATIVE MICROSATELLITE [GT] REPEAT ON A CHROMOSOME 14 COSMID ALSO CONTAINING E2K. Ghazala Ali,[1] Wilma Wasco,[2] Rudolph Tanzi,[2] and John Blass.[1]* [1]Burke Medical Research Institute, Cornell Medical College, White Plains, NY 10605 and [2]Laboratory of Genetics & Aging, Massachusetts General Hospital, Charlestown, MA 02129.

Previous studies have indicated that the activity of the α-ketoglutarate dehydrogenase complex (E2k) is reduced in Alzheimer (AD) brain (Gibson et al, *Arch Neurol* 45:836,1988; Mastrogiacomo et al, *J Neurochem* 61:2007,1994)) and fibroblasts (Cooper et al, *Ann Neurol* 35:312, 1994). KGDHC consists of 3 proteins, E1k, E2k, and E3. We have localized E1k to chromosome 7 (Szabo et al, in press) and E2k to chromosome 14q24.3 (Ali et al, in press); E3 had previously been localized to chromosome 7. In order to test for association of the E2k gene with Alzheimer's disease, we have identified a microsatellite repeat region on a chromosome 14 cosmid which also binds the full-length E2k probe which we have isolated and sequenced previously (Tanzi et al, in press).

Ten cosmids derived from a human chromosome 14 library were tested for cohybridization with E2k and with a GT repeat 30-mer. The one cosmid which was found to bind both probes was subcloned and sequenced. The sequence flanking the GT_{21} microsatellite region was used to design PCR primers amplifying a 401 bp fragment. This is an informative polymorphism giving rise to at least 10 separate common alleles. This polymorphism is being tested for association with familial and apparently sporadic AD.

510.16

MOLECULAR CHARACTERIZATION OF BRAIN ANTIGENS AND THEIR INVOLVEMENT IN NEUROPATHOLOGY OF ALZHEIMER DISEASE. R. Fukuyama, K. Hatanpää, C.R. Jones, S.I. Rapoport and D.R. Brady*.
Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892

We have hypothesized that the selective vulnerability found in Alzheimer disease (AD) brain is based on differential expression of unknown molecules in target brain regions. To test this hypothesis, we employed a conventional and a new monoclonal hybridoma technique, SOFISTIC (Fukuyama et al. *Neurosci. Abstr.* 1993), to raise monoclonal antibodies (MAbs) against antigens extracted from selected human and rat brain areas, hippocampus, entorhinal cortex and amygdala. We characterized molecular sizes and subcellular localization of antigens that are recognized by our MAbs and examined their distribution in rat, monkey and human brain. The immunohistochemical staining pattern of MAbs on brain sections fell into 6 categories; neuronal, dendritic, axonal, synaptic, astroglial and nuclear. Some of the MAbs showed region-specific staining. Five MAbs showed distinct immunoreactive bands on Western blots of cultured cell and brain extracts. Molecular sizes of antigens ranged from 14 to 230 kDa. BG5 antigen was localized to the mitochondria, and AE2 antigen to the cytosolic fraction. We screened λgt11 rat hippocampal and human amygdala cDNA libraries by MAbs to isolate cDNA clones that could encode epitopes of these antigens. We isolated 5 distinct cDNA clones. Except for clone *BG5*, partial sequence data obtained from 2-3 clones for each gene showed no obvious similarity to known sequences reported in GenEMBL database (released in Dec. '93). One of *BG5* clones, *BG5i-2*, detected a single mRNA band about 10 kb in size. Among tissues tested, the *BG5* gene showed the highest expression in human brain. It also weakly cross-hybridized to rat brain mRNA under stringent conditions. We further hybridized *BG5i-2* clone to the human-mouse hybrid panel and assigned the *BG5* gene to human chromosome 15. Its partial cDNA sequence showed a homology to rat microtubule associated protein (MAP) 1A. Western blot and immunohistochemical staining with our MAbs and with SY38, a MAb to synaptophysin, showed a marked decrease of BG5 and AH9 antigens, and synaptophysin, in temporal cortex of AD brains. We suggest that our MAbs are useful tool for analyses of AD and brain structure.

510.17

APOPTOSIS IS INDUCED BY HYDROGEN PEROXIDE IN CULTURED CENTRAL NERVOUS SYSTEM NEURONS E.R. Whittemore* D.T. Loo, and C.W. Cotman. IRU in Brain Aging, Univ. of Calif., Irvine, CA 92717.

The accumulation of β-amyloid peptides (AβP) in senile plaques is a principal event in the neuropathology of Alzheimer disease. We previously reported that CNS neurons undergo apoptosis following exposure to AβP in vitro. A link between AβP-mediated apoptosis and oxidative damage has been suggested by studies reporting that antioxidant agents protect neurons from AβP-induced cell death. We have pursued this possiblity and demonstrated that exposure of neurons to 100 μM hydrogen peroxide for 24h induced apoptosis as assessed by toxicity assays, nuclear staining, and assessment of internucleosomal DNA fragmentation.

We have begun to examine in more detail the mechanism of induction of apoptosis in neurons by hydrogen peroxide. We report that a reduction in cell viability and mitochondrial function occurs within 3h following hydrogen peroxide exposure, and parallels the induction of DNA fragmentation. In other models of apoptosis, increases in $[Ca^{2+}]_i$ have been linked with the activation of endonucleases responsible for DNA fragmentation. Measurement of $[Ca^{2+}]_i$ using fura-2 imaging following exposure of neurons to hydrogen peroxide indicates that the $[Ca^{2+}]_i$ increases steadily over time, consistent with the idea of activation of a Ca^{2+}-dependent endonuclease. Vulnerability to hydrogen peroxide is cell type specific within the CNS, with neurons being most sensitive, followed by microglia, then astrocytes. These results suggest that direct oxidative injury may serve as a general trigger for neuronal apoptosis in the CNS, and that apoptotic cell death may be induced by oxidative injury and/or stress that accompanies many neurodegenerative diseases .

510.18

ABNORMAL LYSOSOMAL HYDROLASE mRNA EXPRESSION IN NEURONS FROM ALZHEIMER DISEASE BRAIN. A.M. Cataldo*, D.J. Hamilton, S.A. Berman, C. Lippa, R.A. Nixon. McLean Hospital, Harvard Med. Sch., Belmont, MA and Dept. Neurol., U. Mass. Med. Ctr. Worcester, MA.

Our cytochemical studies demonstrate progressive disturbances of endosomal-lysosomal function in Alzheimer Disease (AD) pathogenesis. Lysosomal alterations were identified in normal-appearing, at-risk AD neurons and progress with advanced degeneration. Following cell lysis, lysosomal hydrolases are present extracellularly in amyloid-containing senile plaques (SP). To establish whether cytochemical alterations could be explained by changes at the molecular level, we examined the gene expression of cathepsin D (CD) in flash frozen, AD and control brains (PMI ≤ 6.5 hrs). Serial sections of the prefrontal cortex were analyzed with CD antiserum and by *in situ* hybridization (ISH). Changes in the levels of CD expression were evaluated by semiquantitative densitometric and Northern blot analyses. SP were identified by silver stain and thioflavin S. In AD brains, ≥ 75% of neocortical perikarya in layers III and V displayed 2 fold higher levels of CD mRNA by ISH compared to control brains ($p < 0.001$). The same neuronal populations contained increased CD immunoreactivity. Elevated levels of CD mRNA were confirmed by Northern blot. CD mRNA signal was localized in the soma and dendrites, but not in SP immunoreactive with CD antiserum. Our results indicate that lysosomal system alterations occur early in AD pathogenesis. Activation of the lysosomal system is manifested by increased CD gene expression and immunoreactivity in "at-risk" AD neurons. Changes in hydrolase expression may be one of the earliest indicators of selective cell vulnerability in certain AD neurons preceding other histological and cytochemical evidence of degeneration and may relate to the formation of amyloid-containing SP.

510.19

MEMBRANOUS LIPODYSTROPHY WITH ALZHEIMER'S SENILE PATHOLOGY. Y. Takamaru[1], R. Fukatsu[3], K. Tsuzuki[4] T. Yokoo[5], H. Takahashi[2], N. Itoh[5], N. Fujii[4], I. Kusumi*[1], T. Koyama[1], K. Nagashima[2] and N. Takahata[3] Depts of [1]Psychiatry and [2]Pathology, Hokkaido Univ, Depts of [3]Neuropsychiatry and [4]Microbiology Sapporo Medical Univ, [5]Nakamura Memorial Hospital, Sapporo 060, Japan.

Membranous leucodystrophy (ML) is a rare disease characterized by multiple bone lesions with repetitive fractures and progressive neuropsychiatric deterioration. We had an opportunity to examine two cases of the disease with Alzheimer's senile change. In present study, we compare the senile changes in ML with those in Alzheimer's disease.

case 1. 50 yro female. Brain weight 740 g. Severe generalized atrophy diffuse and profound demyelination and gliosis, sudanophilic granules, axonal swelling and spheroids were present. Wide spreaded senile plaques (SPs)and neurofibrillary tangles (NFTs) in the cortex.

case 2. 53 yro female. Brain weight 1030 g. Marked cerebral atrophy, severe demyelination and gliosis, numerous SPs and NFTs were found.

SPs were distributed in cerebral cortices, predominantly in parietal and occipital lobes. The classical or compact plaques were predominant, but diffuse or subpial plaques were rare. Amyloid angiopathy were not recognized SPs were stained with anti β antibodies, and anti amyloid associated proteins Western blot analysis of brain extracts showed similar results.

In conclusion, the differences in senile alteration suggest that there are differences in pathogenesis of these senile changes between two cases extremely unusual cases and Alzheimer's disease.

DEGENERATIVE DISEASE: OTHER—NEUROTOXIC EFFECTS AND HUNTINGTON'S

511.1

MAGNETIC RESONANCE IMAGING OF IBOTENIC ACID LESIONS IN THE PRIMATE CAUDATE NUCLEUS. E.C. Gower* and R.N. Samaraweera. Boston VAMC, Boston, MA 02130.

Monkeys (*Macaca fascicularis*) with ibotenic acid (IA) lesions of the head of the caudate nucleus are characterized by anterograde and retrograde memory defects, and *in vivo* evidence of atrophy of the injected nucleus and ventricular enlargement on MRI. As in Huntington's disease, overt signal change in regions of presumptive hypocellularity is elusive on visual inspection. In this study, we mapped the distribution of signal intensity on proton density images (TR = 2000-2500, TEef = 42; in plane resolution = 0.47 mm). The region containing the caudate nucleus was located by identifying pixel intensity transitions at the border of the caudate in the lateral ventricle, corpus callosum, corona radiata and internal capsule. Dorso-ventral columns of pixels were averaged after subtracting boundary-adjacent pixels to reduce the weighting of the means by heavily myelinated areas, CSF and edge enhancement artifacts. In the normal caudate, signal intensity rose moderately from lateral to medial. In contrast, a local zone of hypointensity caused each IA injected caudate to depart from the linearity of the normal distribution. Regional deviations were approximately in register with the planned coordinates of the lesions. Loss of signal intensity may reflect neuronal depletion and gliosis, and thus the distribution of signal across the caudate nucleus appears to be sensitive to the local anatomic and neurochemical consquences of neurotoxic tissue injury. However, hemosiderin deposits associated with microhemorrhage may also contribute. The findings may have relevance in assessing early state neuropathology in HD with structural imaging.

511.2

3-NITROPROPIONIC ACID NEUROTOXICITY IN STRIATAL AND NON-STRIATAL CULTURES IS MODULATED BY ENERGY SUBSTRATE AVAILABILITY BUT NOT SIGNIFICANTLY BY GLUTAMATE RECEPTOR ANTAGONISTS. S.L. Fink, D.Y. Ho*, and R.M. Sapolsky. Depts. of Neurosciences and Biological Sciences, Stanford Univ., Stanford, CA 94305-5020.

3-Nitropropionic acid irreversibly inhibits the activity of the mitochondrial enzyme succinate dehydrogenase. When administered systemically to rats or ingested accidentally by humans or livestock, it causes selective striatal lesions. All reports thus far on 3-NP's neuronal effects have used whole animals or cortical explants. We studied the effects of 3-NP on dissociated cultures of neurons and glia. We found that, despite its selective toxicity in vivo, incubation with 3-NP leads to comparable dose-dependent neuron death in cultured striatal, hippocampal, cerebellar and hypothalamic neurons. In addition, 3-NP's effects were remarkable energy substrate-dependent, with its toxicity being potentiated by low glucose conditions to a greater extent than was the case for glutamate. Furthermore, infection of the cultured neurons with a herpes simplex virus vector carrying GLUT-1 glucose transporter significantly protected them against toxicity caused by moderately damaging doses of 3-NP in low glucose media.

Finally, the striatal damage caused by 3-NP administration has been theorized to be partially mediated by excitotoxins. This is supported by one report in which glutamate receptor antagonists attenuated 3-NP-induced damage in cortical explants [A. C. Ludolph, *et al., Neurodegen* **1**, 155-161 (1992)]. We found that incubation with the non-specific glutamate receptor antagonist, kynurenic acid, increased neuronal survival from 15% to 61% of control in low glucose conditions, but did not significantly increase survival of neurons exposed to 3-NP. We are currently studying whether 3-NP toxicity can be decreased by co-incubation of the cultures with kynurenic acid and DL-2-amino-7-phosophonoheptanoic acid (APH), an NMDA-selective glutamate receptor antagonist. If so, this would support a role for excitotoxin-induced cellular cascades in neuron death caused by this energetic toxin; however, pilot experiments indicate that most of the toxicity is caused by other cellular processes.

511.3

NEURONAL DEGENERATION INDUCED BY 3-NITROPROPIONIC ACID IN THE FOREBRAIN: ANALYSIS WITH A SILVER METHOD. P.J. Miller* and L. Zaborszky. Center for Molecular & Behavioral Neuroscience, Rutgers, The State University of NJ, Newark, NJ 07102

3-Nitropropionic acid (3-NP) is a mitochondrial toxin which has been suggested (Beal et al., 1993) to replicate many of the histological and neurochemical characteristics of Huntington's disease upon systemic or local striatal application. The subsequent neuronal loss in other brain areas is less well understood. 3-NP was injected stereotaxically (500nmol, 0.1-0.5ul) into various forebrain areas (striatum, internal capsule, thalamus, lateral hypothalamus). Animals were perfused 5-15 days after the lesion and brain sections were impregnated according to a sensitive silver method (Gallyas et al., 1990). This method produces selective, Golgi-like staining of cells whose cytoskeletal system is damaged by various pathological events. While injections of 3-NP into the striatum resulted in local neuronal degeneration, an equally large number of medium-sized spiny striatal neurons degenerated if their axons in the forebrain were exposed to this toxin. Injections of 3-NP also resulted in topographically organized neuronal degeneration in various thalamic nuclei, corresponding to the damage of their axons and/or terminals. Thalamostriatal neurons were always heavily affected. Moderate neuronal degeneration was also observed in the pars compacta and in some cases in the pars reticulata of the substantia nigra. In spite of the fact that corticofugal axons were exposed to the toxin, little cell death was observed in the cortex. These studies suggest that intracerebral injection of 3-NP can cause substantial retrograde degeneration in basal ganglia circuits, with different projections showing different vulnerability. This data should be taken into consideration when using 3-NP in an animal model of Huntington's disease.

Supported by USPHS grants NS23945, NS30024.

511.4

PROGRESSIVE BEHAVIORAL CHANGES DURING ONE-MONTH LOW DOSE ADMINISTRATION OF 3-NITROPROPIONIC ACID. T.K.Kotouzis, C.V.Borlongan, T.B.Freeman, D.W. Cahill* and P.R. Sanberg. Div. of Neurological Surgery, Depts. of Surgery, Neurology, Psychiatry and Pharmacology, Univ. of South Florida Coll. of Med., Tampa, FL 33612.

Excitotoxic animal models have been widely utilized to define the mechanisms involved in the abnormalities observed in Huntington's disease (HD). Recently, 3-Nitropropionic acid (3-NP) has been proposed as an excitotoxic model that closely mimics the anatomical pathology associated with HD based on the striatal atrophy following mitochondrial impairment induced by 3-NP. Since impaired oxidative energy metabolism has been implicated in HD patients, 3-NP seems to reflect a similar mechanism leading to striatal pathology. Very few studies have investigated the consequent behavioral pathology following 3-NP injections in animals. In our previous studies with low dose systemic 3-NP-injected animals (Koutouzis et al. Brain Res., in press), we observed significant hypoactivity, and accordingly suggested that this reflected advanced or juvenile onset HD cases. In the present study, the previous dosing regimen of 3-NP (15 mg/kg, i.p., once every 4 days for 28 consecutive days), was administered but instead of testing only after 28 days, the animals were tested following each day of injection. The present results were very interesting; initially, the 3-NP-injected animals exhibited significant hyperactivity, reaching a plateau after the third injection (day 12), then showing significant hypoactivity from the fourth injection (day 16) onwards. The close resemblance between systemic 3-NP's behavioral effects in rodents and the ongoing behavioral pathology of HD in humans supports the suitability of 3-NP as an animal model of HD. (Supported, in part, by STRC, USF President's Council, and T. Larsen and friends.)

511.5

HISTOLOGICAL AND BEHAVIORAL EFFECTS OF CHRONIC 3-NITROPROPIONIC ACIC TREATMENT IN OLD RATS. E. Brouillet* , M.-C. Guyot, R. Dolan, M. Mazière and P. Hantraye. Service Hospitalier Frédéric Joliot, CNRS URA1285, CEA, 4 place du Général Leclerc, 91401 Cedex Orsay, FRANCE

Chronic administration of 3-nitropropionic acid (3NP) in rats has been shown recently to produce bilateral striatal lesions, closely replicating the neuropathology of Huntington's disease (HD) (Beal et al. 1993, J. Neurosci.13:4181-92). In the present report, we studied the behavioral and anatomical effects of a chronic 3NP treatment (10 mg/kg/day s.c. for 4 weeks). Four months post-lesion, 60-70 % of 3NP-treated animals showed mild motor symptoms including a wobbling gait, an apparent decrease in the length of their steps and an abnormal paw placement during locomotion. Quantitative analysis of the locomotor behavior (under spontaneous and after methamphetamine administration) using video recording showed significant changes in several parameters as compared to saline-treated animals, consistent with stable impairment of locomotion. Histological evaluation of the animals showed small lesions (30-60 % neuronal loss based on Nissl with NADPH-diaphorase interneurons sparing). These lesions were located consistently in the dorso-lateral part of the striatum in 70 % of the 3NP-treated group. These data confirm that chronic 3NP treatment leads to NMDA receptor-mediated like-lesions which are associated with stable motor symptoms.

511.6

CHRONIC BILATERAL INTRASTRIATAL DIALYTIC ADMINISTRATION OF QUINOLINIC ACID PRODUCES ABNORMAL MOTOR BEHAVIOR IN RATS. T. Bazzett*, C. Trahey, S. Ugarte, J. Becker and R. Albin. Depts of Neurology and Biopsychology, University of Michigan, Ann Arbor, MI 48104-1687

We have previously reported that chronic intrastriatal dialytic administration of quinolinic acid (QA) in the rat produces selective neurodegeneration similar to that seen in Huntington's disease (HD). In addition to neuronal loss, we have reported that chronic QA administration induces reversible changes in some areas of the striatum. The present results show that bilateral administration of QA using this technique, produces behavioral changes that may be analogous to motor abnormalities associated with HD. QA treated rats showed increased rearing behavior, oral movements, and nocturnal activity compared to vehicle treated animals. Unlike rats receiving acute injections of QA, animals exposed to chronic QA show a more progressive development of abnormal behaviors.

Additional behavioral measures will be presented including changes in motor behavior following acute stress. Results will also show if any QA induced changes in behavior improve after cessation of QUIN infusion correlating with transient changes in striatal anatomy.

511.7

DIFFERENTIAL RESPONSE OF EXTRACELLULAR GABA IN THE STRIATUM AND NUCLEUS ACCUMBENS IN THE QUINOLINIC ACID RAT MODEL FOR HUNTINGTON'S DISEASE. W. LIN, D.L. Roerig and N.C. REYNOLDS*. Neurology Dept., Med. College of WI and VA Med. Center, Milwaukee, WI 53295.

This study examines the changes in extracellular GABA in the nucleus accumbens (NA) following quinolinic acid (QA) infusions into the striatum to determine whether striatal degenerative change can effect the NA to provide a physical basis for behavioral changes in Huntington's disease (HD). The NA constitutes a major functional interface between the limbic and motor systems in locomotor processes but has also been implicated in positive reinforcement and psychiatric disorders. Although Huntington's disease (HD) is a neurodegenerative disorder with severe effects on striatal neurons, post-mortem GABA content in the NA is significantly decreased in HD patients with psychotic features. Despite the common use of "dementia" as an explanation for behavioral problems in HD in clinical practice, cortical atrophy is a late finding and mental status changes are quite dissimilar to changes seen in classical dementia (i.e., Alzheimer's disease) and often involve psychotic features or emotional lability with violent outbursts.

30 min. after intrastriatal perfusion of QA (0.24M at 2uL/min for 10 min.) laboratory rats displayed overt hypermotility with contralateral rolling movements and rigidity in the neck and forelimbs for 90 to 120 min. Over the next three days increased stereotypic grooming behavior was observed. Irritability to touch and piloerection was apparent from the second day through sacrifice on day 8.

On the 1st day after QA perfusion, extracellular GABA was decreased in striatum (67% of baseline) and increased in NA (156% of baseline). On day 2, GABA returned to baseline in the NA and then declined gradually to 40% of baseline by day 7. Striatal GABA slowly declined to this same level by day 7. These results show that QA perfusion of the striatum causes a slow decline in extracellular GABA in both nuclei but early transient increases in the NA. An extrapolation of such changes to the more protracted time course in HD may help to explain several locomotor and behavioral changes in humans with HD.

511.8

TIMING THE TRANSCRIPTIONAL RESPONSE OF THE IT15 GENE (HUNTINGTIN) DURING THE EARLY STAGES OF THE NMDA RECEPTOR-MEDIATED EXCITOTOXIC CASCADE. L.R. Carlock, K. Gutridge, Y. Shan & P.D. Walker*. Departments of Molecular Biology/Genetics and Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

NMDA receptor overstimulation produces a pattern of neuronal death within the rodent striatum similar to Huntington's disease (HD). However, does the excitotoxic rodent model provide information that can elucidate the function of the IT15 (Huntingtin) gene in the chronic HD process? We sought to examine the transcriptional response of the rodent IT15 gene during the initial stages (1-72 hours) of the excitotoxic cascade and compare it to changes in immediate-early, neurotransmitter-specific, and glial genes. Adult male Sprague-Dawley rats (175-200g) received unilateral intrastriatal injections of quinolinic acid (QA; 1µl of a 240mM concentration) and were sacrificed at various times from 1 to 72 hours later. Following QA injection, striatal induction of immediate early genes (IEGs; c-fos, jun-B, c-jun, and zif/268) extended from 1-12 hours while neurotransmitter mRNAs for GABA, tachykinin and enkephalin synthesis began to decline within the lesion site after 6 hours and were maximally reduced by 24 hours suggestive of neuronal death. Glial transcripts were increased after 3-6 hours and remained high within the lesioned striatum at 72 hours. Interestingly, the transcriptional response of the IT15 gene temporally mimicked the IEGs and also followed the decline of neurotransmitter mRNAs from 6-24 hours. These results suggest that the rodent IT15 gene is transcriptionally responsive to NMDA receptor overstimulation and is mostly expressed by neurons that degenerate during the excitotoxic cascade. Supported by NS24236 (LRC) and WSU Office of Neuroscience Programs.

511.9

MITOCHONDRIAL ENZYME ACTIVITIES IN HUNTINGTON'S DISEASE S.E. Browne*, Allen C. Bowling and M.F. Beal. Neurology Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

An attractive hypothesis of how the gene defect in Huntington's disease (HD) leads to neuronal degeneration is that a defect in mitochondrial energy metabolism may render cells more vulnerable to endogenous levels of glutamate, resulting in slow excitotoxic neuronal death. In support of this hypothesis, PET studies have shown reduced glucose utilization in the basal ganglia of HD patients, while a recent NMR spectroscopy study found increased lactate concentrations in the occcipital cortex and basal ganglia of HD patients (Jenkins *et al.*, Neurology 1993, 43, 2689-2695). In addition, chronic systemic administration of mitochondrial toxins to rodents have been found to produce striatal lesions exhibiting close neurochemical and histological similarities to HD lesions (Beal, Ann. Neurol. 1992, 31,119-130).

The aim of the present study was to investigate whether activities of electron transport chain enzymes are altered in HD patients, relative to levels in control subjects. Activities of citrate synthase and the oxidative phosphorylation enzyme complexes I, II-III and IV, were measured in mitochondrial preparations by spectrophotometric assay procedures. Citrate synthase-corrected complex I activity was found to be significantly increased in frontal cortex of HD patients (+28%, $p < 0.05$), and showed a trend towards an increase in HD caudate. A trend towards decreased complex II-III activity was also evident in caudate. This study is presently being expanded to other brain regions.

(Supported by the Huntington's Disease Society of America).

511.10

EXPRESSION OF THE ALPHA ISOFORM OF PROTEIN KINASE C (PKCα) IS INCREASED IN CAUDATE NEURONS IN PRESYMPTOMATIC HUNTINGTON'S DISEASE. G. Figueredo-Cardenas* and A. Reiner. Dept. Anat. & Neurobiol., UT-Memphis, Memphis, TN 38163.

Protein kinase C (PKC) is a calcium/phospholipid dependent kinase and numerous isoforms have been found in nervous tissue. We have found that the PKCα isoform, a cytosolic isoform normally found in very low levels in striatal projection neurons, is upregulated to immunohistochemically detectable levels in surviving rat striatal neurons subjected to an excitotoxic or ischemic insult. We therefore immunohistochemically examined fixed striatum from a presymptomatic Huntington's disease (HD) victim to determine if PKCα expression was increased and might reveal the early expression of the HD disease process. We found high expression of PKCα in the presymptomatic HD caudate and low expression in the presymptomatic HD putamen. Normal striatum was largely devoid of PKCα labeling. In presymptomatic HD caudate, double-label studies showed that PKCα was expressed by 80% of the Calbindin (Calb) neurons (i.e. projection neurons), a small number of glial cells, but no somatostatin- neuropeptide Y containing interneurons. Using Calb-immunlabeling to distinguish the patch and matrix compartments of caudate (low Calb neuropil = patch) in one section and examining an adjacent PKCα-labeled section, we found that a higher percentage of neurons in patch labeled for PKCα than in matrix. Our results show that enhanced PKCα expression specifically occurs in neurons destined to die in HD, seemingly much before they actually die. This expression occurs first in the neurons that will die first (i.e. caudate neurons). The slightly greater expression in patch than matrix suggests that patch neurons may be affected earlier than matrix neurons in HD. Since the functions of PKCα are diverse, it is uncertain if the enhanced PKCα expression serves to promote or combat the HD process. Supported by NS-28721, NS-19620 and the Hereditary Disease Foundation.

511.11

PURIFICATION AND CHARACTERIZATION OF THE HUNTINGTON'S DISEASE PROTEIN FROM RAT BRAIN SYNAPTIC VESICLES. *J.P. Steiner* T.M. Dawson, A.H. Sharp, R.K. Barrow, C.A. Ross and S.H. Snyder. Depts. Neurosci., Neurol. and Psych., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.*

The identification and cloning of the IT15 gene and the subsequent development of high affinity antibodies raised against two peptide sequences contained within the open reading frame of IT15 has provided us with the necessary reagent to attempt to isolate and purify the Huntington's Disease protein (HDP). Using the affinity-purified antibodies raised against amino acid residues 1-17 of HDP, we probed various membrane and soluble protein subfractions isolated from rat forebrain. HDP is enriched in the synaptic vesicle-containing subfraction, but is readily dissociated from the vesicles by increasing ionic strength. The extracted HDP was fractionated by gel filtration, ion exchange chromatography and differential centrifugation on sucrose gradients and revealed two high molecular weight proteins of 300 and 350 KDa, which cross-react with our anti-HDP Ig. We are obtaining primary amino acid sequence of both proteins in order to identify them. Preliminary sequence analysis indicates that the 350 KDa protein is rat brain HDP. The 300 KDa protein may be a structural homolog of HDP as indicated by the cross-reactivity to HDP antibodies. Both proteins reassociate specifically with synaptic vesicles as well as cytoskeletal elements, suggesting that HDP may play a role in neurosecretion and vesicle recycling.

511.12

CELLULAR LOCALIZATION OF HUNTINGTON'S DISEASE (HD) GENE PRODUCT: ENRICHMENT IN NERVE TERMINALS. A.H. Sharp*, S. Loev, G. Schilling, J. Steiner, A. Lo, J. Hedreen, S. Sisodia, T. Dawson, S.H. Snyder, C.A. Ross. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave., Ross 615, Baltimore, MD 21205.

Huntington's disease (HD) is an inherited neurodegenerative disorder with autosomal dominance and nearly complete age dependent penetrance. The causative gene contains an expanding CAG repeat presumably coding for glutamine in a long open reading frame yielding a 348 kDa predicted protein (HD Collaborative Group, 1993). The disease is notable for selective neuronal loss in the basal ganglia and deep layers of the cerebral cortex which has been proposed to result from excitotoxicity. However, the mRNA for the gene causing HD is widely expressed in both the brain and periphery, with no enrichment in the basal ganglia (Li et al., 1993; Strong et al., 1993). To examine the expression of the protein product, we have now developed affinity-purified polyclonal antibodies against peptides corresponding to amino acids 1-17 and 650-663 of the deduced sequence of the human HD gene product which react specifically with a 350 kDa band on Western blots. Using immunochemical and immunohistochemical methods, we find that the HD protein product, like the mRNA, is widely expressed and enriched in brain. Within the brain, it is more highly expressed in neurons than glia and present in cytoplasm, dendrites, and axons. Within several brain regions, particularly the striatum, it is enriched in nerve terminals and loosely associated with synaptic vesicles and other membranes. These results are compatible with a role for the HD gene product in regulation of neurotransmitter release or reuptake and, perhaps, involvement in excitotoxicity.

511.13

A CONTROLLED TRIAL OF THE GLUTAMATE ANTAGONIST REMACEMIDE HYDROCHLORIDE IN HUNTINGTON'S DISEASE K Kieburtz, M McDermott, A Feigin, P Como, D Abwender, C Zimmerman, C Hickey, C Orme, K Bordwell, J Sotack, C Dunn, JT Greenamyre, I Shoulson* Dept of Neurology, Univ of Rochester, and Fisons Pharmaceuticals, Rochester, NY 14642

The neuropathology of Huntington's disease (HD) is characterized by selective degeneration of striatal medium-sided spiny GABA-ergic neurons, a pattern of neuronal loss which can be reproduced in experimental animals by striatal injections of glutamate or NMDA agonists, suggesting that excitotoxic neurotransmission may play a role in the neurodegenerative process. Glutamate receptor antagonists may therefore ameliorate or slow HD, but such drugs (e.g. MK-801) in humans have been associated with intolerable side effects. We conducted a randomized, double-blind, placebo-controlled tolerability trial of remacemide hydrochloride, a non-competitive NMDA channel blocking agent. Subjects were randomized to receive remacemide 200 mg/d, 600 mg/d or placebo and were assessed by standardized neurological and cognitive tests for 6 weeks, including a 1-2 week blinded staggered withdrawal of drug. The primary outcome measure was the proportion of subjects able to complete the study on the assigned treatment regimen. Thirty-one HD patients in early to moderate stages of illness (18 men, 13 women) were enrolled in the study; and twenty-nine completed treatment. One subject was terminated for non-compliance and one subject elected to discontinue drug because of nausea and vomiting (both in 600 mg/d group). There were no significant differences in the primary measure of tolerability between treatment arms. No severe adverse experiences or adverse cognitive effects were reported. Remacemide appears to be safe and well tolerated in patients with HD at dosages up to 600 mg/day. Larger long-term study of the impact of remacemide on HD is warranted. *(Supported by Fisons Pharmaceuticals and the University of Rochester Clinical Research Center RR00044.)*

511.14

HUNTINGTON'S DISEASE AND CRANIAL DYSTONIA DIFFERENTIALLY AFFECTS JAW MOTOR CONTROL. L.T.Robertson* and J.P.Hammerstad, Dept. of Biol. Structure & Function & Dept. of Neurology, OR Health Sci. Univ., Portland, OR 97201.

Parkinson's disease (PD) and Huntington's disease (HD) are frequently cited as examples of hypokinetic and hyperkinetic disorders of the basal ganglia, respectively (DeLong, 1990). The present study examines the effects of HD and cranial dystonia, another hyperkinetic basal ganglia disorder, on a series of jaw motor tasks and then compares the deficits with those of previously studied PD subjects. We expected subjects with HD and dystonia to have jaw motor deficits reciprocal to those with PD. The patients and matched control subjects were tested on a series of jaw motor tasks including simple voluntary movement, isometric clenching, and natural and paced rhythmic movements. Jaw movements were measured by changes in electromagnetic fields and electromyographic activity of the masseter and temporalis muscles.

HD markedly affected most voluntary and rhythmic jaw movements. HD resulted in a decrease in vertical jaw opening and in velocity during opening and closing and produced low force levels during clenching. Particularly striking was the large trial-to-trial variability that occurred on every task. Both normal and paced chewing were abnormal and included large fluctuations in the cycle frequency, vertical amplitude, and occlusal duration. The performance abnormalities for subjects with cranial dystonia were usually the opposite of those of HD. The subjects with dystonia typically had increased muscle activity, involvoing increased vertical amplitudes during voluntary jaw opening and high levels of force during clenching. Although no abnormalities occurred during normal chewing, vertical amplitudes were increased during paced chewing. The jaw clenching and paced chewing tasks were also associated with fatigue and intermittent spasms.

The jaw motor deficits for HD were similar to the deficits produced by PD subjects, although HD subjects had greater variability than did the PD subjects. Although HD is associated with chorea, HD is best characterized as a hypokinetic disorder for jaw movements whereas cranial dystonia is best characterized as a hyperkinetic disorder.

511.15

Posteroventral Pallidotomy for the Treatment of Dystonia.

T. Kondoh, J.P. Blount, E.P. McDaniels, T.J. Ebner, W.C. Low, and R.E. Maxwell.* Department of Neurosurgery, University of Minnesota Medical School, Minneapolis, MN 55455.

Recent advances in the understanding of the functional anatomy of the basal ganglia have led to the development of new approaches for the surgical treatment of movement disorders. The neural circuitry in the basal ganglia generates excitatory and inhibitory outputs to the pallidum via direct and indirect striatopallidal pathways. Under various pathological conditions, the balance between these excitatory and inhibitory projections can be disrupted, thus resulting in motor dysfunction. Lesions of the pallidum have therefore been proposed to eliminate the outflow of abnormal motor/sensory information. Such lesions in parkinsonian patients have been shown to ameliorate bradykinesia, rigidity, and tremor. In the present study, we have examined the effects of pallidotomies for the treatment of dystonia. Four cases of secondary dystonia resulting from different causes were treated by posteroventral pallidotomy. Dystonia resulted from either intracerebral hemorrhage, cerebral palsy, head injury or viral encephalitis. Each patient had a long history of spasticity and uncontrolled dystonic movements of the extremities (3 - 18 years). In addition, preoperative MRIs revealed lesions extending to the basal ganglia in each patient. MRI guided stereotactic surgeries were performed using the BRW MRI stereotactic frame. The posteroventral pallidum contralateral to the affected extremity was chosen as the target site. Several radiofrequency lesions were made in the targeted pallidum after stimulation to verify the appropriate target area. Following surgery all patients exhibited marked reduction in spasticity. Postoperative MRI showed that the surgical lesions were confined to the posteroventral pallidum. These results suggest that surgical lesions of the pallidum can ameliorate dystonia induced by damage to the basal ganglia, and further support the notion of ablative cancellation therapy for the treatment of basal ganglia-mediated movement disorders.

INFECTIOUS DISEASE

512.1

EVIDENCE FOR APOPTOSIS IN THE CENTRAL NERVOUS SYSTEM IN PATIENTS WITH AIDS. F.J. Denaro*. Department of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The precise mechanism for the neuropathology which has been observed in HIV-infected individuals is now believed to be due to a complex mechanism involving HIV-infected lymphocytes and the neuronal tissue. An indirect mechanism involving viral peptides and/or other cellular agents is believed to be causative in producing cell death in the CNS. Recent studies of the HIV-infected immune system suggests that HIV can induce apoptosis in lymphocytes, and this mechanism is in part responsible for cell death. In the present study, a commercial marker for apoptosis has been used to identify this process in postmortem CNS tissue. With this marker, it has been possible to identify cells histologically in the nervous system which are undergoing apoptosis. Most of these cells are lymphocytes, but some glial cells were also labeled. However, it was not possible to identify them as microglia, astrocytes, or oligodendrocytes at this time. The present observations suggest that the mechanisms which cause cell death in the CNS may be underscored by use of a marker for apoptosis. Whether this involves HIV and/or other agents is to be determined together with the cell types. Supported by NIH Grant R29NS31857-02.

512.2

TNFα-MEDIATED APOPTOSIS IN SK-N-MC NEUROBLASTOMA CELLS: A MODEL FOR NEURONAL CELL LOSS IN HIV-1 INFECTION OF THE CNS. H. A. Gelbard*, A. Talley, S. Perry, K.A. Dzenko, and L. G. Epstein.
Div. of Ped. Neurology, University of Rochester Medical Center, Rochester, NY 14642.

An important feature of the neuropathogenesis of HIV-1 associated dementia is neuronal loss in discrete areas of the cortex and subcortical regions. Evidence for HIV-1 infection of neurons is controversial, but soluble neurotoxic factors, including tumor necrosis factor alpha (TNFα), produced by activated HIV-1 macrophages, may mediate neuronal death. We have demonstrated that TNFα is neurotoxic to cultures of human fetal cortical neurons in a dose-dependent fashion (Gelbard et al., Dev. Neurosci., in press). Because TNFα has been implicated in apoptotic cell death of non-neural cells, we tested the hypothesis that TNFα could induce programmed cell death (apoptosis) in a neuroblastoma cell line (SK-N-MC) differentiated to a neuronal phenotype with 5 μM retinoic acid. TNFα, at a dose of 2 ng/ml, induces formation of DNA laddering in gel electrophoresis studies. Apoptotic nuclei in SK-N-MC cells were identified by an in situ method to label free 3'-OH ends of newly cleaved nuclear DNA. Apoptotic nuclei in SK-N-MC cultures can be identified 6 hours after exposure to 0.2 ng/ml of TNFα, and reach a maximum by 48 hours. At a dose of 0.05 ng/ml TNFα, ~10% of SK-N-MC cells/culture well have apoptotic nuclei. At a dose of 25 ng/ml TNFα ~66% of SK-N-MC cells/culture well have apoptotic nuclei. This dose-dependent increase in apoptotic nuclei is inversely proportional to a dose-dependent (same dose range) decrease in cell viability as measured by trypan blue exclusion and enzymatic conversion of 3-(4,5-dimethylthiazol-2 yl)-2,5-diphenyl tetrazolium bromide (MTT). Studies are in progress to prevent TNFα-mediated neuronal apoptosis by infection of SK-N-MC cells with a recombinant adenoviral vector that expresses a portion of the extracellular domain of the TNFα receptor. (Supported in part by PAF/AmFAR and NINDS).

512.3

APOPTOSIS, VACUOLATION AND INFECTIVITY IN A NOVEL CELL LINE PRODUCING SCRAPIE PRIONS

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Currently available scrapie-infected cell lines do not show distinct morphological changes related to spongiform degeneration. We infected the GT1-1-trk9 neuronal hypothalamic cell line, which secretes GnRH in a synchronized manner and is transfected with the trk-A gene (NGF-R), with mouse RML prions (ScGT1-1-trk9). Subclones have been in culture for over 9 months and were passaged more than 40 times without any decrease in PrP^{Sc} production. Interestingly, there is a pronounced morphological difference between the ScGT1-1-trk-9 and the parental GT1-1-trk9 cells. EM studies showed two characteristic and very prominent ultratructural changes in ScGT1-1-trk9 cells, which were not detectable in GT1-1-trk9 cells: a) huge vacuoles, and b) the apoptotic features of nuclear condensation and fragmentation and autophagocytosis. Extraction of Triton X 100 soluble DNA from ScGT1-1-trk9 cells revealed a DNA fragmentation pattern typical for apoptosis (not detected in GT1-1-trk9 cells). Although showing these very pronounced apoptotic features, over 80% of ScGT1-1-trk9 cells are viable. Notably, after NGF treatment of ScGT1-1-trk9 cells the DNA fragmentation was reduced. For the first time it was possible to infect GT1-1-trk9 as well as mouse neuroblastoma cells (N2a) with conditioned media from ScGT1-1-trk9 cells, permitting transfer of prions between mouse cell lines. Our findings present a cell culture model for the study of prion pathogenesis. The ScGT1-1-trk9 cells exhibiting intracellular vacuolation and apoptotic cell death maybe an useful model for neurodegeneration in humans and animals dying of prion diseases.

512.4

BIOCHEMICAL CHARACTERIZATION AND TISSUE LOCALIZATION OF PRION PROTEIN LIGANDS. B. Oesch, F. Coufal, E. Gubler, C. Bandlow*, S. B. Prusiner, and S. J. DeArmond. Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland and Dept. of Neurology and Pathology, University of California, San Francisco, CA 94143.

Interaction of the prion protein with other cellular components may be an important step for the function of cellular PrP (PrP^{C}) in the normal animal or for the infection with prions which contain an isoform of the prion protein (PrP^{Sc}). We have investigated the biochemical characteristics of PrP binding proteins resulting in the purification of a PrP ligand of 110'000 kDa (Pli 110). We have used ligand blots as an assay for purification[1]. The sedimentation properties of PrP ligands were analyzed by sucrose density centrifugation. Pli 110 in postnuclear supernatants was found to sediment with high density particles suggesting that it may be associated with macromolecular complexes. Purification to homogeneity was acheived by a combination of subcellular fractionation, ion exchange chromatography and SDS polyacrylamide gel electrophoreses.

As a second, independent line of investigation we have characterized binding sites for the prion protein in normal and scrapie-infected brain. Binding of radiolabeled PrP 27-30 was strongest to granule and pyramidal cells of the hippocampal formation. Competition with unlabeled PrP 27-30 revealed a K_i of 2 nM. A synthetic peptide (designated P5) corresponding to the central portion of the prion was able to reproduce the binding pattern observed with PrP 27-30. Peptide P5 bound with a similar affinity and to the same sites as intact PrP 27-30. Binding sites seem to be mostly localized over cell bodies or proximal dendrites. From previous work it is known that PrP is axonally transported and located at the synapse leading to the conclusion that PrP may serve a signaling function at the synapse.

[1] Oesch et al., Biochemistry 29, 5848

512.5

ASTROCYTOSIS AND PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN BRAIN OF SCRAPIE-INFECTED ANIMALS. X. Ye* and R.I. Carp. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York, U.S.A.

Scrapie is the archetype unconventional slow infection disease. PCNA is a 36-kd, acidic, non-histone, delta accessory protein of the DNA polymerase associated with cell proliferation. One issue is whether the astrocytosis seen in scrapie is a function of an increase in reactivity, or, if there is actual replication of astrocytes. In the current study, we used antibody to PCNA as a determinant of cell replication. Female, weanling Syrian hamsters, strain LVG/LAK, were injected intracerebrally with scrapie strain 139H or 263K or with normal hamster brain homogenates. Paraffin sections from scrapie and control brains were examined with PCNA immunostaining (ir-PCNA). We observed that the intensity of ir-PCNA in hippocampus, thalamus, hypothalamus and cortex of 139H- and 263K-infected hamsters was greater than in controls. Furthermore, by using double-staining analysis, we found that PCNA and glial fibrillary acidic protein were co-localized in brain cells. Our results suggest that the astrocytosis seen in scrapie-infected animals is, at least in part, due to actual replication of astrocytes in these animals.

512.6

CORTICAL NEURONAL LOSS IN ASYMPTOMATIC CATS INFECTED WITH FELINE IMMUNODEFICIENCY VIRUS (FIV). B.A. Thiede, R.B. Meeker* and C. Hall, Dept. Neurology, Univ. North Carolina, Chapel Hill, NC 27599 and R. English and M. Tompkins, Dept. of Microbiology, Parasitology and Pathology, School of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606.

In post mortem studies of humans infected with human immunodeficiency virus (HIV), loss of large cortical neurons has been described which may, in part, explain the cognitive deficits of AIDS dementia. Feline immunodeficiency virus (FIV) produces an HIV-like pattern of disease in cats and has recently been shown to contribute to neuronal excitotoxicity in vitro. To evaluate if adult cats infected with (FIV) show early neurodegenerative changes which mimic the neurodegenerative changes seen in humans, we examined cortical cell densities in seven asymptomatic specific pathogen free cats three years after inoculation with FIV. Cell density in cortical layers II-V, was evaluated for small, medium and large cells and compared to cell densities measured from the brains of six cats infected with FeLV. A 15-44% decrease in the number of large neurons in the frontal cortex was found. The largest decreases in neuron density correlated with low CD4:CD8 ratios. Neuronal loss was also evaluated in two FIV-infected random source cats and compared to two random source controls. A 30-34% decrease in large cell density was found. This pattern indicates that FIV induces similar neurodegenerative changes as HIV and that these changes may begin to appear in relatively early stages of infection, before the appearance of overt behavioral or neurological symptoms.
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512.7

ANTI-MYELIN BASIC PROTEIN ANTIBODY IN PEDIATRIC AIDS PATIENTS. K.M. Weidenheim*, M. Tricoche, A. Rubinstein and W.D. Lyman. Department of Pathology (Neuropathology) and Pediatrics (Immunology), Albert Einstein College of Medicine, Bronx, NY 10461.

Infection with human immunodeficiency virus type 1 (HIV-1) is associated with progressive neurologic impairment. Characteristic white matter changes which are thought to contribute to the neurological problems include a generalized decrease in myelin, leukoencephalopathy, vacuolar myelopathy and corticospinal tract degeneration. Corticospinal tract degeneration is important in pediatric AIDS because it correlates with the progressive motor dysfunction observed clinically, and the clinically observed cognitive dysfunction may correlate with injury to hemispheric white matter during HIV-1 infection. Anti-myelin antibodies have been shown to damage white matter in other disease states, and antibodies to myelin basic protein (MBP) have been identified in adult patients with AIDS. However, anti-myelin antibodies have not yet been identified in children with HIV-1 infection. We have developed an ELISA technique which can detect anti-MBP antibody in microliter quantities of human serum. Serum samples from 4 pediatric AIDS patients with neurologic symptoms were analyzed using this system. ELISA plates coated with human MBP were incubated with test sera from 4 pediatric AIDS patients with neurologic disease. Antibody binding was detected using an ABC technique. Anti-MBP-antibodies were identified in 2 patients. Pre-absorption of sera with MBP decreased antibody reactivity. This suggests that the antibody was MBP-specific. Both children with anti-MBP antibodies had motor dysfunction, and one had neuropsychologic impairment. These results suggest that anti-MBP antibodies are present in the serum of some HIV-infected children neurologic symptoms. These antibodies may contribute, in part, to the white matter pathology observed in pediatric AIDS. Support: USPHS MH47667, MH46815.

512.8

FETAL BRAIN ACCUMULATES GP120 SYSTEMICALLY ADMINISTERED TO PREGNANT MICE. J. Y. Wu,* T. W. Moody, D. E. Brenneman and J. M. Hill, Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892; BPRB, NCI, NIH, Rockville, MD 20850.

Pediatric AIDS is often perinatally acquired and frequently accompanied by extensive neurological damage despite low levels of HIV-infected cells. The HIV envelope protein, gp120, has been shown to be toxic to neurons in culture (*Nature* **335**:639,1988) and to induce neuronal dystrophy and behavioral retardation (*Brain Res.* **603**:222,1993). The present study was designed to determine if gp120 could be transmitted from the maternal circulation to the fetal brain during pregnancy. ^{125}I-gp120 was injected into the tail vein of mice during the 16th day of pregnancy, and fetuses were examined after 1, 24, 48 and 72 hours. The extracts of fetal brain and body were analyzed with gel filtration chromatography. The fraction co-eluting with stock gp120 is shown below in % of total CPM ± S.E.M. eluted:

	1 hr	24 hrs	48 hrs	72 hrs
Brain	1.79±0.36	17.34±4.07	46.97±11.41	27.20±3.58
Body	0.56±0.11	2.29±0.56	4.48± 0.48	4.71

Small amounts of radiolabelled material were found in fractions corresponding to the neurotoxic gp120 fragments (*Brain Res.* **603**:222,1993). After 2 days, approximately 0.01% of the radioactive gp120 injected into a mother appeared in the brain of one fetus. This study has shown that: 1) gp120 crossed the placental barrier; and 2) although gp120 levels increased in both the fetal brain and body with time, gp120 was preferentially sequestered in the brain. These data suggest that the neurological damage occurring in pediatric AIDS may be due, in part, to maternal to fetal transmission of gp120.

512.9

IN VIVO DEVELOPMENT OF HIV INFECTED HUMAN BRAIN GRAFTS. C.L. Achim, P. Sarnacki, E. Bonaroti, G. Wang, R. Hammond and C.A. Wiley*, Div. of Neuropathology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Approximately one quarter of AIDS patients develop severe HIV encephalitis with abundant macrophage infiltration and diffuse neuronal damage. Immune factors secreted by activated/infected CNS macrophages may be mediators of HIV associated neurologic damage. To test this hypothesis we developed an experimental model based on grafting HIV infected human neural cells into the brain of SCID mice. In this model, fetal brain cell aggregates grown in vitro are co-cultured with human macrophages infected with the neurotropic HIV strain YU-2. Neural cell aggregates infiltrated by infected macrophages are transplanted into the brain of SCID mice. In this in vivo environment synaptic formation can be studied and the perturbations caused by addition of HIV infected macrophages can then be temporally monitored. By immunolabeling and laser confocal imaging, in vitro cultures of human brain cells show abundant neuronal survival and differentiation. By EM, neuritic and synaptic formation can be detected in long term cultures. In vivo pilot studies suggest that brain microspheres injected into the fat pad of SCID mice survive for several months. Study of these grafts shows presence of functional neural cells and vascular organization suggesting a blood brain barrier. Grafts with HIV infected macrophages contained multinucleated giant cells and virus. Based on these findings we conclude that transplantation of developing human fetal brain grafts into SCID mice is a feasible model of HIV associated neurologic damage.
(CLA is a Pediatric AmFAR Scholar, 77241-16-PF)

512.10

HIPPOCAMPAL PATHOPHYSIOLOGY DUE TO LYMPHOCYTIC CHORIOMENINGITIS VIRAL INFECTION Pearce, B.D.*, Baldridge, J.R., Steffensen, S.C., Henriksen, S.J. and Buchmeier, M.J., Scripps Research Institute, La Jolla, CA 92037

Lymphocytic choriomeningitis virus (LCMV)-infected rats develop ataxia and distinct retinal, hippocampal and cerebellar neuropathology. We evaluated the pathophysiology of LCMV hippocampal tropism by immunohistochemical staining for viral antigen and by electrophysiological examination of evoked potentials and electroencephalographic (EEG) recordings from the dentate gyrus and CA1 hippocampus of halothane-anesthetized Lewis rats. Animals were inoculated intracerebrally with LCMV at postnatal day 4. Although cleared of infectious virus by post-inoculation day 21, viral antigen was detected in the dentate granule cell layer and hilus through day 41 but was usually absent at 80-90 days. In the dentate gyrus, granule cell loss was extensive (approximately 50 %). In Ammon's horn, no viral antigen nor cell loss was observed in any subfield. Hippocampal evoked potentials were elicited by stimulation of corresponding monosynaptic afferents to the dentate and CA1. While there were no differences in the temporo-spatial properties of the hippocampal field potential waveforms between groups, there was a site-specific suppression of GABA-mediated recurrent inhibition in the dentate gyrus of LCMV-infected rats. CA1 paired-pulse responses did not differ between groups. Hippocampal EEG activity was recorded by microelectrodes oriented in the dentate hilus and CA1 hippocampus and frequency spectra were generated from 4 sec EEG epochs. Age-matched uninfected rat EEG was characterized by episodes of high voltage spontaneous theta (4-6 Hz) alternating with low voltage mixed activity while LCMV-infected rat EEG was dominated by continuous theta. These findings demonstrate ongoing pathophysiology in LCMV-infected rats that persists long after clearance of infectious virus from the CNS. Early mediators of the immune response, excitotoxic factors and/or GABA dysfunction may contribute to the observed post-infection abnormalities in synaptic function.

512.11

PROTECTIVE EFFECT OF THYMOSIN PEPTIDES AND PHENOTHIAZINE NEUROLEPTICS ON SURVIVAL OF MICE TREATED WITH LETHAL DOSES OF ENDOTOXIN. M. Fagarasan*, M. Badamchian , and A. Goldstein, Department of Biochemistry and Molecular Biology, The George Washington University, Washington, DC. 20037

Pretreatment with phenothiazine neuroleptics (chlorpromazine, thioridazine, trifluoperazine), thymosin α_1 and thymosin β_4 protected the mice against the lethality induced by endotoxin. Endotoxin and protective compounds were administered i.p. in Swiss-Webster mice. We investigated the molecular mechanism involved in this effect. We determined that phenothiazine neuroleptics and thymosin peptides inhibit a cascade of pathogical mediators: free radicals, cytokines (interleukin-1 and tumor necrosis factor) platelet activating factor and arachidonic acid metabolites (6-keto-PGF1α and TXβ_2). The effect on free radical formation was determined by measuring blood lipid peroxidation and red blood cell glutathione levels. Serum cytokine levels were measured by ELISA and plasma concentrations of arachidonic and metabolites and platelet activating factor by RIA. Pretreatment of the mice with vitamin E, which is an antioxidant also increased survival against lethal doses of endotoxin. We concluded that free radicals are the first step in the cascade of toxic mediators induced by endotoxin. Phenothiazine neuroleptics and thymosin peptides by inhibiting free radical formation, inhibit this whole cascade which induces lethality.

512.12

ICAM-1 BLOCKADE ATTENUATES THE INCREASE OF REGIONAL CEREBRAL BLOODFLOW (rCBF) DURING THE EARLY PHASE OF EXPERIMENTAL BACTERIAL MENINGITIS. J.R. Weber, K. Angstwurm, K.M.Einhäupl, W. Bürger§, G.Ransmayr*#, U. Dirnagl. Dep. of Neurology, §Inst. of Microbiology Charité, Humboldt University, 10098 Berlin, Germany, #Dep. of Neurology, University of Innsbruck, Austria.

Intercellular adhesion molecule-1(ICAM-1), a cell surface receptor plays an important role in leucocyte endothel interaction in inflammatory disease. High levels of ICAM-1 can be measured in the CSF of patients with bacterial meningitis and provide evidence for a strong immune response, which may cause severe complications in spite of effective antibiotic treatment. We investigated the effect of systemic treatment with ICAM-1 antibodies on rCBF in male wistar rats. Meningitis was induced by intracisternal injection of pneumococcal cell wall components (equivalent of 10^7 cfu Streptococcus pneumonae PnR-527, Jena). rCBF was measured continuously over 6 hours with laser Doppler flowmetry. Rat ICAM-1 antibodies (TM8, Athena Neurosciences, Inc. San Francisco) were injected i.v. (1,5mg/kg) in three animals (n=3). In control animals saline was injected (n=4). There was an increase of rCBF in untreated animals 3 hours (mean=187% of baseline ± 47%SD) and a dramatic increase 6 hours after the induction of meningitis (mean=266% ± 59%). In the ICAM-1 antibody treated group the increase of rCBF was lower 3 hours (mean= 127% ± 15%) and significantly attenuated (mean=134% ± 19, t-test p=0,015) 6 hours after induction. The results demonstrate that ICAM-1 is involved in mediating rCBF changes associated with bacterial meningitis.

512.13

HERPES SIMPLEX VIRUS (HSV) INFECTION INCREASES NERVE GROWTH-ASSOCIATED PROTEIN (GAP-43/B-50) IMMUNOREACTIVITY IN RABBIT CORNEA AND TRIGEMINAL GANGLION (TG). R.E. Martin*[1], D.B. Henken[2], J.M. Hill[3]. [1]Univ. Oklahoma College of Medicine; [2] NIH, NINDS Lab. of Exp. Neuropathology; [3]Louisiana State Univ. Eye Center.

HSV type-1 infection (McKrae strain) is correlated with increased GAP-43 immunoreactivity in rabbit cornea during latency, 52 days post-inoculation (52 dpi). Here we determine GAP-43 immunoreactivity in cornea and TG during the acute stages of HSV-1 infection (5 dpi), in latency (84 and 154 dpi) and after reactivation by iv injections of cyclophosphamide and dexamethasone (Cx/Dx). Tissues were evaluated biochemically and histologically. Preliminary results indicated that, for all conditions, GAP-43 immunoreactivity was greater in cornea than in TG. During acute infection, immunoreactivity in cornea was 48% higher than in non-infected controls and no significant difference was seen in TG. During latency (84 dpi), increases were found in cornea (59%) and TG (119%). This effect was maintained at 154 dpi when cornea and TG demonstrated increased immunoreactivity over controls (36% and 104%, respectively). Reactivating latent virus with Cx/Dx at 152-153 dpi revealed higher GAP-43 in cornea and TG compared to controls (134% and 55%, respectively). The data suggest that establishing and maintaining HSV-1 latency are not silent events in these neurons. Supported by OU College of Medicine and NIH EY06311.

512.14

HERPES SIMPLEX VIRUS (HSV) INOCULATION BY A FOOTPAD ROUTE RESULTS IN INCREASED GROWTH-ASSOCIATED PROTEIN (GAP-43) IN MOUSE DORSAL ROOT GANGLIA (DRG) AND DORSAL ROOTS Henken, DB*[1], Martin, JR[1], Goldstein, ME[2], and Curtis, R[3] [1]LENP, [2]LNC, NINDS, NIH, Bethesda, MD, and [3]Regeneron Pharmaceuticals, Tarrytown, NY, USA.

To obtain evidence on neuronal sprouting following HSV infection in sensory ganglia, GAP-43, a protein induced by growing neurons, was examined. The hind footpads of mice were unilaterally inoculated with HSV type-2 strain MS ($9.3x10^6$ pfu/ml) or medium. At 0 (control), 5, 14 and 28 days post-inoculation (dpi), mice were fixed. Sections of decalcified spinal columns and DRGs, were immunoreacted for GAP-43. Reaction product was quantified in ipsilateral (infected) and contralateral (uninfected) lumbar 4th and 5th DRG and proximal dorsal roots using image analysis. At 14 dpi, there was a 4-fold increase in GAP-43 immunoreactivity in the ipsilateral DRG and 8-fold increase in dorsal root. Both cell bodies and fibers were GAP-43-positive. At 28 dpi, GAP-43 was decreased in DRG, but sustained in dorsal roots. Contralateral tissues showed no increase in GAP-43, nor did ganglia or dorsal roots of mock-inoculated mice. Western analysis also showed induction of GAP-43 in dorsal roots. This result, together with selective neuropeptide alterations that we have previously shown, indicate that a sprouting response may be mounted in ganglia in reaction to herpesvirus infection.

512.15

MURINE NEUROPATHOGENIC ts_1 - RETROVIRUS REPLICATION IN MYOBLASTS AND ASTROCYTES IN VITRO T. Powers, P. Stanko, A.J. Goldman, J. Vann, P. Szurek, A. Waclawik, B.R. Brooks*. Dept. of Neurology, UW Medical School, Madison, WI 53792 and Neuromuscular Research Laboratory VAMC Madison, WI 53705

The pathogenesis of the neurodegenerative disease caused by ts_1 retrovirus is not fully understood. This virus can only cause spinal cord degeneration during postnatal development and not in adult mice. ts_1 retrovirus replication in affected animals occurs to very high levels in spleen, CNS and muscle tissue. It is unknown what the role of the replication of ts_1 retrovirus in muscle tissue is in the spinal cord degeneration caused by this virus. We are characterizing this model to determine whether both muscle and CNS infection are required to produce disease. Integrated and unintegrated viral specific DNA forms are present in various tissues post infection. A novel low molecular weight, unintegrated virus specific DNA is seen in CNS tissues but not spleen or muscle.

We studied the replication of ts_1 retrovirus in differentiated astrocytes and muscle cells (CRL 1772) from C3H mice. Virus titers in supernatants from infected myoblasts ($2x10^7$/ml) were of the same magnitude as seen following infection of astrocytes (1-10 x 10^6/ml).

Virus specific DNA species in infected cells were characterized by Southern blot hybridization techniques at various times after infection. Cellular specific gene (actin, glut-1) expression was compared with virus specific gene (env) expression. No new low molecular weight virus specific DNA species have been identified following ts_1 retrovirus infection in vitro. [Neurology 1994;44(Suppl 2):A396]

512.16

A PCR-BASED ANALYSIS OF HSV-1 LATENCY IN THE TRIGEMINAL GANGLION OF THE RAT. D.J. Fink*, R. Ramakrishnan and M. Levine. Departments of Neurology and Human Genetics, and GRECC, VAMC, University of. Michigan, Ann Arbor, MI 48105.

Competitive quantitative PCR and RT-PCR was used to quantitate DNA and RNA from an attenuated ribonucleotide reductase (RR)-deleted herpes simplex virus type 1 (HSV-1) mutant in the rat trigeminal ganglion after peripheral inoculation following corneal scarification. Amplification of ganglionic DNA with oligonucleotide primers specific for the HSV gB gene and for the LAT gene indicated that there were approximately 2 x 10^5 genome equivalents per ganglion at 2, 7 and 56 days after inoculation. Amplification of ganglionic RNA with primers specific for HSV LAT indicated that there were approximately 1 x 10^7 LAT molecules per ganglion at 2 and 7 days post inoculation, and 1.4 x 10^7 LAT molecules per ganglion at 56 days. *In situ* hybridization with a digoxigenin-labeled riboprobe specific for LAT detected an average of 1-2 LAT positive cells in each positive 6 micron section of trigeminal ganglion. *In situ* PCR detection of HSV genomes in similar sections, using digoxigenin-labeled nucleotides with primers specific for HSV gB, identified 27-150 genome-positive cells per section.

These results indicate that there are approximately 50 LAT molecules per latent HSV genome in the trigeminal ganglion, compared to 15 LAT molecules per latent HSV genome in the CNS (Ramakrishnan *et al., J. Virol.*, 1994), but that cells with detectable LATs by *in situ* hybridization represent only a small proportion of those ganglionic neurons containing HSV genomes. The presence of latent HSV genomes in a large number of neurons suggests that HSV may be more efficient in establishing the latent state than would be anticipated from previous reports.

512.17

IMPROVED BRAIN DELIVERY AND IN VITRO EFFICACY OF ZIDOVUDINE (AZT) USING A REDOX CHEMICAL DELIVERY SYSTEM. Y. Mizrachi, A. Rubinstein, Z. Harish, A. Biegon¶, W. Anderson*,§ and M. Brewster§. Div. of Allergy and Immunol., Albert Einstein College of Med., Bronx, NY 10461, §Pharmos, Corp. Alachua, FL 36215 and ¶Pharmos, Corp. Rehovot, ISRAEL.

Improved therapy for AIDS dementia and related encephalopathies may be achieved through enhanced delivery of effective anti-retroviral agents to the brain and CNS. One approach for attaining this goal is the chemical delivery system (CDS) which has been applied to a number of drugs including zidovudine (AZT). The current evaluations were aimed at further defining the pharmacokinetic profile of the AZT-CDS as well as establishing its in vitro antiviral efficacy against HIV in both lymphocytes and a neural cell line. AZT or AZT-CDS at a dose of 130 μmol/Kg was administered to Sprague Dawley rats (i.v., tail vein) using a cyclodextrin-based vehicle. The AZT-CDS produced significantly higher brain levels of AZT (AUC = 425 μg•min/g) than did equimolar AZT dosing (AUC = 13.5 μg•min/g). In addition, blood levels were reduced (AZT from AZT-CDS, AUC = 583 μg•min/mL; AZT from AZT, AUC = 1580 μg•min/mL). In culture systems (CEM lymphocytes and a SKNMC neuroblastoma cell line), AZT uptake after co-incubation of cells with AZT for 1.0 hour was minimal. By contrast, addition of the AZT-CDS to tissue cultures resulted in large increases in the uptake of the AZT-CDS followed by biochemical conversion of the CDS to its main metabolites (the AZT-CDS quaternary salt, AZT-Q^+, as well as AZT itself). These improved uptake profiles were associated with greater in vitro efficacy. Thus, AZT-CDS at 0.5 μM was 80% more effective than AZT in suppressing p24 production (at day 3) in a lymphocyte culture infected with 6000 $TCID_{50}$'s of the N1T strain of HIV and 50% more effective at 0.05 μM. Furthermore, syncytia formation was completely suppressed at an AZT-CDS dose of 0.5 μM (600 $TCID_{50}$'s) while AZT at the same dose was ineffective. Finally, while AZT (at 0.5 μM) is not active in reducing viral replication in an SKNMC neural cell line, the AZT-CDS provided for significant reductions in p24 expression.

MENTAL ILLNESS: SCHIZOPHRENIA II

513.1

KINDLING WITH CLOZAPINE. J.R. Stevens* and D.D. Denney. Depts of Neurology & Psychiatry, Oregon Health Sciences University, Portland OR 97201,

The atypical neuroleptic clozapine improves symptoms and behaviors of many patients with schizophrenia who have failed to respond to other neuroleptics. In addition to blockade of central nervous system monoamine, muscarinic and other receptors, clozapine often causes bilateral spike-wave activity in the EEG and generalized seizures occur in 3-5% of patients treated with the drug. Acute administration of clozapine in rats induces dose related myoclonic jerks. Although considered an undesirable side effect of clozapine, this evidence for epileptogenicity could relate to the therapeutic effect of this drug. The experiments reported here demonstrate that repeated fixed sub-convulsive doses of clozapine (1 mg/kg) that initially caused no behavior change, caused seizure-like activity manifest as myoclonic jerks (MJs) after the third injection of clozapine, and reached 150 MJs/hour by the 15th injection. These results are consistent with *kindling*, i.e., a progressive sustained increase of brain excitability following repeated administration of a fixed subconvulsive dose of a proconvulsive agent. If excitation of neurons in critical brain areas is an important aspect of the therapeutic effect of clozapine and other neuroleptics, *kindling* with repeated fixed low dose administration of clozapine and related drugs might be effective clinically and should decrease undesirable side effects associated with conventional high dose treatment regimens.

513.2

FRONTAL DYSFUNCTION IN SCHIZOPHRENIA. J. Gold, B. Hermann, C. Randolph*, G. Chelune, M. Trenerry, D. Loring, T. Goldberg, D. Weinberger. NIMH Neuroscience Center at Saint Elizabeths, Washington, D.C. 20032

Prior neuropsychological and neuroimaging studies had implicated dysfunction of the frontal cortex in schizophrenia (SC). However, this hypothesis has not been examined directly using a between-patient group design. In this study, we contrasted the neuropsychological performance of 66 SC patients with that of 91 patients with frontal lobe epilepsy (36 with a left frontal seizure focus, 51 with a right frontal focus) who were drawn from 4 epilepsy surgery programs. The epilepsy patients had a mean age of onset of chronic seizures at age 12 and were refractory to conventional pharmacotherapy. All test data was obtained prior to any eventual surgical treatment. The three patient groups did not differ in age, education, or Full Scale IQ. The SC group performed significantly better than either frontal group on measures of semantic memory such as single word reading and vocabulary. The SC patients did not differ from either frontal group on measures of attention and motor speed, which we have previously found to distinguish SC from temporal lobe epileptic patients. SC patients performed significantly better than either frontal group on phonemic fluency, a task which does distinguish frontal from temporal lobe epilepsy patients, but significantly worse than the right frontal group on the Wisconsin Card Sorting Test. The data suggest the limitations of focal lesion models of schizophrenic cognitive impairment, and demonstrate differential impairment of frontal function in schizophrenia with maximal impairment in attention and working memory and a relative sparing of frontally mediated verbal fluency.

513.3

D2/D3 AND D4 RECEPTOR DENSITIES ARE NOT ALTERED IN RATS WITH NEONATAL HIPPOCAMPAL DAMAGE. Michael B. Knable, Angela M. Murray, Barbara K. Lipska, Farouk Karoum*, Daniel R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center, Washington, DC 20032.

Previous studies demonstrated that rats with excitotoxic ventral hippocampal (VH) lesions postpubertally develop DA-related behavioral changes. These effects were similar to some aspects of schizophrenia. We sought to determine if these behavioral effects were associated with changes in the distribution or number of striatal DA receptors. On postnatal day 7 (PD7), rats received infusions of ibotenic acid or vehicle into the VH. They were sacrificed on PD35 and PD56. Brain sections (20μm) were incubated with 1.0nM [3H]YM-09151-2 (containing 50nM 8-OH-DPAT). Adjacent sections were preincubated with 200μM Gpp(NH)p and incubated with 6.0nM [3H]raclopride. Butaclamol (10μM) was used to define non-specific binding in both assays. Mean optical density in the dorsolateral (DLS) and ventromedial (VMS) striatum, and nucleus accumbens (NAC) were converted to fM/mg of tissue. Specific raclopride binding was subtracted from specific YM-09151-2 binding to estimate D4 receptor density. There was decreased binding of YM-09151-2 and raclopride in both control and lesioned rats in DLS and VMS on PD56 compared with PD35. In contrast, D4 analysis did not show an age effect in DLS or VMS. There were no effects of lesion for either ligand or for the calculated D4 receptor density in any region. These results suggest that delayed hyperdopaminergic behaviors in rats with neonatal VH lesions are not mediated by abnormalities in density of striatal D2/D3 or D4 receptors.

513.4

C-FOS EXPRESSION IN RATS WITH A NEONATAL HIPPOCAMPAL LESION IS ALTERED AFTER AMPHETAMINE CHALLENGE

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A neonatal (day 7 after birth, PD7) ibotenic acid lesion (large size) in the ventral hippocampus results in postpubertal (PD56) onset of a variety of abnormal behaviors that have been related to corticolimbic and striatal dopaminergic dysfunction. A more restricted lesion (small) does not induce behavioral hyperactvity on PD56. To further characterize this potential animal model of schizophrenia, we studied the expression of c-fos mRNA in rats with two sizes of hippocampal lesions. C-fos has been shown to be induced by catecholaminergic stimuli. Rats were previously given amphetamine (AMP) (1.5 mg/kg) twice (on PD35 and PD56) and their motor activity was tested. C-fos mRNA expression using an oligonucleotide probe was assesed six weeks later 30 and 90 minutes after AMP (10 mg/kg) or saline. A significant effect of drug treatment was seen in the ventromedial (VMS) and dorsolateral striatum (DLS), in superficial and deeper bands of parietal cortex (SPC and DPC, respectively) as well as the medial prefrontal cortex (MPFC). C-fos expression was decreased in MPFC in rats with a large lesion 30 min after AMP, but not in the striatum. AMP produced a significant time effect in the DPC in rats with both lesions and in DLS after a large lesion. These preliminary results demonstrate that an early hippocampal damage alters the cortical c-fos response to AMP in adult rats.

513.5

NEONATAL EXCITOTOXIC HIPPOCAMPAL DAMAGE ALTERS DOPAMINE RESPONSE TO MILD CHRONIC STRESS AND CHRONIC HALOPERIDOL

Barbara K. Lipska, Stanislaw J. Chrapusta, Michael F. Egan, Richard J. Wyatt*, Daniel R. Weinberger. Clinical Brain Disorders Branch and Neuropsychiatry Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The effects of neonatal (day 7 after birth, PD7) ibotenic acid lesions of the ventral hippocampus (VH) on dopamine release in response to chronic stress (saline injections for 21 days) and haloperidol treatment (0.4 mg/kg for 21 days, i.p.) were investigated in adult (PD56) Sprague-Dawley rats. Tissue 3-methoxytyramine (3-MT) accumulation after MAO inhibition with pargyline measured by GC/MS was used as an index of dopamine release. At baseline (no injection), 3-MT was reduced in striatum (STR) of lesioned rats. Repeated saline injections resulted in a further 3-MT reduction in STR, nucleus accumbens (NAc) and frontal cortex (FC) in lesioned rats, and had no effect in controls. Chronic haloperidol, compared with vehicle, increased 3-MT accumulation in STR, NAc and FC in control and lesioned rats. The 3-MT increase was enhanced in FC and NAc of haloperidol-treated lesioned rats vs controls. These data show that a developmental lesion of VH alters dopamine responsivity to environmental (chronic mild stress) and pharmacological (chronic haloperidol) challenge.

513.6

STRAIN DIFFERENCES IN BEHAVIORAL ABNORMALITIES ASSOCIATED WITH NEONATAL EXCITOTOXIC HIPPOCAMPAL DAMAGE IN THE RAT

Ingrid Phillips, Barbara K. Lipska, Llewellyn B. Bigelow*, Daniel R. Weinberger, Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Neonatal ventral hippocampal (VH) lesions in male Sprague-Dawley (SD) rats do not affect dopamine related behaviors before puberty (35 days of age, PD35). After puberty (PD56), lesioned rats express a variety of behavioral disturbances indicative of increased mesolimbic/nigro-striatal dopamine function. In the present study, we compared the effects of VH damage in two genetically inbred strains of rats - Fisher 344 (F) and Lewis (LEW) with outbred SD rats. It was shown previously that LEW and F rats differ genetically in a number of ways, including stress responsiveness and preference for drugs of abuse, suggestive of different functional states of the mesolimbic dopamine systems. We demonstrate that the patterns of behavioral abnormalities following neonatal VH lesions differ markedly between the strains in terms of severity of disturbances and the age at which they appear. In SD VH lesioned rats, spontaneous and amphetamine-induced locomotion are enhanced at PD56, but not at PD35. In F and LEW VH lesioned rats, exaggarated hyperlocomotion appears early (PD35). More restricted lesions of VH result in the delayed onset of hyperlocomotion (PD56) in F rats, while in SD rats they do not affect locomotor behaviors on PD35 or PD56. These data suggest that the developmental lesion may interact with genetic factors involved in dopaminergic function. They may have implications for studying pathophysiology of schizophrenia in which structural hippocampal defects and genetic liability have been indicated.

513.7

QUANTIFICATION OF MESOPONTINE CHOLINERGIC NEURONS IN THE HUMAN BRAIN.

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Karson et al. (1991) reported that schizophrenics have significantly more NADPH-diaphorase-containing, putative cholinergic, pedunculopontine nucleus neurons than controls (Psychiat. Res.: Neuroimaging, 40:31-48). The purpose of the present study was to use a specific marker for cholinergic neurons, an affinity purified human antibody against choline acetyltransferase (ChAT), and computer imaging techniques to map the locations of cholinergic mesopontine neurons in the human brain. Four cases were collected from the Medical Examiners Office, 22-47 years of age. Cells were mapped on one side of the brain, in 50 μm thick horizontal sections, sampled from 0.5-0.8 mm intervals (n=7-10 sections/case), and stained with 1:200-2,000 antibody concentration. The cell counts were corrected for split-cell counting error. There was an average of 14,986 ± 1,593 neurons (mean ± SEM) (11,661-17,933) within the compact and diffuse sectors of the Ch5 region, and the Ch6 region (nomenclature of Mesulam et al. [J. Comp. Neurol., 281:611-633, 1989]). Future studies will determine whether differences in mesopontine cholinergic neuron numbers exist in schizophrenic cases.

513.8

EXPRESSION OF SYNTROPHIN IN THE BRAIN POSTSYNAPTIC DENSITY OF THE RAT, MDX MOUSE AND A DUCHENNE MUSCULAR DYSTROPHY PATIENT.

Kim, T.W.*[1,2], K. Wu[1,2], Y. Huang[3,4] and I.B. Black[1,2]. [1]Dept. Neurosci. and Cell Biol., UMDNJ/RWJ Med. Sch. and [2]Program in Physiol. and Neurobiol., Rutgers-The State Univ. of NJ, Piscataway, NJ 08854; [3]Div. Neurosci., NYSPI and [4]Dept. Phsychi., Columbia Univ., New York, NY 10032

Duchenne muscular dystrophy (DMD) is an inherited fatal disease affecting one in 3500 boys with progressive muscle degeneration. Although cognitive impairment is a common feature of DMD, mechanisms underlying mental changes are unknown. DMD is characterized by a defect in dystrophin, a 427 kDa protein that is located predominantly in muscle, and that has recently been detected in brain. We have previously found that brain dystrophin is a component of the postsynaptic density (PSD) and is absent in the *mdx* mouse, an animal model of human DMD. Recently, syntrophin, a 58 kDa dystrophin-associated protein, was reportedly concentrated at postsynaptic sites at mouse neuromuscular junction. One postulated function of syntrophin is involvement in clustering of acetylcholine receptors. Colocalization of syntrophin with dystrophin in muscle prompted us to examine the protein in the rat brain by focusing on the PSD. Our results, obtained by Western blot analysis using a specific antibody against syntrophin, revealed that brain syntrophin is an intrinsic component of PSD. Moreover, it is differentially expressed during cortical development and is expressed in a region-specific manner. Despite complete deficiency of the 427 kDa dystrophin in *mdx* mouse and dystrophic human brain, syntrophin was detectable but decreased in cortical PSDs from the *mdx* mouse and from one case of human DMD. Our results indicate that dystrophin and syntrophin are colocalized in the PSD, but are differentially expressed in the diseased state.

513.9

"PERINEURONAL NETS OF EXTRACELLULAR MATRIX" AROUND NERVE CELLS IN VARIOUS NEUROPSYCHIATRIC DISORDERS.

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"Perineuronal nets of extracellular matrix" (PNs) are observed around subpopulations of nerve cells in the adult brain and may control their interactions with surrounding cells. In the cerebral cortex, PNs are associated with interneurons containing the calcium-binding protein parvalbumin (Celio and Blümcke, Brain Res. Rev. 1994;19:128-145). In the present study, the distribution of PNs was analyzed in several areas of the cerebral cortex in the normal human brains and compared to cases affected by psychiatric (Alzheimer's disease, Pick's disease, Down syndrome and schizophrenia) and neurologic disease (multiple sclerosis, epilepsy). Brains were obtained at autopsy (4-24 hours postmortem delay). PNs were visualized using *Vicia villosa* lectin and chondroitin sulfate proteoglycan immunohistochemistry. No obvious changes in distribution and staining intensity of PNs were observed in Alzheimer's disease, Pick's disease, Down syndrome, epilepsy, and multiple sclerosis cases. However, a decrease of PNs around neurons was found in the cerebral cortex of schizophrenic cases. This finding may reflect the fact that disruption of the extracellular matrix or loss of certain components of PNs occur in schizophrenia. Such changes in PNs around select interneuronal cell classes such as parvalbumin-containing cells may cause functional alterations in this population of inhibitory neurons in schizophrenia.

513.10

PHARMACOLOGICAL CHARACTERIZATION OF THE BINDING OF [3H]YM09151-2 TO D4 DOPAMINE RECEPTOR SUBTYPES IN BASAL GANGLIA AND CORTEX OF SCHIZOPHRENICS AND CONTROLS.

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The identification of 5 distinct dopamine (DA) receptor sub-types has important implications for the DA hypothesis of schizophrenia. The D4 receptor is particularly intriguing because it binds clozapine with high affinity. Seeman (1993), subtracting the binding of [^{3}H]Raclopride (labels D2 and D3 receptors) from that of [^{3}H]YM 09151-2 (labels D2, D3 and D4 receptors) for identification of D4 receptors, demonstrated a six fold increase in D4 binding in schizophrenic putamen compared to the controls. We characterized the pharmacology of [^{3}H]YM 09151-2 binding and the density and distribution of D4 receptors using quantitative receptor autoradiography in the striatum, entorhinal and prefrontal cortex, in schizophrenic brains compared to normal controls. Adjacent sections were incubated with 1 nM [^{3}H]YM 09151-2, 1 nM [^{3}H]YM 09151-2 in the presence of 50 nM 8-OH DPAT (to block 5-HT1$_A$ receptors) or 6.0 nM [^{3}H]Raclopride. Butaclamol (10μM) was used to define non-specific binding. In the basal ganglia, schizophrenics had significant increases in D4 receptors in all regions (60-80%) labeled with [^{3}H]YM 09151-2 alone compared to controls. A similar trend was observed in the presence of 8-OH-DPAT, although the variability in binding was increased. No significant changes were observed in entorhinal cortex in schizophrenics. However, 8-OH-DPAT significantly decreased [^{3}H]YM 09151-2 binding (>50%) in the subiculum and entorhinal cortex compared to [^{3}H]YM 09151-2 alone. This suggests that [^{3}H]YM 09151-2 binds to 5-HT1$_A$ receptors in cortical regions.

513.11

RESPONSE OF NEUROTENSIN SYSTEMS SUGGESTS THAT THE 5HT-2 ANTAGONIST, MDL 100,907, HAS CLOZAPINE-LIKE ANTIPSYCHOTIC EFFECTS. G.R. Hanson*, L.G. Bush, J.W. Gibb, and C.J. Schmidt. Dept. Pharmacol. and Tox., University of Utah, Salt Lake City, UT 84112 and Marion Merrell Dow Inc., Cincinnati, OH 45215.

Drugs such as haloperidol (HA) are classified as *typical* antipsychotics because of their prominent extrapyramidal side effects and their lack of effectiveness against the negative symptomology of schizophrenia. In contrast, drugs like clozapine (Cloz) are considered *atypical* antipsychotics because of their lack of extrapyramidal effects and their ability to relieve schizophrenia-related negative symptoms. Although the difference between typical and atypical antipsychotics is not known, the antagonistic effects of Cloz on 5HT-2 receptors, an effect not shared by HA, suggests that a serotonergic mechanism may be important. Based on this hypothesis, the highly selective Marion Merrell Dow 5HT-2 antagonist, MDL 100,907 (MDL), may have atypical antipsychotic effects. This was tested by comparing the responses of caudate and nucleus accumbens neurotensin (NT) systems (a neuropeptide dramatically altered by antipsychotic drugs) to 1, 2 and 4 doses of HA, Cloz and MDL. These studies revealed that the pattern of changes caused by MDL in neurotensin-like immunoreactivity in 6 caudate regions was much more like the effects of Cloz than HA. In contrast, the responses of the NT systems in the anterior and posterior nucleus accumbens were very similar for all three drugs. We conclude from these findings that (1) antagonism of 5HT-2 receptors plays a critical role in the mechanism of atypical antipsychotics, (2) the MDL compound has atypical antipsychotic effects like Cloz and (3) 5HT-2 receptors contribute to the regulation of both caudate and accumbens NT systems. (This work was supported by Marion Merrell Dow Inc. and USPHS grants DA 04222 and DA 00869.)

513.12

Reduced striatonigral activity related to vacuous chewing movements induced by acute haloperidol treatment: a possible model for dystonia. J. N. Ferguson, M. F. Egan, S. E. Bachus, T. M. Hyde, R. J. Wyatt, D. Grega*#, J.E. Kleinman, NIMH at St. Elizabeths, 2700 M.L.King Ave. S.E., Wash., D.C., 20032 and #Boehringer Mannheim, Indianapolis, IN, 46250.

Long term haloperidol treatment produces a syndrome of vacuous chewing movements (VCMs), a putative model of tardive dyskinesia. Increased striatal dynorphin and enkephalin mRNA levels in rats with VCMs suggest overactivity of both D1 mediated striatonigral and D2 mediated striatopallidal pathways. Several studies have indicated that short term intraperitoneal (i.p.) injections of haloperidol can also produce VCMs, raising doubts about the validity of the VCM model. To investigate this issue, we measured mRNA for dynorphin and substance P co-localized in D1-mediated striatonigral neurons. Rats received i.p. haloperidol or vehicle (0.4 mg/kg/d) for three weeks. Significant increases in VCMs were seen shortly after the initiation of treatment. Using in situ hybridization, we found reduced dynorphin and substance P mRNA levels in the dorsolateral and ventromedial striatum in haloperidol treated rats compared to the control group. Substance P mRNA was also reduced in the nucleus accumbens. These findings indicate that "acute VCMs" induced by short term i.p. haloperidol are related to reduced D1-mediated striatonigral activity. "Acute VCMs" appear to be neurochemically distinct from VCMs induced by long term haloperidol treatment, and may be a model for acute neuroleptic-induced dystonia.

513.13

Effects of D1 antagonist SCH23390 on vacuous chewing movements and striatal neuropeptide mRNA following long term haloperidol decanoate M. F. Egan, S. E. Bachus, T. M. Hyde, J. N. Ferguson, R. J. Wyatt, J.T. Noga*, J. E. Kleinman, NIMH at St. Elizabeths , 2700 M.L.King Ave. S.E., Wash., D.C., 20032.

Long term haloperidol treatment produces a syndrome of vacuous chewing movements (VCMs) in rats , a putative model of tardive dyskinesia. Increased striatal dynorphin and enkephalin mRNA levels in rats with VCMs suggest overactivity of both D1 mediated striatonigral and D2 mediated striatopallidal pathways. We attempted to reduce VCMs using the D1 antagonist SCH 23390 (0.5 mg/kg s.c. twice daily for three weeks) in rats treated previously with haloperidol decanoate (n=34) or vehicle (n=13) for 7 months and withdrawn for 5 months. Paradoxically, SCH 23390 markedly increased VCMs in both haloperidol (12.5 vs. 22.5 VCMs/2min) and vehicle (0.5 vs. 6.3 VCMs/2min) treated groups. Activation of striatonigral and striatopallidal pathways was assessed using in situ hybridization for enkephalin, dynorphin, and substance P mRNA. Increased VCMs from SCH 23390 treatment were associated with elevated enkephalin, dynorphin and substance P mRNA in the dorsolateral and/or ventromedial striatum. These results demonstrate that SCH 23390 increases D1 mediated striatonigral and D2 mediated striatopallidal neuropeptide gene expression during long term neuroleptic treatment. Furthermore, they suggest that increased activation of both pathways underlies increased frequency of VCMs.

513.14

IMAGING CEREBRAL BLOOD FLOW IN SCHIZOPHRENICS BY SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY WITH TECHNETIUM-99M-HMPAO. Jin-Sook Cheon*. Department of Neuropsychiatry, Kosin University, School of Medicine, Pusan 602-702, South Korea.

The single-photon emission computed tomography(SPECT) is an unique method to monitor the regional cerebral blood flow three dimensionally in clinical field. In psychiatry, it has a limited value to be applied to the research field rather than the clinical purpose. This is the first study of regional cerebral blow flow mapping in twelve schizophrenic patients using 99mTc as a radiotracer in Korea. Most of the SPECT results except one revealed hypoperfusion pattern(91.7%). The most prevalent loci of the lowest perfusion were bilateral parietal areas($P<0.05$), next, left parietal, left temporal and bilateral frontal, and then cerebellum, and the last basal ganglia and thalamus. Comparing a spectrum produced by enlisting eight cases of a neurosis, a major depression with psychotic features, a schizotypal personality disorder, a residual schizophrenia, subchronic paranoid schizophrenia with acute exacerbation, subchronic paranoid schizophrenia without acute exacerbation, chronic undifferentiated schizophrenia with acute exacerbation, chronic undifferentiated schizophrenia without acute exacerbation in decrease order of perfusion, the cerebral blood flow changes tended to be related with the intensity of psychotic symptoms and duration of illness.

513.15

SMOOTH PURSUIT EYE MOVEMENTS IN PARENTS OF PROBANDS WITH SCHIZOPHRENIA: AN OBLIGATE CARRIER APPROACH. R.G. Ross*, J. Harris, C.M. Cullum, L.M. Rilling, A.D. Radant, L.E. Adler, and R.F. Freedman. Schizophrenia Research Center, Univ. of Colorado Health Sci. Center, Denver, Colorado, 80262.

Smooth pursuit eye movement (SPEM) abnormalities have been found in patients with schizophrenia and in a large percentage of their nonschizophrenic relatives, suggesting that SPEM abnormalities may be an inherited marker of a biologic risk for schizophrenia. This study used infrared oculography and computerized pattern recognition software to study SPEM during a 16.7°/second constant velocity task. Subjects were 7 families with (a) schizophrenic probands, (b) both biological parents available for study, and (c) where only one of the biologic parents had a positive family history for schizophrenia (making that parent the "obligate carrier" for any inherited risk for schizophrenia).

Although 7/14 (50%) of parents had abnormal SPEM (smooth pursuit gain less than 0.85), parents with and without positive family histories did not differ in smooth pursuit gain (t=0.26, n.s.). Sources of error in SPEM include (1) deficiency at accurately tracking the target (requiring "catchup" saccades as compensation) and (2) problems with anticipating target movement (and having an "anticipatory" saccade intrude upon accurate tracking). Comparison of parents with and without a positive family history for schizophrenia demonstrated no difference in SPEM error due to catchup saccades (t=0.40, n.s.), but a significant difference in SPEM error due to anticipatory saccades (t=2.35, p<.04).

Although SPEM abnormalities are common in parents of schizophrenic probands, examination of the sources of error may be necessary to define the biologic marker of schizophrenic risk.

513.16

RAPID SMOOTH PURSUIT EYE MOVEMENTS AND THE ACOUSTIC STARTLE RESPONSE M.D. Brady, A.A. Freeman, E.L.F. Ho, T.L. Rawls and K.A. Young* Neuroscience Laboratory, Waco Veterans Administration Medical Center, 4800 Memorial Dr., Waco, Texas 76711

In eye movement desensitization therapy (EDT) for the treatment of post-traumatic stress disorder (PTSD), patients are instructed to concentrate on mental images which are associated with their stress response while making rapid smooth pursuit eye movements (rSPEM). Patients often report that after sessions of this therapy, they no longer experience the intense psychophysiologic and psuedopsychiatric responses previously generated by the target mental image. One psychophysiological response that is abnormal in many PTSD patients is the startle response. We investigated the possible relationship between rSPEM and the startle response in normal controls. Subjects made 30 rSPEM movements in a 60 sec period followed by 3 startle stimuli in the next 60 seconds. The pattern was repeated 6 times for a block (SPEM). A corresponding block had the same startle stimuli, but the SPEM target moved very slowly in the center of the subjects visual field (NoSPEM). Each subject was exposed to one of 2 treatment orders (NoSPEM-SPEM, SPEM-NoSPEM). Startle response amplitude, prepulse inhibition and within-session habituation was not significantly influenced by rSPEM movements. However, there was a significant interaction of block treatment order with startle amplitude changes between blocks, suggesting that rSPEM may have had a relatively subtle effect to suppress startle amplitude when the SPEM block preceded the noSPEM block. This experiment suggests that the mechanism of EMD therapy may be related to influences of rSPEM on basic neural processes responsible for abnormal psychophysiological responses in PTSD patients.

513.17

QUANTITATIVE MEASUREMENT OF SPEM IN SCHIZOPHRENIA USING THE SEARCH COIL TECHNIQUE. H.P. Goldstein and R.C. Alexander*. Thomas Jefferson University, Philadelphia, PA 19107

Impaired smooth pursuit eye movement (SPEM) in schizophrenia appears to be one of the most consistent biological correlates of schizophrenia. Most previous studies, however, have used non-quantitative methods which do not allow specification of the defects involved. In our study, 9 schizophrenic and 5 normal subjects underwent measurement of initiation and maintenance of smooth pursuit using the search coil technique. Eye movement targets were small spots of light projected onto a tangent screen 1 m in front of the subject and attention was maintained by asking subjects to detect periodic brightening of the target. For some trials involving horizontal target motion, the target was stabilized on the retina for 300 ms (open loop condition) so that the target would follow eye movements. Detailed quantification of eye movements showed considerable overlap between the gain and variability scores of the schizophrenic subjects relative to controls. Further, schizophrenic subjects successfully predicted changes in target direction in the open loop condition, indicating that they were able to construct an "internal model" of target movements.

513.18

IMPROVED REVERSAL LEARNING FOLLOWING L-NAME INHIBITION OF NOS ACTIVITY IN AN ANIMAL MODEL OF SCHIZOPHRENIA M. Lyon*, C. N. Karson, B. L. Freeman. Center for Brain and Behavioral Research, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

One animal model of schizophrenia (Lyon and McClure, 1994) includes abnormal behavioral effects as well as increased numbers of nitric oxide synthase (NOS)-containing cells in the brainstem (Nielsen et al., 1992), as also reported in schizophrenia (Karson et al., 1991). Such animals, exposed to maternal treatment with d-amphetamine during mid-pregnancy, are behaviorally more sensitive to increased NOS activity produced by nitroglycerin or to inhibition of NOS by NG-nitro-L-arginine methyl ester (L-NAME) (Lyon et al., 1994).

Offspring of Sprague-Dawley dams treated in mid-pregnancy with either 5.0 mg/kg d-amphetamine s.c. (AM), or saline s.c. plus the reduced food intake of AM dams (US), were used. N=19 AM, and N=18 US, young received either 20 mg/kg L-NAME or saline s.c. 5 minutes before holeboard foraging trials. Each animal was given 4 baits only in the right-side, or only in the left-side, holes in the field. Three training trials were followed by a right/left reversal trial, and then by extinction. RESULTS: Under saline, right/left lateral turning preference was significantly greater in AM animals than in US controls, and AM animals spent more time in far-field exploration. L-NAME reduced rearing and locomotion, but sharpened correct holepoking in both AM and US rats. L-NAME produced significant improvement in reversal learning in AM rats, which was not found in US animals nor under saline conditions. NOS inhibition by L-NAME may have significant therapeutic value for cognitive dysfunctions, including deficient reversal learning, which are found in schizophrenia.

513.19

NEUROANATOMICAL CORRELATES OF AN EVENT-RELATED POTENTIAL (ERP) INDEX OF AUDITORY SENSORY MEMORY IN SCHIZOPHRENIA. P.B. Ward, C. Loneragan, B. Liebert, S. V. Catts, P. T. Michie, S. Andrews, G. Dixon*, & N. McConaghy. Schools of Psychiatry and Psychology, University of New South Wales, Sydney, NSW 2052 & School of Behavioural Sciences, Macquarie University, NSW 2109 AUSTRALIA.

To investigate the functional significance of subtle neuroanatomical changes in temporal lobe neuroanatomy frequently reported in post-mortem and neuroimaging studies of schizophrenic patients, we recorded an ERP index of auditory sensory memory, termed mismatch negativity (MMN), from 15 DSM-III-R schizophrenic patients and age-, sex- and handedness-matched healthy controls. Patients were aged between 21 and 49 years (mean age 34.3 $\pm$ 8.2 years), and included 9 females and 6 males. MMN was elicited by infrequent 100ms tone pips interspersed among 50 ms standard tones during a visual distraction task. 3D volumetric MRI scans were obtained from all subjects, and Heschl's gyrus (HG) and planum temporale (PT) areas were measured on coronal slices using the BRAINS package (Andreasen et al, 1993). MMN amplitude at the Fz electrode was significantly reduced in schizophrenic patients, as was HG area, particularly in the left hemisphere. PT asymmetry (L>R) was significantly greater in male compared to female subjects, and there was a significant diagnosis by sex by side interaction for PT areas, reflecting significantly reduced right hemisphere PT areas in male schizophrenics. MMN amplitudes were significantly correlated with HG areas (rho = .43 to .67) in both controls and schizophrenics. Supported by the NH&MRC (Australia) and the Rebecca L Cooper Medical Research Foundation.

N.C. Andreasen et al, J Neuropsychiatry Clin Neurosci, 5, 121-130, 1993.

513.20

Regional Cerebral Blood Flow in Schizophrenia: Classification Using Pixel-Wise Discriminant Analysis. J.D. Van Horn*, K.F. Berman, and D.R. Weinberger Unit on PET, Neurosciences Center at St. Elizabeth's, 2700 Martin Luther King Jr. Ave. SE, Washington DC 20032

Regional cerebral blood flow (rCBF) differences between patients and controls have been widely reported as between-subject comparisons of normalized mean values derived from cognitive activation paradigms. Differences in activation between groups are often used to identify affected regions in the pathological subject sample. More recently, multivariate approaches have been applied to region of interest as well as pixel-based data (e.g. principle component analysis). Here we employed a pixel-based discriminant analysis approach to the comparison of schizophrenics and control subject rCBF that enables one to analyze two or more groups of subjects performing two or more tasks, maximizing the statistical difference between the groups and displaying the results as images. The advantages of this method include the ability to localize brain areas most responsible for variation between groups, while partitioning these differences into orthogonal images to reflect potential functional network differences. Additionally, the posterior probability of an individual subject's group classification may be measured to determine, in this case, whether or not he/she demonstrates a pattern of rCBF similar to controls or to schizophrenics.

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SYMPOSIUM. STRUCTURE, FUNCTION, AND REGULATION OF GLUTAMATE RECEPTORS. R.L. Huganir, HHMI, Johns Hopkins Univ., (Chairperson); P.H. Seeburg, Univ. of Heidelberg; M.L. Mayer, NIH-NICHHD; S. Tonegawa, HHMI, Mass. Inst. of Technology.

Glutamate receptors mediate excitatory synaptic transmission in the central nervous system and play critical roles in synaptic plasticity, neuronal development and neurological disorders. These receptors can be divided into two major classes: metabotropic receptors, which are coupled through G-proteins to intracellular second messengers; and ionotropic receptors, which are ligand-gated ion channels. The ionotropic receptors can be further subdivided based on their pharmacological and physiological properties into AMPA, kainate, and NMDA receptors. This symposium will review the recent advances in our understanding of the structure, function and regulation of these receptors and their role in brain function. Peter Seeburg will discuss studies on the molecular cloning of subunits for the AMPA, kainate, and NMDA glutamate receptors including recent studies on the role of RNA editing in the regulation of glutamate receptor function. Mark Mayer will present data comparing the physiological properties and allosteric regulation of native and recombinant AMPA and kainate receptors. Richard Huganir will discuss the role of protein phosphorylation of glutamate receptors in the regulation of their function and in certain forms of synaptic plasticity such as LTP and LTD. Finally, Susumu Tonegawa will present studies using homologous recombination to generate mutant mice to analyze glutamatergic synaptic function. Mutant mice that are deficient in models of synaptic plasticity such as LTP and LTD will be discussed.

516

SYMPOSIUM. GENERAL ANESTHETIC EFFECTS ON SOMATOMOTOR PROCESSING. P. Mason, Univ. of Chicago (Chairperson); J.G. Collins, Yale Univ.; J. Kendig, Stanford Univ.; E. Puil, Univ. of British Columbia.

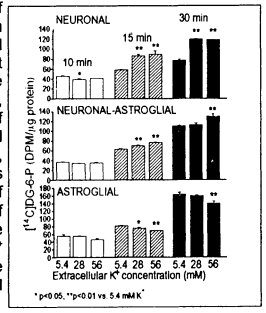
This symposium will highlight our current understanding of how general anesthetics affect excitatory and inhibitory neurotransmission in specific neuroanatomical sites important in somatomotor processing. The relative contributions of actions within the spinal cord, brainstem and thalamus to the somatic unresponsiveness observed during general anesthesia will be outlined. The symposium's aim is to increase our understanding of how anesthetics produce surgical immobility while stimulating further investigations that address this important question. **Collins** will describe the effects of diverse general anesthetics on the evoked responses and receptive field characteristics of spinal dorsal horn cells. These findings will be discussed with reference to the loss of sensation associated with general anesthesia. **Kendig** will review the effects of a variety of anesthetic agents on receptor-specific spinal reflex pathways. Particular attention will be paid to variations in the importance of $GABA_A$ effects among agents and to the extent to which actions on a single receptor can account for somatic unresponsiveness. **Mason** will describe the effect of general anesthetics on pain modulatory neurons, and propose mechanisms that may account for the observed changes in neuronal firing. Predictions will be made of how these brainstem actions may affect somatomotor processing within the spinal cord. **Puil** will discuss the effect of inhalational anesthetics on neurons in the rat ventrobasal thalamus. Anesthetic blockade of the transitions to the thalamic firing modes characteristic of specific behavioral states may serve to interrupt somatosensory perception.

518.1

EFFECTS OF EXTRACELLULAR POTASSIUM ION CONCENTRATION ON RATES OF PHOSPHORYLATION OF DEOXYGLUCOSE IN CULTURED ASTROGLIA AND NEURONS.

S. Takahashi, B.F. Driscoll, M.J. Law and L. Sokoloff*, Lab. of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20892.

The effects of increasing extracellular K^+ concentration on glucose metabolism in primary cultures of rat astroglia and neurons were examined. Cells were incubated with bicarbonate buffer containing 2 mM glucose, tracer amounts of 2-deoxy-D-[^{14}C]glucose ([^{14}C]DG), and various concentrations (5.4, 28, 56 mM) of K^+ for 10, 15, or 30 min followed by a 5 min incubation in [^{14}C]DG-free buffer for an efflux of unphosphorylated [^{14}C]DG. Cells were digested and assayed for labeled metabolites. K^+ caused significant elevations in rates of phosphorylation of [^{14}C]DG in both neuronal and neuronal-astroglial mixed cultures at 15 and 30 minutes, but not in astroglial cultures (Fig.). Veratridine (75 μM), which opens Na^+ channels, significantly raised rates of phosphorylation in astroglial and neuronal cultures (132 and 116%, respectively), and these elevations were completely blocked by 1 mM of ouabain, a specific inhibitor of Na^+,K^+-ATPase. These results indicate that elevated extracellular K^+ concentration does not stimulate energy metabolism in cultured astroglia.



518.2

DIRECT MEASUREMENT OF INTERSTITIAL GLUCOSE AND LACTATE IN THE CONSCIOUS HUMAN BRAIN. E. Wallace, D. Maggs, S.S. Spencer, D. Spencer, C.C. Duncan*, R.S. Sherwin and M.J. During. Departments of Medicine and Surgery, Yale School of Medicine, 330 Cedar St., New Haven CT 06510.

Estimates of brain glucose and lactate can be made by NMR spectroscopy. However, the actual concentrations of these metabolites in human extracellular fluid (ECF), and their relationship to fluctuations in the vascular compartment are unknown. To address this issue, we used microdialysis to monitor glucose and lactate concentrations in human brain ECF, while manipulating plasma glucose (glucose-insulin clamp technique). Methods: Microdialysis catheters were placed bilaterally in the hippocampi of subjects (n=4) undergoing intracerebral depth electrode monitoring for intractable epilepsy. Studies were carried out 3 days after surgical placement, subjects had been seizure free at least 3 days. Measurements were made from both epileptogenic and non-epileptogenic hippocampi. Arterialized plasma and dialysate, glucose and lactate levels were measured sequentially under plasma glucose conditions of fasting (5.5+/-0.5mmol/l), hyperglycemia (11.5+/-0.5 mmol/l) and hypoglycemia (3.0+/-0.5 mmol/l). Absolute glucose ECF concentrations were measured by net flux calculation. Results: At each level of plasma glucose, brain ECF glucose concentrations were substantially lower than concurrent plasma levels (~30%). In addition ECF glucose reached equilibrium 30-40 mins after plasma glucose. In comparison fasting brain ECF dialysate lactate levels were higher than fasting plasma lactate measurements (~x2), and did not change with plasma glucose. Conclusions: 1. Neurons and glia are consistently exposed to glucose concentrations that are substantially lower than those of arterial blood. 2. Brain ECF glucose shows a delayed response to changes in plasma glucose. 3. The marked glucose gradient, from blood to brain ECF, implies that neuronal metabolism may be unexpectedly vulnerable to even small decrements in glucose levels, and that, in this context lactate might serve as an alternative fuel source.

518.3

MECHANISMS UNDERLYING THE INTRINSIC SIGNAL DURING OPTICAL IMAGING OF RAT SOMATOSENSORY CORTEX M. M. Haglund*, D. W. Hochman, J. R. MENO, A. G. NGAI, AND H. R. WINN. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Optical imaging of intrinsic signals has been accomplished *in vitro* in hippocampal slices (MacVicar & Hochman, 1991) and *in vivo* in monkey visual cortex (Frostig et al.,1990). The source of the *in vitro* signal is primarily due to postsynaptic activation and cellular swelling, while the *in vivo* signal from monkey visual cortex is dominated by blood volume changes and oxygen delivery from capillaries.

In rat sensory cortex, sciatic nerve stimulation elicited localized cortical potentials (N1-20 msec; P2-35 msec) that correlated with a 25-50% dilation of the pial arterioles feeding the hindlimb cortex. Optical imaging of intrinsic signals showed changes in areas fed by pial arterioles that dilated. Cerebral blood flow/volume was uncoupled from increases in cortical activity by topically applying theophylline (10uM; A1/A2 adenosine antagonist), decreasing pial arteriole dilation by 70-90% and eliminating the P2 wave. Even though blood volume changes are highly sensitive to changes in arteriole diameter, the intrinsic signals from areas activated in controls increased in its spatial extent and magnitude in the presence of theophylline. Topical application of CPX (1uM; 8-cyclo-pentyl-1,3-dipropylxanthine; A1 antagonist) blocked the P2 wave and potentiated arteriole dilation, while intrinsic signals changes were similar to that found with theophylline.

Uncoupling the increases in blood flow/volume from increases in cortical activity did not effect the intrinsic signal changes. These findings suggest that changes related to blood flow/volume are not the main contributors of the intrinsic signal changes in our preparation.

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518.4

EXPLORING THE TEMPORAL BOUNDARIES OF FMRI: MEASURING RESPONSES TO VERY BRIEF VISUAL STIMULI. R.L.Savoy*, K.M.O'Craven, R.M.Weisskoff, T.L.Davis, J.Baker, B. Rosen. The Rowland Institute for Science, 100 Edwin H. Land Blvd, Cambridge, MA 02142; MGH-NMR Center, 149 13-th St., Charlestown, MA 02129

There is little doubt that fMRI will improve the *spatial* resolution of functional brain imaging; but can fMRI, on its own, yield sufficient *temporal* resolution to be of value in studying the timecourse of perceptual and cognitive processes? In the present study, we begin to address this question by looking at the response to very brief visual stimuli...stimuli of much shorter duration than the hemodynamic response to which fMRI is sensitive. The goals were two-fold. First, to see if such brief temporal stimuli would elicit measurable fMRI responses. Second, to examine the variability of response to temporally precise stimuli, especially the latency of response.

Three normal subjects participated in the experiment. We imaged a single 7mm oblique slice through the calcarine fissure of each individual using Gradient Echo imaging with a 1.5 T GE Signa modified by Advanced NMR, Inc. (Surface coil, TR=400, TE=50, Flip Angle=53°)

In a given run, we presented a full-field circular checkerboard for 1000ms, 100ms, or approximately 17ms. Stimulus onset was synchronized with the scanner. A bite bar minimized subjects' head movements both within and between runs. This combination permitted us to average 10 runs for each subject in each condition.

The stimuli of 1000msec duration yielded clear fMRI responses even on single runs. The responses to the briefer stimuli were apparent in the averaged data. In all three conditions, the response latency was consistent. An increase in the averaged fMRI signal occurred 2 seconds after stimulus onset. These experiments (and other experiments with TR=100) suggest a variability of the averaged response latency of not more than 300msec.

518.5

THE ROLE OF NEURONAL NITRIC OXIDE SYNTHASE (nNOS) IN THE CEREBRAL HYPEREMIC RESPONSE TO HYPERCAPNIA (HC) IN RATS. Q. Wang*, D.A. Pelligrino, V.L. Baughman and R.F. Albrecht. Dept. of Anesthesiol., Univ. of Ill.-Chicago, Chicago, IL 60612

NO has been shown to play a role in the cerebral vasodilatory responses (CVDRs) to a wide variety of stimuli, including HC. NO is produced by the action of NOS. There are 2 constitutive NOS isoforms in the brain, the endothelial (eNOS) and nNOS. Previous studies have used non-specific NOS inhibitors (e.g., nitro-L-arginine) to reveal NO influences, and found, in rats, that such agents attenuated the CVDR to HC ($PaCO_2$ = 60-80 mmHg) by 40-80%. However, the cellular source of that NO is not known. 7-nitroindazole (7-NI) is reported to be a selective inhibitor of the nNOS. In the present study, we monitored cortical cerebral blood flow (CBF) in rats, under normo- and hypercapnic conditions, using laser-Doppler flowmetry (LDF), in the presence and absence of 7-NI. To confirm that eNOS was unaffected by 7-NI, we assessed the CBF responses to the muscarinic acetylcholine agonist, oxotremorine (OXO), which activates eNOS exclusively. The experiments were performed on male Sprague-Dawley rats (~400g, n=9), anesthetized with fentanyl/70% N_2O and mechanically ventilated. Cortical CBF was measured via LDF. In all experiments, an initial CBF response to 5 min HC was obtained prior to 7-NI or vehicle (corn oil) injection. Three ml of the 7-NI solution (at 80 mg/kg) or vehicle were injected ip. CBF responses to 5 min HC were repeated at 30 and 60 min post-injection. After an additional 15 min, the CBF response to a 7 min iv infusion of OXO (1 μg/kg/min) was measured. At the end of the study, the brains were rapidly removed and the cortical tissue was frozen and subsequently analyzed for NOS activity. 7-NI inhibited brain NOS activity by 60% and reduced baseline CBF by 19%, but did not affect MAP or the CBF response to OXO. This confirmed that eNOS is unaffected by 7-NI. The CBF responses to HC at 30 and 60 min were identically attenuated (~50%) by 7-NI, when compared to controls. That reduction is within the range observed by us and others using non-specific NOS inhibitors. The results imply that a major portion of the NO released during HC is nNOS-derived. This presumably neuronally-released NO also contributes to the maintenance of basal CBF.

518.6

THE LOWER LIMIT OF CEREBRAL BLOOD FLOW AUTOREGULATION WITH NITRIC OXIDE SYNTHASE INHIBITION. S.C. Jones* and C.R. Radinsky. Cerebrovascular Res. Lab., Cleveland Clinic Found., Cleveland, OH 44195.

Current evidence indicates that nitric oxide is not involved in CBF pressure autoregulation in spite of other evidence that endothelium influences autoregulation. Because systemic NOS inhibition raises MABP, we investigated cortical NOS inhibition with superfusion.

Four Sprague-Dawley rats (345±15 g, SEM) were anesthetized with 0.5-1% halothane and 70% N_2O in O_2 with body temperature maintained at 37°C. The first day a cranial window was placed over a 6 mm diameter craniotomy (no dura matter) and the animal was allowed to recover. The second day, under anesthesia, a tracheotomy was performed, permitting artificial ventilation, and femoral arterial and venous catheters were placed. Physiological variables (MABP, $PaCO_2$, PaO_2, and pH) were stabilized. CBF was determined using laser Doppler flowmetry through the window. Animals with low CO_2 reactivity were excluded. After superfusion with artificial CSF (aCSF), MABP was sequentially lowered by exsanguination to 100, 85, 70, 55, and 40 mm Hg, followed by reinfusion. After cortical superfusion with 10^{-3} M N^{ω}-nitro-L-arginine (NNLA) for 103±2 m, the sequential pressure drops were repeated. The lower limit (LL) of autoregulation was identified visually as a change in the slope of the CBF vs MABP plot or as the maximum pressure if the plot was linear. The LL was 59±7 during aCSF and 81±10 mm Hg during NNLA superfusion ($p<0.05$). In one of these animals, a 3rd withdrawal sequence with NNLA plus 10^{-2} M L-arginine did not decrease the LL.

Thus, NNLA superfusion raises the LL of autoregulation, but the increase is not reversed by L-arginine. Although our finding is in contrast to those of previous workers, these data suggest 1) that there is a local autoregulatory response that is undetectable with the global CBF techniques of others, 2) that the length of NNLA exposure allowed inhibition of a NOS source that mediates autoregulation, or 3) that the effects of systemically administered NOS inhibitors somehow override their effect on the LL of autoregulation.

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518.7

NITRIC OXIDE MODULATES LOCAL INCREASES OF BLOOD FLOW IN RAT CEREBELLUM. M.Lauritzen*,N. Akgören, and M. Fabricius. Dept. Medical Physiology & Clin. Neurophysiology, Univ. Copenhagen, DK-2200 Copenhagen, Denmark.

We explored the possibility that NO was of importance for increases of cerebral blood flow (CBF) associated with activity of the well-defined neuronal circuits of rat cerebellar cortex. Laser-Doppler flowmetry was used to measure increases of CBF evoked by trains of electrical stimulations of the dorsal surface. The evoked increases of CBF were frequency-dependent, being larger on than off parallel fiber tracts, suggesting that conduction along parallel fibers, and synaptic activation of target cells were important for the increased CBF. This was verified experimentally since the evoked CBF increases were abolished by TTX, and reduced by 10 mM Mg^{2+} and NBQX and CNQX, antagonists for non-NMDA receptors. The cerebellar cortex contains high levels of NO synthase. This raised the possibility that NO was involved in increases of CBF associated with neuronal activation. NO synthase inhibition by topical application of N^G-nitro-L- arginine, attenuated evoked CBF increases by about 50%. This effect was partially reversed by pretreatment with L-arginine, the natural substrate for the enzyme, while N^G-nitro-D-arginine, the inactive enantiomer, had no effect on the evoked CBF increases. Simultaneous blockade of non-NMDA receptors and NO synthase had no further suppressing effect on the CBF rise than either substance alone, suggesting that the NO-dependent flow rise was dependent on postsynaptic mechanisms. The findings are consistent with the idea that local synthesis of NO is involved in the transduction mechanism between neuronal activity and increased CBF.

518.8

Stimulation of endothelial α_2 adrenoreceptors relaxes the rat middle cerebral artery by a mechanism involving endothelium-derived nitric oxide and a pertussis toxin-sensitive G protein. R.M. Bryan*, Jr., M.Y. Eichler, and T.D. Johnson, Department of Anesthesiology, Baylor College of Medicine, Houston, TX 77030

Changes in the diameter of rat middle cerebral arteries (MCAs) were studied after stimulation of α_2 adrenergic receptors with UK14304. Rat MCAs were isolated, cannulated at each end with a glass micropipet, and pressurized to 85 mm Hg. The MCAs were immersed in a bath (37° C) containing physiological saline solution (PSS), and luminally perfused with PSS (100 µl/min). Changes in the vessel diameter were measured after magnification of the MCAs. Resting diameter for the MCAs was 250 ± 4 µm. When UK14304 was added extraluminally to the solution bathing the MCAs, a dose-response dilation was produced with a 13 ± 2 % increase in diameter occurring at a concentration of 10^{-4} M. Luminal administration of UK14304 had no effect on the vessels. The dilations produced by UK14304 were blocked with phentolamine (10^{-6}M), a combined α_1 and α_2 antagonist, and idazoxan (10^{-6}M), a selective α_2 antagonist, but were not blocked by prazosin (10^{-6}M), an α_1 antagonist. The dilations could be blocked by removal of the endothelium, or the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME, 10^{-5} M). The inhibitory effects of L-NAME were reversed with the addition of 10^{-3} M L-arginine, but not 10^{-3} M D-arginine. Furthermore, the dilation produced by UK14304 was completely abolished with pertussis toxin (100 ng/ml). We conclude that the stimulation of α_2 adrenergic receptors with UK14304 produces a relaxation in the rat middle cerebral artery which (a) is endothelium dependent, (b) involves endothelium-derived nitric oxide, and (c) stimulates nitric oxide synthase via a pertussis toxin-sensitive G protein. (Supported by PHS grant PO1 NS 27616.)

518.9

A NOVEL SET-UP TO STUDY CEREBROVASCULAR RESPONSES UNDER WELL-CONTROLLED PHYSIOLOGICAL CONDITIONS IN THE MOUSE. T. Dalkara, K. Irikura, Z. Huang, N. Panahian*, M.A. Moskowitz. Stroke Research Lab., M.G.H., Harvard Medical School, Boston, MA, USA.

Control of physiological parameters such as respiration, blood pressure and gases has proven difficult due to the lack of microtecnology required to monitor these parameters. Here we report that mice can successfully be maintained under anesthesia for 5-6 hours without deterioration in physiological parameters and with normal cerebrovascular reactivity. This can be achieved by a ventilator having an inspiration time below 0.1 second, which appears to be critical to maintain adequate cardiac output and blood pressure. Monitoring end-tidal CO_2 is also indispensable because several manipulations require frequent readjustment of the respiratory parameters.

SV-129 mice were anesthetized with urethane (750 mg/kg, i.p.) and alpha-chloralose (50 mg/kg, i.p.), intubated, paralyzed and ventilated. Body temperature was kept at 36.5-37.5°C. End-tidal CO_2 was maintained to give a p_aCO_2 of 35±4 mm Hg (mean±s.d.). Mean arterial pressure was 95±8 mm Hg, heart rate was 545±77/min and arterial pH was 7.27±0.15. SV-129 maintained a normal cerebral perfusion between 40-120 mm Hg of systemic arterial pressure. Hypercapnia led to a 38±15 (5%CO2) and 77±34% (10%CO2) increase in rCBF (laser-Doppler flowmetry). Mechanical stimulation of contralateral vibrissae increased rCBF by 14±3%. After placement of a closed cranial-window, normal rCBF and pial vessel responses to hypercapnia and whisker stimulation could be maintained.

518.10

GENE KNOCKOUTS FOR THE NEURONAL FORM OF NITRIC OXIDE SYNTHASE HAVE A NORMAL rCBF RESPONSE TO CO_2 INHALATION. K. Irikura, T. Dalkara, Z. Huang, P.L. Huang, W.S. Lee*, M. Fishman, M.A. Moskowitz. Stroke Research Lab., M.G.H., Harvard Medical School, Boston, MA, USA.

The vasodilator, nitric oxide has been proposed as an important mediator in the regulation of cerebral circulation. We compared rCBF responses to hypercapnia in wild-type and mutant SV-129 mice which lack the neuronal form of nitric oxide synthase. Mice were anesthetized with urethane (750 mg/kg, i.p.) and alpha-chloralose (50 mg/kg, i.p.), intubated, paralyzed and ventilated. Respiratory end-tidal CO_2 was monitored and maintained to give a p_aCO_2 of 35±4 mm Hg (mean±s.d.). Baseline mean arterial pressure, blood gases and pH were not significantly different in wild (19) and mutant (12) animals. Hypercapnia was induced by inhalation of 5 or 10 % CO_2 balanced with O_2 and N_2 and led to a 38±15 and 77±34% increase in rCBF (laser-Doppler flowmetry) in both the mutant and wild-type. A robust increase in whole brain cGMP was observed in 5 wild mice sacrificed at the end of a 10-min 5% CO2 inhalation. Mutants (5) had a lower basal level of cGMP and did not show any change after hypercapnia. Topical application of L-nitro-arginine (1 mM) by closed cranial window diminished the hypercapnic response in an hour in both wild and mutant mice. These data suggest that neuronal NO is not the principle mediator of hypercapnic hyperemia. However, the cGMP data implicate that there is an increased production of parenchymal NO in response to hypercapnia in the wild-type.

518.11

CEREBRAL CORTICAL ACTIVATION STUDIES WITH [^{99m}Tc]-BICISATE (ECD) SPECT. E. Matthew* and T. Hill. Deaconess Hospital and Harvard Medical School, Boston, MA 02215.

This study investigates the feasibility of using [^{99m}Tc]-Bicisate (ECD; Neurolite®), a new perfusion agent, to demonstrate changes in regional cerebral blood flow (rCBF) in cortical activation studies.

Normal volunteers (n=25) were studied under baseline conditions (resting in a darkened room), and during activation by either full-field (n=10; mean [SD] age, 32.8 [8.0] years; male, 6; handedness, 7 right)), or right hemi-field (n=10; age, 30.4 [5.7] years; male, 5; handedness, all right)) visual stimulation with a flashing checkerboard, or repetitive right hand finger movements (n=5; age, 33.2 [9.4] years; female, 4; all right handed). These paired scans were realigned and co-registered with an automated program. Baseline and activation studies were compared in each subject. Regions of interest normalized to whole-slice activity were used to determine differences between resting and activated states, as well as left-right differences .

Right hemi-field stimulation caused significant rCBF change only in the left occipital cortex (mean [SD] % change from baseline: left, 4.9 [3.9]; right -0.6 [7.5], $p < 0.05$, t-test, 2-tail), while full-field visual stimulation produced bilateral activation in the occipital cortex. During repetitive right hand finger movements, rCBF was significantly higher in the left precentral area compared to the right (mean % left-right difference [SD], 9.0 [2.1], $p < 0.001$, t test, 2-tail). No other significant regional changes were demonstrated under baseline conditions or during activation.

These data demonstrate that [^{99m}Tc]-Bicisate SPECT scans can be used for cortical activation studies.

518.12

SEROTONERGIC ACTIVITY CONTRIBUTES TO RESTING CEREBRAL BLOOD FLOW BUT NOT THE RESPONSE TO DORSAL RAPHE NUCLEUS STIMULATION IN RAT. M.D. Underwood*, M.J. Bakalian, V. Arango, A.V. Gubbi and J.J. Mann. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Electrical stimulation of the caudal dorsal raphe nucleus (DRN) elicits an increase in cerebral blood flow (CBF). Paradoxically, stimulation of rostral DRN decreases CBF. Because serotonin (5-HT) has both vasoconstrictory and vasodilatory effects on the cerebral vasculature, we sought to determine whether 5-HT contributes to the maintenance of resting CBF and mediates the decreased CBF response to DRN stimulation.

Adult male Sprague-Dawley rats were anesthetized (α-chloralose, 40 mg/kg), paralyzed and ventilated. Arterial pressure (AP), heart rate and blood gases were monitored and controlled and did not differ between groups ($p>0.05$, ANOVA). 5-HT neurons were lesioned by microinjection of 5,7-DHT (25 µg/0.5 µL) into the DRN. Three weeks later the DRN was stimulated (100 µA) and CBF was measured autoradiographically in 32 regions using ^{14}C-iodoantipyrine. 5-HT and 5-HIAA in frontal cortex were determined by HPLC.

In unstimulated, unlesioned controls (n=14) CBF ranged from 48±4 ml/min/100g in corpus callosum to 152±12 ml/min/100g in the nucleus tractus solitarius. DRN stimulation in unlesioned animals (n=12) increased 5-HT (20%) and 5-HIAA (29%) in frontal cortex and reduced CBF globally from 3-24% ($p<0.05$). In lesioned controls (n=6), 5-HT and 5-HIAA were reduced 48% and 52%, respectively and resting CBF was less than in unlesioned controls in 30 of 32 regions by 14±1% ($p<0.05$). In unstimulated animals, resting CBF positively correlated with 5-HIAA ($r=0.61$, $p<0.01$), but not 5-HT ($r=0.24$, $p>0.05$). DRN stimulation in lesioned animals (5HT: 18% of control; 5-HIAA: 17% of control; n=5) resulted in a proportionally similar decrease in CBF as in unlesioned animals ($p<0.05$).

We conclude: 1) intrinsic serotonergic activity contributes a vasodilatory action on CBF and 2) rostral DRN stimulation elicits widespread CBF decreases which are not mediated by 5-HT. (Supported by NARSAD and MH46745.)

519.1

POLYMORPHISM AND GENETIC MAPPING OF SIX HUMAN SEROTONIN RECEPTOR GENES. D. Goldman*, J. Lappalainen, N. Ozaki, B. Nakhai, U. Pesonen, M. Koulu, B. Giblin, D. A. Nielsen, M. Linnoila and M. Dean. Lab. of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Polymorphisms were identified within coding sequences of six 5HT receptor genes. Variants were detected using single strand conformational polymorphism (SSCP) analysis and then converted to PCR-RFLPs. Synonymous substitutions were identified at $5HT_{1A}$, $5HT_{1E}$, $5HT_{1D\alpha}$, $5HT_{1D\beta}$, and $5HT_2$. A relatively abundant Cys ---> Ser substitution was identified at $5HT_{2C}$. The 5HT receptor genes (some previously localized by *in situ* hybridization or in somatic cell hybrids) were then genetically mapped on a fine-scale basis by linkage analysis in CEPH/NIH pedigrees, as follows:

$5HT_{1A}$	Chromosome 5	Previously linkage mapped.
$5HT_{1D\alpha}$	Chromosome 1p35	
$5HT_{1D\beta}$	Chromosome 6q13	
$5HT_{1E}$	Chromosome 6q13	
$5HT_2$	Chromosome 13	
$5HT_{2C}$	Chromosome Xq21	

The $5HT_{1D\beta}$ and $5HT_{1E}$ genes are genetically linked on 6q13, providing the first example of physical clustering of two members of the 5HT receptor gene family. The $5HT_{2C}$*Ser*/$5HT_{2C}$*Cys* amino acid substitution is being evaluated for functional significance and for association to serotonin-influenced traits.

519.2

PHARMACOLOGICAL CHARACTERIZATION OF TWO SPLICE VARIANTS OF THE CLONED RAT 5-HT_4 RECEPTOR COUPLED TO STIMULATION OF ADENYLATE CYCLASE. N. Adham*, C. Gerald, P.J.-J. Vaysse, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

We have isolated two splice variants of the pharmacologically-defined 5-HT_4 receptor, 5-HT_{4L} and 5-HT_{4S}, differing in the length and sequence of their carboxy termini. In rat brain, the 5-HT_{4S} transcripts are restricted to the striatum, but the 5-HT_{4L} transcripts are expressed throughout the brain, except in cerebellum. When either receptor is transiently expressed in COS-7 cells, each of them stimulates adenylate cyclase activity and is sensitive to the benzamide derivative cisapride. Although 5-HT_{4S} and 5-HT_{4L} clones exhibit very similar pharmacological profiles, some differences were noted: The expression levels of 5-HT_{4S} were approximately 2-fold greater than the 5-HT_{4L} clone when transiently transfected in COS-7 cells and compared in parallel experiments. Despite the lower expression levels, 5-HT_{4L} clone produced significantly greater stimulation of adenylyl cyclase relative to 5-HT_{4S} clone. These data indicate that although 5-HT_{4S} and 5-HT_{4L} are splice variants and differ only at their C-terminal tails, they may nevertheless possess different coupling efficiencies to elicit functional responses. Whether 5-HT_{4S} and 5-HT_{4L} couple to different isoforms of G_s and/or adenylyl cyclase remains to be investigated. Interestingly, 5-HT_{4L} clone has 4 protein kinase C phosphorylation sites, whereas 5-HT_{4S} clone has only 3. This additional phosphorylation site could lead to differences in the regulation of these two receptors. Alternative splicing may provide a mechanism by which functional diversity is established for 5-HT_4 receptors.

519.3

THE 5-HT_4 RECEPTOR: MOLECULAR CLONING AND PHARMACOLOGICAL CHARACTERIZATION OF THE HUMAN 5-HT_{4L} RECEPTOR. C. Gerald, N. Adham, P.J.-J. Vaysse*, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

Recently, we described the molecular cloning and pharmacological characterization of two splice variants (5-HT_{4S} and 5-HT_{4L}) of the rat 5-HT_4 receptor. We now report the identification of a human 5-HT_{4L} cDNA clone from a human hippocampal cDNA library. The amino acid sequence deduced from the longest open reading frame is 91.8% identical to the rat sequence, revealing 31 amino acid changes of which 11 are non conservative, including 2 in TMI, 1 in TMII and 1 in TMIV. Interestingly, the human cDNA contains one deoxynucleotide insertion at the 3' end of the coding region, creating a reading frame shift and introducing a stop codon 16 deoxynucleotides downstream in the reading frame. Due to this nucleotide insertion, the carboxy terminal tail of the human 5-HT_{4L} receptor is 16 amino acids shorter than its rat counterpart. The human clone displays 5 fold higher affinity than the rat clone for the 5-HT_4 antagonist [^{3}H]GR113808. Benzamide derivatives like cisapride, BRL-24924 and zacopride, partial agonists at the 5-HT_4 receptor, all potently inhibited binding. The biggest differences between the human and the rat clones were observed with α-Me-5-HT and zacopride which exhibited at least 5 to 10 fold higher affinity for the human 5-HT_{4L} receptor.

519.4

MICE LACKING 5-HYDROXYTRYPTAMINE 1B RECEPTORS DISPLAY AGGRESSIVE BEHAVIOR. [1]R. Hen*, [2]F. Saudou, [3]D. Ait Amara, [4]C. Belzung, [2]A. Dierich, [2]M. LeMeur, [1]S. Ramboz, [3]L. Segu, [4]R. Misslin, [3]M.C. Buhot. [1]Center for Neurobiology and Behavior, Columbia University, 10032 New York, [2]CNRS, INSERM, 11 rue Humann, 67085 Strasbourg, [3]Lab. de Neurobiologie du CNRS, 13402 Marseille; [4]Lab. de Psychophysiologie, U.L.P., 67000 Strasbourg.

The $5HT_{1B}$ receptor, which is the rodent homologue of the human $5HT_{1D\beta}$ receptor, has been suggested to be involved in a number of physiological states such as appetite, locomotor activity and agressiveness, as well as in certain pathological states such as migraines.

In order to study the functions of the $5HT_{1B}$ receptor in the mouse, we have generated by homologous recombination, mutant mice lacking the gene encoding the $5HT_{1B}$ receptor. Such homozygous mutant mice develop, move, feed and breed apparently normally. When analyzed in a light-dark choice test and in an open field, the $5HT_{1B}$-minus mice displayed the same behavior as their wild type littermates, suggesting that this mutation does not affect base-line levels of locomotion and anxiety. However, the hyperlocomotor and anorectic effects of the $5HT_1$ agonist RU24969 (5mg/kg, i.p.) were absent in mice lacking the $5HT_{1B}$ receptors.

In the resident-intruder aggression test where isolated test mice are faced with a wild-type intruder, the mutants attacked the intruder faster and more intensely than wild type mice. This increased aggressiveness of $5HT_{1B}$-minus mice might be related to the fact that a class of 5-HT1 agonists termed serenics (eltoprazine) have antiaggressive properties, and with the finding that certain impulsive aggressive behaviors are associated with deficits in central serotonin.

519.5

SUDDEN DEATH IN 5-HT_{1C} RECEPTOR "KNOCKOUT" MICE. L.H. Tecott*, L. Sun and D. Julius. Depts. of Pharmacology and Psychiatry, University of California, San Francisco, CA 94143-0450.

The 5-HT_{1C} receptor is an abundant serotonin receptor subtype that is restricted to the central nervous system. It has been implicated in many of the physiological effects of serotonin and psychotherapeutic drugs, however, the lack of specific agonists and antagonists of this receptor hinders analysis of its functional significance. To study the roles of this receptor, we have generated, through homologous recombination, mutant mice lacking a functional 5-HT_{1C} receptor gene. To verify the loss of functional receptor protein, brain polyA+ RNA from mutant and wild type mice were injected into *Xenopus* oocytes. A loss of serotonin-induced depolarization was noted in oocytes injected with RNA from the mutant animals. In addition, 5-HT_{1C} receptor immunocytochemistry revealed a loss of immunoreactivity in brain sections from mutant animals. Most mutant mice appear healthy and evidence no reproductive abnormalities. However, approximately 25% of males hemizygous for this X-linked mutation display an apparent sudden death syndrome. Death is preceded by no apparent signs of illness such as loss of weight or reduced levels of activity. The age range of animals at death is from 3 to 13 weeks, with a peak incidence at 6-8 weeks of age. Studies are underway to determine the cause of death in these animals. In particular, the possibilities that deaths of mutant mice are due to seizures or cardiac arrhythmias are under examination.

519.6

TARGETED DISRUPTION OF THE $5HT_2$ RECEPTOR GENE RESULTS IN DEVELOPMENTAL ABNORMALITIES IN MICE. M. Toth*[1,3], T. Robinson[2], D. Benjamin[3], and T. Shenk[2]. [1]Dept. of Pharmacology, Cornell Univ. Med. Coll., New York, NY 10021; [2]Dept. of Mol. Biol., Princeton University, NJ 08544; [3]Center of Alcohol Studies, Rutgers Univ. Piscataway, NJ 08855.

Although 5HT is considered as a neurotransmitter, recent findings indicate that it is also a developmental and mitogenic signal. In order to study the developmental function of the receptor directly, we have disrupted the $5HT_2$ receptor gene by homologous recombination in D3 embryonic stem (ES) cells. These ES cells express the $5HT_2$ receptor, thus it was possible to determine the extent of receptor loss as the result of the gene knock-out. Interestingly, inactivation of one allele of the $5HT_2$ receptor gene resulted in a more than 50% loss in receptor binding. In parallel, transcripts encoded by the intact receptor were also reduced more than twofold, upto 20% of the level measured in normal ES cells. In contrast, large amount of transcripts encoded by the truncated receptor allele was produced in the knock-out cells (8-10 fold of the expected 50% level). We speculate that the truncated mRNA is more stable than the intact receptor RNA, and the high steady state level of the truncated RNA may suppress the expression of the wild type allele, a phenomenon resembling to allelic exclusion. Alternatively, the receptor allele which remained intact in the knock-out cells may have been parentally imprinted. Presently, we are performing experiments to distinguish between these two possibilities. Suppression of the intact allele was observed not only in ES cells. Chimeras with high extent ES cell contribution displayed negligible receptor binding in tissues derived from ES cells demonstrating that suppression is maintained through development. Chimeras showed disturbances in postnatal development, probably as the result of low receptor expression. A reduction in weight gain was evident starting at postnatal day 5-10. Feeding was also reduced, particularly at the age of 3-5 weeks resulting in cahexy and eventually death.

519.7

CHARACTERIZATION OF HUMAN 5-HT2 RECEPTOR GENE PROMOTER Q.S. Zhu*, K. Chen and J.C. Shih. Dept. Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033.

The 5' flanking sequence of human 5-HT2 receptor gene has been characterized. Anchor PCR mapped multiple transcription initiation sites between nucleotide -1157 to -1127 in human brain and two cell lines SHSY-5Y (neuroblastoma) and HeLa (cervix carcinoma). Promoter activity was detected in a 0.35 kb HaeIII/BamHI fragment which encompasses these initiation sites in a transient transfection assay in the two cell lines. Sequence analysis revealed Sp1 and PEA3 binding sequences in this fragment. Gel retardation assay detected Sp1 and two other protein factors. DNase1 footprinting analysis detected a 30 bp protected region which contained a Sp1 binding sequence and a novel protein binding sequence, possibly for the new factor found in gel retardation assay. A 0.39 kb BamHI/PvuII fragment, 3' of the 0.35 kb fragment, exhibited the highest promoter activity. It contains a cyclic AMP response element (CRE)-like sequence, a PEA3 sequence and E-boxes. DNase1 footprinting assay found a protected E-box and a new protein binding sequence next to the CRE element. In addition, a transcription initiation site was found downstream of this fragment (at -496) in brain, suggesting that the 0.39 kb fragment could be an additional promoter. A 0.45 kb PvuII/Sma fragment, 3' of the 0.39 kb fragment, strongly down regulated the promoter activity. This new data is essential for further studying the regulation of 5-HT2 receptor gene expression. (Supported by NIMH grants R37 MH39085 (MERIT Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin Professorship.)

519.8

TRANSCRIPTIONAL CONTROL OF THE SEROTONIN-2 RECEPTOR GENE. S.J. Garlow*, R.D. Ciaranello. Dept. of Psychiatry and Beh. Sci., Stanford Univ Med Ctr, Stanford, CA 94305.

Analysis of the DNA sequence in the promoter region of the rat serotonin-2 (5-HT_2) receptor gene reveals a variety of consensus and near consensus response elements for known transcription factors. There are potential response elements for NF-1, AP-1, AP-2, SP-1, EGR-1, and two very degenerate versions of the glucocorticoid response element(GRE). We have been testing the functionality of these elements in a co-transfection assay system. In this system a promoter-reporter plasmid, with the 5-HT_2 promoter driving the firefly luciferase gene, is transfected into cells along with the cDNA for the particular transcription factor carried on the CMV5 expression vector.

There are three potential EGR-1 sites in the 5-HT_2 promoter, which overlap the major site of transcription initiation. The effect of EGR-1 on the 5-HT_2 promoter has been tested in three different cell lines including CCL-39, A7r5, and Neuro2A. In all three cell types the EGR-1 factor seems to inhibit transcription from the 5-HT_2 promoter.

There are four potential AP-1 sites in the 5-HT_2 promoter, with two located 5' to the site of transcription initiation, and two located 3' to the site of transcription initiation. The AP-1 complex is introduced into the cells by transfecting in equal amounts of the Fos and Jun cDNAs on the CMV5 expression vector. The AP-1 factor seems to have a cell specific effect on transcription of the 5-HT_2 promoter. In the CCL-39 cell line, the AP-1 factor inhibits expression from the 5-HT_2 promoter. In the Neuro2A line, the AP-1 factor stimulates expression from the 5-HT_2 promoter.

The potential GREs have been tested in two different paradigms. In the first, the cDNA for the GR is co-transfected into CCL-39 cells along with the luciferase reporter plasmid, then challenged with dexamethasone. In the second paradigm, the luciferase reporter plasmids are transfected into RS-1 cells which are a steroid responsive sub-line of PC12 cells, then challenged with dexamethasone. In both paradigms, there is a dexamethasone mediated inhibition of expression of the 5-HT_2 promoter.

519.9

A BRAIN-SPECIFIC PROTEIN THAT BINDS TO 5-HT_{1C} AND 5-HT_2 mRNA. H.T. Kao* and R.D. Ciaranello. Nancy Pritzker Laboratory of Molecular and Developmental Neurobiology, Stanford University Medical Center, Stanford, CA 94305.

The 5-HT_{1C} and 5-HT_2 receptors are members of the serotonin 5-HT_2 family of receptors, sharing similar pharmacology, signal transduction pathways, and sequence homology. The RNAs for both receptors contain untranslated regions more than twice the length of the coding region, suggesting that post-transcriptional regulation may be an important mechanism for regulating these receptors.

Many post-transcriptional processes, such as the control of RNA stability or translation, are exerted by the action of RNA binding proteins. We thus began our investigations by searching for brain proteins that bind to RNAs encoding 5-HT_{1C} and 5-HT_2 receptors.

We observed several proteins capable of binding to both 5-HT_{1C} and 5-HT_2 receptor RNAs, when binding was detected by crosslinking radioactive RNA to rat brain cortical extracts. Most of the binding is confined to the 3' UTR and coding region of the RNA. Of all these proteins, we have focused on one, a 60 kd protein, that binds in a sequence-dependent manner and recognizes AU rich domains. 5-HT_{1C} RNA sequences can compete against binding of this protein from 5-HT_2 RNA, suggesting that the same protein is capable of binding to both RNAs. Kinetic studies also suggest that the affinity of the interaction is high (Kd = 3 nM).

The 60 kd protein is expressed specifically in the central nervous system, with highest levels in the cortex and hippocampus. The protein is also expressed in a cell line of neuroectodermal origin, Neuro2A cells. We hypothesize that this protein is an important post-transcriptional regulator of a class of brain RNAs, including RNAs encoding neurotransmitter receptors. The Neuro2A cell line will facilitate studies aimed at examining the function of this protein.

519.10

STRUCTURAL AND MECHANISTIC BASIS OF ALLOSTERIC REGULATION IN THE 5-HT_2 RECEPTOR. H. Weinstein, D. Zhang and B.J. Ebersole* Mount Sinai School of Medicine, NY, NY 10029

A structural model of the 5-HT_2 receptor we described recently [J. Med. Chem. 36:934,1993] was used to explore the mechanism of allosteric regulation of G-protein coupled receptors (GPCRs) by cations. Ligand binding and effector coupling had been shown to be subject to such regulation. Mutations in α_2-adrenergic and dopamine-D_2 receptors have implicated a conserved Asp in transmembrane helix (TMH) 2 in this process, but the mechanism remained unclear. The 5HT_2 receptor model in which we studied the mechanistic relation between the TMH2-Asp and the ligand binding site, had been shown to be similar to the structure of rhodopsin, and to behave in computational simulations of ligand binding in a manner consistent with the known pharmacological properties of the ligands. In this model, the conserved Asp98 in TMH2 (TMH2:Asp98) directly interacts with two other conserved residues TMH7:Tyr380 and TMH7:Asn376 which, in turn, create with TMH7:Ser372 a hydrogen-bond network connecting TMH2:Asp98 to the ligand binding pocket. Structural insights from the model, amplified by mechanistic conclusions from the computational simulation of receptor activation by agonist binding, led to a hypothesis for the mechanism of allosteric regulation, and to a quantitative assessment of the interaction between various ligands and the 5-HT_2 receptor model. A significant correlation emerged between the experimentally determined ligand affinities and computed interaction energy terms. The nature of this correlation connects ligand binding to the conformational changes induced in the 5HT_2 receptor, and suggest specific interactions between TMH2 and other TMHs involved in the allosteric regulations. *Supported by NIH DA-06620 and DA-00060.*

519.11

MOLECULAR REQUIREMENTS FOR HALLUCINOGEN ACTIONS AT 5-HT2A RECEPTORS. BL Roth*, A Uluer and MS Choudhary, Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

There is now considerable evidence that 5-HT2A receptors are a major site of action for hallucinogens in the central nervous system. We investigated the molecular details responsible for hallucinogen-induced regulation of 5-HT2A receptor distribution and activity using native and mutant 5-HT2A receptors. Chronic (14 hr) DOI exposure (10 μM) caused a desensitization of 5-HT and quipazine stimulated phosphoinositide (PI) hydrolysis. Chronic exposure to DOI (1-10 μM) also induced a redistribution of immunolabeled 5-HT2A receptors to intracellular organelles; this change in subcellular receptor distribution was abolished by the 5-HT2A antagonist clozapine. By contrast, overnight exposure to clozapine *alone* shifted the overall distribution of immunolabel to the cell surface with minimal intracellular staining evident; an apparent *decrease* in cell surface staining was also evident after clozapine treatment. Point mutations (F340L, F340A) greatly diminished the affinities of several phenethylamine and tryptamine hallucinogens at the 5-HT2A receptor, but did not alter the intrinsic efficacy of the phenethylamine hallucinogens DOI and DOB. Despite DOI's ability to maximally activate PI hydrolysis (>20-fold), DOI was unable to induce down-regulation of [^{3}H]-ketanserin binding sites or to desensitize PI hydrolysis after chronic exposure in cells expressing the F340L mutation. These results suggest that DOI causes a intracellular redistribution of 5-HT2A receptors and that desensitization of 5-HT2A receptor activity after long-term agonist exposure occurs via a process *independent* of second messenger production.

519.12

ACTIVATION OF TWO DISTINCT CLASSES OF PROTEIN KINASE REGULATES EXPRESSION OF 5-HT_{2A} RECEPTOR mRNA IN P11 CELLS. **R. C. Ferry*, L. Cass, and P. B. Molinoff. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.**

Exposure of P11 cells to 5-HT leads to down-regulation of 5-HT_{2A} receptors and to a transient increase in levels of mRNA encoding the receptors. The increase in receptor mRNA appears to represent a counter-regulatory mechanism since the increase was associated with attenuation of agonist-induced receptor down-regulation and acceleration of receptor recovery following chemical inactivation of receptors with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). The roles of protein kinase C (PKC) and tyrosine kinase(s) (TK) in regulating levels of 5-HT_{2A} receptor mRNA were investigated. Exposure of P11 cells to 5-HT (10 μM; 90 min) or phorbol 12-myristate 13-acetate (PMA, 100 nM; 105 min), an activator of PKC, caused a two-fold increase in receptor mRNA levels. Preincubation with 100 nM PMA for 24 h, which down-regulated PKC activity, or with the selective PKC inhibitor bisindolylmaleimide (5 μM; 15 min) antagonized the effects of both 5-HT and PMA. These findings suggest that activation of PKC is sufficient to increase levels of 5-HT_{2A} receptor mRNA and indicate that the effects of 5-HT on receptor mRNA levels require activation of PKC. Exposure of P11 cells to genistein (150 μM; 15 min), a nonselective inhibitor of cellular TK, prevented 5-HT and PMA from increasing receptor mRNA levels, whereas the inactive analog daidzein (150 μM; 15 min) did not. The cell membrane-impermeable TK inhibitor lavendustin A (10 μM; 15 min) had no effect on basal, 5-HT-, or PMA-stimulated increases in receptor mRNA levels. The finding that genistein specifically blocked 5-HT- as well as PMA-induced increases in receptor mRNA indicates that 1) a TK lies downstream from PKC in the transduction pathway that regulates 5-HT_{2A} receptor mRNA expression, and/or 2) PKC- and TK-dependent phosphorylation of a common intermediate protein is required to increase receptor mRNA levels. Incubation with the tyrosine phosphatase inhibitor orthovanadate (1 mM; 15 min) prior to exposure to 5-HT prolonged the time that receptor mRNA levels were elevated. Collectively, these data suggest that activation of PKC and modulation of cellular phosphotyrosine levels are involved in regulating levels of 5-HT_{2A} receptor mRNA in P11 cells. (Supported by USPHS grants MH 48125 and NS 18591.)

519.13

EXPRESSION OF 5-HT2 RECEPTOR IMMUNOREACTIVITY ON GABA-ERGIC INTERNEURONS IN RAT CORTEX. D. A. Morilak*, R. Lujan-Miras, P. Somogyi and R.D. Ciaranello, Nancy Pritzker Lab, Dept. Psychiatry, Stanford Univ., Stanford, CA 94305 and MRC Anatomical Neuropharmacology Unit, Oxford University, Mansfield Road, Oxford OX1 3TH, UK

We have previously demonstrated 5-HT2 receptor immunoreactivity in the rat brain using a polyclonal antibody raised against the rat 5-HT2 receptor. The distribution and morphology of labelled neurons in cortex, hippocampus, basal forebrain and other regions led us to speculate that they may be GABAergic interneurons. In the present study, we addressed this question using in situ hybridization histochemistry for GAD67 mRNA, and post-embedding immunocytochemistry for GABA. We observed a substantial overlap in distribution of neurons expressing GAD67 mRNA and 5-HT2 immunoreactivity. This correlated distribution was seen in cortex, hippocampus and basal forebrain, supporting the hypothesis that 5-HT2 expressing cells in these regions synthesize GABA. To test this hypothesis directly, we employed a double-immunoperoxidase approach. Animals were perfused with 4% para-formaldehyde/0.1% glutaraldehyde. This fixation protocol represented a compromise between the requirement for glutaraldehyde for GABA processing, and the sensitivity of 5-HT2 immunoreactivity to over-fixation. Following 5-HT2 immunoprocessing on 50 μm sections, serial 0.5 μm semithin sections were cut through identified 5-HT2-positive cells in cortex, and subjected to post-embedding GABA immunoprocessing. Even though the fixation protocol resulted in a severely attenuated GABA labelling., we still observed a small number of double-labelled, 5-HT2 positive GABAergic interneurons. From these data, together with the distribution and morphology of 5-HT2 positive cells, we conclude that 5-HT2 expressing neurons in cortex, and perhaps also in hippocampus and other forebrain regions, represent subpopulations of GABAergic interneurons.

REGENERATION

520.1

NEURONAL REGENERATION IN HOLOTHURIA GLABERRIMA J. E. García-Arrarás*, I. Torres, E. Arroyo, W. Cruz, L. Jiménez, L. Estrada, K. Rivera and L. Díaz-Miranda, Biology Dept., Univ. of Puerto Rico, Rio Piedras, P.R. 00931.

Among higher metazoans, echinoderms exhibit the most impressive capacity for regeneration. Holothurians, or sea cucumbers, respond to adverse stimuli by autotomizing and spewing out their visceral organs which are then replaced by a regeneration process. Neuronal fibers and cell bodies are known to be present within the viscera, however, previous regeneration studies do not account for the nervous component. We have used immunocytochemistry at the light microscope level (antibodies to acetylated α-tubulin, to the neuropeptides GFS, galanin and CCK and novel monoclonal antibodies) and the electron microscope to describe the enteric nervous system of holothurians. We show here, that the enteric nervous system of the sea cucumber, *Holothuria glaberrima,* regenerates following evisceration and in 3-5 weeks is virtually identical to that of non-eviscerated animals. The regeneration of the enteric nervous system occurs parallel to the regeneration of other organ components, fibers are observed prior to the formation of the mucosal layer and the first neurons appear shortly after. The regeneration of the nervous system commands high interest in view that members of the closely related phylum, Chordata, either lack or have a very limited capacity to regenerate their nervous system. Thus, holothurians provide a model system to study nervous system regeneration in deuterostomes. [Supported by grants from EPSCoR, the Whitehall Foundation, and the University of Puerto Rico.]

520.2

STIMULATION OF REGENERATIVE SPROUTING ENHANCES PREFERENTIAL MOTOR REINNERVATION (PMR)
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Motor axons regenerating after transection of mixed nerve preferentially reinnervate distal motor branches. Collaterals of a single motor axon often enter both sensory and motor Schwann cells of the distal stump; specificity is generated by pruning collaterals from sensory pathways while maintaining those in motor pathways (J. NSci. 13:2730). Stimulation of collateral formation should therefore allow increased pathway sampling and greater regeneration specificity. Experiments were performed on the proximal femoral nerves of 250gm female SD rats. Forty nerves were sharply transected and sutured; 40 others were crushed with jewlers forceps proximal to the repair site both 2 and 4 weeks before suture to stimulate collateral sprouting. Twenty nerves in each group were evaluated at 3 weeks, and 20 at 3 mos., by applying HRP to one terminal branch and Fluoro Gold to the other. The mean number of motoneurons projecting axons into the femoral sensory and motor branches at 3 weeks was similar after routine suture (M=149, S=170), but was significantly different after crush/crush/suture (M=244,S=103, p=.001). Specificity was apparent 3 mos after routine suture (M=242, S=130, p=.001), but was greatly augmented 3 mos after crush/crush/suture (M=263, S=81, p=.001). The number of motoneurons projecting collaterals to both branches at 3 wks, the substrate for specificity generation by collateral pruning, was significantly greater after crush/crush/repair (72 vs 53, p=.01). Nerve crush before suture thus increases the number of motoneurons projecting collaterals to both sensory and motor branches, and also results in substantial augmentation of PMR. This finding mandates a search for non-invasive techniques of stimulating collateral sprouting to enhance regeneration specificity.

520.3

DIRECT INVOLVEMENT OF p53 IN PROGRAMMED CELL DEATH OF OLIGODENDROCYTES. O. Eizenberg, A. Faber-Elman, E. Gottlieb, M. Oren, V. Rotter, V. Lavie* and M. Schwartz, Department of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel.

Recent studies have shown that dimeric interleukin 2 produced enzymatically *in vitro* by nerve-derived transglutaminase is cytotoxic *in vitro* to mature oligodendrocytes, known to inhibit axonal growth. The cytotoxic effect operates via apoptosis. Cells showed commitment to apoptosis after 3–4 h of treatment with dimeric interleukin 2. This interval could reflect a necessary rate limiting process of activation or suppression of specific genes, or modification of specific regulatory proteins. A protein that appears to act as a positive regulator of apoptotic cell death in a number of cell types is the p53 tumor-suppressor gene product. The aim of the present study was to determine whether p53 is involved in the death of mature rat brain oligodendrocytes in response to dimeric interleukin 2. We demonstrate here that the dimeric interleukin 2 induces in oligodendrocytes translocation of endogenous wild-type p53 from the cytoplasm to the nucleus, as early as 15 min after exposure. Moreover, oligodendrocytes infected with a retroviral vector encoding a p53 miniprotein expressing the dimerization site of p53 and by that acts as a negative dominant inhibitor of endogenous wild-type p53 activity, are effectively protected from apoptosis induced by dimeric interleukin 2. These findings led to the conclusion that p53 is directly involved in the apoptotic mechanism induced by dimeric interleukin 2.

520.4

THE EFFECTS OF MACROPHAGES ON PERIPHERAL NERVE REGENERATION IN NERVE GRAFTS IN THE ABSENCE OF LAMININ, FIBRONECTIN OR COLLAGEN IV. G.Wang, Y.Xin*, Y.Hu, J.Qin, S.Zhong and K.Hirai, Dept. of Anatomy, First Military Med. University, Guangzhou, China, Ctr. for Neurobiology and Behavior, Columbia University, 722 W. 168 St. New York, NY 10032 and Dept. of Anatomy, Kanazawa Medical University, Ishikawa, 920-02, Japan.

We previously studied the effects of laminin (LN), fibronectin (FN) and collagen IV (Col.IV) on peripheral nerve regeneration. To investigate the effects of macrophages (MPHs) on peripheral nerve regeneration in the absence of LN, FN or Col.IV, nerve transplantation experiments were done by using 151 male adult Wistar rats. Donor sciatic nerve grafts (N=98) were obtained from 49 rats and denatured by repetitive freezing and thawing. After treatment with anti-rat LN (N=24), FN (N=20), Col.IV (N=30) serum or control serum (N=24), the nerve grafts were implanted into the recipient rats to bridge sectioned sciatic nerves. The grafts were excised and examined under light and electron-microscope at different postoperative days. The results showed that MPHs were recruited from blood and appeared earlier than Schwann cells. The number of MPHs in the anti-FN, anti-Col.IV and control groups increased sharply from postoperative day 2 and peaked at day 7, then quickly decreased. In the anti-LN group, increase of MPHs was delayed to postoprative day 7, peaking at day 10. The number of MPHs then decreased slowly. Rate of axon growth and mylination was fast in the control group, followed in turn by anti-Col.IV, anti-FN and anti-LN groups. Our results indicate that in the early stage of nerve regeneration, increased MPHs eliminate degenerated tissues effectively and thereby promote nerve regeneration. In the absence of LN, howerer, both increase and decrease of MPHs are delayed, resulting in delayed axon growth and mylination.

520.5

CREATION OF A NEURON INHIBITORY ENVIRONMENT MEDIATED BY A SPECIFIC ASTROCYTIC PROTEOGLYCAN. DR. Canning*, A. Höke & J. Silver. Dept of Neurosciences, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106-4975.

CNS astrocytes normally support the growth and maintenance of axons. One aspect of this tropic behavior is the supplying of favorable substrates which allow axons to grow on their surfaces and the extracellular matrix. We have recently discovered an *in vitro* method of converting the behavior of astrocytes from supplying neuronal growth promotory environments into those which are inhibitory. This change in behavior can be activated by exposure of the astrocytes to small peptide sequences of the beta-amyloid protein in an insoluble form. This activation results in the localized deposition of a matrix containing chondroitin sulfate, similar to that seen at sites of reactive gliosis after trauma *in vivo*.

Amongst the various proteoglycans manufactured by astrocytes, we have found that the experimentally induced inhibitory environment is mediated by the upregulation and deposition of a specific chondroitin/heparan sulfate proteoglycan. This proteoglycan has properties inhibitory to axonal outgrowth when combined with other molecules of the extracellular matrix. These inhibitory properties can be experimentally reversed by removal of chondroitin sulfate glycosaminoglycans from the matrix created by activated astrocytes, but not by enzymatic removal of other glycosaminoglycans. Affinity purification of this proteoglycan has revealed that it is lightly glycosylated and carries the carbohydrate epitopes 7D4, CS-56 and 3B3. Data from molecular weight estimations, preliminary peptide analysis and amino acid composition determinations suggest that this may be a novel proteoglycan to the CNS, which is upregulated in areas of reactive gliosis.

520.6

ESTABLISHMENT OF A NEURON-GLIAL CO-CULTURE SYSTEM IN CHEMICALLY DEFINED MEDIUM; A TOOL FOR STUDIES ON CNS REGENERATION. C. Johansson, K. Fried, M. Risling, S. Cullheim*, and J. Frisén. Department of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden.

The lack of axonal regrowth after injury in the brain and spinal cord is not primarily a result of weak intrinsic neuronal regenerative capacity. Rather, it is a result of an unfavourable environment for neurite extension in the CNS. It is known that astrocytes as well as oligodendrocytes may inhibit axon growth. CNS glial cells are however known to support axonal growth and guidance during embryogenesis. Moreover, after CNS injury, reactive glial cells can support axonal sprouting in the lesion area. This demonstrates that CNS glial cells can support axonal growth in the adult under certain circumstances. If the signals which induce a switch in glial pheno-type to one which supports axonal growth were known, strategies to induce such prop-erties *in vivo* after nervous system injury could be developed. In order to search for factors which may determine glial phenotype, we have developed an assay to measure the effect of different molecules on the ability of astrocytes to support neurite growth.

Mixed glial cultures were obtained from new-born rat brain and grown in DMEM with 10% fetal calf serum. Pure astrocytes were isolated by shaking confluent cultures. Sensory neurons were prepared from new-born rat dorsal root ganglia. The astrocyte medium was changed to L15 with essential supplements 0, 1, 3, 5, 7, 9, or 11 days before the neurons were plated on astrocyte monolayers. Serum-free medium may provide an environment which is close to physiological, since astrocytes *in vivo* are separated from serum by the blood-brain barrier. A vast number of neurons adhered and grew elaborate neurites on astrocytes which had been in serum containing medium short before the neurons were plated. In contrast, significantly less neurons attached to astrocyte cultures which had been grown for longer time in serum-free medium, and many of the neurons had no or only very short processes.

The data show that serum can induce axon-growth permissive features in astrocytes. Since astrocytes give poor support for adhesion and neurite out-growth under serum-free conditions, the co-culture system presented here can be used to assay the capacity of different molecules to induce an axon-growth promoting astrocytic phenotype.

520.7

SHORT- AND LONG-TERM DEGENERATIVE CHANGES IN PERIPHERAL NERVE AND THEIR ABILITY TO SUPPORT AXONAL REGENERATION. A. K. Gulati* Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

The present study was designed to define acute and chronic changes in peripheral nerve undergoing in situ degeneration and their ability to support axonal regeneration after transplantation. Peripheral nerves were degenerated in situ for a period of 2,4, 8 and 48 weeks for analysis and transplantation in an surgically created gap of the host rat peroneal nerve. During early phase of degeneration the injured axons and associated myelin is broken down and absorbed by proliferating Schwann cells and infiltrating macrophages. The Schwann cell basal laminae persisted throughout degeneration, however were progressively reduced in size and at 12 months were occluded. Such long-term degenerated nerves were reduced in diameter as compared to short-term degenerated nerves (2 and 4 weeks) that exhibited increased diameter. In order to study axonal regeneration through predegenerated nerves, 2 cm long segment was transplanted in host rats. Grafts and distal host nerves were analyzed to determine axonal regeneration. Axonal regeneration was rapid and by 12-weeks many myelinated axons were observed in distal nerve with establishment of innervation of target muscle. Although many regenerated axons were observed in the 48-week degenerated transplant group, the nerves remained small in diameter and the axons were thinly myelinated. In addition buildup of connective tissue was observed in such grafts. The results show that, short-term degeneration is beneficial as compared to long-term degeneration in supporting axonal regeneration (supported by NIH grant NS-24834).

520.8

INJURY INDUCED PROTEOGLYCANS INHIBIT THE POTENTIAL FOR LAMININ MEDIATED AXON GROWTH THROUGH AN ASTROCYTIC SCAR. Robert J. McKeon*, Ahmet Hoke, and Jerry Silver. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Following injury to the adult CNS, a number of extracellular matrix molecules are upregulated in areas of glial scarring. These molecules include laminin and a chondroitin sulfate proteoglycan (CS-PG) which, when used individually as substrates in *in vitro* assays, can promote or inhibit neurite outgrowth, respectively. We have hypothesized that the balance of the expression of these molecules ultimately determines the potential for neuronal regeneration *in vivo*. In order to test this hypothesis, we have begun to characterize the expression of CS-PG's after cortical injury. Using Western analysis, we have identified a 3B3-positive CS-PG with an apparent molecular weight of 180-400 kd that is specifically upregulated in gliotic tissue after injury to adult, but not neonatal animals. This CS-PG inhibits the ability of astrocyte produced laminin to promote neurite outgrowth. Following digestion with chondroitinase ABC, neurite outgrowth on glial scars formed *in vivo* on nitrocellulose implants is significantly increased. However, treatment of chondroitinase treated glial scars with antibodies to the β1,β2 chains of laminin dramatically reduces neurite outgrowth compared to tissue treated with chondroitinase alone. These data suggest that the timing and relative amount of different ECM molecules produced by reactive astrocytes is a critical factor in determining the amount of axonal outgrowth which takes place after CNS injury. Experiments designed to purify, and further characterize this injury induced CS-PG, and analyze the mechanism of its interaction with laminin are currently in progress. Supported by a grant from the Paralyzed Veterans of America Spinal Cord Research Foundation and NS 25713.

520.9

RECOVERY FROM SPINAL CORD INJURY IN NEONATAL S.AMERICAN OPOSSUMS. N.R. Saunders, L.Weller*, A. Deal and G.W. Knott. Depts of Physiology & Anatomy, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001.

Recovery from spinal cord crush has been studied in neonatal (P5-7) and postnatal (P14-20) S.American opossums (*Monodelphis domestica*). Mothers were anaesthetized with intraperitoneal pentobarbitone; the young, still attached to the mother received additional inhaled Metofane anaesthesia. Spinal cords were crushed at the upper thoracic level via a skin incision using fine forceps. Completeness of the lesion was assessed by application of a minor nociceptive stimulus to the hind limbs below the lesion both at the time of lesion and on the day following; absence of a forelimb response was taken as indicating a complete lesion. Control littermates were unoperated. Video recordings were made of operated and control animals at different intervals after surgery up to and beyond weaning (P60). Spinal cords were removed under terminal anaesthesia at different times after operation and in controls. Conduction across the crush was tested for *in vitro* by electrical stimulation of the central end of the cord and recording caudally. In 12 neonates the spinal cord was isolated 24-48h after crushing. Ten out of 12 showed complete block of conduction. In the other 2 the amplitude of the mass action potential was less than 1% of controls. Conduction across the crush by recovered 5 to 10 days post crushing (n=14). Bouin's fixed spinal cords were examined morphologically using silver staining and examination of thick (30-50μm) sections using an Edge Scientific Stereomicroscope. Complete crush at P5-7 was followed by fibre growth across the crush by 5 to 10 days after operation. Substantially normal locomotor function was present towards the time of weaning (P50-60). In neonates operated at P14-20 recovery of conduction did not occur.

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520.10

CORRELATION OF THE FINE STRUCTURE OF LESIONS WITH NEURAL REPAIR IN ISOLATED SPINAL CORDS OF NEONATAL OPOSSUMS AGED 5 DAYS AND 12 DAYS. Z.Varga, J.Fernandez and J. Nicholls*, Biozentrum, Univ. of Basel, Basel Switzerland, 4056.

Profuse outgrowth of neurites has been shown to occur into and beyond the site of a crush in the spinal cord isolated from a 5 day old opossum. We have now analyzed repair at various stages after injury, by light and electron microscopy. During the first 1-3 days, the site of the lesion as well as rostral and caudal segments were totally disrupted, containing no identifiable elements. After 5 days in culture axons and growth cones unaccompanied by glia entered the lesion. Growth occurred along the basal lamina of the pia mater on which synaptoid structures were made. By contrast in lesioned spinal cords in culture isolated from older animals aged 12 days, no repair occurred. The lesion produced by a crush was restricted in its length to the actual site of damage and involved neighboring segments to a lesser extent. Axons and growth cones approached the edge of the crush but did not grow through it. Antibodies against glial fibrillary acidic protein labeled material in these lesions of cords from 12 day old animals which contained only debris and no astrocytes or oligodendrocytes. Our results confirm the existence of a critical period for repair in the neonatal opossum. As yet, no cellular or molecular component has emerged as being responsible on its own for the failure of repair in older animals.

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520.11

PATTERNS OF NEURITE OUTGRWOTH ACROSS TRANSECTED SPINAL CORD OF NEONATAL OPOSSUM IN CULTURE

H.A. Vischer*, Biozentrum, Univ. of Basel, Switzerland

Profuse neurite outgrowth occurs across a lesion made by a crush in the isolated spinal cord of neonatal opossum. Such lesions break axons while the pia mater remains intact (Woodward et al., J. exp. Biol., 1993, 176: 77-88). In this study entire central nervous systems (CNS) of 3-6 day old animals were placed into sylgard molds and completely transected at C5-C7 levels. The two parts were tightly fit together and held in in place by Matrigel. To visualize growing fibers, the rostral parts of the preparations were labeled with the lipophilic marker DiI and incubated for up to 6 days in BME. In 27 out of 35 preparations, fibers in the ventrolateral segment of spinal cords grew up to 500 µm beyond the cut. To determine whether the local environment might influence axonal pathfinding, the spinal cord were cut and the caudal parts were rotated by 180°. In 13 out of 17 experiments ventrolateral fibers turned rostrally on the site of the cut. In 4 out of those 13 experiments fibers grew across the cut into the caudal part at its organotypic ventral segment and thus entered the "correct" area of the spinal cord. This behavior suggests that they use local cues for guidance. We now are using molecular biological approaches to learn more about the nature, distribution and temporal expression of factors that might be involved in axonal guidance.

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520.12

EVIDENCE FOR GROWTH OF SUPRASPINAL AXONS THROUGH HEMISECTED AND TRANSECTED SPINAL CORD IN THE DEVELOPING OPOSSUM, DIDELPHIS VIRGINIANA.

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We have shown previously that at least some supraspinal axons grow around a hemisection of the thoracic cord in developing opossums and that a critical period exists for that plasticity. In such experiments, normal spinal cord was left and axons used it to circumvent the lesion. More recently, we hemisected the thoracic cord at earlier stages of development (postnatal day 5). When the animals were sacrificed as adults, normal-appearing spinal cord was present at the lesion site and orthograde transport methods showed that brainstem axons grew through the lesion, not around it. In a second series of animals operated at the same age, the spinal cord was transected at the same level and approximately 30 days later, Fast Blue was injected several segments caudal to the lesion. In such cases, relatively normal appearing spinal cord was present at the lesion site and supraspinal neurons were labeled in all of the areas expected from controls. Our results suggest that the mammalian spinal cord regenerates after lesions at early stages of development and that descending spinal axons grow through the lesion site to more caudal levels. (Supported by NS-25095 and 10165).

COGNITION: HUMAN I

521.1

HUMAN OSCILLATORY BRAIN ACTIVITY NEAR 40-HZ COVARIES WITH COGNITIVE TEMPORAL BINDING.

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The minimal time interval required to identify auditory stimuli as separate was correlated with the reset of the 40-Hz magnetic signal in the human brain, in order to test the hypothesis that 40-Hz activity may relate to temporal binding by the C.N.S.

Neuromagnetic recordings of coherent oscillatory activity near 40-Hz were obtained over auditory areas of the right hemisphere from nine healthy adults, using a 37-channel Magnetoencephalography (MEG) system (BTi). Subjects attended to auditory stimuli organized in ten blocks consisting of one or two clicks (10 kHz, 60dB SPL) presented binaurally at varying interstimulus intervals (3, 6, 9, 12, 15, 18, 24 or 30 ms). 1000 epochs were recorded for each block with inter-pair intervals of 130 ± 10 ms, the transient responses were averaged and filtered between 20-50 Hz. The average response to a single stimulus consisted of a 2.5 oscillatory cycle 40-Hz response. A set of two stimuli were then presented and the perceptual threshold for identifying the stimuli as two events was established. Experimental and modeling results indicate a stimulus-interval dependent response with a critical time interval of 12-15 ms. At shorter intervals, at which the subjects reported the perception of only one auditory event, only one 40-Hz response was observed. As the interval increased a second 40-Hz wave appeared, abruptly. This second 40-Hz correlated significantly with the recognition by the subjects that two distinct auditory stimuli had been delivered.

These results indicate that oscillatory activity near 40-Hz represents a neurophysiological correlate to auditory temporal processing. It also supports the view that 40-Hz activity relates not only to primary sensory processing, but also to the temporal binding underlying cognition.

521.2

EVENT-RELATED POTENTIAL CORRELATES OF WORKING MEMORY PROCESSES.

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In an initial study of event-related potentials (ERPs) associable with operationally defined working memory processes, subjects viewed randomly arranged series of 2 to 5 single digits (fixed 1.5 sec inter-stimulus interval). Digit pairs were interposed between series. ERPs were recorded from the scalp (Fz, Cz, Pz, Oz). In the Control condition, subjects were instructed to detect digit pairs. The Update condition used the same stimuli and motor requirements but subjects were asked to indicate whether digit pairs matched the preceding two single digits. Success in the Update task requires retention of the first two single digits and their order of presentation. Beyond two, this representation must be successively "updated". ERPs to single digits in the Control condition were digitally subtracted from Update condition ERPs for corresponding serial positions, resulting in Difference waveforms for single digit positions 1 to 5. Subtraction was intended to remove perceptual, response-related and other processes common to the two conditions, thereby isolating the ERP's attributable to cognitive processes required for "updating". ERPs for positions 1 & 2 were relatively small and did not differ. A large positive potential was observed at position 3 which became significantly larger in the Difference traces for positions 4 and 5. These data are consistent with Baddeley's model of working memory, which postulates distinct mechanisms for short term information storage and on-line processing. Two supplementary studies support the ability of this novel ERP paradigm to distinguish between storage and processing, as well as processing types.

521.3

ELECTROPHYSIOLOGICAL AND BEHAVIORAL SIGNS OF HEMISPHERIC ASYMMETRIES OF ATTENTION IN A "SPLIT-BRAIN" PATIENT.

A. M. Proverbio [1], A. Zani [2], G. R. Mangun*[1], M. S. Gazzaniga[1]. [1] Center for Neuroscience, University of California, Davis, CA 95616, [2] Istituto di Psicologia del CNR, 00137 Rome, Italy.

Neuropsychological data have shown that the two cerebral hemispheres differ in the control of spatial attention. The present study investigated hemispheric asymmetries and visuomotor integration in a patient who has undergone complete resection of the corpus callosum, thereby disconnecting the cerebral hemispheres at the cortical level. Simple reaction times (RTs) and Event-related potentials (ERPs) were recorded to lateralized visual stimuli in the patient J.W. and in an age-matched normal control subject (both right-handed). The ERPs were recorded from 61 scalp sites referred to a balanced non-cephalic sterno-vertebral reference. The subjects's eyes were monitored by means of an infrared video-camera and horizontal and vertical electro-oculogram. Stimuli were randomly presented at 6° and 10° of eccentricity to the left or right of a centrally located fixation point. The ISI varied between 1400 and 2800 msec. A response to all stimuli was required. The control subject showed no asymmetries in RTs or P3 amplitude. In contrast, in the split-brain patient RTs were 20 msec slower for more eccentric stimuli in the LVF, but no effect of eccentricity was evident for RVF stimuli. P3 amplitude measures showed that both hemispheres produced a strong response to RVF stimuli, while LVF stimuli elicited a much greater response over the contralateral, right hemisphere. The results support previous theories (e.g., Kinsbourne, 1987) proposing that the right hemisphere allocates attention broadly over left and right hemispace, while the left hemisphere is strongly biased toward the right.

521.4

WORDS AND PSEUDOWORDS EVOKE DIFFERENT PATTERNS OF GAMMA-BAND (30 HZ) RESPONSES.

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Words and pronounceable pseudowords are physically similar, but invoke different cognitive processes. If gamma-band responses of the brain are related to cognitive processing, these stimuli may induce distinct patterns of gamma-band responses in humans. Words and pseudowords were presented in lexical decision and memory tasks. EEG and MEG signals were recorded from both hemispheres. Analysis of spectral responses around 30 Hz revealed differences between stimulus classes. Over the left, language-dominant hemisphere words evoked stronger 30 Hz responses compared to pseudowords. While word-evoked responses did not significantly deviate from the baseline, pseudowords elicited a *reduction* of spectral power.

A tentative explanation of this effect is the following: Word presentation invokes ignition of a cortical cell assembly corresponding to the word. The ignition process includes fast periodic and coordinated activation of large numbers of neurons which is visible in the EEG and MEG. After pseudoword presentation, a variety of assemblies are (pre-) activated leading to uncorrelated activity in numerous assemblies. This results in a reduction of spectral power in the EEG or MEG.

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521.5

LOCALIZATION OF SPONTANEOUS OSCILLATORY CORTICAL ACTIVITY FROM MAGNETOENCEPHALOGRAPHIC DATA RECORDED DURING THE IMAGINATION OF MOVEMENT AND SILENT SPEECH. Claudia D. Tesche*, Mikko A. Uusitalo, and Matti J. Kajola Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland. Risto J. Ilmoniemi BioMag Laboratory, Helsinki University Central Hospital, 00290 Helsinki, Finland

Does mental imagery in the absence of external stimuli alter the spectral characteristics of spontaneous oscillatory activity in humans? We measured spontaneous activity in six subjects with a 122-channel whole-scalp magnetoencephalographic (MEG) array. The subjects performed two contrasting tasks: the imagination of the self-performance of a motor activity and the silent generation of a chain of words. We developed a novel analysis method, frequency-domain signal-space projection (FDSSP), to compute components of ongoing spontaneous activity at specific cortical sites from this data. Although intersubject differences were significant, highly reproducible spectra of oscillatory activity were obtained within subjects in the primary somatomotor and parietal association areas which showed variability in the spectral features with task including variations in amplitude and, in some subjects, task-dependent changes in the frequency of sharply defined spectral peaks.

FDSSP analysis may also be applied to electroencephalographic (EEG) data. Objective evidence of the constancy of the state of a subject obtained from FDSSP may facilitate the interpretation of psychophysical measurements. FDSSP analysis of rhythmic EEG and/or MEG data recorded during epileptic activity and sleep may lead to the localization of sources of activity in specific neuronal populations.

521.6

THE EFFECTS OF BROMOCRIPTINE, A D-2 DOPAMINE RECEPTOR AGONIST, ON THE COGNITIVE ABILITIES OF HUMAN SUBJECTS WITH DIFFERENT WORKING MEMORY CAPACITIES. D. Y. Kimberg, M. D'Esposito and M. J. Farah*. Dept. of Psychology, Carnegie Mellon Univ., Pittsburgh, PA 15213; Depts. of Neurology and Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Recent research in monkeys and humans has suggested that the prefrontal cortex (PFC) subserves working memory (WM), and that dopamine plays an important role in the PFC WM system. In order to provide a new test of this hypothesis, and to investigate the role of dopamine and WM in a variety of cognitive tasks, we administered a battery of tasks to normal human subjects twice, on and off bromocriptine, a D2 dopamine receptor agonist, in a double blind procedure. The battery of tasks included: working memory tasks (spatial working memory, verbal working memory, and the fan effect paradigm), other tasks thought to depend on PFC (The Wisconsin Card Sorting Test, Stroop Task, a context memory task, and a dual task paradigm), and a control task (visual search) not hypothesized to depend on WM or PFC. Although there was no significant main effect of bromocriptine over all subjects, those with lower verbal WM capacity benefitted from the drug on both WM tasks and other PFC tasks and, more surprisingly, those with higher WM capacity were reliably impaired by it on these same tasks. No effects of bromocriptine were observed for the visual search control task. These results demonstrate a selective effect of bromocriptine on PFC tasks, which depends on subjects WM capacity. Of course, the precise nature of the relations among individual differences in WM, PFC function and cognition remains to be understood. Nevertheless, these results strengthen the evidence for the role of dopamine in WM and PFC function, and also suggest a possible mode of therapy for PFC-related cognitive impairment.

521.7

DISCORDANCE FOR PLANUM TEMPORALE ASYMMETRY IN HANDEDNESS-DISCORDANT MONOZYGOTIC (MZ) TWINS. H. Steinmetz*, A. Herzog, G. Schlaug, Y. Huang and L. Jäncke[‡]. Departments of Neurology and [‡]General Psychology I, Heinrich-Heine-University, PO Box 101007, D-40001 Düsseldorf, F.R.G.

Studies comparing biological with adoptive families have shown that handedness is influenced by prenatal factors. It is unclear whether these factors are genetic or non-genetic. Using *in vivo* magnetic resonance (MR) morphometry we measured the degree of planum temporale (PT) asymmetry $\delta PT=(R-L)/[0.5(R+L)]$ in 20 MZ twin pairs concordant (10 right-handed pairs) or discordant (10 pairs) for handedness reporting no birth complication, neuropsychiatric illness, learning disability or failure in elementary school (3 MR observers blind for twins *vs.* 60 controls, and right *vs.* left hemisphere). As previously described for singletons, right-handed twins showed leftward PT asymmetry ($P<.05$) whereas the left-handed co-twins, as a group, lacked asymmetry ($P>.10$). Intraclass correlations were low ($R<.25$). This discordance for structural asymmetry can be accounted for by models assuming cleavage of an intrinsically asymmetric blastomere or differential action of other non-genetic factors within MZ twin pairs *in utero*. The findings confirm a coupling of lateralized brain structure and function. At least in MZ twins, early epigenetic factors must play a role in laterality development. [Support: DFG, H. & L. Schilling Foundation]

521.8

PET STUDIES OF COGNITIVELY RELATED CEREBRAL BLOOD FLOW IN MENSTRUAL-RELATED MOOD DISORDER PATIENTS AND CONTROLS: EFFECTS OF GONADAL STEROIDS. K. F. Berman,* P.J. Schmidt, J.L. Ostrem, M.A. Danaceau, G. Esposito, J.D. Van Horn, D.R. Rubinow, D.R. Weinberger. NIMH/IRP, Clinical Brain Disorders and Biological Psychiatry Branches, Bethesda, MD 20892.

Despite inferential evidence from cognitive and other behavioral studies and from animal experiments, there is little direct information about the effects of hormones on human neurophysiology during cognition. We used the oxygen-15 water PET method to measure regional cerebral blood flow (rCBF) in four women with menstrual-related mood disorder (MRMD) and four controls during three pharmacological conditions: 1) ovarian suppression induced by gonadotropin releasing hormone agonist (Lupron), 2) Lupron plus estrogen (E), and 3) Lupron plus progesterone (P). During each drug condition subjects were scanned at rest and while performing the Wisconsin Card Sorting Test and a simple matching task. Absolute CBF (ml/min/100g) was determined pixel-by-pixel, and the three PET data sets were each registered to a single MRI scan on which regions were drawn.

Global CBF was lower in the patients than the controls during the resting Lupron baseline (45 vs. 55, $p=0.04$) and tended to increase with P and decrease with E for the group as a whole. The region most affected by hormone treatment was the superior left anterior cingulate (ANOVA drug effect $F=4$, $p<0.05$), which was increased by P and decreased by E during the tasks despite unaltered performance scores. These data provide direct insight into the effects of gonadal steroids on brain function during cognition and may also suggest a neurophysiologic characteristic of patients with MRMD.

521.9

PET ACTIVATION IN ALZHEIMER'S DISEASE DEMONSTRATE THE ROLE OF LEFT ANGULAR GYRUS IN SEMANTIC MEMORY. M. Grossman*, E. Hughes, K. Onishi, N. Biassou, M. D'Esposito, K.M. Robinson, X.-S. Ding, A. Alavi, M. Reivich. Cognitive Neurology Section, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

We sought to define the cognitive and physiologic basis for semantic memory. Cognitive assessments were used to characterize semantic memory deficits in patients with probable Alzheimer's disease (pAD) during comprehension and expression of words and pictures. An equilibrium technique used the constant infusion of ^{15}O-H_2O to quantify regional cerebral blood flow (rCBF) in individual patients and controls during a semantic category membership recognition task (identifying exemplars of a familiar superordinate category). An individualized anatomic atlas based on each subject's MRI localized rCBF activation. Cognitive assessments revealed that several pAD patients had a supramodal semantic memory deficit. These patients demonstrated characteristic difficulty understanding and expressing word and picture information about a target concept that could not be attributed to a memory deficit or non-specific cognitive limitations. PET assessment revealed that individual control subjects consistently recruited left angular gyrus during the PET semantic challenge. By comparison, pAD patients failed to recruit left angular gyrus during a semantic challenge while PET monitored rCBF. Instead, pAD patients recruited right inferior parietal structures. pAD patients without supramodal semantic memory deficits were able to recruit left angular gyrus during a PET semantic challenge. We hypothesize that left angular gyrus contributes importantly to semantic memory by integrating information from multiple, material-specific and modality-specific sources.

521.10

IMPAIRED RECOGNITION MEMORY IN AGING IS ASSOCIATED WITH REDUCED ACTIVATION OF HIPPOCAMPUS AND FRONTAL CORTEX. CL Grady*, AR McIntosh, B Horwitz, LG Ungerleider, P Pietrini, MJ Mentis, JV Haxby. Lab. of Neuroscience, NIA, Lab. of Neuropsychology, NIMH, and Lab. of Psychology and Psychopathology, NIMH, Bethesda, MD 20892.

We measured rCBF in 10 young (25 ± 2 yrs) and 10 old (69 ± 6 yrs) healthy subjects using positron emission tomography and [15-O]water during learning of and recognition memory for faces. Three tasks were carried out by all subjects in the following order: learning, face matching, and recognition. Old subjects performed significantly worse than young subjects during the recognition condition (66% v. 80% correct, $p<0.001$). During learning, compared to face matching, young subjects showed increased rCBF ($p<0.001$) in the right hippocampal region, left inferior frontal and medial frontal cortex. Old subjects did not show significant activation of the hippocampus during learning. They did show activation in left frontal cortex ($p<0.001$), but in a location different from the one seen in young subjects. During recognition, compared to face matching, the young subjects had rCBF increases in right frontal and parietal cortex, and anterior cingulate ($p<0.001$), and the old subjects had rCBF increases in right frontal cortex (again, in a different location). These results support earlier PET studies that found right frontal activation during recognition memory, and provide evidence for the role of the hippocampus in stimulus encoding. In addition, they suggest that reduced recognition in older subjects is due, at least in part, to a failure to adequately activate the hippocampal area during learning, and thus to adequately learn the list of faces. Alterations of rCBF in prefrontal cortex also appear to play a role in age-related memory impairment.

521.11

LOCALIZATION OF LANGUAGE OPERATIONS WITH $H_2{}^{15}O$ PET: INDIVIDUAL DIFFERENCES IN FUNCTIONAL ANATOMY AND THEIR RELATION TO IMAGE SMOOTHING. S.Y. Bookheimer* and T.A. Zeffiro. Brain Mapping Division UCLA, Los Angeles, CA 90024 and National Institute on Aging, NIH, Bethesda, MD.

Sixteen normal volunteers underwent positron emission tomography while performing simple language tasks (reading words and naming objects). Images were stereo-tactically normalized and smoothed using Statistical Parametric Mapping (MRC Cyclotron Unit)to an effective FWHM of 12 or 21 mm. Although the same critical threshold was used in both analyses, the results differed markedly: activation in left inferior frontal (IFG) cortex (Broca's area) was identified only with the broad-filter while significance in cerebellum, striatum and extrastriate cortices were detected with both filters. However, spatially separate maxima were identified in these regions only with the narrow filter. A second experiment with left handers-who are known to have greater spatial variability in language organization- confirmed the difficulty in obtaining reliable anterior but not posterior results. High between-subject variability in the structural and functional anatomy should favor broad band filters, which are more sensitive to changes occurring over a wider spatial extent; Inferior frontal language areas are among the most variable cortical structures. Our data suggest that the optimum smoothing kernels will differ as a function of the known anatomic variability. Interpretation of PET language data is critically dependent on how this variability is treated in image analysis.

DEGENERATIVE DISEASE: ALZHEIMER'S—MECHANISMS OF DEGENERATION

522.1

NONLINEAR EEG DYNAMICS IN AGING AND DEMENTIA. KL Coburn*, WS Pritchard, DW Duke, NC Moore, RM Hostetler, MW Jann. Mercer Univ. Sch. of Med., Macon, GA 31207.

A series of 5 studies investigate topographic aspects of nonlinear EEG dynamics and their changes with information load, aging, and dementia. Major findings are that dimensional complexity (DCx; estimated correlation dimension) topography corresponds to EEG patterns of different cortical areas; some areas are more "chaotic" than others. Topographic patterns change in response to stimulation, and DCx offers a single-variable approximation of information load. Increasing visual information load increases DCx, especially over occipital areas in young subjects, but this effect is reduced in aging and nearly lost in dementia. Addition of nonlinear measures to traditional linear (FFT) measures improves the discrimination of demented patients from controls. For both types of measures, nonlinear neural net prediction offers significant advantages over linear analysis methods. Thus, nonlinear EEG measures and analysis methods offer unique perspectives for understanding both normal and pathological aging.

522.2

INITIAL CLINICAL APPLICATIONS OF "BURST" fMRI IMAGING D.R. Weinberger*, V.S. Mattay, K.J. Korula, J.H. Duyn, C.T. Moonen, R. Sexton, T. Sunderland, F.A. Barrios, J.A. Frank. Clinical Brain Disorders Branch, National Institute of Mental Health, Washington, D.C. 20032

Functional neuroimaging using MRI techniques (fMRI) have unique capabilities for mapping the physiological correlates of behavior; their potential clinical applications are less clear. In this study, we evaluated the utility of a fast 3-dimensional T2* sensitive method, called Frequency Shifted BURST (FS-BURST) in various neurologic disorders. This novel method has the capability to scan the whole brain on a conventional 1.5 T MR scanner in only two seconds. Nine normal volunteers, three patients with mild to moderately severe dementia of the Alzheimer's type (DSM-III-R diagnosis), one patient with a subacute cortical infarct in the left middle cerebral artery distribution and one patient with a high grade lymphoma were studied in the resting condition. Scans were performed on a conventional 1.5T GE/SIGNA MR scanner. Modified (FS) BURST MRI was performed during intravenous bolus administration of Gd-DTPA (0.13 mmol/kg). 3D datasets were acquired with an effective spatial resolution of 4.3x4.3x6.4mm within 2.2 seconds. 3D maps of relative cerebral blood volume (rCBV) and bolus arrival time were created by fitting a synthetic curve to the intensity time course on a pixel by pixel basis. The rCBV maps of the Alzheimer' s patients revealed focal areas of hypoperfusion in a frontotemparoparietal distribution identical to their SPECT perfusion scans. In the patient with the subacute infarct, the transit time maps showed arrival time delays of 5-7 seconds within and around the infarct clearly confirming the diagnosis of left middle cerebral artery occlusion. In the patient with CNS lymphoma decreased CBV around the tumor correlated with a mass effect. No focal defects were identified in the normal controls. These results illustrate the technique's potential as a clinical tool in the diagnosis and management of various brain disorders. This technique can be added to routine clinical MR scanning, requiring additional imaging time of only 3 - 5 minutes.

522.3

ANALYSIS OF STAGED AUTOPSY CASES UNCOVERS PACE OF ALZHEIMER'S DISEASE-RELATED NEUROFIBRILLARY CHANGES. J. Bohl (1), H. Müller (2), H. Braak (2)* T.G. Ohm (2). (1) Abt. Neuropathologie, J.Gutenberg Universität, 55131 Mainz, (2) ZMorph., J.W.Goethe Universität, 60590 Frankfurt; Germany.

The speed of progression of Alzheimer's disease (AD)-related neurofibrillary changes is unknown. This is because of the impossibility to histopathologically follow-up one and the same individual over decades of its life. The present approach is based on a recently introduced staging system which differentiates between six stages of AD-related neurofibrillary changes and analyzes a staged sample of 887 autopsy brains. The time needed to attain respective stages of pathology for 5% of a given cumulative sample is determined. The resulting 5%-percentiles are a measure for the average pace by which the disease-related changes progress assuming that the underlying stages represent a sequence of events and do not independently "pop-out". Advancing age and the prevalence of AD-related changes of a given stage show a nonlinear positive correlation with only slight acceleration above the age of 65. Statistically, it takes at least 16 years to shift from stage I to stage II, 14 years from stage II to III, 13 years from stage III to IV and 5 years from stage IV to V (=AD) for 5% of a given cumulative sample. Obviously, the deep roots of AD-related neurofibrillary changes can be traced about 50 years back and may thus even extend into adolescence. The unveiling of the duration of the shift between selected stages provides a powerful tool for epidemiological studies. A possible risk factor should shorten this period whereas beneficial influences would result in a delayed shift even if the considered cohorte dies "too early" for the full development of AD.

522.4

LAYER-SPECIFIC DENDRITIC ALTERATIONS OF HIPPOCAMPAL GABAERGIC NEURONS SUGGEST TRANSNEURONAL CHANGES IN ALZHEIMER'S DISEASE. S. Münch, R. Nitsch* and T. G. Ohm Center of Morphology, University Clinic Frankfurt, F.R.G.

Neurons in layer II and III of the entorhinal cortex are the first to exhibit neurofibrillary changes in Alzheimer's disease (AD). In this early stage of the disease, the target area of axons arising from these neurons, i.e., the hippocampus, remains devoid of these changes. Experimental studies have demonstrated layer-specific transneuronal changes on dendrites in the termination zone of entorhinal fibers, e.g., the outer zones of the dentate molecular layer. The present study was designed to unravel whether similar transneuronal dendritic changes occur during the spread of neurofibrillary tangles from the entorhinal cortex to the hippocampus as shown in a staging analysis by Braak and Braak (Acta Neuopathol. 82:239, 1991). We analyzed the hippocampal formation of 33 staged Alzheimer cases obtained from autopsy. The entire dendritic tree of parvalbumin-immunostained hippocampal GABAergic neurons was monitored using an interactive neuron tracing system. This cell population is known to remain devoid of neurofibrillary tangles even in severe AD. The quantitative analysis revealed a statistically significant reduction of the mean dendritic length, the segment number and branch order of dendrites in the course of AD. These changes were confined to the apical dendritic tree which extends into the termination zone of entorhinal fibers. In contrast, basal dendrites extending into the hilus remained unchanged in their morphology. Our data demonstrate that layer-specific dendritic alterations in hippocampal GABAergic neurons appear together with the spread of histopathological changes in AD. This suggests the occurrence of transneuronal changes on the hippocampal targets of entorhinal neurons.
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522.5

DYSTROPHIC PERIKARYA AND NEURITES INTERSPERSED WITH NORMAL NADPH DIAPHORASE-/NITROGEN MONOXIDE SYNTHASE (NOS)-CONTAINING NEURONS (NOSN) IN THE CEREBRAL CORTEX OF PATIENTS WITH NONALZHEIMER DEMENTIA AND MOTOR NEURON DISEASE (MND), BUT NOT WITH MND WITHOUT DEMENTIA. R.O. Kuljis* and R.L. Schelper. Depts. Neurology and Pathology, The University of Iowa and VAMC, Iowa City, IA 52242.

Many adult patients with MND develop cognitive impairment, which may antedate motor manifestations, and that is most frequently not attributable to superimposed conditions such as Alzheimer's disease. The precise basis for such cognitive deterioration in patients presumed to have an insult selective to motor neurons remains elusive. In the course of a series of studies on the role of NOSN in neurodegenerative disorders, we discovered that these neurons exhibit clearly dystrophic features in MND. Five patients with sporadic MND were studied, two with neuropsychologically verified dementia (MND+d; both 65 years old; 1-23 hours of postmortem autolysis) and three without dementia (64-68 years old; 4-18 hours of autolysis). Tissue blocks from nine primary sensory, motor, association and limbic areas of the neocortex were sectioned frozen and reacted for NADPH diaphorase histochemistry, which reveals NOSN and their neurites. A conventional histopathologic analysis failed to show a cause for the cognitive symptoms in MND+d, but verified the clinical diagnosis of MND in all cases. Cases with MND+d, but not those without dementia, exhibit numerous markedly dystrophic NOSN perikarya and neurites, interspersed with normal NOSN in all areas examined. An additional series of control tissue sections from four age-matched and two younger patients -- with a wide range of autolysis intervals and fixation parameters -- failed to reveal similar dystrophic changes, indicating that they may be selective to MND+d. These observations suggest that cognitive impairment in MND+d may be related to a pancortical deterioration of the ubiquitous NOSN network, indicating a novel role for these neurons in neurodegenerative disorders. Supported by PHS grant NS29856.

522.6

A DECREASE OF GROWTH INHIBITORY FACTOR (GIF) IN THE TEMPORAL CORTEX OF ALZHEIMER'S DISEASED (AD) BRAIN IS INVERSELY RELATED TO GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) EXPRESSION AND GLIOSIS. N.J Cartel*, W.H. Yu, C.A. Mizzen, E.T. Jaikaran, D.R. McLachlan
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GIF, or metallothionein-3 (MT-3), a member of the metallothionein family, is localized predominantly in astrocytes of the molecular layer of the dentate gyrus, the pyramidal cell layer of the hippocampus, and layers 2-6 of the neocortex. GIF is expressed in mature astrocytes but is down-regulated during fetal development and in AD brain.

Western blots for GFAP, GIF, and total metallothionein (MT-1, MT-2 and GIF; MT_{TOT}) were performed to quantitate these proteins in temporal lobe of adult human control and AD brains. Levels of MT_{TOT} in control and AD temporal cortex were indistinguishable. However, GIF in AD brain was decreased by 84.1% when compared to control. Conversely, there was an 83.4% increase of GFAP expression in AD brain when compared with control.

Immunocytochemical detection of the same proteins was performed on sections of control and AD from the middle temporal gyrus. Double immunostaining suggested that GIF is down-regulated in the cytoplasm, nucleus and processes of astrocytes eventhough reactive gliosis was found in AD brains. This was reflected in heavy GFAP staining of astrocytes in AD sections. Furthermore, even though no differences in MT_{TOT} expression were apparent when assessed by blotting, immunostaining suggested diminished staining per glial cell. Presumably, absolute levels determined by blotting were unaltered due to increases in cell number concomittant with gliosis. Thus, it seems that basal MT_{TOT} expression is not altered in large regions of temporal lobe in AD. GIF is the first astrocytic protein found to be down-regulated during gliosis in AD.

522.7

DISTRIBUTION OF NGF IN THE NORMAL AGED HUMAN BRAIN AND IN ALZHEIMER'S DISEASE.

SA Scott*, J Weingartner, and KA Crutcher. Department of Neurosurgery, University of Cincinnati School of Medicine, Cincinnati OH 45267-0515.

Using the two-site ELISA, both the rodent and monkey brain have been examined for NGF content with high levels found in the hippocampus and neocortex. Recent data from our laboratory suggest a similar distribution in the aged human brain (mean 74 yr) and, moreover, that the levels are comparable to those observed in other species. The highest levels of human NGF are within the dentate gyrus (1200 pg/g) whereas the lowest are found in cerebellum (80 pg/g), brainstem (40 pg/g) and spinal cord (0 pg/g). Between these extremes, neocortical areas contain 300-500 pg/g NGF with little variation among the principal gyri, although the temporal and insular regions generally contain the highest levels. The basolateral amygdala and the nucleus basalis of Meynert are rich sources of NGF (~ 700-800 pg/g). Other areas with notably high levels include the choroid plexus (> 1000 pg/g), entorhinal cortex (750 pg/g), medial thalamus (600 pg/g), and neostriatum (450 pg/g), whereas the pineal gland, olfactory bulb/tract, hypothalamus/mammillary bodies, and globus pallidus contain generally lower levels (< 350 pg/g). This broad spectrum of NGF localization in brain, together with the known distribution of high-affinity trkA receptors, suggests that brain NGF is not localized simply in relation to the basal forebrain cholinergic system.

NGF levels were at least moderately higher with Alzheimer's disease in all regions examined (frontal and occipital poles, amygdala, putamen, hippocampus, cerebellum, superior temporal gyrus, and inferior parietal lobule), although the distribution was similar to that in aged controls.

Supported by NIH #NS31410 and AGO5605

522.8

SELECTIVE ABNORMALITIES IN MULTIPLE NEUROTRANSMITTER RECEPTOR DENSITIES IN NEURODEGENERATIVE DISEASES.

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This study investigates the densities of multiple Neurotransmitter Receptors, in ten different cortical regions of human brain, obtained from patients: Normal, Schizophrenia(Sch), Alzheimer's disease(AD) and Multi Infarct Dementia(MID).

Aliquots of membrane suspension obtained from each cortical region were analyzed simultaneously for the binding of radiolabeled nicotine, ketanserin and SCH 23390 to nicotine,serotonin 5HT2 and dopamine D1 receptors respectively. The receptor binding assays were done in the absence and presence of cold ligands to obtain specific and non-specific binding respectively of radiolabeled ligands. Important differences in the densities of the three different neurotransmitter receptors were noted in the ten cortical regions of Sch, AD, MID brains as compared to normal.

-5HT2 receptor showed a significant **increase** in **Sch brains** and a remarkable **decrease** in **AD and MID brains.**

-Nicotine receptor underwent significant **reduction in AD and MID brains** , and in 9 out of 10 cortical regions in **Sch** brains.

- D1 receptor was **enhanced** in the cortical regions studied in **Sch. brains.**

Selective alterations in the densities of the above multiple neurotransmitter receptors in brain, appear to be present in aforementioned Neurodegenerative diseases.

522.9

ALZHEIMER'S DISEASE PATHOGENESIS: EVIDENCE OF INCREASED PROOXIDANT ACTIVITY IN THE INFERIOR TEMPORAL LOBE. A.M. Palmer* and M.A. Burns. *Departments of Psychiatry and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.*

The concentration of a product of lipid peroxidation (malondialdehyde) was determined in six areas of neocortex of 8 subjects with Alzheimer's disease and 8 control subjects, matched for age (74 ± 7 and 73 ± 8 years, respectively) and postmortem delay (10 ± 6 and 11 ± 7 hours, respectively). Malondialdehyde (MDA) concentration was significantly increased by incubation with iron and ascorbate in all samples. Basal and iron/ascorbate-stimulated malondialdehyde concentration were 19-40% higher in the inferior temporal cortex of Alzheimer subjects than corresponding controls; other regions were unaffected. In order to assess antioxidant capacity, we determined the activities of glutathione peroxidase and glutathione reductase in addition to two enzymes of the pentose phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphonogluconate dehydrogenase) in three areas of neocortex. The only difference was an increase in the activities of glucose-6-phosphate dehydrogenase (to 126% of controls) and 6-phosphonogluconate dehydrogenase (to 162% of controls) in the inferior temporal cortex of Alzheimer subjects. Together these data indicate that lipid peroxidation is increased in Alzheimer's disease because of increased prooxidant activity rather than diminished antioxidant defenses. Moreover, it appears that the activity of pentose phosphate pathway is increased in response to increased prooxidant activity since 6-phosphonogluconate dehydrogenase activity significantly correlated (R_S = -0.89, $p < 0.01$) with the stimulated formation of MDA. This inverse relationship suggests that elevations in the activity of pentose phosphate enzymes reflect a compensatory change in response to increased prooxidant activity. Although it is not clear if increased prooxidant activity is a cause or a consequence of early pathological change, it is consistent with the involvement of oxidative stress in the maintenance and progression of the cascade. Antioxidant therapy can therefore be expected to stop, or at least slow, the progress of this debilitating disease of late life.

522.10

NEUROTOXIC EXTRACTS OF ALZHEIMER'S DISEASE BRAIN ARE ASSOCIATED WITH REACTIVE MICROGLIA. L.J. Haverkamp* and J.B. Kirkpatrick. Depts. of Neurology and Pathology, Baylor College of Medicine, Houston, TX 77030.

We have adopted a triple stain for plaques (thioflavine-S), cell nuclei (bisbenzimide), and reactive microglia (immunohistochemistry for HLA-DR) in tissue obtained after relatively short post-mortem intervals (<6 hr.), lightly fixed in paraformaldehyde. Using this technique, we note as especially prominent in Alzheimer's disease (AD), clusters of three or more reactive microglia, invariably in association with all (99%) core senile plaques, a majority (40-85%) of neuritic plaques, and none of the diffuse plaques which are often found in the brains of non-demented elderly. Confocal scanning laser microscopy comparisons of the microglia at a distance from plaques and those associated with core plaques, show the latter to exhibit up to three times the level of HLA-DR immunoreactivity, six times the cell body volume, and 70-fold decreases in process length. In vitro, microglia stimulated with ß-amyloid assume a reactive morphology and produce a potent neurotoxin whose effects can be blocked by NMDA receptor antagonists. A neurotoxin, apparently identical in properties to this poison produced by microglia in vitro (Giuilian et al., 1993, J. Neurosci. 13:29), can be extracted from AD brain autopsy material. Extracts of normal (N=5) and neurological control (ALS; N=6) brains (matched for age and post-mortem interval) lacked toxic effect. Toxic activity was regionally distributed within AD brains (N=7), with hippocampus invariably containing high levels of toxin while cerebellum and white matter had very little. Correlations of histopathology and toxicity of adjacent tissue, from 8 regions in each of the control and AD brains, showed a close relationship between neuron-killing activity and the density of microglial clusters. Lesser degrees of association were found between toxicity and neuritic or diffuse plaques. These findings suggest participation of activated microglia-derived neuronal poisons in the neuron death which occurs in AD.

522.11

APOε4 GENE-DOSE DEPENDENT DEVELOPMENT OF ALZHEIMER-TYPE NEUROFIBRILLARY TANGLES - A PCR-AIDED AUTOPSY ANALYSIS. T.G. Ohm* (1), M. Kirca (2), J. Bohl (3), H. Scharnagl (2) and W. März (2,4); (1) ZMorph. & (2) ZBioChem J.W. Goethe-Universität, 60590 Frankfurt/M, (3) Neuropath. J. Gutenberg-Universität, 55131 Mainz, (4) Klin. Chemie, Albert Ludwigs-Universität, 79106 Freiburg, Germany.

The final diagnosis of Alzheimer's disease rests on the presence of a sufficiently high number of neurofibrillary tangles and neuritic plaques. We based our study on a staging system (Braak and Braak, Acta Neuropathol 82: 239, 1991) which considers the gradual development of AD-related changes over time and correlates highly with the cognitive decline assessed ante mortem. Our analysis revealed that the mean stages for NFTs as well as for A4/ß-amyloid deposition get significantly shifted upwards in ε4-carriers. A NFT-stage shift represents an earlier onset of the pathological process of about one decade. The fact that both NFT and extraneuronal amyloid formation coincidently correlate positively with the gene-dose for apoE suggests an metabolic link to apoE-metabolism associated with apoE isoform-specific alterations.

ApoE	Observed Means		Age-adjusted means	
genotype	NFT	A4/ß	NFT	A4/ß
ε2/2	2.00	1.00	3.23	1.80
ε3/2	3.89	2.54	3.33	2.18
3/3	3.25	2.42	3.07	2.30
ε4/2	4.00	3.50	3.44	3.14
ε4/3	4.37	3.09	4.05	2.88
ε4/4	5.17	4.00	5.56	4.25
univariate F	*4.26*	*4.92*	*4.98*	*5.78*
p	*<0.001*	*<0.001*	*<0.001*	*<0.001*

CELL LINEAGE AND DETERMINATION III

523.1

An identified neuron necessary for induction of segment-specific neurons in the leech CNS. G. W. M. Bothe*, T. Becker and E. R. Macagno. Dept. Biol. Sci., Columbia Univ., New York NY 10027

Whereas a set of 400 neurons is found in most ganglia in the leech, *Hirudo medicinalis*, ventral nerve cord, each of the two ganglia innervating the sex organs contains an additional 350 small neurons. The birth of these neurons is induced by the male organ during a critical period from day 13 to 16 of embryogenesis. They were therefore named "Peripherally Induced Central" (PIC) neurons. The inductive signal leading to the birth of these cells is conveyed via the nerves that connect the CNS to the male organ (the sex nerves). In the absence of these nerves, induction does not take place. The sex nerves contain the axons of 10 - 11 identified neurons in each sex ganglion.

To test whether any of these neurons are involved in the induction process, we used single and pairwise cell ablations. One pair of cells, the mediolateral (ML) neurons, were found to play a key role in the induction process. Ablation of a single ML neuron resulted in a 50% reduction of dividing PIC precursors on the side of the ganglion where the neuron was killed. Ablations of other neurons had no significant effect. Ablation of both ML neurons in one ganglion did not totally abolish cell divisions, suggesting that other neurons can partly substitute for the ML neurons in inducing PIC neurons.

The effect of the ML neuron on proliferation in the sex ganglia is consistent with the idea that their axons convey the inductive signal from the target to the CNS. We are currently investigating the spatial relationship of the ML neuron and the PIC neuron precursors at the time of induction.

523.2

MESOTOCINERGIC NEURONS ARISE FROM A REGION PREDICTED BY A NEURAL PLATE FATE MAP FOR *XENOPUS* FROG EMBRYOS. K.M. Conway* and C. Thaler. Dept. Biol., The American Univ., Wash., D.C. 20016

We used Eagleson and Harris' fate map (*J. Neurobiol.* 21, 427) to identify a region comprising left anterior medial neural plate and adjacent medial anterior neural ridge likely to contain all precursors of left side mesotocinergic neurons. Reciprocal grafts of this region were exchanged among embryos of the *Xenopus* species *laevis* and *borealis*. The experimental design included sham operated and unoperated controls. Early limb bud stage animals were processed for immuno-cyto-chemistry (ICC) using a primary anti-mesotocin antibody reagent (VA-10: Conway and Gainer, *J. Comp. Neurol.* 264, 494), and ultimately labelled with the fluorochrome Texas Red (TR). Detergent treatment then permitted nuclei to be stained with 4'-6-diamino-3-phenylindole (DAPI).

Borealis host embryos subject to surgery had poor survival rates, statistically fewer mesotocinergic cells, and fuzzy DAPI staining, and were thus not further considered. Slides with tissue from *laevis* hosts and unoperated *borealis* controls were examined using a 40x oil immersion objective lens. They clearly showed mesotocinergic neurons labelled by TR and, where appropriate, regions of speckled DAPI stained nuclei characteristic of *borealis* tissue.

Resolution of DAPI stained speckles in individual *borealis* nuclei using a 100x oil immersion objective lens was obtained by digital contrast enhancement of video images. A few known *borealis* nuclei were artifactually scored as *laevis* as their nuclear speckles were not resolved. However, analysis of mesotocinergic cells on the operated side of chimeric *laevis* hosts showed statistically significant numbers of *borealis* cells and statistically insignificant numbers of *laevis* cells.

Thus, the grafted region contains the precursors of all mesotocinergic neurons on the operated side in early limb bud stage *Xenopus* tadpoles. We thank NICHD and TAU for support and Tom Miele (Opelco) for equipment demonstrations.

523.3

NEURONAL PHENOTYPE DETERMINATION IN THE HINDBRAIN: CONSECUTIVE ASSIGNMENT OF AXIAL POSITIONAL VALUES. A.Lumsden*, A.Hornbruch and H.Simon. (SPON: Brain Research Association) MRC Brain Development Programme, UMDS, Guy's Hospital, London SE1 9RT, England.

A working hypothesis is that cell pattern in the neural tube is controlled by a coordinate system of positional information, set up along the anteroposterior (AP) and dorsoventral (DV) axes. During development, the AP axis of the chick hindbrain becomes subdivided into a series of compartments (rhombomeres) that underlie its segmented architecture. Coincident with delineation by interfaces where cell mixing is transiently restricted, the rhombomeres become determined for expression of a specific combination of selector genes (Hox genes) that may encode rhombomere identity. Rhombomere phenotype is determined at this stage. Thus, when presumptive rhombomere 4 (r4) is transposed for r2 (at 6 somites), the graft expresses an r4-specific gene (Hoxb-1) and develops r4-specific neurons. We now find that only the AP and not the DV positional value of the rhombomere is determined at this time. Thus, when r4 is grafted to the r2 position with its DV polarity inverted (the alar plate positioned ventrally, abutting the floor plate) r4-specific motor neurons appear medially, within the original alar plate. Cells are committed to their fates according to position on a Cartesian grid whose coordinates are set sequentially; DV positional values are labile when AP values are already fixed.

523.4

PHYLOGENETIC DIVERSIFICATION WITHIN EMBRYONIC HINDBRAIN RHOMBOMERES AND THE NEURONAL ORGANIZATION UNDERLYING POSTURAL CONTROL. E. Gilland and R. Baker*. Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016

Comparative ontogenetic models have great potential for establishing how gene expression confers positional identity to cells, an identity that in turn largely predetermines early embryonic axonal pathways and innervation patterns. Despite the diversity of adult brainstem organization, recent data suggest that the overall segmental, rhombomeric (Rh) blueprint of the hindbrain has been genetically conserved in vertebrates. The phylogenetic diversification of cellular identity related to derived sensory-motor circuits responsible for vertebrate posture can now be addressed within this Rh framework. Dye labeling demonstrates that cranial and spinal somatomotor neurons arise as largely non-overlapping segmental populations arrayed rostral to caudal along the Rh neural axis with homeotic-like shifting of cranial nerve efferents occurring in different species. Surprisingly, the hindbrain areas identified as afferent to eye, head and spinal cord limb/axial motor nuclei are ordered posterior to anterior in the medulla of diverse vertebrates. In particular, we have documented the morphology and physiology of individual, non-overlapping nuclei extending through regions arising from Rh 8 to 4 in teleosts. Notably, the two most evolutionarily derived oculomotor centers, the horizontal eye position and velocity integrators, are located just rostral to the spinal cord and are presumed to originate from Rh 8. Two other gaze-related nuclei are located further rostral, likely arising from Rh 7. Comparison of homologous postural circuitry between taxa suggests that gene expression within a given embryonic hindbrain compartment must coordinate the developmental plans of diverse neuronal types that were acquired and combined through stepwise phylogenetic elaboration. We propose that the need to insert novel, derived circuitry (e.g. the integrators) into established, more primitive, hindbrain sensory-motor blueprints constrains the functional organization of downstream targets of homeotic selector genes. As a result, gene deletion/ectopic expression experiments involving high level selector genes can be expected to produce a mosaic pattern of variation altering both ancient and recently acquired species-specific neuronal circuitry. We conclude therefore that to incisively address the molecular correlates of neuronal development, experimental analyses will require consideration of the phylogenetic diversity of neuronal structure and function observed in embryonic rhombomeres.

523.5

CELL LINEAGE IN THE FORMATION AND REGENERATION OF THE OLFACTORY PLACODES. G.D. Burd*, A. Collazo, and S.E. Fraser. Dept. of Molecular and Cellular Biology, Univ. of Arizona, Tucson, AZ 85721 and Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

Previous studies in *Xenopus* demonstrated that the olfactory placodes are derived from cells in the anterior neural ridge (Eagleson and Harris, 1990). We and other laboratories have also observed that the olfactory placodes can regenerate (Byrd and Burd, 1993). The goals of the current study were to demonstrate the origin of the olfactory receptor neurons during normal development and to determine the origin of olfactory receptor neurons in regenerated olfactory placodes. The lineage of cells was determined by labeling small groups of cells with DiI(5)C18 or single cells with lysinated rhodamine dextran (Molecular Probes), and the fate of these cells was followed *in vivo* using low light level video microscopy. Cells in the anterior-lateral region of the anterior neural ridge (labeled at stages 14-16) give rise to olfactory placodes and form the olfactory and vomeronasal epithelia in tadpoles (stage 48). At later stages (stages 22-24), cells in the sense plate prior to and during olfactory placode formation give rise to olfactory receptor neurons and supporting cells. Following removal of the olfactory placodes (stages 33-34), we found that undifferentiated cells surrounding the olfactory placodes in the sense plate form new olfactory placodes and give rise to new olfactory receptor neurons. In summary, undifferentitated cells in the sense plate retain the potential to form olfactory receptor neurons well after olfactory placodes have formed. The molecular signals and genes involved in this process remain an exciting avenue for future investigation. Support: NSF, MDA, and NIMH.

523.6

GENESIS OF OLFACTORY RECEPTOR NEURONS PROCEEDS THROUGH DISTINCT PROGENITOR CELL STAGES AND IS REGULATED BY FIBROBLAST GROWTH FACTORS. A.L. Calof*, J.L. Guevara, K. Hannon†, B.B. Olwin†, and M.K. DeHamer. Department of Biological Sciences, University of Iowa, Iowa City, IA 52242, and †Department of Biochemistry, Purdue University, West LaFayette, IN 47907.

In vivo, neurogenesis in the olfactory epithelium (OE) proceeds continually, but *in vitro*, in minimally supplemented defined medium, Immediate Neuronal Precursors (INPs) of olfactory receptor neurons (ORNs) divide only once. We found that Fibroblast Growth Factors (FGFs) promote extended proliferation of INPs in OE cultured from E14-15 mouse embryos, while other polypeptide growth factors tested do not. Expression of FGF receptors, detected by RT-PCR in RNA isolated from E14-15 OE, suggests that FGFs may stimulate INP proliferation *in vivo* as well. Labeling INPs through successive S phases with bromodeoxyuridine (BrDU) and ^{3}H-thymidine (^{3}H-TdR) demonstrated that FGF allows INPs to undergo a second division before differentiating into ORNs. Varying the interval between BrdU and ^{3}H-TdR pulses allowed length of the INP cell cycle to be estimated as 17 hr with an S phase of ~8 hr; this finding enabled us to estimate that FGF exerts its effects in late G2/M or early G1. Neurogenesis usually ceases by 48 hr in culture, even in FGF, because >90% of INP progeny differentiate into postmitotic ORNs. However, in rare explants grown in FGF, large groups of proliferating INPs are seen after this time, suggesting that a progenitor cell--possibly a stem cell--is producing new INPs in these explants. Genetic experiments suggest that the transcription factor Mammalian Achaete Scute Homolog 1 (MASH1) may be required at an early stage of genesis of ORNs (Guillemot et al., Cell 75:463-476 [1993]). We find that a subpopulation of proliferating migratory cells in early (t=6 hr) OE cultures expresses MASH1, but expression declines rapidly; this decline is not due to cell death. MASH1+ cells are immunologically and morphologically distinct from basal cells and ORNs, and their high ^{3}H-TdR labeling index suggests that they are not stem cells. The data are consistent with a model of olfactory neurogenesis in which the MASH1-expressing cell is an early stage of, or progenitor to, the INP, which functions as a neuronal transit amplifying cell.

523.7

NOTCH IS A DETERMINANT OF RETINAL GANGLION CELL FATE IN THE CHICK. C.P. Austin*, D. Feldman, J. Ida, S. Fields-Berry, and C.L. Cepko. Dept. of Genetics, Harvard Medical School, Boston, MA 02115.

The factors affecting cell type determination and differentiation in the vertebrate retina are largely unknown. Ganglion cells (GC) are the first cell type to be produced in the chicken retina, and we have used an in vitro system to study the factors influencing their development. The presence of an inhibitor of GC differentiation was suggested by the finding that when E4 chick retinal cells were dissociated and cultured at low density, the percentage of cells expressing GC-specific markers increased 5-fold over 24 hours (Austin and Cepko, Soc. Neurosci. Abstr. 519.13, 1993). The *Notch* gene family has been hypothesized to code for cell surface receptors that mediate inhibitory signals influencing cell fate in a number of organisms. We have now found that *Notch* plays a similar role in the chick retina, as an inhibitor of GC differentiation. When antisense oligonucleotides directed against any of 3 regions of the *Notch* sequence are added to retinal explant cultures, the number of GC's in the explant increases by 50% over 24 hours relative to explants incubated with sense oligo controls. To test the specificity of the oligo sequences in bringing about GC overproduction, we have used antisense oligonucleotides mismatched at 1, 3, or 5 bases, and found that the extent of GC overproduction decreases with increasing mismatch. Western blots showed that Notch protein level is greatly diminished in the explants treated with antisense oligonucleotides, relative to sense oligonucleotide, or non-oligo-treated explants. Expression of a truncated form of the human *Notch* homologue (*TAN-1*), corresponding to the intracellular portion of the protein, via retroviral transduction reduced the number of GC produced in vivo and prevented the overproduction of GC previously seen in dissociated culture. These findings suggest that *Notch* is an inhibitor of ganglion cell differentiation in the early chick retina, and supports the emerging hypothesis that this gene family may play a central role in neuronal determination in vertebrates. *Supported by K11 EY 00321 (C.P.A.) and RO1 EY 09676 (C.L.C.) from the National Eye Institute.*

523.8

PROLIFERATION AND CELL TYPE CHOICE ARE MODULATED IN RAT RETINAL CELLS EXPRESSING VIRALLY TRANSDUCED EGF RECEPTORS. L.E. Lillien*. Dept. of Anatomy and Neurobiology, Medical College of Pennnsylvania, Philadelphia, PA 19129.

TGFα is expressed in the developing retina and affects several aspects of retinal development in vitro. These effects are age- and dose-dependent: low levels of TGFα (0.01–0.1 ng/ml) stimulate the proliferation of late embryonic and early post-natal retinal cells, but not younger retinal cells; higher concentrations of TGFα (1-10 ng/ml) affect cell type choice, inhibiting rod development and enhancing bipolar, Muller, and amacrine cell development. To study the contribution of limitations in EGF receptor (EGF-R) expression to the age- and dose-dependence of retinal cell responses to TGFα, a retroviral vector was used to introduce additional copies of wild-type human EGF-Rs into rat retinal progenitor cells (human EGF-Rs are immunohistochemically distinct from endogenous EGF-Rs). The vector also contains an IRES sequence, which allows a second gene encoding the marker B-gal to be co-expressed at high levels in infected cells. For example, 98-100% of retinal cells that express virally transduced EGF-Rs also express B-gal. The co-expression of B-gal facilitates analysis of the proliferation, survival, and differentiation of infected cells. Explants of E18 rat retina were infected with virus expressing EGF-Rs and B-gal, or with virus expressing B-gal alone. Proliferation (measured by BrdU incorporation) was analyzed 6-7 d after infection, and differentiation into rods (determined by opsin expression) was analyzed 11-16 d after infection, using rabbit anti-B-gal together with either mouse anti-BrdU or mouse anti-opsin (Ret-P1) antibodies. Proliferation of retinal cells infected with EGF-R virus was enhanced by 30-300% in response to endogenous ligand (no TGFα added) or exogenous TGFα (0.1 ng/ml). Development of retinal cells infected with EGF-R virus into rods was reduced by 50-75%. These observations suggest that limited levels of EGF-R expression contribute to the regulation of proliferation and differentiation in the retina, and that these restrictions can be overcome by manipulating levels of receptor expression.

523.9

ORIGIN OF SUBVENTRICULAR ZONE CELLS
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In the late stages of cortical neurogenesis, the subventricular zone (SVZ) is a prominent layer containing numerous mitotically active cells. It is believed that these cells give rise to the astrocytes and oligodendrocytes of the cerebral cortex and the subcortical white matter. In order to investigate whether SVZ cells originate from ventricular zone (VZ) cells, we made intraventricular injections of a recombinant retrovirus carrying the reporter gene for *E. Coli* β-galactosidase at different embryonic ages. Rats were killed after 3 days or at birth, and at postnatal days (P) 3, 7 and 14. All animals, irrespective of the age of injection, showed β-gal+ SVZ cells when examined at birth or later. Therefore, we concluded that cells present in the VZ between E15 and E19 give rise to some, if not all, SVZ cells. β-gal+ cells were present in the SVZ at least until P14, although their number was greatly reduced by this age. In order to ascertain whether SVZ cells originate from a dormant population of neuroepithelial cells or if they are stem cells left behind by their migrating progeny, we used bromodeoxyuridine (BrdU) to distinguish between mitotically active or quiescent cells. BrdU was given every two hours over a period of 24 hours starting 2 days after retroviral injection. Animals were killed shortly after the last injection or at birth. It was found that the majority of β-gal+ SVZ cells had incorporated BrdU, thus indicating that they were mitotically active during the period of BrdU injection. This finding suggests that stem cells in the VZ give rise to the glial progenitors present in the SVZ.
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523.10

A DEVELOPMENTALLY REGULATED, NEURAL SPECIFIC PROTEIN EXPRESSED BY EARLY POST-MITOTIC NEURONS IN THE EMBRYONIC RAT CORTEX J.E. Minturn*, H.J.L. Fryer, D.H. Geschwind, and S. Hockfield. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Using two-dimensional gel electrophoresis we previously identified a 64 kD protein (protein 310) that is neural specific, abundantly expressed in the embryonic cerebral cortex, and down-regulated in the adult (J. Neurosci. 9:4304). Antibodies to synthetic peptide fragments from protein 310 label cells in the developing rat brain as early as embryonic day 12 (E12) when only a few neurons have been born. In double immunofluorescent labeling experiments all cells in the developing cortex that express class the neuronal marker class III β-tubulin also express protein 310 at all ages examined. Double label studies with protein 310 and an antibody to proliferating cell nuclear antigen, a nuclear protein expressed by dividing cells, demonstrate that protein 310 is only expressed by non-mitotic cells. These data indicate that protein 310 is expressed by early postmitotic neurons.

Further support for the expression of protein 310 by cells as they make a commitment to a neuronal phenotype was obtained by examination of two cell lines. PC12 cells exposed to NGF differentiate into cells with neuronal properties, including neurite extension and membrane polarization. Western blot analysis shows that protein 310 levels are markedly increased in PC12 cells induced into a neuronal phenotype by NGF addition. Similarly, in the P19 embryonal carcinoma cell, different concentrations of retinoic acid (RA) cause cells to assume different phenotypes (either muscle or neural). Northern analysis shows that the mRNA for protein 310 is present only when P19 cells are induced into a neural phenotype.

The expression of protein 310 in early post-mitotic neurons and in cell lines that are induced to express neural properties, make protein 310 one of the earliest proteins expressed after cells have undergone the commitment to a neuronal phenotype. (Supported by NS22807 and the HHMI).

523.11

REGULATION OF THE RADIAL GLIA-ASTROCYTE TRANSFORMATION PATHWAY IN THE DEVELOPING FOREBRAIN K.E. Hunter* and M.E. Hatten, Laboratory of Developmental Neurobiology, The Rockefeller University, 1230 York Avenue, New York, New York 10021.

Two classes of astroglial cells underly establishment of structure in the developing cerebral cortex. In the embryonic period, a system of radial glial cells serves to support neuronal migration: these cells are process-bearing and specifically express the RC2 antigen. In the late embryonic and perinatal period, radial glia are believed to transform irreversibly into astrocytes, which are stellate in form and express GFAP but not RC2. Astrocytes are not supportive of neuronal migration, instead providing neurotrophic and metabolic support for maturing neurons and contributing to the maintenance of the mature cortical cytoarchitecture.

We previously showed that expression of radial glial cell identity *in vitro* requires extrinsic soluble signals which are present in embryonic neocortex, and that these signals can induce mature astrocytes to assume a radial glia-like form and express RC2 antigen *in vitro* in the presence of embryonic signals, and *in vivo*, following transplantation into embryonic neocortex (Soc. Neurosci. Abs. 256.12, 1993). These observations suggest that, when provided with appropriate inducing signals, astrocytes can express some of the features of radial glia, their precursors, and that this developmental pathway is therefore reversible and regulated at least in part by availability of these inducing signals. The inducing signals are absent from mature forebrain, suggesting that the progression from radial glia to astrocyte is a bidirectional pathway, which is extrinsically regulated by these signals. Preliminary characterization indicates that the inducing signals are protein in nature, and represent either a previously unidentified role for a known peptide, or a potentially novel neural growth factor.

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523.12

CO-LOCALIZATION OF CDK-5, MUNC-18 AND SYNTAXIN DURING DEVELOPMENT OF THE RAT CEREBELLUM. P. Grant*, Veeranna, K.T. Shetty and H.C. Pant. LNC, NINDS, National Institutes of Health, Bethesda, Md. 20892.

Neuronal cdk-5 phosphorylates the high molecular weight neurofilament protein (NF-H). During the purification of this kinase, we identified a molecule of 67 kd (P67) which is associated with and activates cdk-5 activity (Shetty, et al, 1993 Abst. Soc. Neurosoc. 19: 62). A molecule, called Munc 18, with a sequence identical to P67, was shown to be associated with syntaxin, one of the key molecules of the synaptic vesicle fusion protein complex (Hata et al, 1993, Nature 366,347). To further explore the function of Munc 18, either as a regulator of cdk-5, or as a component of the synaptic vesicle complex, (or both), we investigated the immunohistochemical expression of cdk-5, Munc 18, syntaxin and NF-H during development of the rat cerebellum from P2 to the adult. We assumed that if these molecules were functionally related, then they should: (1) co-localize within the same cells and tissues, and (2) undergo similar patterns of developmental regulation. Using antibodies to cdk-5, NF-H, syntaxin and Munc-18, we could show that at all stages, the epitopes for cdk-5, Munc-18 and syntaxin were co-localized in the developing molecular layer (parallel fibers), the synaptic glomeruli in the inner granule cell layer, and in afferent and efferent fibers (climbing fibers, mossy fibers and Purkinje cell axons) within each folium. Only cdk-5 and Munc-18 antigens were detected within the Purkinje cell bodies. The pattern of NF-H expression, however, showed differences since it was absent from the molecular layer through most of development, but was primarily restricted to axons and fiber bundles within each folium. At late developmental stages, NF-H stained fibers were seen within the dendritic and surrounding soma regions of Purkinje cells. The expression patterns of CDK-5 and Munc-18 during development, as seen immunohistochemically and in immunoblots, are similar, which is consistent with the hypothesis that they are functionally related.

NEUROTRANSMITTERS: NEUROTRANSMITTER INTERACTIONS

524.1

EVIDENCE FOR A ROLE OF STRIATAL GABA IN THE ANTAGONISTIC DOPAMINE - GLUTAMATE REGULATION OF LOCOMOTION. AN *IN VIVO* MICRODIALYSIS STUDY IN THE AWAKE FREELY MOVING RAT. **W.T. O'Connor*, M. Morari, U.Ungerstedt and K. Fuxe. Departments of Pharmacology and Neuroscience, Karolinska Institute, Stockholm, Sweden.**

The effect of local perfusion with tetrodotoxin (TTX) in the substantia nigra pars reticulata (SNr) on behavior and basal dopamine (DA), GABA, and glutamate (GLU) levels in the dorsolateral striatum was monitored using *in vivo* microdialysis in the awake rat. Basal striatal DA, GABA and GLU levels (nM) were 1.7 ± 0.9, 10.4 ± 0.7 and 307 ± 40 respectively. Nigral perfusion (70min) with TTX (10uM) produced a strong contralateral rotation (8.5 +/- 4 per min after 30 min); a decrease in ipsilateral striatal DA release (-70% of control) and an increase in striatal GABA (+40%) and GLU (+80%) release. Intrastriatal perfusion with MK-801 (10uM) alone did not affect behavior or striatal neurotransmitter release but counteracted the contralateral rotation (1.3 +/- 0.5 per min after 30 min); abolished the increase in striatal GABA release and delayed the increase in striatal GLU release associated with perfusion with TTX in the ipsilateral SNr. The reduction in striatal DA release associated with nigral TTX was not affected by intrastriatal MK-801. Thus, the counteraction of the nigral TTX induced increase in both rotational behavior and striatal GABA release following intrastriatal perfusion with MK-801 strengthens the evidence for the striatal GABA neuron as a major target for the antagonistic DA-GLU interaction in the regulation of locomotion.

524.2

ADENOSINE ANTAGONIST DELAYS THE SUPPRESSION OF SYNAPTIC FUNCTION INDUCED BY CYANIDE (CN) IN RAT HIPPOCAMPAL SLICES. **P.J. Zhu* and K.Krnjević Anaesthesia Research and Physiology Departments, McGill University, Montréal, PQ, H3G 1Y6, Canada.**

Acute exposure to cyanide causes a variety of CNS symptoms. The exact mechanisms are not understood. In the present experiments, evoked population spikes were recorded in the CA1 region. CN rapidly, but reversibly inhibited population spikes: with 5 μM CN and 10 μM CN (for 5 min, in the bath), population spikes were reduced by 19 ± 2.9% (± S.E., n=3) and 29 ± 2.5 % (n=9), respectively; with 50 μM and 100 μM CN, they were fully blocked in 5 out of 9 slices and 9 out of 9 slices, respectively; and the respective 50% reduction times were 138 ± 6.9 s and 115 ± 6.4 s. Though field EPSPs were also decreased, they were more resistant to CN. The adenosine receptor antagonist 8-sulphophenyl theophylline (8-SPT) strongly delayed the CN-induced suppression of population spikes: the 50% reduction times being 119 ± 6.7 s before, and 189 ± 14.3 s during the application of 1 μM 8-SPT (n=7); in 10 μM 8-SPT, 5 min of CN reduced population spikes by a maximum of only 41 ± 4.4 % (n=8). 8-SPT's effects could be washed out. Conversely, the action of CN was potentiated by the adenosine transport blocker dipyridamole (1 μM, n=5). The ATP-sensitive K-channel blocker glyburide did not alter the CN-induced suppression of population spikes (n=8). The present results indicate that adenosine release contributes to the suppression of synaptic transmission induced by cyanide.

Supported by Medical Research Council of Canada.

524.3

SEIZURE PROTECTION WITH IB-MECA, A SELECTIVE AGONIST OF THE NOVEL ADENOSINE A3 RECEPTOR. D.K.J.E. von Lubitz[a], M.F.Carter[a], R.C.-S. Lin[b], K.A.Jacobson[a]*, [a]NIDDK/NIH/Molecular Recognition Section, Bethesda, MD 20892; [b]Dept. of Physiology and Biophysics, Hahnemann University, Philadelphia, PA, 19102.

The biological function of adenosine A3 receptors is virtually unknown. We have, therefore, investigated the effect of acute and chronic preischemic stimulation of these receptors on the outcome of NMDA or pentylenetetrazole (PT) induced seizures in C57Bl/J5 mice (N=10/group). Animals were injected I.P. with N6-(3-iodobenzyl)-adenosine-5'-N-methylcarboxamide [IB-MECA (10, 50, 100 μg/kg)] 15 min prior to either NMDA (60 or 125 mg/kg) or PT (75 mg/kg). In the chronic regimen, IB-MECA (100 μg/kg/day) was injected I.P. for 4 weeks followed by NMDA (60 mg/kg) administered 24 h after the last administration of IB-MECA. The onset of neurological symptoms following injection of NMDA (60 and 125 mg/kg) was significantly delayed by acute and chronic IB-MECA at doses >50 μg/kg. At 100 μg/kg both acute and chronic IB-MECA significantly reduced postictal mortality in both NMDA and PT groups. Acute injection of 120 μg/kg IB-MECA improved survival of PT induced seizures even further. Our results indicate that, contrary to its effect in cerebral ischemia, acutely injected IB-MECA is highly protective against seizures elicited by different epileptogenic drug classes and that, as in stroke, chronic treatment with IB-MECA offers an equally significant seizure protection. In view of our results, further exploration of the therapeutic value of adenosine A_3 receptor stimulation is highly warranted.

524.4

K^+-INDUCED ATP RELEASE FROM ORGAN OF CORTI OF THE INNER EAR MEASURED BY LUCIFERIN/LUCIFERASE BIOLUMINESCENCE ASSAY IN VITRO. P. Wangemann* and D.C. Marcus. Cell Physiology Lab and Biophysics Lab, Boys Town National Research Hospital, Omaha, NE 68131

Purinergic receptors for ATP have been identified in several cell types within the organ of Corti including outer and inner hair cells, Deiter's and Hensen's cells. The presence of purinergic receptors suggested that there is a source of ATP within the organ of Corti. However, stimulus-induced release of ATP from the organ of Corti has never been demonstrated. In the present study, we measured ATP as rate of photons released by a luciferin/luciferase bioluminescence assay. Organ of Corti was microdissected from the inner ear of the gerbil and placed into a perfusion chamber on the stage of an inverted microscope. Addition of luciferin/luciferase bioluminescence assay caused virtually no increase of the baseline photon count. However, substitution of 40 mM K^+ for an equimolar amount of Na^+ caused a rapid increase in the rate of photons counted. These data suggest that the organ of Corti contains a source of ATP which can be released upon depolarization with K^+. Even though it has been speculated, based on analogy to other cholinergic systems, that ATP is coreleased with acetylcholine from cholinergic efferent nerve terminals, the source of ATP within the organ of Corti remains to be determined.

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524.5

CHARACTERIZATION OF MAMMALIAN ARGININE DECARBOXYLASE (ADC) AND ITS DISTRIBUTION IN BRAIN. G. Li*, S. Regunathan & D.J. Reis. Div. of Neurobiol., Dept. of Neurol. and Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

The amine agmatine (Agm) (decarboxylated arginine), is an endogenous ligand for imidazoline (I-) and α_2-adrenergic receptors present, with its biosynthetic enzyme ADC, in bovine and rat brain (Li et al., *Science* 263:966, 1994). While prevalent in bacteria, neither Agm nor ADC have previously been found in mammalian tissue. We investigated whether mammalian ADC has unique properties. ADC was assayed by measuring conversion of ^{3}H-arginine to $^{14}CO_2$. Unlike bacterial ADC, which is cytosolic, brain ADC is membrane associated, synaptosomal, enriched in the inner membranes of mitochondria and unevenly distributed with activity greatest in cerebral cortex and hippocampus, in agreement with immunocytochemical distribution of Agm (see Wang et al., *Soc. Neurosci. Abstr.*, 1994). To partially purify ADC, rat liver mitochondria were isolated by differential centrifugation and Percoll gradients. Mitochondrial enzymes were solubilized by detergent and further purified by chromatography. Rat hepatic ADC is also mitochondrial, utilizes arginine and ornithine as substrates (K_ms of 0.75 and 0.25 mM), has pH and thermal optima of 8.23 and 25°C, respectively, and is facilitated by Mg^{2+} and inhibited by Ca^{2+}, with other ions ineffective. In contrast to most decarboxylases, ADC does not require pyridoxal phosphate (PLP) as co-factor. Difluoromethylarginine (DFMA) or -ornithine (DFMO), selective inhibitors of ADC or ornithine decarboxylase, or inhibitors of nitric oxide synthase (NMA, NMMA), an enzyme also utilizing arginine as substrate, were ineffective. We conclude: mammalian ADC is a mitochondrial-associated enzyme which differs in many characteristics from cytosolic forms of ADC in bacteria. Since many I-receptors are mitochondrial, the findings indicate that Agm interacts with receptors close to its site of biosynthesis.

524.6

FLUOXETINE AND MOUSE BRAIN NOREPINEPHRINE TURNOVER: A PRELIMINARY STUDY RELEVANT TO THE MECHANISM OF DRUG-INDUCED AKATHISIA. L.M. Hall*, G.M. Anderson, and D.J. Cohen, Child Study Center, Yale Univ. School of Medicine, New Haven, CT 06520.

Akathisia, or restlessness, can be a debilitating side effect in patients treated with neuroleptics, tricyclics, and serotonin selective reuptake inhibitors. The amelioration of akathisia by propranolol or clonidine, and the development of akathisia-like symptoms after administration of ß-agonists, suggest that the noradrenergic system may play a critical role in the pathophysiology of akathisia. Reports of fluoxetine (FL)-induced akathisia, and a possible relationship between drug-induced akathisia and suicidality, make the study of FL's effects on the noradrenergic system of particular interest.

In limited previous neurochemical research, acute FL has been reported to increase the norepinephrine (NE) metabolite MHPG-SO$_4$ in rat hypothalamus by 50-60% (Perry and Fuller, Neurosci Abst., 1991, p. 1178), while G.A. Smythe et al. (1988) have reported increased hypothalamic NE turnover after combined treatment with FL and 5-hydroxytryptophan. There are also several recent reports of increased extracellular fluid NE after acute FL.

We have examined FL's acute (2 hr) and subacute (4 day) effects on mouse forebrain, hindbrain, and hypothalamic NE turnover. No significant changes in regional MHPG levels or MHPG/NE ratios were observed after 2 hr or 4 day treatment with either 1 or 10 mg/kg ip FL. The noradrenergic agents clonidine (1 mg/kg) and yohimbine (5 mg/kg) were observed to markedly and significantly alter MHPG levels and MHPG/NE ratios in the expected manner. Additional data will be presented regarding the effects of acute and chronic haloperidol and imipramine, and chronic FL, in mice, as well as the acute effects of FL in rats. The preliminary results do not provide support for the idea that acute and subacute FL may produce akathisia through increases in central NE functioning.

524.7

SITE-DIRECTED MUTAGENESIS OF THE HISTAMINE H_1-RECEPTOR INDICATES A SELECTIVE INTERACTION OF Asn^{207} WITH SUBCLASSES OF H_1-RECEPTOR AGONISTS. R. Leurs*, M.J. Smit, C.P. Tensen#, A.M. Ter Laak and H. Timmerman. Leiden/Amsterdam Center for Drug Research, Department of Pharmacochemistry and Graduate School for Neurosciences, Department of Biochemistry and Molecular Biology#, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

The cloning of gene encoding the H_1-receptor made it possible to study the interaction of subtype specific ligands with the receptor protein. In this study we investigated the role of the Asn^{207} and the Thr^{203} residues in TM5 of the guinea-pig histamine H_1-receptor by site-directed mutagenesis to non-functional alanines.

After stable expression of the receptor mutants in Chinese Hamster Ovary cells the pharmacological properties of the receptor proteins were investigated by [^{3}H]mepyramine binding studies and [^{3}H]inositol phosphate accumulation. Whereas the Thr^{203} is not important for the action of histamine, the Asn^{207} residue appears to be involved in the binding of the N^{τ}-nitrogen atom of histamine and its 2-methyl analogue. For the 2-(3-bromophenyl)-analogue and the non-imidazole H_1-receptor agonists 2-pyridylethylamine and 2-thiazolylethylamine the Asn^{207} residue is not essential for binding. On the basis of this study we conclude that different histamine H_1-receptor agonists interact in different ways with the receptor proteins. Moreover, we speculate that the interaction with the N^{π}-nitrogen atom is essential for receptor activation.

524.8

THE GUINEA PIG HISTAMINE H_2 RECEPTOR : GENE CLONING, TISSUE EXPRESSION, AUTORADIOGRAPHIC MAPPING AND CHROMOSOMAL LOCALIZATION OF ITS HUMAN COUNTERPART. E.Traiffort, M.L.Vizuete, M. Ruat, J. Tardivel-Lacombe, V. Dimitriadou*, M.L. Bouthenet and J.C. Schwartz.U.109 de l'INSERM, Centre Paul Broca, 75014 Paris.

The screening of a guinea pig genomic library, using DNA probes derived from the sequence of the rat histamine H_2 receptor, allowed us to isolate an intronless gene encoding a protein of 359 amino acids displaying all major features of G protein-coupled receptors and a 84% homology with the rat histamine H_2 receptor. Northern blot analysis, performed with a specific C-terminal tail DNA probe derived from the guinea pig sequence revealed a 4.6 kb transcript in various guinea pig tissues. In brain, a high expression was found in striatum, brainstem, olfactory tubercles and bulb. A signal was also present in hippocampus, cerebellum, hypothalamus, thalamus, substantia nigra. In peripheral tissues, the expression was detected in stomach, lung and heart, where the labelling was very high in ventricles but hardly detectable in atrium. *In situ* hybridization studies showed the highly contrasted guinea pig cerebral expression of the H_2-receptor gene transcripts which was compared to a detailed autoradiographic mapping of the receptor using ^{125}I-iodoaminopotentidine. There was a rather good agreement between the distribution of the two markers. A high labelling was detected in striatum, Calleja islands, olfactory tubercles, cortex, hippocampus, pontine nuclei, inferior olive. Amygdaloid complex, lateral geniculate nucleus, superior colliculi and median thalamus were also labelled. An interesting discrepancy exists, however, in the hippocampal complex where the binding sites were high in the molecular layer, whereas the mRNA were localized in the pyramidal or granular cell layers. Finally, using a chromosome mapping panel constructed from somatic cell hybrids, we assigned the human histamine H_2-receptor gene to chromosome 5 showing that the genes encoding the H_1- and the H_2-receptor are not clustered.

524.9

HISTAMINE DYSFUNCTION IN THE RED NUCLEUS CAN PRODUCE TORSIONAL DYSTONIA WHICH IS RELIEVED BY DIPHENHYDRAMINE, PYRILAMINE, OR CIMETIDINE. M.R. Stone, J.L. Vant Groenewout, V. Vo, D.D. Truong*, R.R. Matsumoto. Department of Neurology, Univ. of California Irvine, Irvine, CA 92717.

It has previously been shown that diphenhydramine hydrochloride (Benadryl) eliminates or reduces symptoms of dystonia in human patients with acute dystonic reactions and idiopathic torsion dystonia. This study demonstrates that beneficial effects of diphenhydramine in the treatment of idiopathic torsion dystonia are related to the H1-selective antihistaminergic actions of the drug. In addition, the H1 antagonist pyrilamine and the H2 antagonist cimetidine effectively reduce the symptoms of idiopathic torsion dystonia. Our data also provides support to previous research which has implicated the red nucleus in the pathophysiology of dystonia. The effects of drugs injected into the red nucleus of rats were observed by measuring the angle of deviation of the head from the horizontal plane. A significant rotation of the head is indicative of dystonia. Histamine (dose range 0-10 nmol) produced dose dependent dystonia in rats ($P<0.002$). The dystonia produced by a high dose of histamine (10 nmol) could be antagonized by coadministration of diphenhydramine ($P<0.01$), pyrilamine ($P<0.05$), and cimetidine ($P<0.04$). Significantly more dystonia was observed when histamine was injected into the red nucleus rather than midbrain areas surrounding the structure ($P<0.002$). These results indicate that the beneficial effects of diphenhydramine, pyrilamine, and cimetidine in reducing symptoms of idiopathic torsion dystonia occur via a histaminergic pathway involving the red nucleus.

524.10

ADENOSINE-DOPAMINE INTERACTION IN THE VENTRAL STRIOPALLIDAL SYSTEM: A NEW FOCUS FOR THE TREATMENT OF SCHIZOPHRENIA. S. Ferré[1,2], W.T. O'Connor[3], P. Snaprud[2], U. Ungerstedt[3] and K. Fuxe[2]*. [1]Dept. of Neurochemistry, C.S.I.C., 08034 Barcelona, Spain. [2]Department of Neuroscience and [3]Department of Physiology and Pharmacology, Karolinska Institute, S171 77 Stockholm, Sweden.

Recent studies have shown the existence of a specific antagonistic interaction between adenosine A_{2a} receptors and dopamine D_2 receptors in the brain. This A_{2a}-D_2 interaction seems to be essential for the behavioural effects of adenosine agonists and antagonists, like caffeine. In the present study quantitative receptor autoradiography and brain microdialysis were combined to demonstrate a powerful antagonistic A_{2a}-D_2 interaction in the ventral striopallidal system. In the presence of the A_{2a} agonist CGS 21680, dopamine exhibited a lower efficacy in displacing the radiolabelled D_2 receptor antagonist [^{125}I]iodosulpiride from the rat ventral striatum, specially in the nucleus accumbens. A tonic dopaminergic modulation of the striopallidal neurons from the ventral striopallidal system was demonstrated by a dual-probe approach, by infusing selective dopamine agonists and antagonists in the nucleus accumbens and by measuring dopamine extracellular levels in the nucleus accumbens and GABA extracellular levels in the nucleus accumbens and in the ipsilateral ventral pallidum. The infusion of CGS 21680 in the nucleus accumbens induced the same postsynaptic changes as the D_2 antagonist raclopride, i.e., an increase in pallidal GABA extracellular levels, without changing those levels in the nucleus accumbens. Furthermore, the coinfusion in the nucleus accumbens of low concentrations of CGS 21680 and raclopride, which were ineffective when administered alone, induced a significant increase in pallidal GABA extracellular levels. These results suggest that A_{2a} agonists, alone or in combination with D_2 antagonists, could be advantageous antischizophrenic drugs, as blockade of D_2 receptors in the ventral striopallidal system appears to be associated with the antipsychotic activity of neuroleptics but not with their extrapyramidal motor-side effects.

524.11

EFFECT OF CHRONIC NEUROLEPTIC ADMINISTRATION ON RAT STRIATAL ADENOSINE-2 (A-2) RECEPTOR DENSITY. B. Parsons, D. Togasaki*, S. Kassir, S. Przedborski. Depts. of Psychiatry and Neurology, Columbia University, New York, NY 10032.

We have demonstrated synergistic interactions between adenosine-2 (A-2) and dopamine-2 (D-2) receptors in a behavioral rat model. Because A-2 and D-2 receptors are co-localized on the same type of striatal neuron, we studied the effects of chronic neuroleptic administration (typical and atypical) on striatal A-2 and D-2 receptors in rat brain. Rats received 21 daily injections (i.p.) of haloperidol (1.5 mg/kg), fluphenazine (1.5 mg/kg), sulpiride (100 mg/kg), clozapine (20 mg/kg), or saline. Striatal receptor density (B_{max}) and affinity (K_d) values for [^{3}H]CGS21680-labeled A-2 receptors and [^{3}H]sulpiride-labeled D_2 receptors were measured using homogenate binding. Haloperidol and fluphenazine significantly increased (>30%) striatal A-2 and D-2 receptor density, whereas sulpiride and clozapine had no significant effect. K_d values for [^{3}H]CGS21680 were unchanged in all groups. Thus, we observed significant increases in the density of A-2 receptors in rats striatum following chronic administration of typical, but not atypical, neuroleptics. The findings may be relevant to the mechanisms by which typical neuroleptics produce tardive dyskinesia, and may be of relevance to other neurologic and psychiatric disorders.

524.12

STIMULATORY EFFECTS OF SUB-ANESTHETIC DOSES OF KETAMINE, A PSYCHOTOGENIC NMDA RECEPTOR ANTAGONIST, ON THE OUTFLOW OF DOPAMINE AND GLUTAMATE IN THE PREFRONTAL CORTEX. B. Moghaddam and M.L. Bolinao*, Dept. of Psychiatry, Yale Univ. Sch. Med. and VA Med. Ctr. 116A/2, West Haven, CT 06516.

Sub-anesthetic doses of ketamine, a non competitive NMDA-receptor antagonist, cause several schizophrenia-like symptoms in non-schizophrenics including impaired performance on frontal lobe sensitive tests (Krystal et al., Arch. Gen. Psychiat. 1994). In this study, intracerebral microdialysis in conscious rats was used to assess the effect of ketamine on the extracellular levels of dopamine and glutamate in the prefrontal cortex. A thorough dose-response study indicated that sub-anesthetic doses (10, 20, and 30 mg/kg, i.p.) of ketamine increase glutamate levels while the anesthetic dose of 100 mg/kg decreases these levels. An intermediate dose of 50 mg/kg was without an effect. This finding is similar to our previous data with other types of NMDA receptor antagonist, and may be attributed to activation of an NMDA-type autoreceptor on glutamatergic terminals. Administration of 30 mg/kg ketamine, and not 100 mg/kg ketamine, also increased the release of dopamine in the prefrontal cortex. Local infusion of the non-NMDA receptor antagonist, CNQX, blocked the stimulatory effect of ketamine on dopamine release. Although the behavioral effects of the sub-anesthetic doses of ketamine have been attributed to post-synaptic *inhibition* of the NMDA receptor, this study indicates that ketamine may exert some of its effect by increasing the levels of endogenous excitatory amino acids, thus, causing a *stimulation* of post synaptic (non-NMDA) excitatory amino acid receptors. Supported by MH48404, MH44866, WHVA Center for Schizophrenia and VA Merit Award.

EXTRASTRIATE VISUAL CORTEX: PARIETAL AREAS II

525.1

EFFECTS OF VESTIBULAR AND NECK PROPRIOCEPTIVE SIGNALS ON VISUAL RESPONSES IN POSTERIOR PARIETAL CORTEX. L.H. Snyder*, R.A. Andersen, Division of Biology, Caltech, Pasadena CA, 91125.

Eye, head and body position modulate the discharge of retinotopically tuned units in posterior parietal cortex (PPC). The conjunction of retinal and extraretinal signals may aid coordinate transformations from visual to body- or world-centered reference frames. We investigated the sensory modalities responsible for extraretinal signals in areas 7a and LIP of macaque monkeys.

Vestibular signals mediated effects of body position in space on single units in PPC. Animals were seated on a vestibular turntable in a light-proof room. Saccades to retinotopically identical locations were elicited after whole-body displacement in the dark. The discharge of single units was influenced following rapid (10 d/s/s) but not gradual (0.04 d/s/s) rotations (7/8 cells), ruling out unintentional auditory, visual or other cues to body position in space.

Neck proprioceptive signals mediated effects of head-on-body position. Proprioceptive, vestibular and visual signals may all be affected by head displacement. To show that changes in proprioceptive signals alone were sufficient, whole-body rotation in the light was followed by counter-rotation of the head back to the starting position in the dark, resulting in body but not head displacement. Identical saccades subsequently elicited in the dark revealed single unit modulation identical to that obtained by merely rotating the head on a stationary body (11 units).

Vision influenced the effect of position in space on single units. Following whole body rotation **in the light**, responses to identical saccades elicited in the dark were often modulated by body position (8/22 units). In contrast, body position was much less likely to effect saccadic responses following whole body rotation **in the dark** (7/58 units). Our results suggest that in the absence of vision, the PPC boosts its reliance on vestibular signals.

525.2

MAPPING VISUAL SPACE AROUND THE ARM WITH BIMODAL VISUAL-TACTILE NEURONS. Michael S. A. Graziano* and Charles G. Gross. Department of Psychology, Princeton University, Princeton, NJ 08544.

Neurons in the ventral portion of area 6, in the frontal lobe, may help to encode the locations of objects in nearby, extrapersonal space. In monkeys, these neurons often respond to both visual and tactile stimuli. They have tactile receptive fields (RFs) on the face or limbs, and corresponding visual RFs, which extend outward from the tactile RFs about 20 cm into the space surrounding the body.

We recorded from neurons in the arm representation of area 6 in awake fixating macaques, and studied the effect of placing the animal's arm in different locations. For most cells with tactile RFs on the arm or hand, when we moved the arm, the corresponding visual RF moved with it. However, when we moved the fixation point, the visual RF remained stationary, "attached" to the arm. Thus, these neurons appear to code the location of visual stimuli with respect to the arm, independent of eye position. These "arm centered" neurons may be useful for hand-eye coordination.

525.3

EGOCENTRIC MOTION FROM OPTIC FLOW AND EYE POSITION IN AREA 7A OF THE BEHAVING MACAQUE. R.M. Siegel* and H.L. Read. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Eye position and optic flow can be combined to determine heading in head-centered coordinates. Recordings were made from area 7a while the monkey was performing a reaction time visual perceptual task. The random dot motion stimuli elicited percepts of translation, planar rotation, radial expansion or compression, or three-dimensional shape. No neurons were found to be selective for the three-dimensional shape from motion stimulus. The absence of neurons in area 7a selective for three-dimensional shape is in accordance with the proposed division of visual processing into a what and where pathway (Ungerleider and Mishkin, 1983).

Area 7a neurons were found with translation, radial, and planar rotational selectivity or various combinations (Read *et al.*, 1994). For example single neurons could be selective to both clockwise rotation and radial expansion. This suggested that there might be cells selective to spiral motion as predicted earlier on psychophysical grounds (Anderson and Siegel, 1993). Neurons were found with selectivity to particular spiral motions (e.g. clockwise compression). Thus there seems to be a coarse coding for the type of structured motion in area 7a which could in principle be used to determine heading.

The dependence of the response of these optic flow selective neurons on the retinal locus of the stimulus was examined. The 20^0 diameter patterns were moved to different locations on a 10^0 by 10^0 grid. Area 7a neurons did **not** respond with equal activity at all locations. This retinotopic tuning in dissimilar to the spatial invariance for position reported in some MST neurons (Tanaka *et al.*, 1986; Duffy & Wurtz, 1991; Lagae *et al.*, 1994).

Combination of these retinotopic position dependent signals with eye position signals could be used to determine egocentric motion in body coordinates. To test this, a particular optic flow was presented while eye position was systematically varied; the retinal locus of the center of the pattern remained constant under these conditions. Although the relative motion selectivity of area 7a neurons did not change with eye position, the strength of the responses was modulated by eye position. Using multi-variate linear regression it was found that about half of the cells exhibited a linear dependence on the horizontal and/or vertical eye position at the $p<0.05$ level. In summary, using carefully controlled psychophysical stimuli, neurons in area 7a were shown to be selective to many different optic flow patterns. These visual responses were dependent on eye position providing signals needed to compute egocentric heading. Support: NIH EY09223 and ONR N00014-93-1-0334.

525.4

SPACE CELLS IN THE DORSAL STREAM: OPTIC FLOW SELECTIVITY IN AREA 7A H.L. Read*, C.A.M. Nogueira, K.C. Anderson and R.M. Siegel. Center for Molecular and Behavioral Neuroscience., Rutgers University, Newark, NJ 07102.

The opponent vector selectivity and angle of gaze sensitivity of area 7a neurons lead to the hypothesis that these cells are involved in the construction of an egocentric space (Anderson *et al.*, 1985; Motter *et al.*, 1987). We have examined the response properties of area 7a neurons in three hemispheres of two monkeys to a host of egocentric motion patterns to further test this hypothesis. Single unit recordings of neurons in area 7a were obtained from monkeys performing a reaction time task. Trials were initiated when the monkey pulled a lever and fixated a 3^0 square. A visual stimulus appeared 2000 msec after trial initiation; 1500 to 4000 msec later a change in visual stimulus or fixation point was the cue to release a lever and end the trial. Visual receptive fields were mapped with a 10^0 bar of light in a 40^0 x 40^0 grid. Light sensitive neurons responded to a bar of light with a brief increase in firing rate following stimulus onset and offset. RFs covered from 100 to 1600 deg^2 of the test area and often included the central region of fixation. The response selectivity to radial, rotation and spiral motion was assessed at one position within a neuron's RF. Test displays were presented in a *structured motion* to *unstructured motion* sequence or *vice versa*. Neurons were selective for stimulus type if the change in firing rate in the 500 msec interval following stimulus onset varied with stimulus type (ANOVA; $p<.05$). Selectivity for both structure (translation, radial, rotation) and direction of motion (e.g. expansion vs. compression) were observed. When the initial stimulus was a structured motion of the preferred type, tonic responses often lasted for the 1500 to 4000 msec period prior to unstructuring of the display. In contrast, the response following onset of non-preferred or unstructured motion was phasic as seen with a stationary bar of light. The transition from unstructured motion to structured motion evoked a phasic response that occurred either before or on the key release. The selectivity at this transition was the same as that selectivity associated with the structured motion onset. For the majority of neurons, the RF for optic flow stimuli overlapped with that of the classical RF. The magnitude of response varied with retinotopic position, but the selectivity did not. These findings suggest that the structure and/or direction of optic flow is indeed a salient feature for area 7a neurons. Supported by NIH EY09223 and ONR N00014-93-1-0334.

525.5

OPTIC FLOW RESPONSES OF MST NEURONS DURING PURSUIT EYE MOVEMENTS. C. J. Duffy* and R. H. Wurtz. Dept. of Neurology, Univ. of Rochester Med. Ctr., Rochester, NY 14642, and Lab. of Sensorimotor Research, National Eye Inst., NIH, Bethesda, MD 20892.

The retinal image of an optic flow field is altered by concurrent smooth pursuit eye movements, complicating the task of interpreting that image. Information about pursuit, if available to the visual system, might simplify that interpretation. We recorded the responses of 117 MST neurons to optic flow stimuli, during fixation and smooth pursuit, to determine whether the response of these cells is altered by smooth pursuit.

We recorded from 117 single neurons in area MST in two monkeys. We first determined which optic flow pattern (radial or circular) evoked the strongest response when the center of motion (COM) was centered on the screen. We then presented the preferred pattern, with 8 other stimuli created by combining it with 8 directions of planar motion, shifting the COM to 8 different positions in the stimulus. Ninety-four percent of the neurons responded differently to the centered and shifted COMs. Each neuron was then tested with the centered COM during smooth pursuit in 8 different directions. Eighty-five percent of the neurons showed different responses to different pursuit directions. We compared the shifted COM response with those to pursuit across the centered COM, and found that most neurons (61%) had the same response to the retinal image, whether it was created by optic flow, or optic flow with pursuit. However, 39% responded differently when pursuit effects contributed to the retinal image. In addition, over half of the neurons (56%) responded during smooth pursuit across a blank screen.

These studies suggest that some MST neurons access both optic flow and smooth pursuit signals, potentially contributing to optic flow analysis during smooth pursuit eye movements.

525.6

RESPONSES OF LIP NEURONS DURING A MOTION DISCRIMINATION TASK: A DECISION PROCESS IN ACTION? MN Shadlen*, JM Groh, CD Salzman & WT Newsome. Depts. of Neurobiology and Neurology, Stanford University, Stanford, CA 94305

Visually guided behavior requires selection or decision processes that identify one among many possible objects as the target of a movement. We investigated one such decision mechanism in a rhesus monkey trained to discriminate the direction of motion in a visual display and to indicate its decision by executing a saccade to one of two possible targets. During performance of the task, we recorded from neurons in LIP, a parietal area that receives projections from MT and MST and participates in the planning of saccades.

In each experiment, we placed one potential saccade target within, and the other outside, the movement field of the neuron under study. Each trial contained a 2 sec stimulus presentation followed by a 0.5-1 sec delay period prior to the saccade. Not surprisingly, delay period activity of LIP neurons reliably predicted the monkey's eye movement: activity was stronger on trials in which the monkey chose the direction of motion toward the movement field. More interestingly, LIP neurons also signaled the monkey's impending choice during the stimulus presentation interval, well before the saccade. This predictive activity built up earlier and was more reliable for easy than for hard discriminations (strong vs. weak motion signals). In other words, LIP neurons appeared to accumulate evidence regarding the direction of motion in the stimulus and to maintain a response suitable for guiding the appropriate saccade. Little evidence for this kind of activity was observed in control blocks of trials in which the same stimuli were presented, but the animal was not required to perform the direction discrimination. Thus the activity of LIP neurons may reflect an active decision process in which sensory signals are evaluated to inform target selection. Supported by NEI (EY05603), training grant NS 07158-14, and HHMI.

525.7

RELIABLE TEMPORAL MODULATION IN CORTICAL SPIKE TRAINS IN AWAKE MONKEY W Bair*[1], C Koch[1], WT Newsome[2], KH Britten[3]. [1]Computation and Neural Systems, Caltech, Pasadena, CA 91125; [2]Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305; [3]Center for Neuroscience, UC Davis, Davis, CA 95616.

We analyzed the repeatability and precision of temporal structure in spike trains recorded from area MT in behaving rhesus monkeys which viewed a dynamic random dot stimulus that did not vary from trial to trial (no-var data; Newsome, Britten, and Movshon, 1989). We find a surprising degree of regularity in the temporal modulation of the response of many cells—the most reliable cells will fire a few isolated action potentials or show a period of elevated firing which begins and ends fixed in time relative to stimulus onset with a standard deviation of less than 4 msec in recording periods which may last many minutes to hours. Spike count during these short periods of elevated firing rate is more reliable, i.e. has a lower variance to mean ratio, on average than the spike count taken over the entire 2 sec stimulus. Onset and offset of high firing periods can occur with very similar and fast time constants. Autocorrelation analysis reveals that deviations from the mean response are rarely substantially correlated beyond 100 msec, and in half of cells no correlation exists between consecutive interspike intervals beyond that predicted from the time-varying mean firing rate. Reliable temporal modulation disappears or diminishes for completely coherent motion stimuli, and we propose an experiment to determine whether this is due to saturation in firing rate or due to a qualitative change in the way MT cells respond to the stimulus.

525.8

INTEGRATION OF MOTION AND STEREO CUES IN MACAQUE VISUAL AREA MT. D.C. Bradley and R. A. Andersen. Division of Biology, California Institute of Technology, Pasadena CA 91125.

Most neurons in primate middle temporal cortex (MT) are highly sensitive to motion in a particular direction, but are inhibited when an opposing movement occurs simultaneously in their receptive field. An important benefit of this "motion opponency" is that random motion signals created by non-motion stimuli (e.g. flicker) tend to cancel each other out. However, motion opponency may also lead to impaired MT responses under conditions of transparent motion, where motion direction signals are often locally opposed and as such tend to negate each other in MT. We hypothesized that this problem might be overcome if opponency between signals from different visual depths (and thus different surfaces) did not occur. To test this idea, we stimulated 146 MT neurons with a random dot pattern moving in the preferred direction and at the optimal disparity (i.e. depth) for a given cell. A second pattern moving in the antipreferred direction was introduced, and the suppression due to this pattern was measured as a function of its disparity. In ~40% of the cells, suppression was greatest when the antipreferred disparity was similar to the disparity of the preferred pattern and decreased substantially as the patterns became separated in depth. Further studies in which the disparity of the preferred pattern was altered showed that the maximally-suppressive antipreferred disparity tended to coincide with the cell's optimal disparity (as defined for preferred motion) regardless of the actual disparity of the preferred pattern. Our findings thus suggest that motion opponency in MT occurs mainly within a given disparity channel. This disparity-specific opponency favors the cancellation of random motion signals from a given depth, while allowing independent representation of surface movements at different depths.

525.9

SINGLE NEURONS IN POSTERIOR PARIETAL CORTEX MAY REPRESENT BASIS FUNCTIONS FOR SPATIAL TRANSFORMATIONS. A. Pouget* and T. J. Sejnowski. Howard Hughes Medical Institute, Salk Institute, La Jolla, CA 92037

Many neurons in the monkey posterior parietal cortex have gaussian visual receptive fields that are modulated by a monotonic function of eye position, called the gain field. Similar gain fields have been observed in neural networks that compute the head-centered position of objects (Zipser an Andersen, Nature, 1988), suggesting that populations of parietal neurons may form a distributed representation of the egocentric position of objects.

There is an alternative interpretation for these gain fields in the context of sensori-motor transformations. Motor commands generated from the retinal location of an object and the current eye position are nonlinear functions of these inputs. Such transformations can be approximated by using a single intermediate representation in which neurons compute basis functions of the sensory inputs. We propose that the parietal cortex contains such an intermediate representation. A gain field can be modeled as a gaussian function of retinal location multiplied by a sigmoidal function of eye position, which is known to form a basis function. This raises the possibility that the parietal cortex does not attempt to compute the positions of objects in a particular frame of reference but instead computes a general purpose representation of the retinal and eye position from which any transformation can be synthesized by direct projection. This representation predicts that hemineglect, a neurological syndrome produced by parietal lesions, should not be confined to egocentric coordinates but should be observed in multiple frames of reference in single patients, a prediction supported by several experiments.

525.10

EXPLICIT SPATIAL REPRESENTATION IN PARIETAL CORTEX IS NECESSARY FOR PROPER CONJOINING OF VISUAL FEATURES S.R. Friedman-Hill[1*], L.C. Robertson[1,2], and A. Treisman[3]. [1]Center For Neuroscience, UC-Davis, Davis, CA 95616; [2]Veterans Administration, Martinez, CA; [3]Princeton University, Princeton, NJ

Research with human and non-human primates implicates parietal cortex in spatial attention. Our studies of a patient with remarkably symmetrical, bilateral parietal lesions due to embolic infarcts suggest that spatial deficits may also contribute to impaired object perception.

The patient, RM, is frequently unable to make explicit spatial judgments. When asked to judge the position of a single object (left, right, or center, in one condition; up, down, or center in another), RM was incorrect for 30% of the trials. This deficit in judging the location of one item was not further increased by the presence of an irrelevant distractor. However, if asked to judge the location of the first item relative to the second item, RM's performance fell completely to chance.

Concomitant with these spatial deficits, RM has a tendency to misconjoin features of objects. Errors of this type are referred to as "illusory conjunctions." For example, when presented with a red X and a blue O, he may report seeing a red O, even with free viewing (display times up to 5 sec) and undivided attention. Neurologically normal subjects produce illusory conjunction errors only under divided attention conditions with very short exposures. RM makes fewer illusory conjunction errors with sequential presentation of stimuli than with simultaneous presentation. In the absence of reliable spatial information, temporal information can be used to organize which features should be conjoined.

The data suggest that there are important interactions between the "what" and the "where" pathways. Spatial information plays an integral role in the construction of our percepts of objects from neurally distributed features.

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525.11

A MODEL FOR COMBINED EYE-HEAD TRACKING INCORPORATING THE PROPERTIES OF MSTI VT-NEURONS. P.W. Dicke and P. Thier*. Dept. of Neurology, University of Tübingen, 72076 Tübingen, Germany

Combined smooth eye-head-tracking (CEHT) is carried out by primates in order to stabilize the retinal image of a moving object on or close to the fovea. We have tested the idea that the generation of CEHT is based on a signal related to target velocity in space, reconstructed in a spatially distributed manner at the level of parietal area MSTI. Two 3-layered connectionist networks were used in order to represent area MSTI on the left and on the right side of the brain respectively. The input layer of each of the two "MSTI" networks was made up of units, sensitive to retinal image slip, eye velocity and head velocity with identical directionalities. Further experimental findings build into the networks were the speed-tuning of VT-neurons and the distribution of their preferred directions, characterized by the fact that every direction between 0 and 360 deg may be found on either side of the brain with a possible bias for ipsiversive directions. Using error backpropagation the "MSTI" networks could be trained successfully so that the sum of the outputs of the two networks equalled target velocity in space. Assuming a fully activated VOR and furthermore taking into account the established properties of the eye and head motor systems, this signal was then used to generate coordinated eye and head movements. The overall model has proven able to fit measured combined smooth eye and head movements under a wide variety of conditions. Furthermore it is able to predict the effects of experimental lesions of MSTI in monkeys: Unilateral MSTI lesions were mimicked by removing one of the two connectionist networks involved. This manipulation resulted in an ipsiversive eye-head tracking deficit, which, with respect to the eye movement part, is in full accordance with reported experimental findings. *Supported by Graduiertenkolleg Neurobiologie Tübingen and DFG KFG "Neuroophthalmologie".*

NEUROTRANSMITTERS IN INVERTEBRATES IV

526.1

EIGHT NOVEL FMRFamide-LIKE PEPTIDES (FLPs) FROM ASCARIS SUUM. C. Cowden* and A.O.W. Stretton. Zoology Dept., Univ. of Wisconsin-Madison, 53706.

We are investigating the role of FLPs in the control of locomotion in Ascaris. AF1 and AF2 were isolated from extracts of 10,000 heads. Confirmation of additional sequences has been limited by the low yield of purified peptide (2-10 pmoles).

We are now isolating FLPs from an acid methanol extract of 62,000 heads and male tails. After 7 HPLC steps, 8 FLPs have been isolated and their sequences confirmed by MALDI-MS. The yield of purified peptide was 70-500 pmoles. Peptides AF1-AF12 range from 4-14 amino acids and have six C-terminal tetrapeptides: FIRF-, YLRF-, YMRF-, FLRF-, VLRF-, and PLRFamide. On the basis of their effects on muscle tension, 3 classes of bioactivity have been found.

This is the most heterogeneous family of FLPs reported from one species. Additional active peaks from this purification are being isolated at this time.

526.2

NEUROPEPTIDE PRODUCTS OF THE *FMRFamide* GENE MODULATE THE *DROSOPHILA* NEUROMUSCULAR JUNCTION. R.S. Hewes* and P.H. Taghert. Department of Anatomy and Neurobiology, Washington University Medical School, St. Louis, MO 63110.

The *Drosophila FMRFamide* gene encodes a complex, neuropeptide precursor protein encoding many diverse FMRFamide-related sequences. In the larval CNS, this gene is expressed in a small, stereotyped pattern of neurons, including 6 that project to the thoracic dorsal neurohemal organs. From there, FMRFamide-related peptides may be released to act on peripheral targets via the circulation. Three synthetic peptides--with sequences deduced from the FMRFamide precursor (DPKQDFMRFamide, MDSNFIRFamide, SVQDNFMHFamide)--enhanced nerve-stimulated contractions of internal and external abdominal muscle fibers by increasing peak tension, contraction rate, and relaxation rate. These peptide effects began within 1-2 min of the onset of peptide application, reached peak strength after 3-7 min, and ceased after 10-30 min. The threshold concentrations were 10 nM for DPKQDFMRFamide and MDSNFIRFamide and were 10-100 nM for SVQDNFMHFamide. These results suggest that 1) receptors with high affinity for FMRFamide-related peptides are located on several skeletal muscles and/or on motoneuron terminals, and 2) multiple sequences contained in the FMRFamide precursor may be functionally equivalent at the neuromuscular junction. The design of labeled peptides will be important for receptor localization and for cloning of FMRFamide receptors by functional expression. Towards this end, we found that biotinSGSGDPKQDFMRFamide, an N-terminally extended form of DPKQDFMRFamide, also enhanced nerve-stimulated contractions at a threshold of about 10 nM. (Supported by NIH Grants #NS21749 and #NS07071).

526.3

PHYSIOLOGICAL ACTIONS OF NINE MEMBERS OF THE MYOMODULIN FAMILY OF PEPTIDE COTRANSMITTERS AT THE B16-ARC NEUROMUSCULAR JUNCTION OF *APLYSIA*. V. Brezina*, B. Bank, E.C. Cropper and K.R. Weiss. Dept. of Physiology & Biophysics, Mt. Sinai School of Medicine, New York, NY 10029.

Neuromodulation by multiple related peptides with different spectra of physiological effects appears an effective way to integrate complex physiological functions. A good opportunity to study this issue is provided by the intrinsic network of modulatory peptides that regulates output of the simple ARC neuromuscular circuit of *Aplysia*. Under appropriate behavioral circumstances, ACh-induced contractions of the ARC muscle are modulated in several ways by members of at least three families of peptide cotransmitters released from the ARC's own motorneurons. In this work we have focused on the myomodulins (MMs), released from motorneuron B16. Nine MMs have now been identified, either purified from the ARC system, found encoded by a MM gene, or both (Cropper et al., *PNAS* 84:5483, *Peptides* 12:683; Miller et al., *Soc. Neurosci. Abstr.* 16:307, *J. Neurosci.* 13:3358). Previous work with MM_A and MM_B has shown that they both potentiate the size as well as the relaxation rate of ARC-muscle contractions at low concentrations, but only MM_A depresses the contractions again at higher concentrations. We have proposed that the potentiation is due to enhancement of the L-type Ca current in the muscle, and the depression to activation of a K current (Brezina et al., *J. Neurosci.*, in press). We have now extended this analysis to all nine MMs. All similarly enhance the Ca current and potentiate the size, as well as the relaxation rate, of ARC-muscle contractions. In contrast, their ability to activate the K current varies greatly, and their ability to depress the contractions varies correspondingly. These results further support the modulation of the Ca and K currents as the mechanisms of the potentiation and depression, and suggest differential modulation of the size and relaxation rate of the contractions as one possible important role of the MMs at the B16-ARC neuromuscular junction.

526.4

cAMP DEPENDENT PHOSHORYLATION OF *APLYSIA* TWITCHIN MAY MEDIATE MODULATION OF MUSCLE CONTRACTIONS BY NEUROPEPTIDE COTRANSMITTERS. W. C. Probst, E. C. Cropper*, J. Heierhorst, S. L. Hooper, H. Jaffe, F. Vilim, S. Beushausen, I. Kupfermann, and K. R. Weiss. Dept. Physiol. & Biophys, Mt. Sinai Med. Schl., NY, NY 10029; Ctr. Neurobio., Columbia Univ., NY, NY 10032, LNC-NINDS Protein/Peptide Sequencing Facility, and the Laboratory of Neurobiology, NINDS, Bethesda, MD 20892.

Acting via a cAMP-cAPK cascade, members of two neuropeptide families, the small cardioactive peptides and myomodulins, modulate contraction amplitude and relaxation rate in the accessory radula closer muscle of the marine mollusc *Aplysia californica*. A ~750 kDa phosphoprotein was identified in the ARC muscle as the major substrate for cAMP-dependent protein kinase activated either by application of neuropeptides or by peptides released by motor neuron stimulation at physiological frequencies. Immunoblot and immuno-electronmicroscopy experiments revealed the widespread presence of this protein in *Aplysia* muscles, and its colocalization with contractile filaments in the ARC muscle. Sequence analysis of proteolytic peptide fragments derived from the protein indicated that it is structurally related to the muscle protein twitchin. Finally, the level of neuropeptide-induced phosphorylation of the protein correlated well with peptidergic modulation of the relaxation rate of the muscle. We propose that twitchin in *Aplysia*, and perhaps in other species, may mediate the modulation of the relaxation rate of muscle contractions.

526.5

NITRIC OXIDE MEDIATES CHEMOSENSORY ACTIVATION OF FEEDING IN *LYMNAEA STAGNALIS* M.R. Elphick, G. Kemenes, K. Staras and M. O'Shea* Sussex Centre for Neuroscience, Sch. of Biol. Sci., Sussex Univ., Brighton, BN1 9QG, U.K.

We have used NADPH diaphorase histochemistry to establish the distribution of NO synthase in the CNS of the mollusc *Lymnaea stagnalis*. A dense population of stained fibers are present in the lip nerves and these are derived from a band of sub-epithelial bipolar cells located at the margins of the lips. These could be chemosensory neurons and NO may be involved in activating feeding. To test this hypothesis we used a semi-intact lip-CNS preparation where a sucrose stimulus can be applied to the lips while recording from identified neurons that control feeding. Sucrose activation of the feeding network was inhibited when the CNS was pre-incubated with the NO scavenger haemoglobin (Hb), the NO synthase inhibitor N^{ω}-Nitro-L-arginine or the guanylyl cyclase inhibitor methylene blue (MB). Central activation of the feeding network by current injection into the slow oscillator (SO) interneuron was not affected by Hb. Application of NO donors or 8-bromo-cGMP to the CNS also activated the feeding network. We tested the behavioural significance of these physiological experiments by injecting animals with Hb or MB before placing them in a sucrose-rich environment and monitoring feeding behaviour. Both Hb and MB delayed the onset of feeding compared to met-Hb (which should not bind NO) and distilled water, respectively. Our results indicate that NO is released in the *Lymnaea* CNS by sucrose-activated chemosensory neurons and causes cGMP-mediated activation of feeding behaviour. This is the first description of a specific behavioural role for NO in the CNS. [Supported by BBSRC grant no. GR/J33234]

526.6

NITRIC OXIDE AS A PUTATIVE TRANSMITTER AT SYNAPSES BETWEEN IDENTIFIED CENTRAL NEURONS OF APLYSIA. J.W. Jacklet* Dept. Biol., SUNY Albany, NY 12222.

NADPH-diaphorase staining of the CNS of Aplysia was used to identify neurons that may contain nitric oxide synthase (NOS) and produce nitric oxide (NO). There are a few densely stained neurons in all ganglia and ca 30 bilaterally paired neurons in the cerebral ganglion, including 2 in each E-cluster. Their axons and neurites are stained and form well defined fiber tracts and synaptic gloneruli, including the lateral terminus, which is also stained by antiserum to myomodulin, a neurotransmitter / modulator. NADPH-diaphorase stains terminal processes on cell bodies of some unstained E-cluster neurons, suggestive of axo-somatic synapses. NO generated using 1 mM SIN-1 (3 morpholino-sydnonimine) and S-NC (S-Nitrosocysteine) depolarizes these neurons, induces action potentials and increases input resistance. Degassed SIN-1 and S-NC solutions have no effect. A membrane permeant cGMP analogue, 8-bromo-cGMP mimics the effect of SIN-1 and S-NC. E-cluster neurons stained by NADPH-diaphorase, and marked by dye injection, synaptically depolarize neurons that receive NADPH-diaphorase stained terminals. Consistent with NO effects, the response is calcium dependent and blocked by nitro-arginine. These results suggest that NO released from NADPH-diaphorase stained neurons acts like a neurotransmitter and depolarizes postsynaptic neurons by activating guanylate cyclase and increasing cGMP, which alters ionic conductance.

526.7

SEROTONIN STIMULATION OF CILIA BEAT FREQUENCY IS MEDIATED BY AN INCREASE IN INTRACELLULAR CALCIUM IN EMBRYOS OF *HELISOMA TRIVOLVIS*. J. J. Goldberg*, K. J. Christopher, C. Neumann and J. P. Chang. Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

Prior to the development of crawling, *Helisoma trivolvis* embryos display a cilia-driven rotational behavior within the egg capsule. One component of this behavior, rotational surges, results from the transient stimulation of pedal ciliated cells by serotonin that is released from the identified embryonic neurons, ENC1. In order to examine the signal transduction mechanism underlying the serotonin-induced stimulation, ciliated cells were isolated from *Helisoma* embryos, plated under cell culture conditions and monitored in slow motion using time-lapse videomicroscopy. In the absence of serotonin, cilia beat spontaneously and regularly at frequencies ranging from 5-9 cycles per second. Serotonin induced a sustained, dose-dependent increase in cilia beat frequency with an EC_{50} around 1 μM. This stimulation was not mimicked by either potassium-induced depolarization or addition of 100 μM forskolin and 200 μM isobutylmethylxanthine, a treatment that elevates the level of intracellular cyclic AMP. In contrast, addition of thapsigargin (1 μM) or A23187 (10 μM), treatments that increase the concentration of cystolic free calcium, caused an increase in cilia beat frequency similar to that produced by 100 μM serotonin. Furthermore, the response to serotonin was prevented when cells were exposed to calcium-free medium or the voltage sensitive calcium channel blockers, nifedipine or verapamil (1 μM). These data suggest that serotonin stimulates cilia beating by causing a calcium influx in a depolarization-independent manner. Supported by NSERC of Canada.

526.8

NEUROTRANSMITTER 5-HT AND DA LEVELS AND THEIR SYNTHESIS ACROSS THE LIFE SPAN OF *APLYSIA*. C. Hong*, J.M. Flinn, R. Holt, V. Chandhoke and Tom Capo.† George Mason University, Fairfax, VA 22030, † Univ. of Miami, FL 33149.

The levels of serotonin (5-HT) and dopamine (DA) in ganglion tissue were examined for animals ranging in age from 3 to 13 months post-hatch, the tissue tryptophan and tyrosine levels were also measured using HPLC. The results indicated that 5-HT increased slowly in the ganglion from 3 to 4 months and from 7 to 13 months, however it increased rapidly in the 4 to 6 month age range. In contrast, the increase in DA was predominantly constant across the age span. The ratio of 5-HT to DA showed a strong age dependency, with the lowest values at 3, 4, 12, and 13 months and the highest ratio at 6 months. 5-HT/DA represents the excitatory to inhibitory neurotransmitter ratio. These results suggest that DA may dominate 5-HT in very young and very old animals where previous studies have shown behavioral sensitization of the Gill-siphon withdrawal reflex to be weaker. The analysis of the ratios of neurotransmitter to its precursor in the ganglion (5-HT/TRP and DA/TYR) suggests that the synthesis of neurotransmitters may be one of the factors contributing to the changes in the net neurotransmitter levels. The 5-HT/TRP ratio increased at the 6 to 8 months period and then leveled off, while the synthesis of DA/TYR ratio had a slight increase after 7 months. Further studies on the metabolic enzymes and degradation courses are underway.

526.9

AGE RELATED CHANGES IN SEROTONIN AND DOPAMINE NEUROTRANSMITTERS AND RECEPTORS IN APLYSIA CALIFORNICA. M.D. Southall, S. Shirazi, V.Chandhoke*, P.W. Royt and J.M. Flinn. George Mason University, Fairfax, VA 22030

The age associated changes in dopaminergic and serotonergic receptors were examined in *Aplysia californica*. In earlier studies (Hong, 1994) it has been shown that the levels of serotonin (5-HT), dopamine (DA) and their ratio vary with age. In this study the levels of neurotransmitter receptors were examined in the ganglia obtained from 4, 5, 6, 10 and 12 month old animals. Receptor analysis was performed using radiolabeled bound ligands specific for the receptor of interest. ^{3}H-LSD was used to determine the saturation binding for total 5-HT and DA receptors. We report here the results for the 5 month (young) and 10 month (old) animals. Specific binding in the 5 month animals was found to be 9.9 fmol/mg of total protein for 5-HT and 8.4 fmol/mg protein for DA. For the 10 month animals, the specific binding for 5-HT was 20.9 fmol/mg protein and 16.4 fmol/mg protein for DA. This represents a two fold increase in specific binding for both 5-HT and DA in the 10 month as compared to the 5 month animals.

526.10

EVIDENCE FOR INSULIN-LIKE PEPTIDES IN *APLYSIA* AND POSSIBLE LOCALIZATION IN THE CEREBRAL GANGLIA. L. Stadtmauer, J. Koester, N. M. McKay, D.H. Solomon, and J.H. Schwartz*. Departments of OB-GYN and Pharmacology and the Center for Neurobiology & Behavior, Columbia University College of Physicians & Surgeons, New York, NY 10032.

Vertebrate insulin can stimulate tyrosine autophosphorylation of a vertebrate insulin-like receptor in *Aplysia* bag cell neurons. We have therefore searched for peptides in *Aplysia* similar to vertebrate insulin using HPLC purification and a placental insulin-receptor autophosphorylation assay. Fractions from *Aplysia* embryos and total nervous system were purified by HPLC. One fraction both stimulates placental insulin-receptor kinase activity and competes with insulin in receptor-binding assays and in radioimmunoassays at nanomolar concentrations of the vertebrate peptide. The material in this fraction has been purified and its amino acid sequence is being determined. Input to the bag cells originates in the cerebral ganglion (Ferguson et al, *J. Comp. Physiol. A* **164**: 849), which contains cells that we find may be homologous to the light green cells (LGCs) of *Lymnaea* (LGCs produce molluscan insulin-like peptides -- MIPs -- which control growth and reproduction): We find that an antiserum against R15α2 peptide cross-reacts with vertebrate insulin as well as with the material that purifies as insulin. Immunocytochemical studies with this antiserum stain two bilateral clusters of neurons in the cerebral ganglion that appear homologous to the LGC clusters in *Lymnaea*. Since these cells do not express R15α2, this antiserum may be recognizing another peptide. As in *Lymnaea*, the neurons in *Aplysia* that exhibit this insulin-like immunoreactivity send processes to neurohaemal organs at the base of the lip nerves. Further studies are in progress to determine whether these LGC-like clusters are the source of the insulin that acts on bag cell neurons.

526.11

ASSAY FOR CYCLIC AMP LEVELS, SYNTHESIS AND DEGRADATION AT THE MEMBRANE OF LIVE MOLLUSCAN NEURONS. Sudlow, L.*, and Gillette, R. Dept. of Physiology and Biophysics, Univ. of Ill, Urbana, IL 61801

Neurons exhibiting cyclic AMP-gated current ($I_{(Na,cAMP)}$) allow *in vivo* assay of cyclic AMP levels, synthesis and degradation (Huang and Gillette, '91, J.Gen.Physiol. 98:835, '93, J.Physiol.462:307; Sudlow, et al., in prep.). We have estimated 1) the concentration of cAMP due to stimulation by 5-HT; 2) 5-HT stimulated adenylate cyclase activity; and 3) phosphodiesterase rate constants. Occlusion of $I_{Na,cAMP}$ by 5-HT-induced current, and the voltage-dependent inactivation associated with both cAMP injection and neuromodulators, indicate common modes of action. The procedure requires establishing: 1) the dose response relationship for $I_{(Na,cAMP)}$ with steady-state cAMP iontophoretic current; 2) occlusion assay of $I_{(Na,cAMP)}$ with neuromodulator; 3) analysis of phosphodiesterase rate constants; and 4) measurement of cell diameter. Calculations yield an estimate of adenylate cyclase activity per sec per unit membrane area. We have estimated the adenylate cyclase activity in pedal ganglion G neurons in *Pleurobranchaea* during 10 μM 5-HT stimulation to range from 0.98 to 2.77 pmol*$cm^{-2}sec^{-1}$, averaging 1.59 ± 0.31 (n=5). 5-HT-induced cAMP concentrations at the membrane ranged from 4.94 to 12.14 μM, averaging 7.68 ± 1.22 μM (n=5). Supported by NIH grant RO1 NS26838-01 to R.G.

526.12

A SUBDIVISION OF OCTOPAMINE NEUROSECRETORY CELLS IN THE LOBSTER. P. Bräunig, I. Walter, H. Schneider* & E.A. Kravitz; Neurobiol. Dept., Harvard Med School, Boston, MA 02115.

Octopamine (OA) and serotonin (5HT) appear to participate in the regulation of aggressive behavior in lobsters (*Homarus americanus*). Injections of OA generate extended postures resembling those seen in submissive lobsters, while 5HT injections trigger flexed postures typical of dominant lobsters. We have mapped OA- and 5HT-immunoreactive neurons in the lobster CNS and identified 4 5HT and 28 OA neurosecretory cells likely to be important in this postural regulation. Within the OA group, immunostaining appears gradually throughout embryonic and larval development. This suggests that the OA system might be divided into subsets showing coincidental appearance of immunostaining and function. Here we describe one such subset. It includes 3 pairs of cell bodies located in the 3rd and 4th thoracic ganglia that selectively innervate the claws. Projections of these cells were traced through the paramedial nerves that run parallel to the nerve cord into the 1st roots of 1st thoracic ganglia, supplying the claws. The identity of the cells as octopaminergic was confirmed by combining iontophoretic injections of Lucifer Yellow with immunostaining. Large overshooting action potentials are recorded from their somata. These are characteristic of arthropod neurosecretory cells, and suggest that the claw octopamine cells (CLOCs) might form neurosecretory endings in claw muscles. During development OA - immunostaining appears in CLOCs at the 2nd larval stage, shortly before functional claws first appear.We are exploring the role served by the CLOCs in fighting behavior in lobsters. Supported by NIH, DFG, and Gottlieb-Daimler and Karl-Benz Stiftung.

AXON GUIDANCE II

527.1

OUTGROWING RETINAL AXONS GUIDED BY RADIAL (GLIA) CELLS. B. Schlosshauer* and H. Stier. *Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen (NMI), 72762 Reutlingen, FRG.*

In order to analyse the cellular mechanisms that govern oriented axonal outgrowth within the retina, we employed cryocultures of the embryonic chicken. Viable retina strips were explanted onto cryosections of retina and axonal outgrowth was monitored. In vivo only ganglion cell axons and neuroepithelial/radial glia endfeet form the optic fiber layer; radial Müller glia is the only nonneuronal cell type in the chicken retina. *1) Ganglion cell axons are directed by a dual mechanism: the retina tissue is composed of an inhibitory zone (all outer layers) and a permissive zone (optic fiber layer).* a) In vitro, axons grow preferentially on the innermost layer of retinal cryosections even after coating sections with laminin. b) Axons approaching outer retina layers respond partially with a growth cone collapse (time lapse video recording). c) In contrast, prior heat inactivation of cryosections allows axonal outgrowth even in outer retina layers. *2) The permissive zone is formed by endfeet of neuroepithelial/radial glia.* a) After elimination of ganglion cells and their axons due to prior optic nerve transection, explant axons still prefer the innermost retina layer of in vivo pretreated cryosections. b) Isolated glia endfeet provide an excellent growth substrate. *3) The inhibitory zone is formed by somata of radial glia.* a) Axonal outgrowth is inhibited on radial glia somata purified from retina cell cultures by complement mediated neuronal cytolysis. b) The restricted number of outgrowing axons on glia somata are highly fasciculated therefore avoiding contact with glia somata (scanning electron microscopy). c) Heat inactivation of purified glia somata converts glia cells into a permissive substrate. **The data suggest that ganglion cells axons are guided into the innermost retina layer by polarized neuroepithelial/radial glia cells with their endfeet being permissive and their somata being inhibitory for outgrowing axons.** Supported by DFG.

527.2

RETINOIC ACID AND PEPTIDE GROWTH FACTORS INFLUENCE OLFACTORY NEURITE OUTGROWTH. I. G. Whitesides* and A-S. LaMantia. Department of Neurobiology, Duke University, Durham, NC 27710.

We have evaluated the role of retinoic acid and various peptide growth factors in the initial formation of the olfactory nerve in the mouse. Immunohistochemical and *in situ* localization has shown that several retinoic acid receptors (RARα and RXRγ) as well as trk-B and FGF receptors are present in olfactory epithelial cells and/or axons at embryonic day 10-11. Furthermore, local sources of retinoic acid, FGF, and other peptide hormones are available in the fronto-nasal mesenchyme (where olfactory axons grow toward the brain) and the anterior neural tube (their ultimate target). Thus, it is possible that retinoic acid and various growth factors may influence the outgrowth of developing olfactory axons as well as their initial trajectory to the ventrolateral forebrain.

To assess the effects of these signaling molecules on olfactory axon outgrowth, we used a neurite outgrowth assay in which explants of the early olfactory epithelium are placed on a laminin substrate in serum-free media. We then added all-*trans* retinoic acid (10^{-7}M) and allowed the explants to extend neurites for 48 hours. Retinoic acid significantly increased both the number and length of neurites that grew out from these explants compared to those grown on the laminin substrate alone. The effects of peptide growth factors whose receptors are found on olfactory epithelial neruons (BDNF, b-FGF, and NT-4) have also been examined. Apparently, a variety of signaling molecules and their receptors that are normally found in and around the developing olfactory epithelium and nerve can influence aspects of olfactory neurite outgrowth *in vitro*. These factors may similarly contribute to the growth and guidance of olfactory axons to their telencephalic targets *in vivo*. Supported by HD29178 to A-S. L.

527.3

NEURONAL ORGANIZATION OF THE EMBRYONIC FORE- AND MIDBRAIN IN WILDTYPE AND MUTANT MICE. G.S. Mastick* and S.S. Easter, Jr., Dept of Biology, U of Michigan, Ann Arbor, MI 48109

The fluorescent axon tracers dil and diO were used on fixed E10.5 embryos to map groups of early neurons and pioneer axons with respect to interneuromeric boundaries. The spatial and temporal relationship between central neurons, tracts, and cranial nerves defines the coordinates of the rostral embryonic brain, revealing several general features: **a) Groups of neurons can straddle major transverse interneuromeric boundaries.** dtmesV and mlf somata were found in both mesencephalon and synencephalon, implying that these neuronal types are not simply defined by neuromere boundaries. In contrast, oculomotor (nIII) somata were confined to the ventral mesencephalon, abutting but not crossing the synmes border. **b) Position and timing may specify neuronal identity, at least in terms of axon projections.** A double-labelling strategy showed that mlf and nIII somata were both in ventral mesencephalon, but in non-overlapping nuclei. In contrast, the late-appearing tectobulbar neurons were interspersed with the very early dtmesV neurons in dorsal mesencephalon. **c) Two major axon systems develop, marking the dorsal and ventral halves of the rostral brain.** dtmesV (dorsal) and mlf (ventral) appeared first, projecting caudally into the rhombencephalon. Axons of the tract of the postoptic commissure (tpoc) extend from the base of the optic stalk into dtmesV, suggesting that the tpoc is dorsal, and the "front" end of the brain is ventral to the optic stalk. **d) The pioneer axons do not follow interneuromeric borders.** Instead, broad zones of the neural tube are filled with axons sharing trajectories.

We examined the altered organization of the embryonic brain in *Wnt-1* mutant mice using neuronal landmarks at E9.5. The loss of this secreted growth factor causes deletion of mesencephalon and rhombomere 1, loss of nIII and nIV, and creates a new boundary between the forebrain and rhombomere 2. Surprisingly, pioneer axons navigate accurately across the new boundary.

527.4

ARREST OF AXONAL GROWTH *IN VITRO* BY LIVING, SECTIONED OR FIXED TARGET NEURONS. D.H. Baird* and B. Kruk. Department of Anatomy and Neurobiology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

The regulation of axonal growth by CNS targets was examined using an *in vitro* system in which the extension of cerebellar mossy fibers from explants of basilar pontine nuclei is interrupted by their targets, granule neurons. This "stop-growing signal" is both target- and axon-specific, and its regulation involves NMDA receptors. In the presence of NMDA antagonists, mossy fibers are not arrested by granule neurons, and the growth of long (>300 μm) neurites is 3-4 times that of controls. NMDA has an opposing effect; it enhances the stop signal provided by granule neurons, resulting in shorter mossy fibers. Neither NMDA nor its antagonists alter the growth of mossy fibers in the absence of granule neurons.

To determine if functional, pre- or postsynaptic NMDA receptors are involved in the stop-growing signal, mossy fibers were cultured on granule neuron monolayers that had previously been fixed in 4% paraformaldehyde, and on unfixed, frozen sections of cerebellum. In both cases, mossy fibers extended less than 100 μm from their explant of origin, while mossy fibers extended more than 500 μm on fixed laminin or on sections of brainstem. These results suggest that molecules which directly mediate the stop-growing signal are insensitive to fixation, and are located on the granule cell plasma membrane, or are part of a matrix produced by granule neurons. In addition, the results suggest that functional granule neuron NMDA receptors are not strictly required for the arrest of axons by target neurons, and if they are involved at all, they must regulate this process indirectly.

527.5

EVIDENCE FOR GUIDEPOST CELLS IN AXON OUTGROWTH DURING DEVELOPMENT OF *DROSOPHILA*. L.S. Wang* and H. Keshishian, Department of Biology, Yale University., New Haven, CT 06511

The characteristic pattern of the bodywall innervation in *Drosophila* embryos provides an ideal system to study axon guidance and synapse formation. Segmental nerve branch A (SNa) grows out laterally in the anterior half of the segment and makes a stereotypical posterior turn at mid-bodywall to innervate muscle fibers 5 and 8. Cash et al. (J. Neurosci. 12:2051, 1992) showed that in the absence of these target muscle fibers, SNa still turns posteriorly. Therefore, cues other than the targets are present to guide SNa growth. Although the SNa trajectory can be altered by laser ablation of the muscle fibers in its path, it always makes the posterior turn to innervate its target muscle fibers (Wang and Keshishian, Neurosci. Abst. 18:1273, 1992). We observed the presence of 2 cells (possibly glial) at the corner of muscle fibers 12 and 5. Their locations correspond to the site at which SNa usually makes endings on muscle fiber 5. In mutants of the *spitz* family, such as *pointed,* which affect ventrolateral patterning, 20% of the SNa nerves fail to turn (Wang et al., Neurosci. Abst. 19:235, 1993). Examination of *pointed* mutants showed: (A) In 6 out of 8 cases SNa failed to turn when the 2 glial cells were missing. (B) In 20 out of 22 cases SNa turned normally when these cells were present. To test whether these cells are in fact guidepost cells, we are surgically removing them in filleted embryos at late stage 15, when SNa has not yet turned. The embryos are then cultured until late stage 16, when SNa normally has reached its targets. These experiments will critically define the role of these putative guidepost cells in inducing the posterior turn of SNa.

527.6

THE DROSOPHILA *DERAILED* GENE ENCODES A PUTATIVE RECEPTOR TYROSINE KINASE REQUIRED FOR BOTH AXONAL FASCICULATION AND MUSCLE INSERTION SITE SELECTION. C. A. Callahan[1,2] and J. B. Thomas*[1], [1]The Salk Institute, La Jolla, CA 92037, [2]Dept. of Neurosciences, Univ. of California, San Diego, CA.

In order to establish synaptic connections with their appropriate targets, neurons must choose specific axon pathways during development. Similarly, developing somatic muscles choose stereotyped insertion sites within the epidermis. To begin understanding the molecular mechanisms underlying these recognition events, we have carried out an enhancer-trap screen using a novel fusion reporter, tau-b-gal, which effectively labels the projections of expressing neurons (PNAS, in press). From 2500 enhancer-trap lines, we have isolated approximately 40 that express tau-b-gal in subsets of interacting embryonic CNS neurons and/or in somatic muscle subsets.

The *derailed (drl)* gene was isolated from one line by plasmid rescue and shows the same pattern of expression as tau-b-gal. *drl* is expressed in a subset of approximately 20 interneurons that fasciculate into 2 major axon bundles, as well as in somatic muscles 21-23. From sequence analysis, the *drl* gene product is a member of the receptor tyrosine kinase (RTK) family, and shows the highest similarity to the vertebrate *RYK* RTK. Individuals homozygous for the P element insertion, which severely reduces CNS *drl* transcription, show defasciculation of the *drl* expressing interneurons. Transcriptional nulls for *drl* were generated by P element excision. In embryos lacking *drl* expression, the muscles that normally express *drl* fail to insert at their appropriate locations within the epidermis. These results suggest that mutations in *drl* disrupt specific recognition events both between neurons and their axonal pathways and between muscles and their insertion sites. They also implicate the RTK gene family in these developmental processes.

527.7

SPECIFICITY OF SYMPATHETIC PREGANGLIONIC PROJECTIONS IN THE CHICK IS INFLUENCED BY THE MATURATION STATE OF THE SOMITIC MESODERM. J.W. YIP*. Dept. of Neurobiology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

The spatio-temporal patterns of sympathetic preganglionic projections in the chick are segmentally specific. For example, T1 preganglionic neurons enter the sympathetic trunk at stage 27 and project rostrally. In contrast, T4 preganglionic neurons enter the sympathetic trunk at stage 28 and project caudally. Previous studies from this laboratory have shown that the somitic mesoderm influences these patterns. This study examines whether the maturation state of the somitic mesoderm is responsible for that influence.

Two series of experiments were designed to confront preganglionic neurons with somitic mesoderm of an older maturation state. In one, the T1-T4 neural tube segments of a stage 14 embryo were transplanted to the upper cervical (more mature) region of the same embryo. Orthograde HRP labeling showed that outgrowth of T1 preganglionic neurons translocated to the cervical region occurred much earlier than normal, such that by stage 26 the translocated T1 preganglionic neurons have already projected extensively into the sympathetic trunk. This indicates that preganglionic neurons will send their axons out earlier when they encounter a more mature somitic mesoderm. In another series of experiments, the T1-T4 neural tube segments from a stage 13 donor were transplanted homotopically to a stage 18 host. In the resultant embryo, the T1 preganglionic neurons projected in both rostral and caudal directions, instead of in their normal, predominantly rostral direction. This result indicates that the direction of preganglionic projections can be altered by the maturation state of the somitic mesoderm.

The maturation of the somitic mesoderm may therefore relate to the formation of the specific pattern of preganglionic projections.

527.8

MUTATIONS IN *sidestep* LEAD TO DEFECTS IN PATHFINDING AND SYNAPTIC SPECIFICITY DURING THE DEVELOPMENT OF NEUROMUSCULAR CONNECTIVITY IN DROSOPHILA. H. Sink and C.S. Goodman*, HHMI, Neurobiol. Div., Dept. of Mol. and Cell Biol., U. C., Berkeley, CA 94720

We screened over 5000 heavily mutagenized 3rd chromosome lines in Drosophila, and identified 140 mutations that disrupt the embryonic projection of motor axons. Here we focus on mutations in the *sidestep* gene which disrupt specific aspects of motor axon pathfinding, while the muscles appear normal. In the periphery of wild-type embryos, motor axons defasciculate to generate 5 characteristic branches or motor nerves. In *sidestep* mutant embryos, the motor projection frequently has 2 instead of 5 branches, with a resultant lack of innervation of the most ventral muscles.

Several deficiencies delete the chromosomal region containing the *sidestep* gene. The phenotype of *sidestep*/deficiency embryos is the same as homozygous *sidestep* embryos (4 independent alleles), suggesting that this phenotype may represent the null condition. The *sidestep* mutants are semi-viable as transheterozygotes and over deficiency. Despite the lack of innervation of many of the ventral muscles, and inappropriate innervation of certain dorsal muscles (see below), larval responses to touch appear relatively normal.

Motor axons that innervate the most distal muscles (e.g., #12,13) of the ventral muscle group often diverge from the inappropriate pathway and innervate their correct muscle targets. In contrast, other misrouted motor axons continue to extend dorsally. Instead of innervating their normal ventral muscle targets (e.g., #7,6), these axons synapse on dorsal muscle 4. Interestingly, muscle 4 has the same relative orientation within its muscle group as do several of the uninnervated ventral muscles (7,6) within their group. The *sidestep* mutant phenotype suggests that targeting cues may be similar or even duplicated in different regions of the embryonic musculature. Work in progress is aimed at cloning the *sidestep* gene.

527.9

TESTING THE ROLE OF TARGETS IN MOTONEURONAL SPECIFICITY. C.E. Beattie* and J.S. Eisen, Institute of Neuroscience, University of Oregon, Eugene, OR, 97403

We are investigating mechanisms underlying neuromuscular specificity in vertebrates using embryonic zebrafish. During zebrafish development, identified motoneurons with specific soma positions in the spinal cord become committed to innervate particular regions of myotome. Motor growth cones may be guided by myotomal cues. Alternatively, they may grow in a specific direction regardless of myotomal cues, suggesting that guidance cues originate from some other source. To differentiate between these mechanisms, we analyzed motor axonal outgrowth after altering the orientation of the myotome. Labeled donor myotomes were isolated, rotated along the dorsal-ventral axis and transplanted into unlabeled host embryos from which native myotomes had been removed. Following dorsal-ventral reorientation of the myotome and isochronic transplantation at 16h, an identified motoneuron, CaP, extended its growth cone ventrally as it does normally. This result suggests either that dorsal and ventral myotomal regions have become respecified or that CaP extends its axon ventrally independently of myotomal cues. To resolve this issue, heterochronic transplants were performed. Following dorsal-ventral reorientation and transplantation of 19h myotomes into 16h hosts, CaP extended an axon which often grew laterally and appeared to avoid the ventral myotomal region which now contained dorsal muscle. This result supports the idea that younger myotomes respecify their dorsal and ventral regions while older myotomes retain their original polarity after transplantation. Motor axons, therefore, appear to be guided by cues originating from myotome. Supported by ACS PF-3982 and NS23915.

527.10

SCHWANN CELL PROCESSES GUIDE REGENERATING AXONS. Y.-J. Son* and W.J. Thompson. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Terminal Schwann cells (TSCs) at the adult rat neuromuscular junction sprout extensive processes following denervation (J. Neurocytol. 21: 50). We have used an antibody which marks denervated TSCs and an antibody against neurofilament to examine the relationship between TSC processes and regenerating axons in the soleus muscle. Prior to any reinnervation, some TSC processes grow to contact adjacent endplates or fasciculate with the TSC processes emanating from adjacent endplates. Thus, TSC processes come to interconnect many endplates prior to the return of regenerating axons. Most axons arrive at denervated endplates by growing down the old Schwann cell tubes. After re-occupying the endplate, axons extend processes onto the TSC processes, and, in many cases, navigate along these TSC processes to innervate adjacent endplates. Consequently, many endplates come to be polyneuronally innervated, by an axon in the old Schwann cell tube and one or more sprouts guided from adjacent endplates by TSC processes.

To determine whether this guidance of axons is a general property of Schwann cells (SCs), we examined SC outgrowth from the cut end of a denervated nerve stump transplanted to the surface of soleus. SCs and their processes grew out over the muscle in the absence of axons. When a nerve with axons was transplanted, axons and SCs both grew from the nerve and each axon was associated with SC processes. In several cases we found axons navigating along SCs with the SCs apparently leading the axons. The rate at which axons and SCs extended from a transplanted nerve was the same as that at which SCs extended in the absence of axons. On the other hand, when axons were confronted with existing SC processes their growth rate was at least 7 fold faster. Our results suggest that SC processes guide axonal growth during regeneration in the peripheral nervous system.

527.11

MOTOR AXON SPROUTS ARE INDUCED AND GUIDED BY PROCESSES EXTENDED FROM SCHWANN CELLS. W.J. Thompson* and Y.-J. Son. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

In view of the guidance that terminal Schwann cells and Schwann cells (SCs) in the nerve give regenerating axons (Son and Thompson, this meeting), we have examined the possibility these cells play a role in the sprouting of motor nerves in adult muscles.

We first examined nerve sprouting induced by partial denervation (PD). Within 3 days of PD, use of an anti-neurofilament antibody showed that nerve sprouts were extended from about 28% of the terminals remaining in the muscle. Staining with a general SC marker (anti-S-100) showed that all of these sprouts were associated with SC processes. Staining with the antibody 4E2, which marks SCs at denervated endplates and the processes they extend, showed that a large fraction of the terminal sprouts were extended from junctions which had been contacted by processes of SCs from denervated junctions. In these cases the nerve sprouts grew along the SC process to the adjacent denervated endplate. Additional examples were found of nodal sprouts which were growing along SC processes.

We next examined sprouting induced by botulinum toxin-induced paralysis. In all cases, nerve terminal sprouts were associated with Schwann cell processes extended from the endplate. In several cases, SC processes were longer than axonal processes.

Finally, we examined the ability of SCs and their processes growing from the end of a denervated, nerve stump implanted on the surface of the soleus muscle near the endplate band to induce sprouting from intact junctions. Schwann cells growing into intact junctions induced sprouts which then followed the Schwann cell processes. On the other hand, passage of a Schwann cell process near a junction seemed insufficient to induce sprouting.

Our experiments demonstrate a clear association between the extension of Schwann cell processes and the induction and guidance of nerve terminal sprouts in muscle.

527.12

DYNAMICS OF TERMINAL SCHWANN CELL PROCESSES AT NEUROMUSCULAR JUNCTIONS IN LIVING MICE. R. J. Balice-Gordon* Dept. Biology & Inst. for Neurol. Sci., Univ. of Pennsylvania, Phila., PA 19010-6018.

Presynaptic motor nerve terminals at neuromuscular junctions are covered by non-myelinating terminal Schwann cells whose function is poorly understood. In living animals, terminal Schwann cells can be selectively labeled using Calcein Blue (CB; am ester, Molecular Probes, Eugene, OR). CB labels Schwann cell bodies and processes at junctions but does not label nerve terminals, muscle fibers, myelinating Schwann or other cells. In adult mice, CB stained Schwann cell processes were observed to be confined to the endplate region and were often precisely aligned with motor nerve terminal branches. Such alignment was not observed in neonatal mice. Following denervation, terminal Schwann cells rapidly extended processes well beyond the endplate region, often joining with processes of cells from adjacent endplates, consistent with observations made using S-100 immunochemistry (Reynolds & Woolf, 1992). Recently, Thompson et al. have shown that following denervation, regenerating axons are closely associated with Schwann cell processes and suggest that these cells may play a role in axon guidance back to the endplate (Son & Thompson, this vol.). Observations of terminal Schwann cells *in vivo* will allow the dynamics of the response of these cells to denervation and their role in synaptic regeneration and maintenance to be studied.

527.13

EFFECT OF CELL-ELIMINATION IN THE DISTAL PART OF CRUSHED TIBIAL NERVES STUDIED BY IMPLANTED NERVE CUFF ELECTRODES IN THE CAT. K. Fugleholm*, J. Sørensen, H. Schmalbruch, C. Krarup, Inst. of Med. Physiology, Univ. of Copenhagen, and Dep. of Clinical Neurophysiology, Rigshospitalet, Denmark, DK 2200 N.

The importance of Schwann and other cells for nerve regeneration after a crush lesion was studied in the cat tibial nerve. Segments of the distal nerve adjacent to the crush were made cell-free by freezing and thawing. Regeneration was followed by a series of cuff electrodes implanted around the tibial and sciatic nerve. Twice weekly, action potentials were elicited distally and recorded proximally; this allowed to continuously follow axonal elongation. The elongation rate was about 3 mm/day; it was unchanged whether the distal nerve was not frozen or frozen for 20 (*Fugleholm et al., 1994, J.Neurosci. in press*) or 30 mm. Freezing the nerve for 40 mm, however, slowed the elongation to almost stand-still about 35 mm distal to the crush lesion, i.e. inside the frozen segment. The electrophysiological findings were confirmed by morphometric evaluation at four levels distal to the lesion. Removal of myelin debris was delayed in 40- as compared to 30-mm-long frozen segments. The results indicate that axonal regeneration is limited by the length of a cell-free nerve segment distal to the lesion and suggest that invading macrophages may play a critical role for the repopulation of the nerve segments by Schwann cells.

527.14

GROWTH OF AXONS IN ADULT FIBRE TRACTS. G. Raisman*, M. Suzuki, S.J.A. Davies, G.A. Brook, Y. Li and P.M. Field. Norman and Sadie Lee Research Centre, Lab of Neurobiology, NIMR, MRC, London NW7 1AA, UK.

Adult fibre tracts (fimbria, corpus callosum, spinal dorsal columns) have a regular, rectilinear arrangement of astro- and oligodendro-glial cells and processes. Embryonic neurons microtransplanted by minimally traumatic injections into otherwise unlesioned tracts grow profuse axons which rapidly elongate along the planes determined by the longitudinal glial and axonal structure of the host tracts. Both specific and also incorrectly matched neurons (e.g. superior collicular cells in the fimbria, hippocampal cells in the spinal cord) grow equally rapidly, profusely, and with the same topographic distribution. Axons enter terminal fields, but their ultimate fate has not yet been determined. Cultured Schwann cells injected as a suspension rapidly migrate along blood vessels, and become transformed into elongated, thread-like cells which also migrate and become dispersed along the longitudinal axis of adult host tracts. Cultured Schwann cells microtransplanted into otherwise unlesioned adult spinal dorsal columns induce sprouts from both cut and uncut axons. Both motor axons (of the corticospinal tract) and sensory axons (of the dorsal columns) respond. These sprouts arise either from the cut ends of the axons, or as branches of axonal stems (probably from myelin internodes). In contrast to the sprouting from cut axons in the absence of Schwann cells (when profuse sprouts persist for long periods after lesion, but do not elongate), the Schwann cell induced sprouts branch and elongate along the long axis of the host tract, and form local terminal field-like arborisations with varicosities, but their ultimate fate is still not known.

INGESTIVE BEHAVIORS VI

528.1

CORTICOTROPIN-RELEASING FACTOR (CRF) ANTAGONIST BLOCKS THE AUTONOMIC, ENDOCRINE, INGESTIVE & BEHAVIORAL EFFECTS OF CENTRAL BOMBESIN (BN). Z. Merali[1]*, H. Plamondon[1], P. Kent[1] and K. Banks[1], [1]Psychology & *Pharmacology, Univ. of Ottawa, Ottawa, Ont. Canada, K1N 9A9.

Bombesin (BN)-like peptides and their receptors are widely distributed within the mammalian CNS. Central administration of BN has a number of biologically significant actions including elevation of blood catecholamines, glucose and corticosterone, elicitation of grooming and locomotor activity, and suppression of food intake. It is of interest to note that the autonomic and behavioral syndrome elicited by CRF strongly resembles that of BN, and that there is a significant anatomical overlap in the localization of these two neuropeptides. In the present study, we attempted to elucidate the potential role of CRF in the mediation and/or modulation of the BN effects, by utilizing a specific CRF receptor antagonist α-helical CRF $_{9\text{-}41}$ (α-hCRF). In male Sprague-Dawley rats, BN (0.1-0.5 μg; icv) caused a dose- and time-dependent increase in the circulating levels of glucose and ACTH. Although α-hCRF (5-10 μg; icv) by itself was without significant effect, it blocked the BN-induced increase in ACTH and markedly attenuated BN-induced hyperglycemia. BN increased grooming and locomotor activity, both of which were markedly attenuated by α-hCRF. Finally, in rats trained to ingest daily food ration during a 4 hr access period, BN markedly suppressed ingestive behavior for up to 4 hr. This suppression was completely blocked by pretreatment with both doses of α-hCRF (5 or 10 μg). These data indicate that central CRF system may play a significant role in the mediation of behavioral and autonomic effects of BN.

528.2

EXOGENOUS CCK ANALOG AND INTRAGASTRIC GLUCOSE PRELOADS PRODUCE SIMILAR MICROSTRUCTURAL CHANGES IN MEAL PATTERNS. T.H. Moran*, A.R. Baldessarini, and G.J. Schwartz. Department of Psychiatry and Behavioral Sciences, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Ingested food elicits a variety of peripheral and central signals that can determine the pattern and magnitude of food intake within a meal. While the magnitude of intake suppression resulting from two different signals may be identical, the pattern of intake producing those suppressions may not be. If the intake patterns underlying two identical suppressions are similar, it seems likely that the signals produced by the two stimuli are processed in similar ways. Using automated lick detectors, we examined the patterns of glucose intake (0.125 g/ml) over a 30 min test period following an overnight food deprivation in male Sprague-Dawley rats following: 1) 5 ml intragastric glucose preloads (0.125 - 0.25 g/ml), and 2) a dose range of the a norleucine substituted CCK analog. Both intragastric glucose and the CCK analog reduced intake to similar degress and the patterns of intake for similar suppressions did not differ. Analyses of the ongoing behavior revealed: 1) that neither class of stimuli affected the peak interlick interval or the lick rates within a burst, and 2) overall lick rates were slower and the number of bursts were significantly reduced to a similar degree by both classes of stimuli. These similar patterns suggest that the signals arising from exogenous administration of the CCK analog are processed in a similar fashion to those arising from a nutrient preload. Supported by DK19302.

528.3

EFFECTS OF CONTINUOUS NEAR-CELIAC CCK-8 INFUSIONS ON SUCROSE INTAKE AND FEEDING BEHAVIOR. J.E. Cox*, G.S. Perdue, and W.J. Tyler. Dept. Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

Bolus injections of cholecystokinin octapeptide (CCK-8) are more potent for reducing sucrose intake when injected through an aortal catheter with its tip near the celiac artery than when given intravenously (Calingasan et al., Am.J.Physiol. 263:R572-R577). We compared suppression of intake of 30% sucrose produced by continuous near-celiac and intravenous infusions of 100 - 1600 ng CCK-8. Adult, male Spague-Dawley rats received infusions thoughout 20 min feeding tests. Significant reductions in intake were produced by 400, 800, and 1600 ng near-celiac CCK-8. Only the two highest doses reduced intake when administered intravenously. Behavioral observations indicated [1] that CCK-8 had no effect on feeding during the first several minutes but accelerated the decline in incidence of feeding later in the test and [2] that near-celiac CCK-8 had more pronounced effects on feeding behavior than did intravenous CCK-8. Thus during slow infusions, greater potency of near-celiac CCK-8 was accompanied by temporal intake patterns indicative of enhancement of satiety. These data provide further evidence that cholecystokinin acts within the upper gastrointestinal tract to inhibit food intake.

528.4

ROLE OF CCK IN MEDIATING THE SATIETY RESPONSE TO DUODENAL PEPTONE IN RATS. T.A. Woltman and R.D. Reidelberger*. VA Medical Center, Omaha, NE 68105 and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178.

An important role for CCK in mediating the satiety response to duodenal nutrients is supported by data showing that CCK receptor blockade reverses the anorexic effects of duodenal nutrient infusions in *sham* feeding rats. In the present study, we used the CCK-A receptor antagonist devazepide (DEV) to examine the role of CCK in mediating the anorexic effects of peptone infusion into the duodenum of non-food deprived *real* feeding rats at the start of the dark period. Rats (n=15) with duodenal and jugular vein cannulae, received 2-h duodenal infusions of peptone (0, 1.7, 3.5, 7, 14, 28%); meal patterns were measured for 4 h. Peptone suppressed feeding dose-dependently primarily by decreasing meal size. The minimal effective dose was 3.5%; 14% peptone decreased caloric intake by about 50%. Rats (n=15) then received IV injections of DEV (0, .03, .1, .3, 1 mg/kg) 15 min before 2-h duodenal infusions of vehicle or 14% peptone. Data were analyzed by repeated measures ANOVA. When given alone, peptone suppressed feeding and DEV stimulated feeding. There was a significant interaction of peptone and DEV at low but not high doses of DEV. DEV (.03 and .1 mg/kg) had no effect on feeding when given alone, yet reversed the anorexic effect of peptone. In contrast, DEV (.3 and 1 mg/kg) was equally effective in stimulating feeding whether or not peptone was infused and peptone was equally effective in suppressing feeding whether or not DEV (.3 and 1 mg/kg) was injected. These results do not support the hypothesis that CCK mediates the satiety response to duodenal peptone in *real* feeding subjects.

528.5

DEPLETION IN HYPOTHALAMIC NEUROPEPTIDE Y AND DIETARY PREFERENCES IN MSG-TREATED RATS. B. Beck*, A. Stricker-Krongrad, A. Burlet, J.P. Nicolas and C. Burlet. INSERM U.308 - MRCA - 38, rue Lionnois - 54000 Nancy (France).

Neonatal treatment with monosodium glutamate (MSG) induces neuronal degeneration in the arcuate nucleus (ARC). This nucleus is the main hypothalamic site of neuropeptide Y (NPY) synthesis. Hypothalamic NPY preferentially stimulates carbohydrate intake and is influenced by diet composition. In this experiment, we measured the effects of a MSG treatment on NPY levels and dietary preferences. Newborn Long-Evans rats were injected I.P. with either MSG (4 mg/g BW) or saline on the 3rd, 5th and 7th days of life. Rats were fed on a control (C) diet until adulthood. Then, they could choose between a high carbohydrate (HC) diet and a high fat (HF) diet. NPY concentrations were measured in several microdissected hypothalamic nuclei. They were significantly smaller in the MSG rats than in the control rats in the ARC (- 52 % ; $p<0.001$) and in the parvocellular part of the paraventricular nucleus (pPVN) (- 57 % ; $p<0.004$). Energy intake in the food choice situation was greater in the control than in the MSG rats (81.3 ± 5.3 vs 62.2 ± 3.7 Kcal/day ; $p<0.01$). This augmentation was due to a greater intake of HF diet (+ 60 % ; $p<0.001$) and a smaller intake of HC diet in the control rats (- 25 % ; $p<0.01$). These results confirm some of our previous findings showing that rats with a carbohydrate preference are characterized by lower NPY levels in the pPVN. Supported by Fondation B. Delessert (Paris).

528.6

HYPOTHALAMIC NEUROPEPTIDE Y (NPY) UPREGULATION PRECEDES THE ONSET OF DIABETIC HYPERPHAGIA. A. Sahu*, C.A. Sninsky*, and S.P. Kalra, Depts. Neuroscience and *Medicine., Univ. Fla., Gainesville, FL 32610

Recent evidence shows that *in vivo* and *in vitro* NPY release in the paraventricular nucleus (PVN) and NPY gene expression in the arcuate nucleus (ARC) increase in association with hyperphagia in longterm diabetic rats. In the present study we sought the timing of the onset of NPY upregulation and hyperphagia in streptozotocin (STZ)-treated diabetic rats. Adult male SD rats were injected with either STZ (65 mg/kg) or vehicle via tail vein on day 0. Rats were killed on days 2, 3 and 4. PreproNPY mRNA levels in the medial basal hypothalamus which includes the ARC, were determined by solution hybridization/RNAse protection assay using a cRNA probe. NPY levels in the microdissected hypothalamic sites were also estimated by RIA in STZ-treated rats. As expected, STZ treatment induced marked hyperglycemia and hypoinsulinemia along with loss in body weight after STZ treatment. Analysis of NPY gene expression, peptide levels and food intake showed a remarkable temporal sequence of events. PreproNPY mRNA levels were significantly increased (4.5 fold, $p<0.001$) within 48 hours of STZ treatment. Similarly, of the 7 hypothalamic sites examined, NPY peptide levels were increased only in the PVN 2 days after STZ injection. In contrast, food intake was decreased for 3 days and thereafter, a significant hyperphagia was evident on day 4. Collectively, these results show that the enhanced NPY synthesis and release in the ARC-PVN axis precedes hyperphagia in diabetic rats. Since very low levels of insulin can inhibit NPY release from the PVN nerve terminals (Soc. Neurosci. Abst. 19:1821, 1993), we propose that the regulation of food intake by insulin is mediated by PVN NPY innervations of a subpopulation of neurons in the ARC. Supported by UF DSR KG 717 (AS), NIH DK37273 (SPK), VA Merit Review (CAS).

528.7

NPY IMMUNONEUTRALIZATION SUPPRESSES FEEDING IN VMH-LESIONED HYPERPHAGIC RATS. *Satya P. Kalra, M.G. Dube* and Pushpa S. Kalra*, Departments of Neuroscience and Physiology, Univ. of Fla. College of Medicine, Gainesville, FL 32610

It has been known for a long time that destruction of the ventromedial hypothalamus (VMH) induces hyperphagia and increase in body weight. The neural signal(s) responsible for the hyperphagic response have not been identified. Neuropeptide Y (NPY) is the most potent orexigenic signal known and the evidence that passive immunization of rats against NPY suppresses daily food intake implies that NPY may be a physiological appetite transducer. Therefore, we hypothesized that hyperphagia in VMH-lesioned rats may be NPY-dependent. Female rats received electrolytic or sham lesions in the VMH and permanent cannulae in the third ventricle of the brain. The VMH-lesioned rats displayed robust hyperphagia (5.5±1.1 g/2h) as compared to sham-lesioned rats (1.6± 0.3 g/2h) 12-26 days post-surgery. These rats were passively immunized against NPY by intraventricular injections (3 μl) of purified IgG containing a specific NPY-antibody (NPY-Ab) at 0900, 1100 and 1300 h, and food consumption was evaluated between 1300-1500 h. The results showed that food intake was suppressed by greater than 93% in the NPY-Ab injected, VMH-lesioned rats. Similar central injections of purified IgG from normal rabbit serum (control) had no effect on food intake in these VMH-lesioned or sham-lesioned rats. These results implicate for the first time a locally-produced neural signal in the VMH-lesioned syndrome and support the hypothesis that the potent orexigenic effects of hypothalamic NPY may participate in induction of hyperphagia and the attendant body weight gain in VMH-lesioned rats. (Supported NIH, DK 37273).

528.8

THE BENZODIAZEPINE AGONIST MIDAZOLAM MICROINJECTED INTO THE RAT PARABRACHIAL NUCLEUS PRODUCES HYPERPHAGIA. S.J. Cooper* and S. Higgs. Sch. of Psychology, Univ. Birmingham, Birmingham B15 2TT, U.K.

Benzodiazepine (BZ) receptor agonists cause a substantial hyperphagia in rats and numerous other mammalian species, and produce striking enhancements of taste preferences and positive taste reactivity measures. We have located a population of BZ receptors in the rat parabrachial nucleus (PBN) (unpublished data), and have therefore investigated the possible role of the PBN in relation to BZ-induced hyperphagia. Nondeprived male rats were trained to consume either a sweetened palatable mash or a 3% sucrose solution. The water-soluble BZ, midazolam, was microinjected bilaterally into the PBN (3, 10 or 30 μg/μl in 0.5 μl) and compared with a saline-vehicle condition. Microinjection of midazolam (30 μg/μl) into the PBN significantly increased the intake of either mash (N=6) or sucrose solution (N=5) ($p<0.01$). The hyperphagic response was blocked by the selective BZ receptor antagonist, flumazenil. Intra-PBN injections of midazolam (3, 10 or 30 μg/μl in 0.5 μl) did not affect locomotor activity as determined by an activity monitor. These data are consistent with the view that BZ receptors in the PBN may constitute the receptors at which BZs induce their considerable hyperphagic effect.

528.9

TAMOXIFEN PREVENTS NEUROLEPTIC-INDUCED WEIGHT GAIN AND HYPERPHAGIA IN FEMALE RATS. T. Baptista*, E. de Baptista, M. Altemus, and S.R.B. Weiss Biological Psychiatry Branch and Clinical Neuroendocrinology Branch, NIMH, Bethesda, Md. 20892

Elsewhere, we have suggested that the weight gain and hyperphagia induced by chronic administration of neuroleptics in female rats is related to a neuroleptic-induced hyperprolactinemia, which causes a decrease in gonadal estrogen synthesis, ultimately resulting in decreased activity of hypothalamic neurons involved in satiety (Parada et al., 1989). This mechanism has been investigated using the D_2 selective dopamine antagonist sulpiride, and the weight gain was prevented by concomitant administration of either estrogen or bromocriptine. Recent evidence suggests that the antiestrogen tamoxifen may act as an estrogen agonist on food intake regulation because, in the short term, it prevents weight gain and hyperphagia in ovariectomized rats. In addition, tamoxifen and neuroleptics compete for the same receptor in hypothalamic neurons (Gray et al., 1993), which further suggests that tamoxifen might prevent neuroleptic-induced weight gain. To assess this hypothesis, female rats were divided into groups of 10 animals each, and were treated for 21 days with either vehicle, sulpiride (20 mg/kg, i.p.), or sulpiride plus one of the following doses of tamoxifen citrate: 2.5, 5, 10, 20, 50, or 100 mg/kg, i.p. Sulpiride-induced weight gain and hyperphagia were completely prevented by tamoxifen at doses of 5 or more mg/kg. A trend was observed between the dose of tamoxifen and inhibition of weight gain under sulpiride: $r = 0.68$, $F(1,6) = 4.4$, $p < 0.08$. Tamoxifen may be useful to prevent weight gain in obesity-prone women under neuroleptic treatment.

528.10

CARBOHYDRATE OXIDATION PREDICTS FOOD DEPRIVATION-INDUCED FOOD INTAKE. C.R. Park*, R. Seeley, and S.C. Woods, Depts. of Behavioral Neuroscience and Psychology, University of Washington, Seattle, WA 98195

Hypherphagia following fasting is a robust phenomenon. However, the actual signal used by a fasted animal to detect the necessity to ingest calories is still not known. Theories have focused upon glucose or lipid oxidation and cellular energy generation. We conducted the following study to investigate the relationship between several whole-body metabolic parameters and hyperphagia following food deprivation.

Naive, Long-Evans rats (14) were food deprived for periods ranging from 3 to 16 hours. Two hours before refeeding, they were placed in an indirect calorimeter for 2 hours. The rats were then returned to their home cages, food was restored, and food intake was recorded for 3 hours. Multiple regression analysis of several parameters, including respiratory quotient, carbohydrate and fat oxidation rates, energy expenditure, body weight, VO2, and VCO2 was performed. Of all the variables considered, only carbohydrate oxidation rate reliably predicted the amount of food consumed when access to food was restored. Importantly, using linear regression line fitting, a function was derived which predicted 0 grams of intake at a carbohydrate oxidation rate identical to that observed in the post-prandial state. These data indicate that fasting carbohydrate utilization rate may be an important determinant of refeeding hyperphagia.

528.11

CAFETERIA-DIET OBESITY DAMPENS THE MESOACCUMBENS DOPAMINE SYSTEM AND ALTERS ITS RESPONSE TO AMPHETAMINE AND FOOD INTAKE. E. Pothos*, A. C. Krain and B. G. Hoebel, Dept. of Psych., Princeton Univ., Princeton, NJ 08544.

Feeding can increase extracellular dopamine (DA) levels in the nucleus accumbens (NAc) of freely moving rats (Hernandez & Hoebel, *Physiol. & Beh. 44*, 599), but little is known about the effects of obesity on basal DA release in the NAc. In the present study, female albino rats (n=16) developed cafeteria-diet obesity and body weight averaging 23% above controls (n=16). *In vivo* microdialysis showed that basal DA levels in the NAc of the obese rats were 70% lower than in controls ($p<.01$), but *d*-amphetamine (1.5 mg/kg i.p.) increased NAc DA to 759% of baseline in the obese group and only to 285% in the control group ($p<.05$). When both control and obese rats were food deprived for 24-36 hrs, there was no longer any measurable depression of basal NAc DA in the obese group. After a Purina pellet meal, NAc DA increased significantly in the food deprived controls ($p<.01$), but not in the food deprived obese; however, a cafeteria-diet meal did increase DA ($p<.01$). The results suggest that cafeteria-diet obesity (a) dampens basal activity of the mesoaccumbens DA system, but mild food deprivation and highly palatable food restore it, and (b) increases the amount of DA available for release by amphetamine.

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528.12

ANTISENSE OLIGODEOXYNUCLEOTIDES TO G-PROTEIN SUBUNIT SUBCLASSES: CHRONIC INTRACEREBROVENTRICULAR (ICV) INFUSION IDENTIFIES A TRANSDUCTIONAL REQUIREMENT FOR THE MODULATION OF FEEDING. C.R. Plata-Salamán[1,*], C.D. Wilson[1] and J.M.H. ffrench-Mullen[2]. [1]SLHS, Univ. Delaware, Newark, DE 19716 and [2]Dept. Pharmacology, Zeneca Pharmaceuticals Group, Wilmington, DE 19897.

Heterotrimeric guanine nucleotide-binding (G) proteins transduce signals from the membrane G-protein-coupled receptors to cellular effectors. We studied in rats the effects of the chronic ICV (into the third ventricle) infusion for 72 h (through osmotic minipumps) with antisense phosphothio-oligodeoxynucleotides (15 µg/24 h) corresponding to G-protein subunit subclasses (α, β, and γ). The results for nighttime food intake (mean±SE, g) at the 48-60 h period after initiation of the vehicle, antisense or sense infusion were: vehicle, 20.2±1, n=10; Gαs antisense, 20.5±1, n=12; Gαi common antisense (to Gαi1, i2 and i3), 20.0±1, n=8; GαO common antisense (to GαOA and GαOB), 15.8±1, $p<0.01$, n=11 (water intake increased 9% from control); GαOA antisense, 15.4±1, $p<0.01$, n=11; GαOB antisense, 19.2±1, n=10; Gαq antisense, 19.5±1, n=7; Gα11 antisense, 21.3±1, n=7; Gβ common antisense, 20.3±1, n=5; Gγ common antisense, 15.8±0.5, $p<0.02$, n=8. (Gγ1.1, 2.1, 3.1 and 4.1 antisense also decreased feeding, n=6-7/group). Infusion with 15 µg/24 h of GαO common sense or GαOA sense had no effect on nighttime food intake (19.4±1, n=8; and 20.7±1, n=9). Computerized analyses of behavioral patterns demonstrated that the GαOA antisense depresses nighttime feeding by reducing meal frequency (to 4.9±0.3 meals, n=11, $p=0.001$ from control: 7.5±0.6 meals, n=8) without decreasing meal size or meal duration. Immunoblots of hypothalamic ventromedial nucleus from normal rats (n=12) show that the levels of GαO protein subunit are >30-fold higher relative to those of Gαi1-i3 and Gαs subunits. The results suggest that the G-protein subunit subclasses GαOA and Gγ are required for the modulation of normal feeding behavior, and that changes in their activity may be associated with the chemical regulation of feeding.

528.13

CONDITIONED PREFERENCES AFFECT THE TEMPORAL PROPERTIES OF THE TASTE CODE. B. K. Giza[1], K. Ackroff[2], A. Sclafani[2,*], S. A. McCaughey[1] and T. R. Scott[1] ([1]Dept Psychol, Univ Delaware, Newark DE 19716; [2]Dept Psychol, Brooklyn Col, Brooklyn NY 11210)

Conditioned taste preferences (CTP's) may be created by pairing a novel taste (CS) with intragastric infusion of nutrients (US). We recorded taste-evoked activity in the NTS of rats with CTP's to either 0.1 M $MgCl_2$ (Mg^+) or to 0.01 M citric acid (Ci^+) to determine whether the afferent code was modified by this procedure. Forty-seven rats trained in Brooklyn were transported to Delaware for electrophysiological recording. Behavioral tests were conducted before recording to exclude rats that did not show >75% preference for the CS^+ over both the CS^- and water. The two neural data sets were composed of single unit responses from 15 Mg^+ (N=61) and 12 Ci^+ (N=68) rats. Three neural subgroups--oriented toward NaCl, HCl and glucose--were identified by cluster analysis in each data set. There was no significant change in mean evoked activity to either CS^+, either across all neurons or within any subgroup. The positions of $Mg^+$and Ci^+ in taste spaces generated from each data set were unaltered by conditioning. Therefore, the gustatory neural code across neurons was not affected by development of a CTP. Temporal aspects of the evoked activity were then examined. The phasic burst that characterizes responses to non-sweet stimuli was suppressed to citric acid in Ci^+ rats rendering the temporal profile less like that of HCl ($p<0.01$) and more like that of glucose ($p<0.01$). Thus, in the temporal taste space, citric acid abandoned the sour-bitter stimuli and joined the sugars. The time course of activity to $MgCl_2$, however, was unmodified in Mg^+ rats. A phasic burst of activity is generated as the temporal signature of a conditioned aversion (Chang & Scott, 1984). Here the phasic response is selectively reduced for a stimulus to which a preference has been conditioned. The effect was limited to citric acid, which occurs in the natural environment of rats and has been associated with health consequences. Supported by grant DK30964 from the NIDDKD.

529.1

CROSS-TOLERANCE TO D_2 BUT NOT D_1 DOPAMINE RECEPTOR AGONISTS IN RATS TOLERANT TO CAFFEINE-INDUCED ROTATIONAL BEHAVIOR. B.E. Garrett*and S.G. Holtzman. Dept. of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

Caffeine produces contralateral turning in rats with unilateral 6-OHDA (8 µg/4µl)-induced lesions of the nigrostriatal pathway. The present study a) determined if tolerance develops to this effect of caffeine and b) examined the role of dopamine receptors. Male Sprague-Dawley rats with 6-OHDA lesions received scheduled access to either a 0.1 % caffeine solution (average drug intake = 70 mg/kg/day) or to drug-free tap water (controls). Drugs were administered SC to groups of 8 rats and turning was recorded for 3 hr. Caffeine (10-100 mg/kg) and theophylline (3.0-100 mg/kg) produced biphasic increases in contralateral turning in control rats, with the peak increase occurring at 30 and 56 mg/kg, respectively. Rats receiving caffeine chronically were tolerant to this effect of both drugs. The selective D_2 agonists quinpirole (0.01-1.0 mg/kg) and R(-)-propylnorapomorphine (NPA, 0.003-0.1 mg/kg) and the selective D_1 agonists SKF-38393 (0.3-10 mg/kg) and SKF-77434 (0.3-10 mg/kg) also produced dose-dependent increases in contralateral turning in control rats. This effect of the D_2 agonists was reduced significantly in rats receiving caffeine chronically, whereas the effect of the D_1 agonists was unchanged. Therefore, tolerance develops to caffeine-induced rotational behavior and there is cross-tolerance to the methylxanthine derivative theophylline. The differential cross-tolerance among dopamine receptor-selective agonists suggests that tolerance to caffeine-induced rotational behavior is mediated by the D_2 but not the D_1 dopamine receptor. (Supported in part by DA03413, K05 DA00008 and P.R. Harris Pre-doctoral Fellowship).

529.2

Neurobiological bases of methamphetamine action in the hippocampus. E. S. Onaivi*[1], K. A. Parker[1], A. Charkrabarti[1], G. Chaudhuri[3], E. D. Motley[2], C. Bishop-Robinson[1] and S. S. Chirwa[2] Depts of [1]Pharmacology, [2]Physiology and [3]Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208.

The effect of psychoactive drugs such as psychomotor agitation, attentional disturbances and memory dysfunctions suggests the involvement of the hippocampus and temporal lobe. However, the exact mechanisms by which the above effects are mediated are not known. A multidisciplinary approach is being used to investigate the neurobiological bases of membrane bound (DA) receptor function following activation by methamphetamine in the hippocampus of a rodent model. Groups of rats (N=10 per group) were chronically treated with vehicle, 1.0 and 10.0 mg/kg methamphetamine (ip) for about 3 months. The locomotor activities, stereotype behavior and performance in the elevated plus-maze tests were evaluated weekly. A reduction in aversion to the open arms was produced by the 1 mg/kg dose. The animals treated with the 10 mg/kg, also displayed a reduced aversion which reverted to an intense aversiveness towards the open arms of the maze after six weeks of treatment. Locomotor activity and stereotype behavior were significantly increased following treatment with the low and high doses of methamphetamine, the magnitude being greater at the 10 mg/kg dose. The tolerance which developed was more pronounced with the locomotor activity and stereotype behavior than with the performance on the plus-maze test. The neurochemical, molecular and electrophysiological bases underlying these neurobehavioral changes induced by methamphetamine are currently being studied in the hippocampal slices. Supported by NSF-MRCE Grant# HRD-9255157 and RCMI Grant# NIH 5G12RR0303208

529.3

BEHAVIOURAL AND NEUROCHEMICAL STUDIES OF A PHENTERMINE AND FENFLURAMINE DRUG MIXTURE IN RATS. Shoaib M[1], Baumann MH[2], Rothman RB[2], Goldberg SR[1], Schindler CW[1]* [1]Preclinical Pharmacology Branch, [2]Clinical Pharmacology Branch, Addiction Research Center, National Institute on Drug Abuse, Baltimore MD 21224, USA.

Clinical case studies suggest that combined administration of phentermine (PHEN) and fenfluramine (FEN) may be useful in the treatment of alcohol and cocaine addictions (Hitzig, *Maryland Med J* **42**, 1993). The present experiments examined the nature of interaction between the two aminergic agonists using the drug discrimination paradigm to examine subjective effects and the *in vivo* microdialysis technique to assess the neurochemical effects in the nucleus accumbens. Three groups of Sprague-Dawley rats were trained to discriminate saline from (1) FEN (1.0 mg/kg i.p.), (2) PHEN (1.0 mg/kg i.p.) and the mixture (3) PHEN+FEN (1:1 ratio) under a fixed ratio (FR10) schedule of food reinforcement. The majority of rats trained on the mixture acquired the discrimination after 32 ± 4 sessions. In comparison the majority of rats trained to discriminate the individual compounds failed to meet criteria in 60 sessions. The individual compounds (1 mg/kg i.p.) generalized partially to the mixture, and complete generalisation was observed following 3.0 mg/kg of FEN or PHEN. In concious rats, local infusions of FEN (1µM) or PHEN (1µM) into the nucleus accumbens selectively increased serotonin and dopamine respectively. The mixture (1:1 ratio) increased both amines to a similar degree. Therefore, this dual stimulation of the amines by the mixture may be the basis for the cueing effects of the FEN+PHEN drug mixture. Furthermore, these aminergics appear to interact additively at both behavioural and neurochemical levels.

529.4

IS METHYLPHENIDATE LIKE COCAINE? STUDIES ON THEIR PHARMACOKINETICS AND DISTRIBUTION IN HUMAN BRAIN. N.D. Volkow*, Y.-S. Ding, J.S. Fowler, G.-J. Wang, J. Logan, J.S. Gatley, S. Dewey, C. Ashby, J. Liebermann, R. Hitzemann, A.P. Wolf. Medical Department and Chemistry Department, Brookhaven National Laboratory, Upton, New York 11973; Department of Psychiatry SUNY-Stony Brook, Stony Brook, New York 11794; Hillside Hospital, Glen Oaks, New York 11004.

Cocaine and methylphenidate (MP) have very similar pharmacological properties. Yet cocaine is considered one of the most reinforcing and addictive drugs while MP is widely used to treat attention deficit disorder in children. PET was used in conjunction with [11C]methylphenidate to measure the pharmacokinetics and regional brain distribution of methylphenidate in normal volunteers. The relationship between MP's pharmacokinetics and MP-induced "high" were also evaluated. The results were compared with those previously obtained for cocaine as assessed with [11C]cocaine. MP's absolute uptake in brain (7-10%) as well as its regional distribution, was identical to that of cocaine. MP pretreatment (0.5 mg/kg iv) inhibited binding only in striatum suggesting, that as for cocaine, specific binding was limited to striatum. The pharmacokinetics of MP and of cocaine in brain were significantly different. Clearance of MP from striatum (90 minutes) was significantly slower than that of cocaine (20 minutes). For both drugs their fast uptake in striatum paralleled the experience of the "high". For MP the "high" decreased very rapidly despite significant binding of the drug in brain. In contrast for cocaine, the decline in the high paralleled its fast rate of clearance from brain. Because the experience of the 'high' correlated only with the initial fast uptake of the drug in brain, the slow clearance of MP from brain may serve as a limiting factor in promoting its frequent self-administration and may explain the much lower abuse of MP than of cocaine.

529.5

CLORGYLINE POTENTIATES THE TOXICITY OF SUBSTITUTED AMPHETAMINES IN CULTURED FETAL RAPHE NEURONS. E.T. Kokotos Leonardi*, X.P. Hou and E.C. Azmitia, Dept. of Biology, New York University, NY 10003.

MDMA and FEN are substituted amphetamines that bind to the serotonin transporter, promote the release and inhibit the re-uptake of, serotonin in pre-synaptic terminals. The release of serotonin and inhibition of its reuptake and catabolism may work synergistically to raise extracellular levels of serotonin in the brain. A consequence of increased extracellular serotonin is PKC activation (see abstract by Kramer and Azmitia) and the stimulation of glycogenolysis (see abstract by Poblete and Azmitia) which may contribute to the neurotoxic mechanism of substituted amphetamines. We now report that MDMA and FEN toxicity at concentrations ranging from 1-10 µM is potentiated by the addition of clorgyline at concentrations ranging from 0.1 to 10 nM. For example, uptake of ^{3}H-5-HT in 10 µM FEN treated cells= 50% of control; 10µM FEN+ 0.1nM clorgyline=32% of control ($p<0.001$); ^{3}H-5-HT uptake in 5 µM treated MDMA cells=63% of control, 5 µM MDMA+0.1nM clorgyline=57% of control ($p<0.001$). Furthermore, the addition of serotonin at concentrations ranging from 10 nM-10 µM produced inhibition of serotonin uptake in a dose-dependent manner.

Serotonergic cells were obtained from E14 rat pups. A rostral rhombencephalic and caudal mesencephalic slice was dissected from each embryo. Following mincing and trituration, cells were plated on poly-D-lysine coated 96 well plates (1.2×10^5 cells/mL) and were incubated at 37°C. FuDR was added on day 2, and was removed followed by the addition of serum free media on day 3. Drugs were added on day 8 and uptake assay of ^{3}H-5-HT was performed on day 12. Samples were counted by liquid scintillation counting at a counting efficiency of 58%. These results suggest that serotonin may be a component in the neurotoxic mechanism of MDMA and FEN. This work was supported by NIDA contract #271-87-8144 to E.C.A.

529.6

THE ACTIVATION OF PKC BY MDMA IS DEPENDENT ON THE RELEASE OF 5-HT FROM VIABLE NERVE TERMINALS. H.K. Kramer* and E.C. Azmitia. Dept. of Biology, New York Univ., NY, NY 10003.

Substituted amphetamines, such as 3,4-methylenedioxymethamphetamine (MDMA), have been shown to bind with high-affinity to and inhibit the serotonin (5-HT) uptake transporter, promote the release of 5-HT, increase the uptake of extracellular Ca^{2+}, and inhibit the activity of monoamine oxidase-A. Additional studies have shown that many of MDMA's acute and neurotoxic effects arise from an interaction with the central 5-HT_2 receptor. This receptor is coupled to the breakdown of membrane-bound phospholipids and the activation of protein kinase C (PKC). These effects, occurring in concert, appear to underlie MDMA's neurotoxic properties. Our laboratory has previously reported that MDMA induces a prolonged translocation of soluble PKC to the plasma membrane when administered *in vivo*, and this persists up to 5 days after the cessation of treatment. We now report that this metabolic effect is dependent on the release of 5-HT and the stimulation of postsynaptic serotonin receptors.

Male Sprague-Dawley rats were exposed to MDMA (8 x 20 mg/kg, s.c.) and assayed for PKC translocation. A subset of animals were pretreated (21 days earlier) with the 5-HT neurotoxin, p-chloroamphetamine (PCA), to reduce cortical 5-HT content. 72 hrs. following the last MDMA injection, rat corticies from both groups were assessed for PKC translocation and 5-HT terminal density via high affinity ^{3}H-PDBu and ^{3}H-paroxetine binding, respectively.

MDMA induced a lasting translocation of PKC in animals exposed for four days (saline: 7.32 $\pm$ 0.25 pmol/mg prot.vs. MDMA: 10.78 $\pm$ 0.37 pmol/mg prot.; $p \leq 0.05$). Rats pretreated with PCA showed a significant (94.5%) decrease in the density of cortical 5-HT uptake sites, and in those groups, MDMA was unable to elicit an activation of PKC. We believe that the prior loss of cortical 5-HT nerve terminals prevents the cytosolic release of 5-HT by MDMA and the concomitant activation of PKC. These results show that the presynaptic release of 5-HT mediates this metabolic effect of MDMA, and that the activation of PKC and subsequent phosphorylation of its membrane-bound substrates may contribute to amphetamine-induced neuronal death. (NIDA # 271-90-7403 to ECA)

529.7

ACTIVATION OF GLYCOGEN PHOSPHORYLASE BY MDMA(+) IN ASTROGLIAL-RICH PRIMARY CULTURES. J.C. Poblete* and E.C. Azmitia. Dept. of Biology, New York Univ., NY, NY 10003.

Glycogen is the single largest energy reserve in the brain. Neurotransmitters, neuropeptides, and ions regulate glycogen levels in the brain by modulating the activity of glycogen synthase (GSase) and glycogen phosphorylase (GPase). GPase has recently been shown to be co-localized with glial fibrillary acidic protein (GFAP), an astroglia-specific marker, suggesting that glycogen is localized in astroglial cells. Additionally, serotonin (5-HT) has been shown to stimulate glycogenolysis and functional receptors are found in neurons and in glia. It has been reported that 3,4-methylenedioxymethamphetamine (MDMA), a drug of abuse, stimulates the release of, inhibits the reuptake of 5-HT, and selectively inhibits the activity of MAO-A.. These biochemical consequences of MDMA lead to a prolonged 5-HT activity. This study investigates the effects of MDMA and 5-HT on glycogen metabolism in the CNS. A histochemical method was designed to visualize active GPase in an astroglial-rich primary culture. The number of GPase reactive sites were measured using computer-aided densitometric software (OPTIMAS; Bioscan, Inc).

5-HT activates GPase in a concentration-dependent manner. Maximal activation by 5-HT was achieved at 10 μM and resulted in a126% increase in the number of reactive sites. Interestingly, MDMA(+) directly stimulated GPase activity with maximal activation induced by 5 μM and which caused a 70% increase in the number of reactive sites . The effects of 5-HT and MDMA(+) were attenuated by 10 μM mianserin, a 5-HT2 (S2) receptor antagonist. Our results indicate that these effects may be mediated by S2 receptor, and that MDMA leads to increased synaptic 5-HT activity (see abstracts by Kramer and Azmitia, and Kokotos-Leonardi and Azmitia), and consequently, GPase activity. Extended GPase activity can lead to depletion of synaptic energy stores. Thus, compromising the energy state of the synapse will lead to synaptic dyshomeostasis and may contribute to terminal degeneration induced by substituted amphetamines. An astrocyte-neuron metabolic link may be vital for synaptic homeostasis.(NIDA # 271-90-7403)

529.8

MDMA-INDUCED NEUROTOXICITY IN RAPHE PRIMARY CULTURE INVOLVES L-ARGININE-NO AND OXIDATIVE PATHWAYS. C. Cerruti*, P. Sheng, B. Landenheim, and J-L Cadet. Molecular Neuropsychiatry *Section, NIDA/ARC, Box 5180, Baltimore, MD. 21224*

Methylenedioxymethamphetamine (MDMA) is a neurotoxic drug of abuse. Its use is also associated with long-term depletion of serotonin that correlates with morphological damage to serotonergic terminals. The mechanisms by which these drugs destroy 5-HT terminals have yet to be elucidated. It has been pointed out that free radical-mediated events may form a final common pathway in neurotoxic processes. In order to test the role of the free radical, nitric oxide (NO), we have used an *in vitro* model of serotonergic cells cultured from raphe nuclei of rat fetuses. In these cells, MDMA causes dose-dependent cytotoxicity. Cell death was attenuated by the NO synthetase inhibitors, nitro-L-arginine and L-nitro-methylarginine. Moreover, the toxic effects of MDMA were attenuated by benzamide, an inhibitor of ADP-ribosylation. Primary cultures of serotonergic cells obtained from SOD-transgenic mice were also protected against MDMA-induced toxicity. These results suggest that MDMA induces neurotoxicity via NO formation and by the production of superoxide radicals.

529.9

ATTENUATION OF METHYLENEDIOXYAMPHETAMINE (MDA)-INDUCED NEUROTOXICITY IN TRANSGENIC MICE WITH INCREASED CU/ZN-SUPEROXIDE DISMUTASE ACTIVITY (SOD): EVIDENCE FOR INVOLVEMENT OF FREE RADICALS. J.L. Cadet*, B. Ladenheim, R.B. Rothman, E. Carlson, C. J. Epstein. NIH/NIDA, ARC, Baltimore, MD 21224; and Department of Pediatrics, UCSF, San Francisco, CA 94143.

Administration of methylenedioxyamphetamine (MDA) to rodents causes loss of monoaminergic terminals. The mechanism by which MDA causes its neurotoxicity is not known. MDA might kill these terminals by producing oxyradicals due to the increased release of dopamine in the synaptic space (Nash and Nichols, 1991). In order to further evaluate the role of oxyradicals in MDA-induced neurotoxicity, we have tested its effects in transgenic mice (SOD-Tg) which overexpress the human SOD gene. In nontransgenic mice, MDA administration causes a significant loss of DA terminals in the caudate-putamen measured by receptor autoradiography of [^{125}I]RTI-55-labeled dopamine uptake sites. In contrast, there were no significant changes in heterozygous SOD-Tg mice. Homozygous SOD-Tg mice show small decrements in striatal DA terminals which were of much smaller magnitude than those observed in the Non-Tg mice. These results suggest that MDA-induced toxicity in rodents may be secondary to the increased production of superoxide radicals after administration of MDA.

529.10

DIRECT EFFECTS OF METHAMPHETAMINE ON GLUTAMATERGIC NEURONS. S.S. Chirwa* and M. Clayton. Physiology, Meharry Medical College, 1005 D.B. Todd Blvd, Nashville, TN 37208.

Experiments to determine the direct effects of methamphetamine (METH) on glutamatergic neurons were conducted in hippocampal slices (400 μm thick) obtained from male guinea pigs (100-150 g). Briefly, slices were maintained in a chamber that was superfused with oxygenated and warmed ACSF (30° C). METH (0.1-1.0 μM) was superfused for 5-10 min, as appropriate. The bioelectrical parameters of CA1b pyramidal cells were determined, and synaptic efficacy was evaluated via the analyses of dendritic EPSPs associated with single and paired-pulses. Briefly, CA1b neurons exposed to METH showed significant dose-dependent decreases in rheobase (50-83% of controls; n=18 cells; $p<0.05$, ANOVA/Duncans tests in this and subsequent entries) and in afterhyperpolarization (32-97% of control: n=18 cells). Resting membrane potential was unaltered in all cells and, therefore, could not account for the above changes. Similarly, there were insignificant changes to action potential and input resistance. METH caused reversible augmentation of single EPSPs (140-470% of controls; n=6 slices) and attenuation of paired pulse facilitation (65-80% of controls; n=6 slices). A decrease in rheobase could reflect drug-induced changes to specific ionic conductance. An augmented EPSP to single stimuli, taken together with attenuation of paired-pulse facilitation, suggests increases in evoked glutamate release. Supported by NIH grant G12-RR03032.

529.11

A SINGLE INJECTION OF AMPHETAMINE OR METHAMPHETAMINE INDUCES DYNAMIC ALTERATIONS IN c-*fos*, *zif/268* AND PREPRODYNORPHIN mRNA EXPRESSION IN RAT FOREBRAIN. J.Q. Wang*, A.J.W. Smith, D.C. Mayer and J. F. McGinty. Dept. of Anatomy and Cell Biology, East Carolina Univ. Sch. of Med., Greenville, NC 27858-4354, USA

In this study, the effects of a single dose of the indirect dopamine agonists amphetamine (AMPH) and methamphetamine (METH) on mRNA expression of c-*fos*, a member of the leucine zipper family, and *zif/268* (NGFI-A, egr1 and Krox-24), a member of the zinc finger family, and the opioid peptide, preprodynorphin (PPD), in various regions of rat forebrain were investigated with quantitative *in situ* hybridization histochemistry 1, 2, 3, 6 or 30 h after injection. A different and more intensive behavioral syndrome was induced following METH (15 mg/kg, i.p.) as compared with that observed after AMPH (5 mg/kg, i.p.). METH induced a far greater increase in c-*fos* and *zif/268* mRNA in sensorimotor cortex, dorsal striatum (caudoputamen) and ventral striatum (nucleus accumbens) than did AMPH. The increase in c-*fos* mRNA expression peaked at 1 h and returned to basal levels in all regions by 3 h. In contrast, the increase in *zif/268* mRNA expression in the cortical regions was equally strong at 1 and 2 h, gradually returning to basal levels by 6 h after either drug. However, in the striatal regions, *zif/268* mRNA levels peaked at 1 h and declined gradually to basal levels by 6 h. Interestingly, METH caused an actual suppression of *zif/268* gene expression (>50%) in both caudate and nucleus accumbens at 3 h. PPD mRNA expression was increased in a patchy motif in the caudoputamen and nucleus accumbens beginning 2 h and returning to basal levels by 30 h after injection of either drug. This study, together with our recently published observation that PPD mRNA is induced in the caudate 3, 6 and 18 h after AMPH or METH injection (Smith and McGinty, Mol. Brain Res., 21:359-362, 1994), provides a detailed description of the differential modulation of c-*fos*, *zif/268*, and PPD mRNA expression in the cerebral cortex and striatum by amphetamines over time (Supported by DA03982).

530.1

H.M.'s MRI SCAN SHOWS SPARING OF THE POSTERIOR HALF OF THE HIPPOCAMPUS AND PARAHIPPOCAMPAL GYRUS S. Corkin*, D. G. Amaral, K. A. Johnson, and B.T. Hyman. Dept. of Brain and Cognitive Sciences and the Clinical Research Center, MIT, Cambridge, MA, 02139; Center for Behavioral Neuroscience, SUNY, Stony Brook, NY, 11794; Dept. of Radiology, Brigham and Women's Hospital, Boston, MA, 02115; Dept. of Neurology, Mass. General Hospital, Boston, MA, 02114.

Decades of research with H.M. and other amnesic subjects has established that the hippocampus and surrounding medial temporal-lobe structures are necessary for long-term explicit memory. Yet we continue to explore the importance of particular medial temporal areas for memory function, and examine the correspondence between the critical lesion in human amnesia and in the nonhuman primate model. An important step is to document as precisely as possible the extent of the lesions in human amnesic subjects. We therefore performed high-resolution MRI studies in H.M. (at age 66), using a 1.5 Tesla GE Signa System. Axial and coronal T1-weighted (TR=550 msec; TE=16 msec) and T2-weighted (TR=2500 msec; TE=90 msec) images were acquired with a 5-mm slice thickness and a 1-mm interval between slices (matrix=256 x 192 pixels; NEX=1; FOV=22 cm). The bilateral lesion includes the medial temporal pole, amygdala, and entorhinal cortex. Approximately the anterior 2 cm of the dentate gyrus, hippocampus, and subicular complex are removed, but the posterior 2 to 2.5 cm of these fields are intact but shrunken. The rostral perirhinal cortex is also removed, but because the collateral sulcus and its medial cortex are visible, at least some of the posterior perirhinal and parahippocampal cortex may be intact. Neocortical atrophy is slight and consistent with his age. The cerebellum is noticeably atrophic (likely due to antiepileptic drug therapy). Future imaging studies with MRI and PET, respectively, will provide volume measurements of specific brain areas, and will assess the functional status of the remaining hippocampal tissue.

530.2

H.M. SHOWS IMPAIRED WORD-STEM COMPLETION PRIMING WITH NOVEL WORDS B. R. Postle* and S. Corkin. Dept. of Brain and Cognitive Sciences, and the Clinical Research Center, MIT, Cambridge, MA, 02139.

Evidence of intact perceptual priming with novel stimuli (patterns, pseudowords) in amnesic subjects suggests that they may be able to learn novel information when tested with other repetition priming paradigms. To test this hypothesis, we examined word-stem completion priming with novel words in the amnesic patient H.M. (age = 67 yrs; ed. = 12 yrs), who shows normal word-stem completion priming with familiar words. He underwent bilateral medial temporal resection in 1953 to alleviate intractable epilepsy. His performance was compared to that of 10 age- and education-matched control subjects (NCS). Subjects were tested on word-stem completion priming, cued recall, and a vocabulary test (four-alternative forced-choice). The stimuli were common English words that entered the dictionary after 1965, and thus came into general usage after the onset of H.M.'s amnesia, i.e., words for which he presumably lacked a lexical representation. We computed the mean percentage of word stems completed to studied versus unstudied words. As expected, H.M.'s cued recall score (10.5%) was inferior to the NCS's score (35.3%) ($p<.001$). On priming, H.M.'s score (1.3%) was also inferior to the NCS's score (16.1%) ($p=.0001$). His failure to prime with post-1965 words suggests that word-stem completion priming relies on pre-existing lexical representations of the stimulus material. Further support for this view comes from the finding that NCS showed greater priming with words pronounced correctly at study than with mispronounced words ($p=.0001$). Correct pronunciation may be an index of lexical representation because the NCS had higher vocabulary scores for words pronounced correctly at study (89.3%) than for mispronounced words (73.4%) ($p<.03$). H.M.'s mean vocabulary score (43.9%) was inferior to the NCS's (84.8%) ($p=.0001$). We propose that activation of pre-existing lexical representations at study and test is the mechanism for word-stem completion priming. Word-stem completion priming, unlike perceptually dependent forms of priming with words, requires subjects to engage in lexical retrieval.

530.3

COGNITIVE PROCESSES UNDERLYING RECOGNITION MEMORY: EVIDENCE FOR A SPECIFIC IMPAIRMENT OF RECOLLECTION IN AMNESIA. A.D. Wagner*, M. Verfaellie, P. Croce, and J.D.E. Gabrieli. Dept. of Psychology, Stanford University, Stanford, CA 94305 and Memory Disorders Research Center, Boston University School of Medicine and DVAMC, Boston, MA 02130.

Studies of recognition performance in normal subjects suggest that two separable processes mediate recognition memory: controlled recollection and automatic fluency. It has been proposed that recognition failure in amnesia (AMN) reflects a disproportionate impairment of recollection and that fluency may be spared. We examined the status of recollection and fluency in AMN by equating the recognition memory performance of 8 AMN patients and 11 control (CON) subjects. CON subjects were presented single study and yes/no recognition test trials for separate lists of high-frequency (HF) (mean of 135/mil) and low-frequency (LF) (mean of 2.5/mil) words. For each list, AMN patients had the same initial study and test trial followed by seven additional study-test sequences with these same words and new distractors in each test. CON subjects had better recognition than AMN patients in the first trial and also showed superior recognition accuracy for LF versus HF words. The additional study-test trials equated AMN patients' recognition accuracy with that of CON subjects for HF words, but failed to equate the same patients' recognition of LF words. A separate study with 96 college students indicated that recollection may play a greater role in recognition of LF than HF words. Taken together, these results suggest that the improved recognition performance exhibited by AMN patients following extra study-test trials reflects fluency processes and not recollection processes. These findings indicate that the processing basis of recognition performance in AMN patients following extra study is not the same as that of CON subjects, even when recognition accuracy is numerically equated, and that recollection, relative to fluency, is disproportionately impaired in AMN. Supported by grants from the NSF and NIH (NINDS 1P50NS26985 and NIA RO1AG11121).

530.4

THE CORE MEMORY DEFICIT IN AMNESIA IS NEITHER ONE OF CONCEPTUAL PROCESSING NOR ONE OF EXPLICIT RETRIEVAL. C. J. Vaidya, J. B. Demb, M. M. Keane*, L. A. Monti, N. J. Cohen, R. A. Poldrack, and J. D. E. Gabrieli. Dept. of Psychology, Stanford Univ, Stanford, CA 94305; Memory Disorders Research Center, Boston VA, Boston, MA 02130; Dept. of Neurological Sciences, Rush Med Center, Chicago, IL 60612; Beckman Institute, Univ of Illinois, Urbana-Champaign, IL, 61801.

Amnesic patients' memory is often intact on implicit tasks, despite severe deficits on explicit tasks. Because most implicit tasks depend on perceptual processing, and most explicit tasks on conceptual processing, it has been proposed that the core memory deficit in amnesia is one of conceptual processing. By this view, amnesic patients should be impaired on both implicit and explicit tasks that require conceptual processing, and intact on both implicit and explicit tasks that require perceptual processing. To test this view, 23 amnesic and 23 control subjects performed four tasks: implicit-perceptual (word fragment completion), implicit-conceptual (word association), explicit-perceptual (word fragment cued-recall), and explicit-conceptual (word associate cued-recall). A study-phase modality (auditory/visual) manipulation validated the nature of processes (perceptual and conceptual) engaged by each task. Amnesic patients were impaired on both explicit tasks (perceptual and conceptual) and intact on both implicit tasks. Thus, the core memory deficit in amnesia is not one of conceptual processing. Alternatively, amnesia may reflect impaired declarative processes, rather than impaired explicit retrieval per se, because explicit tasks require processing of declarative or relational information. By this view, amnesic patients should be impaired on any task that requires declarative processing, regardless of the retrieval instructions. To test this view, 10 amnesic and 11 control subjects performed the general knowledge task, under implicit and explicit retrieval instructions. Amnesic patients were impaired under both implicit and explicit instructions. Results are consistent with multiple memory systems views positing that amnesia reflects a selective impairment in a limbic-diencephalic memory system that is engaged during declarative processing.

530.5

DISSOCIATION BETWEEN FORMS OF ASSOCIATIVE IMPLICIT MEMORY THAT ARE INTACT OR IMPAIRED IN GLOBAL AMNESIA. E. D. Levy*, J. D. E. Gabrieli, S. L. Reminger, and W. S. Francis. Depts. of Psychology, Stanford University, Stanford, CA 94305 and Northwestern University, Evanston, IL 60208.

Patients with amnesia have a deficit in learning new associations between words when memory is measured by explicit tests. Amnesic patients have a more variable pattern of results when learning of such new associations is measured by implicit tests. They are impaired when the implicit test is one of stem completion (SC), but they have shown normal learning when the test is one of reading speed (RS) or perceptual identification (PI). In the present studies, we examined whether normal subjects show a similar dissociation between different measures of implicit memory for new associations. In the first study, subjects either read aloud word pairs or generated sentences. In the test phase, subjects performed either a SC or a PI test with word pairs identical to those studied (same context), recombinations of studied words (different context), and new items (baseline). The measure of associative learning was the difference in priming between same-context and different-context pairs. For SC priming, associative learning occurred only if subjects performed the more elaborative sentence-generation task. In contrast, subjects showed associative PI priming after both reading and sentence-generation study tasks. Additional studies examined associative priming as measured by RS. In one experiment, subjects read aloud word pairs with or without imagery instructions in the study phase. While explicit memory was superior in the imagery condition, associative priming was similar in both conditions. Further experiments indicated that associative priming, as measured by RS, includes both a perceptual component (it was diminished when words were studied auditorally and tested visually) and a productive component (it was eliminated if subjects read the word pairs aloud in reverse order). Results indicate that different implicit tests of the learning of new associations provide measures of dissociable memory mechanisms. The PI and RS tests may reveal perceptual and productive associative mechanisms that do not require study-phase elaboration and that do not depend upon declarative memory processes. SC tests may reveal lexical or semantic associative mechanisms that require elaborative study and that depend upon declarative memory processes. Supported by grants from the NSF and NIH.

530.6

MAPPING IMPLICIT MEMORY WITH ELECTRICAL BRAIN STIMULATION: DISSOCIATIONS BETWEEN AUDITORY AND VISUAL STUDY MODALITIES. T.A. Blaxton*, B. Malow, C. Figlozzi, S. Sato, C. Kufta, S. Bookheimer and W. Theodore. Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Functional mapping of implicit memory was examined via electrical stimulation of subdural electrodes implanted in the speech dominant hemispheres of eight temporal lobe epilepsy patients. All patients were undergoing speech mapping prior to temporal lobe resection for treatment of intractable seizures. Memory materials were presented as subjects performed language tasks such as confrontation naming of single pictures and identification of objects from an array of four pictures. Auditory presentation of memory materials occurred as patients performed an auditory responsive naming task (e.g., answering questions such as "Tell me what you carve at Halloween"). These and other tasks were interleaved together and electrical stimulation (2-15mA) was delivered periodically to selected brain sites as patients performed the tasks. Implicit memory was assessed as subjects performed a category member production task without stimulation. In this task patients were asked to name three items from a given category and priming was observed when patients were more likely to provide target items recently presented during the language tasks as category exemplars than nonstudied targets. Priming on category member generation was unaffected by stimulation of parietal cortex, which served as a control region. Priming for both visually and auditorily presented items was disrupted from electrical stimulation of lateral temporal cortex. Priming from visually presented targets was eliminated altogether when brain sites in the inferior and basal temporal cortex were stimulated during study, although stimulation in this region did not impair auditory priming nearly as severely. In contrast, priming from auditorily presented items was disrupted by stimulation of frontal cortex more than priming from visually presented items.

530.7

PET ACTIVATION MEASURES REVEAL A DISSOCIATION BETWEEN BRAIN REGIONS UNDERLYING PERCEPTUAL AND CONCEPTUAL PROCESSES IN PICTURE-NAMING PRIMING. S. M. Park*, T. A. Blaxton, J. D. E. Gabrieli, C. M. Figlozzi, and W. H. Theodore. Department of Psychology, Stanford University, Stanford, CA 94305; Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Picture-naming (PN) priming has been shown to be mediated by perceptual and conceptual processes. Priming produced by repeated picture naming (P-P) involves perceptual and conceptual processes; priming produced by prior reading of picture labels (words) (W-P) involves conceptual processes. The neuroanatomical bases for the perceptual and conceptual processes underlying PN priming were examined using positron emission tomography (PET). While performing a PN task, regional cerebral blood flow was measured in 12 normal subjects tested in a Scanditronix II scanner using six injections of ^{15}O. The design employed 3 study-test conditions that were administered twice; scans were taken during each test phase. In the baseline condition, subjects named pictures and then different, nonstudied pictures. In the P-P condition, subjects named pictures and then renamed those same pictures. In the W-P condition, subjects read words and then named pictures corresponding to those word labels. PET images were analyzed using Statistical Parametric Mapping (all tests were set at $p < .01$). Deactivations for P-P priming relative to baseline were found in right lingual gyrus, right inferior occipital gyrus, and bilateral fusiform gyri, and for W-P priming in the left transverse temporal gyrus. A comparison between P-P and W-P naming revealed more activation in right precuneus, right superior temporal gyrus, right middle frontal gyrus, and cingulate for P-P priming, and more activation in left hippocampus, left temporal gyrus, left inferior frontal gyrus, bilateral fusiform, and left inferior occipital gyrus for W-P naming. Across comparisons, perceptual processes tended to involve bilateral posterior regions of the brain, whereas conceptual processes engaged more left temporal and anterior regions. These results suggest that separable neural substrates mediate perceptual and conceptual priming processes.

530.8

A FUNCTIONAL MRI STUDY OF LONG-TERM EXPLICIT MEMORY IN HUMANS. CE Stern*, S Corkin, AR Guimaraes, R Sugiura, CA Carr, JR Baker, PJ Jennings, RG González, BR Rosen. Harvard Medical School and MGH-NMR Center, Charlestown, MA 02129, Dept. of Brain and Cognitive Sciences and the Clinical Research Center, M.I.T., Cambridge, MA 02139.

It has been postulated that during learning an interaction is established between the medial temporal memory system and distributed storage sites in the neocortex. Using functional MRI (fMRI), we studied young normal subjects while they performed picture encoding (n = 7) and picture recognition (n = 5) tasks. High speed echo-planar imaging (EPI) techniques were combined with asymmetric spin-echo imaging sequences to simultaneously evaluate fMRI signal changes thoughout the whole brain. To assess picture encoding, subjects were asked to look at a consecutive series of complex, colored pictures so that they could recognize them later. As a control condition, subjects were scanned while viewing a single repeating picture. Subtraction images (encoding minus control) showed significant signal intensity increases bilaterally in hippocampal and parahippocampal regions and at the temporal-occipital junction. Recognition memory was assessed by subtracting images obtained during a two-alternative, forced-choice recognition test (identify previously viewed pictures) from images obtained during a two-alternative, forced-choice identification test (identify indoor vs. outdoor pictures). The subtraction images revealed significant fMRI changes bilaterally in prefrontal cortex (areas 46 and 10). This fMRI experiment provides new evidence about the participation of the medial temporal system during information encoding and also about the locus of cortical areas that support picture recognition memory.

530.9

HIPPOCAMPAL ACTIVATION IN fMRI EVOKED BY DEMAND FOR DECLARATIVE MEMORY-BASED BINDING OF MULTIPLE STREAMS OF INFORMATION. N. J. Cohen*, C. Ramzy, X. Hu, H. Tomaso, J. Strupp, P. Erhard, P. Anderson, & K. Ugurbil. Beckman Institute & Department of Psychology, University of Illinois, Urbana-Champaign, IL, 61801; Center for Magnetic Resonance Research, University of Minnesota Medical School, Minneapolis, MN, 55455.

Despite abundant evidence linking the hippocampal system to memory, it has been difficult to find reliable hippocampal activation in functional brain imaging studies of memory performance. Here we report robust hippocampal activation in a functional MR imaging (fMRI) study that was based on Cohen and Eichenbaum's (1993) proposal linking hippocampal system function to declarative memory for the relations among perceptually distinct objects. Seven normal volunteers participated in two tasks involving the processing of color images of faces. In different conditions, the faces were presented alone, or with the superimposition of a name, or of a name and an occupation-related icon. In the baseline task, S's made gender decisions about each face, ignoring any names or icons that were presented with them. In the memory task, S's had a series of alternating study and test conditions requiring the binding together of the 3 different streams of information. The declarative-memory dependence of this task was verified in behavioral testing of one well-characterized amnesic patient who performed at chance levels (vs 78% correct for the normal S's). Using a TURBO FLASH gradient-echo pulse sequence, 18 sagittal slices of the brain were collected at the rate of 1 per sec throughout the entire 30-minute (baselines and memory task) session. In each of the 7 S's, activation (increased signal intensity on 99%-confidence T-maps) was observed in left hippocampal and parahippocampal regions in a comparison of baseline task versus memory task. Activation was initiated at the outset of the study phase of the first memory test condition and stayed elevated above baseline levels throughout the 8 memory (study + test) task conditions.

530.10

A FUNCTIONAL MRI (fMRI) STUDY OF SEMANTIC ENCODING AND MEMORY IN THE LEFT INFERIOR FRONTAL GYRUS. J.B. Demb*, J.E. Desmond, A.D. Wagner, M. Stone, A.T. Lee, G.H. Glover, and J.D.E. Gabrieli. Depts. of Psychology and Radiology, Stanford University, Stanford, CA 94305.

Brain imaging (PET) and clinical studies have indicated a role for the left inferior frontal gyrus (LIFG) in semantic processing. We investigated this system using fMRI and a levels-of-processing paradigm. A standard 1.5T GE Signa Magnet was used with a T2* sensitive gradient echo spiral sequence (TR=75 ms, TE=40 ms, flip angle=23°). Two 7-mm functional slices were acquired separately in the Talairach & Tournoux (1988) coronal plane (y= +32, +39; 1.8mm pixel size). Images were acquired every 1.5 seconds for 5.5 minutes. Subjects viewed words continuously and switched between performing semantic judgements (e.g., respond to abstract but not concrete words) and nonsemantic judgements (e.g., respond to words printed in upper but not lower case) every 20 words for four cycles. Data were analyzed by correlating activation over time to a sinusoidal reference vector. Areas in the LIFG, but not in the analogous region in the right hemisphere, showed activation that correlated significantly with the reference vector (r=.30). There was an increase in activation during the semantic task. In a second experiment at the same slices, subjects performed the semantic task on blocks of 20 words shown for a first time (new) and then repeated for a second time (old) in successive blocks. There was less activation in the LIFG for old than for new words. The deactivation may reflect implicit conceptual memory (repetition priming) for old words. Thus, it appears that the same LIFG region that was important for semantic encoding was also the locus for item-specific semantic memory for words. These results indicate that fMRI may be useful in detecting the brain locus of semantic encoding and memory. Supported by grants from the NIH (F32NS09628), the Alzheimer's Association, Stanford Office of Technology and Licensing, and the Lucas Foundation.

530.11

VERBAL MEMORY IMPAIRMENT AFTER RIGHT TEMPORAL-LOBE EXCISION: THE ROLE OF ABNORMALITY ON THE UNOPERATED SIDE AS REVEALED BY ^1H MRS AND T_2 RELAXOMETRY. A. Incisa della Rocchetta, D.G. Gadian, A. Connelly, C.E. Polkey, G.D. Jackson, K. Watkins C.L. Johnson, M. Mishkin, and F. Vargha-Khadem. **SPON: European Brain and Behaviour Society.** Neurosciences Unit, Institute of Child Health, University of London, Mecklenburgh Square London WC1N 2AP U.K

Verbal memory deficits are not usually observed following unilateral *right* anterior temporal excision for the relief of pharmacologically intractable epilepsy. In individuals in whom this pattern occurs, there could be undetected abnormality in the unoperated left temporal lobe. Performance on verbal memory tests was assessed in relation to both side of temporal lobe surgery and the presence or absence of abnormalities in the contralateral, unoperated, temporal lobe as revealed by MR spectroscopy or T2 relaxometry. Forty-eight patients who had undergone either en-bloc temporal lobectomy or selective amygdalo-hippocampectomy were tested on Logical Memory and Paired Associate Learning from the Wechsler Memory Scale Form I. ^1H MRS and T2 Relaxometry were used to detect temporal-lobe abnormalities on the side contralateral to the operated one. Excision in the left temporal lobe impaired immediate and delayed recall of the Logical Memory prose passages as well as performance on the third learning trial and delayed recall of paired associates, irrespective of the presence or absence of contralateral abnormality. Excisions in the right temporal lobe also impaired delayed recall of both the prose passages and paired associates, but only if there was abnormality on the contralateral side. Verbal memory deficits in the right temporal-lobe patients can be attributed to abnormality in the left temporal region, contralateral to the excision; therefore the present results uderscore the usefulness of MRI and MRS in uncovering abnormalities in the temporal lobe that can go undetected using other diagnostic procedures.

530.12

A POSITRON EMISSION TOMOGRAPHY (PET) STUDY OF REGIONAL CEREBRAL BLOOD FLOW (RCBF) DURING LONG- AND SHORT-TERM VERBAL MEMORY. D.S. O'Leary*N.C. Andreasen, R.R. Hurtig. Mental Health Clinical Research Center, Dept of Psychiatry, Univ of Iowa College of Medicine, Iowa City IA 52244

We assessed rCBF with PET using the bolus ^{15}O-labelled water method in 13 normal adult volunteers performing auditory, verbal memory tasks. One week prior to PET imaging subjects learned a 15 word list and a short verbal passage. Subjects practiced the material to 100% correct recall again one day before imaging. During two conditions of the eight condition PET study, the list and passage were read to the subjects immediately prior to image acquisition. During two other conditions a novel word list and novel passage were read to the subjects. Following reading of the material each subject immediately began recall; this was timed for each condition to occur about 10 s prior to arrival of the bolus of ^{15}O-labelled water in the brain. Other conditions in the study were a resting baseline, recall of a personal episode from the subject's past, a verbal fluency task, and a repeat of the familiar verbal passage to assess replicability of rCBF changes. Compared to resting baseline, there were increases in rCBF in left precentral gyrus in all conditions which were related to the quantity of verbal production during each task. There were also large increases in flow in cerebellum in all conditions which were less directly related to vocal output. Subtraction of the familiar from the novel verbal conditions showed increased flow in the short-term memory conditions in right cerebellum for both the word list and passage, and in the left dorsolateral frontal lobe for the list. Flow in mesial temporal lobe structures did not differ significantly from baseline in any memory task. Interestingly, although all activation conditions required the subject to speak, the left frontal region corresponding to Broca's area showed increased flow over baseline only in the verbal fluency task. The present findings as well as other PET studies from our group and other centers suggest that current conceptions of the localization of memory and other functions based on lesion data will require major revision.

530.13

THE ACQUISITION AND RETENTION OF A MOTOR SKILL: A FUNCTIONAL MRI STUDY OF LONG-TERM MOTOR CORTEX PLASTICITY A. Karni*[1], G. Meyer[1], P. Jezzard[2], M. Adams[1], R. Turner[2] and L.G. Ungerleider[1], [1]NIMH & [2]NHLBI, NIH, Bethesda MD 20892.

The speed at which a short sequence of finger-to-thumb-oppositions is accurately performed more than doubles with practice over a period of several weeks. This learning does not generalize to a matched sequence made of identical component movements, nor to the contralateral hand. These results suggest that a discrete, highly lateralized, motor representation of the learned sequence undergoes experience dependent plasticity. We studied the local blood-oxygenation-level-dependent fMRI signal in primary motor (M1) cortex on consecutive weekly sessions by comparing the activation maps induced by 2 matched sequences (A, B) tapped at a fixed rate of 2 taps/sec. Four contiguous parasagittal slices (centered 30mm from midline; slice thickness 5mm) were acquired at 4T using gradient echo EPI (TR-2000ms; TE-26ms; FOV-16cm; 64x64). A rest-X1-rest-X2-rest design was employed where X1 and X2 were, respectively, sequences AB, BA, BB or AA. Subjects were taught both sequences, and initial performance was measured immediately before the first scanning session. Thereafter, one sequence was selected to be practiced daily for several weeks, while the other was performed only during scanning.

Results (4 Ss): 1) Before training, a comparable area was activated in M1 by both A and B. However, a given sequence consistently gave a smaller area if executed second rather than first ("ordering" effect). 2) By week 4 of training - concurrent with superior performance - the extent of cortex activated by the practiced sequence had increased significantly, overriding the ordering effect. This change persisted for at least several weeks, as did improved performance.

These results suggest 2 independent activity-related changes in M1: A short-term, within-session, habituation-like effect, and a slowly evolving, long-term reorganization which may underlie the acquisition of the motor skill. As the latter is highly specific for sequence, not component movements, we conjecture that practice recruits M1 units into the representation of the learned sequence.

530.14

Differences in Processing Spatial Information by Women and Men as Revealed by PET. Lauber, E.*, Jonides, J., Koeppe, R., Awh, E., Schumacher, E., Smith, E., and Minoshima, E.

Re-analysis of work presented previously (Jonides, et al., 1993, Nature, 363, 623-625) allowed us to search for sex differences in processing of spatial information. In the original work seven women and eleven men were given a spatial working memory task while PET blood flow measures were taken. Three dots were presented briefly on a CRT, followed by a brief delay, followed by a probe circle to which subjects indicated if it encircled one of the previous dots. No differences in behavioral performance were observed between males and females. However, after subtracting a control condition that was similar in all respects except for the absence of a delay period, PET blood flow data revealed differences in activation patterns for the two groups. Though both groups showed activation in Right Brodmann's Areas 40 and 6, only women showed activation in Left Area 44, 40, and 6. In contrast, areas unique for the men were Right and Left Areas 47 and Medial Area 32. The data are interpreted as supporting differences in processing spatial information by women and men. Women showed a clear left hemisphere dominance for spatial working - memory while men revealed more right hemisphere activation. Activation of Broca's area (Left Area 44) suggests that the women may have adopted a verbal encoding strategy resulting in performance similar to men.

PROCESS OUTGROWTH AND ADHESION MOLECULES

531.1

DEVELOPMENTAL INCREASE IN POLYSIALIC ACID ON NCAM FACILITATES COLLATERAL BRANCHING OF CORTICOSPINAL AXONS. M.M. Daston*[1], M. Bastmeyer [1], U. Rutishauser [2] and D.D.M. O'Leary [1]. [1] The Salk Institute and [2] Case Western Reserve University.

Corticospinal tract (CST) axons innervate their spinal cord targets by extending collateral branches. These branches begin to arise along the axon on P4, days after the primary growth cones have passed their targets. The neural cell adhesion molecule (NCAM) is often modified by the addition of polysialic acid (PSA). PSA has been implicated as a regulator of axon growth and pathfinding because of its ability to alter cell-cell interactions. We have found that the extension of collateral branches from CST axons is influenced by the PSA moiety on NCAM.

To assess the distribution of NCAM-PSA, we immunostained transverse sections of rat spinal cords over the first postnatal week. At all ages, NCAM is broadly distributed in the spinal cord, found in the spinal grey terminal fields and in the CST. However, PSA staining is weak or absent over most of the spinal cord, with the exception of the CST. The PSA staining in the CST is weak at P0 to P2, but becomes intense by P4, the age when collateral branches begin to form.

To examine the role of PSA in the extension of collateral branches, we injected endo N, which cleaves PSA from NCAM, into the fourth ventricle of neonatal rats. Immunostaining revealed that PSA was completely eliminated whereas NCAM appeared normal. CST axons were labeled by DiI injected in motor cortex. In endo N-treated rats examined at P4, 5 or 6, many fewer axon collaterals extended from the CST into the surrounding spinal grey compared to normal rats or rats injected with inactive endo N. These results suggest that the increase in PSA that occurs preferentially in the CST facilitates innervation of the spinal grey. The role of PSA may be to attenuate axon-axon interactions in the CST and thus enhance an axon's ability to respond to targeting cues.

531.2

L1 EXPRESSION MARKS TRANSLAMINAR AXON SPROUTING IN RAT DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESION Styren, S.D.* S.T. DeKosky, P. D. Miller, G. C. Styren and C.F. Lagenaur
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L1 is a cell adhesion molecule important in axonal growth and fasciculation within the CNS. We examined the role of L1 in reactive synaptogenesis and axonal growth following denervation of the hippocampal dentate gyrus molecular layer (ML) by entorhinal cortex (ERC) lesion. We compared immunohistological and ultrastructural distribution of L1 in the denervated ML and employed image analysis to evaluate lamina-specific changes over time. L1 staining was uniformly distributed over the ML in unlesioned animals. 2 days after ERC lesion there was a paucity of staining in the denervated outer ML, while inner ML staining was unchanged. During the 30 days following lesion, commissural and associational (C/A) afferents from inner ML sprouted partway into the denervated zone. L1 was expressed on these sprouting afferents and exactly corresponded to fiber outgrowth as assessed by Holmes fiber stain. As the L1-bearing axons of the C/A projection expanded, staining for embryonic N-CAM (re-expressed on the dendrites of the denervated zone) appeared to recede; there was never overlap of L1 and embryonic N-CAM staining. Ultrastructural analysis confirmed localization of L1 staining to axonal profiles. These changes in cell adhesion molecule expression closely paralleled the known sequence of reactive synaptogenesis and axonal sprouting, and demonstrate a link between cell adhesion molecule expression and axonal sprouting and attempts at self-repair by the CNS.

531.3

REGIONAL LASER INACTIVATION OF L1 AND NCAM AFFECTS GROWTH CONE MOTILITY IN CHICK DRG NEURONS. K. Takei *, T. Chan and D. G. Jay, Dept. of Cell. and Mol. Biology, Harvard University, Cambridge, MA 02138.

L1 and NCAM are neural cell adhesion molecules that belong to the Ig superfamily and are abundantly expressed in the developing nervous system. We have used regional inactivation of these molecules to investigate the role they play in growth cone motility. Microscale chromophore-assisted laser inactivation (micro-CALI) was performed on chick DRG neurons cultured on laminin and L1 substrates. Using micro-CALI, we are able to produce highly localized damage of target molecules in the growth cone that are bound with malachite green (MG)-labeled antibodies. To inactivate intracellular domains of the molecules, the MG-labeled antibodies were loaded into cells by trituration; a high level of retention of the antibodies was observed up to 8 hr later by immunocytochemistry. Micro-CALI of L1 and NCAM (180kD isoform) in the growth cone of DRG neurons grown on laminin substrate induced filopodial and lamellipodial collapse. When DRG neurons were grown on L1 substrate, micro-CALI of L1 resulted in dramatic retractions of the whole neurite as well as regional retractions of the growth cone. Laser irradiation of neurons loaded with MG-labeled bovine serum albumin, MG-labeled non-specific antibodies or without antibodies did not affect growth cone behavior. These data strongly suggest that L1 and NCAM play a key role in growth cone motility. Our findings may help us to define the physiological role of L1 and NCAM in axonal pathfinding mechanisms.

531.4

REMOVAL OF PSA FROM NCAM ENHANCES NEURITE GROWTH FROM AMACRINE CELLS IN CULTURE. LI. Kljavin*[1], C. Lagenaur[2], M.I. Ignatius[1]. [1]Dept. MCB. U.C.Berkeley, [2]Dept. Neurobiol. U. Pittsburgh.

Amacrine cells are retinal interneurons that project relatively short processes onto neighboring cells exclusively within the inner plexiform layer (IPL). Our studies have focused on defining the factors that regulate amacrine cell neurite growth. Recently, we demonstrated that NCAM functions as a neurite growth-promoting factor for rat amacrine cells in culture. However, NCAM can exist in a number of different forms which can alter its function, one of which involves a decrease in the polysialic (PSA) content as the nervous system matures. We have found similar decreases in PSA levels within the IPL. At postnatal day 3 (P3) in the rat retina the IPL is highly immunoreactive for PSA, whereas at later ages (P25) PSA immunoreactivity is absent. The loss of PSA has been shown to enhance NCAM-NCAM adhesion and inhibit NCAM dependent neurite growth in other cell types. We tested the effectiveness of NCAM to promote neurite growth from amacrine cells in culture after PSA was removed. Amacrine cells derived from P3 rat retina were cultured on affinity purified NCAM rich in PSA. PSA was removed from the culture substrate by using neuraminidase before plating cells or removed from both the culture substrate and amacrine cell surfaces using the enzyme Endo-N. Total neurite lengths for amacrine cells after 24 hours in culture increased by 49% when PSA was removed from the substrate and 78% when PSA was removed from both the substrate and amacrine cell surfaces (Control, 67.4μm±1.9; Neuraminidase, 97.2μm±1.0; Endo-N, 116.7μm±1.6). After 8 hours in culture, 68%±2.1 of the amacrine cells had formed distinct lamellipodia following PSA removal whereas only 8%±4.3 did in control cultures. These results show that removal of PSA causes an increase in the rate of neurite growth from amacrine cells cultured on purified NCAM substrates. Supp. by NIH NS 31366 and the Amyotrophic Lateral Sclerosis Association.

531.5

FUNCTIONAL ANALYSIS OF CELL ADHESION MOLECULE L1 USING DEFECTIVE HERPES SIMPLEX VIRUS VECTORS.

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L1, a member of immunoglobulin superfamily, is a transmembraneous glycoprotein that promotes neurite outgrowth and cell migration. L1 is expressed in neurons and peripheral schwann cells, and potentially involved in neural regeneration through a homophilic adhesion mechanism. Plasmid-based defective herpes simplex virus (HSV) vectors are an efficient means to transfer genes into the central nervous system (CNS). To characterize the functional role of L1, we constructed several defective HSV vectors containing the CMV_{IE} or GFAP promoter upstream of human or rat L1 cDNA.

Primary, cultured rat astrocytes and several cell lines, which do not express L1, were infected with defective HSV vectors containing human or rat L1. At 30 hours after infection, cells were fixed and stained with polyclonal antibody against L1. The CMV promoter efficiently drove L1 expression in both primary astrocytes and cell lines, whereas GFAP promoter drove L1 specifically in primary astrocytes. Primary astrocytes, Vero cells and rabbit skin cells expressing L1, after infection with defective HSV vectors, were morphologically altered. The cells contained long processes with many branches. The morphological change was not observed in cells infected with defective HSV vectors expressing β-galactosidase or alkaline phosphatase.

These defective HSV vectors will be used to study the functional role of L1 in neural regeneration.

531.6

POLARITY AND MORPHOLOGY OF RAT HIPPOCAMPAL NEURONS IN CULTURE IS INFLUENCED BY CELL ADHESION AND ECM MOLECULES.

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Hippocampal neurons grown on poly-lysine (PL) seem to replicate their *in vivo* program of axonal and dendritic development. To see whether this inherent program can be influenced by cell-surface interactions, we plated the neurons on purified cell adhesion molecules L1 or N-cadherin and on the extracellular matrix (ECM) component laminin. We found that cells on L1 extended multiple, extremely long processes that acquired axonal characteristics (i.e. enrichment in L1 and tau, and the exclusion of MAP-2) by 12 hrs in culture. These axons continued to elongate rapidly, did not fasciculate, and branched in perpendicular patterns. Dendrites were short, extended over underlying axons, and rarely grew on L1 itself. In contrast, neurons in sister cultures on PL had only a few minor processes while some had extended a single axon. The morphology of neurons grown on N-cadherin was very similar to that on PL, but the development of both axon and dendrites was greatly accelerated. By 12 hrs in culture cells already had a single, very long axon and well-developed minor processes expressing high levels of MAP-2. In comparison, neurons on laminin only exhibited a selective increase in axonal outgrowth over that seen on PL. Thus, substrates dramatically influence the growth pattern of hippocampal neurons *in vitro*. We show that the interaction of neurons with molecules they may encounter in their *in vivo* environment may influence the program of axonal and dendritic development and may alter the differential distribution of axonal and dendritic components.

531.7

SUBSTRATE-BOUND L1 ACCELERATES AXONAL GROWTH BY CULTURED RAT HIPPOCAMPAL CELLS.

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We examined the influence of the cell adhesion molecule L1 on the development of polarity by embryonic rat hippocampal neurons in culture. Neurons were cultured on nitrocellulose-treated glass coverslips coated with 8D9, the chick homolog of L1, or on coverslips coated with polylysine (PL). L1 selectively stimulated axonal growth and accelerated the development of morphological polarity. After 8 h. in culture, 37% of cells grown on L1 had formed axons compared with less than 1% of cells grown on PL. By 24 h., 99% of cells on L1 and 71% of cells on PL had formed axons, and the axons of cells grown on L1 were about four times longer than those on PL. In addition, 24% of cells on L1 developed two axons after 24 h. The length of minor processes did not differ on the two substrates. Despite these pronounced changes in cell shape, polarity markers segregated similarly on the two substrates. L1 became restricted to axons while MAP2, transferrin receptor, and LDL-receptor related protein segregated to somata and dendrites on both L1 and PL. By 3 weeks in culture, cells grown on L1 had formed well-differentiated dendrites, as indicated by their morphology after MAP2 staining, and they were contacted by numerous synaptophysin-positive presynaptic terminals. The selective effect of L1 on axonal growth is not surprising, since substrate-bound L1 would be expected to bind homophilically to neuronal L1, which is restricted to the axonal surface. The relatively normal development of dendrites on the L1 substrate may have been mediated by small amounts of L1 on the dendrites. Alternatively, an adhesion molecule that interacts with L1 in a heterophilic manner may be present on dendrites. Finally, other proteins secreted by the cultured cells or present in the medium may bind to the nitrocellulose and permit dendritic growth.

This work was supported by NIH grant NS17112, NEI 05285 (V.L.) and by a graduate research fellowship from the NSF.

531.8

SELECTIVE HIPPOCAMPAL NEURITOGENESIS: AXON GROWTH ON LAMININ OR PLEIOTROPHIN, DENDRITE GROWTH ON POLY-D-LYSINE,

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Toward the goal of engineering live neuronal networks, we have begun a search for culture substrates that are selectively adhesive for specific cell types and their neurites, either axons or dendrites. This investigation is possible because of the development of a serum-free medium for growth of isolated neurons without glia (B27/Neuro-basal, GIBCO; Brewer et al. (1993), J. Neurosci. Res. 35:567). Glass substrates were coated with either poly-D-lysine (PL), PL followed by laminin, or pleiotrophin. Neurons from embryonic day 18 rat hippocampus were plated at 80 cells/mm^2. Survival (live/total cells) was equivalent on the three substrates. After 1 day, neuritogenesis was dramatically enhanced on laminin or pleiotrophin, compared to polylysine. After 4 days, the uniform narrow processes on pleiotrophin had continued to grow, while those on laminin largely stopped. In contrast, cells on PL grew shorter tapered processes, characteristic of dendrites. The shorter tapered processes reacted with antibodies against the cytoskeletal dendritic marker MAP2. The long, narrow processes reacted with antibodies recognizing the cytoskeletal axonal marker tau. By selective patterning of substrates, it may be possible to specifically direct axonal and dendritic growth.

PROCESS OUTGROWTH AND CYTOSKELETON

532.1

Neurofilament subunit composition specifies axonal caliber.

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Neurofilaments are neuronal intermediate filaments comprised of three polypeptide subunits NF-L, NF-M and NF-H. Increasing evidence has shown that neurofilaments are required for the development of normal axonal caliber, a crucial determinant of axonal conduction velocity. To test the role of each individual subunit, we have over-expressed different subunits in transgenic mice. Surprisingly, a simple increase in number of filaments by a 2-fold increase in NF-L inhibits radial axonal growth, producing ***smaller*** axons. An increase (by 2 fold) in either NF-H, NF-M or both, (presumably increasing the side arms), inhibits radial axonal growth to an even larger extent. However, combined increase of NF-L with either NF-M or NF-H produces ***larger*** axons. These data demonstrate that radial axonal growth requires not only an appropriate number of filaments but also filaments comprised of a balanced composition of the different subunits and in particular, a normal ratio between the content of NF-L and either of two large subunits, NF-M and NF-H.

532.2

MAP2 EXPRESSION AND NEURITIC BRANCHING ARE REDUCED WHEN SPINAL CORD NEURONS ARE CO-CULTURED WITH WOBBLER MOUSE ASTROCYTES.

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Embryonic normal mouse neurons can be cultured on layers of primary astrocyte culture and are identified by microtubule-associated proteins, MAP2, exhibiting a high degree of neuronal cell-type specificity. With the aid of specific antibodies to MAP2, we studied the neuritic outgrowth and examined the intensity of MAP2 staining which allows a direct visualization of neurite outgrowth. We performed cocultures of embryonic normal neurons with both normal and wobbler astrocytes. These mutant astrocytes are strongly GFAP-reactive and develop, *in vivo* and *in vitro*, morphological modifications in process outgrowth and arrangement (J.Neurocytology, 23, 179-192, 1994). We found that the number of MAP2 negative-neurons coupled to wobbler astrocytes was much higher compared to those coupled to normal astrocytes, pointing towards a defect in neurite outgrowth mediated by wobbler astrocytes. We thus studied the expression and cell distribution of MAP2 proteins in both conditions. Quantitative image analysis showed a reduced number of neurites and neuritic branching points in the wobbler astrocyte-neuron coupled cultures compared to the normal cocultures. These results are in agreement to the recent observation of Weiya and Vacca-Galloway (1991) showing that *in vivo* the extent and branching of neuritic arborization is abnormal in wobbler mice. Similar observations were made when normal neurons and astrocytes were cocultured in the presence of conditioned medium from wobbler astrocyte cultures. Altogether, these data strongly support the hypothesis that neuritic outgrowth is inhibited under the influence of soluble factors secreted by wobbler astrocytes and not normal astrocytes. These observations suggest that wobbler astrocytes possibly produce high amounts of glia-derived growth inhibitors.

532.3

CHARACTERIZATION AND REASSEMBLY OF ALPHA-INTERNEXIN (NF66) ISOFORMS FROM BOVINE SPINAL CORD. M.E. Miller* and B.J. Balin. Med. Coll. of PA, Dept. of Pathology and Lab Med., 3200 Henry Ave., Phila., PA 19129.

We have demonstrated previously that alpha-internexin (NF66), a member of the type IV family of intermediate filaments, can be isolated from bovine spinal cord and that purified NF66 is capable of reassembly (as well as coassembly with other type IV intermediate filaments) into ~10nm-diameter filaments (**Balin & Miller (1994) J. Neurosci. Res., in press**). During purification, a high molecular weight isoform of NF66 was found to co-elute with the high molecular weight neurofilament subunit (NF-H) following anion exchange chromatography of an enriched cytoskeletal fraction. These two proteins were found to be inseparable by gel filtration and a gel elution strategy (**Miller et al. (1994) J. Neurosci. Meth., submitted**) was used to purify NF66 to homogeneity for reassembly studies. We now demonstrate the existence of lower molecular weight isoforms (< 66kD) of bovine NF66 that can be isolated by successive cation and anion exchange chromatography. These isoforms of NF66 are also shown to be capable of reassembly into ~10nm-diameter filaments by negative staining and immunoelectron microscopy. Characterization of these NF66 isoforms by 1- and 2-D gel electrophoresis and Western blotting suggests that these polypeptides are NF66 phosphoisoforms. Supported by PHS grant AG10160.

532.4

ALTERING THE PROTEIN COMPOSITION OF DEVELOPING AXONS: MICROINJECTION OF A NEUROFILAMENT ANTIBODY TO STUDY NEURITE OUTGROWTH IN *XENOPUS* CULTURED SPINAL CORD NEURONS. W. Lin* and B.G. Szaro. Biol. Sci., SUNY-Albany, Albany, NY 12222.

The cytoskeletons of newly differentiating *Xenopus laevis* spinal cord axons contain a middle- (NF-M) and a lower molecular weight neurofilament protein (XNIF). Microinjection of antibodies against NF-M into embryonic blastomeres subsequently results in abnormal distributions of XNIF-immunoreactivity within the neurons of intact embryos and inhibits peripheral nerve development (*J. Comp. Neurol.* 308:576). To better understand these effects, we examined the consequences of anti-neurofilament antibody injection on embryonic spinal cord neurons isolated in culture. Single blastomeres of 2-cell stage embryos were microinjected with an anti-NF-M antibody mixed with FITC-dextran. Injections at the 2-cell stage inoculated only half of the neurons, allowing us to compare the injected neurons, which were identified by FITC-fluorescence, to normal ones within the same culture. After injection, neural tubes were dissociated and cultured at stage 22. Immunocytochemistry with rhodamine-labeled secondary antibodies confirmed that for at least the first 24 hrs in culture, the injected antibody was uniformly distributed throughout somas and within neurites. Furthermore, the neurofilament antibody successfully altered the normal distribution of neurofilaments within neurons. For example, neurites of uninjected neurons are evenly filled with XNIF-immunoreactivity. In contrast, in injected neurons, XNIF accumulated within the soma, leaving neurites devoid of detectable neurofilaments. These results resembled the effects observed in intact embryos. Next, we will measure the rates of neuritic outgrowth in the absence of neurofilaments. Supported by NIH R29-NS30682.

532.5

OVER-EXPRESSED DREBRIN FORMED THICK, CURVING BUNDLES OF ACTIN FILAMENTS, FROM WHICH TROPOMYOSIN WAS DISSOCIATED, IN FIBROBLASTS. T. Shirao[1]*, K. Hayashi[1], K. Isa[1] and R. Ishikawa[2]. [1]Dept. of Neurobiol. & Behav. and [2]Dept. of Pharmacol., Gunma Univ. Sch. of Med., Maebashi 371, JAPAN

Drebrin is an actin binding protein rich in the neuron. During the development of the brain, it is localized at the submembranous region of migrating neuroblasts and growing axons and dendrites. By transfection of fibroblasts with drebrin cDNA, the outgrowth of highly branched, neurite-like cell processes is induced from the cells that the protein is expressed at high levels. We demonstrate by an immunocytochemical method that the drebrin expressed in transfected cells binds to stress fibers and, as a consequence, thick, curving bundles of actin filaments are formed. Such bundles were observed not only in the highly branched, neurite-like cell processes but also in the cell bodies of transfectants. Tropomyosin was dissociated from actin filaments in these cells. Biochemical analysis also revealed that drebrin strongly inhibited the actin-binding activity of tropomyosin by competitive binding. These data suggest that actin filaments that bind drebrin form a novel class of actin filaments, which may play a role in neuronal morphogenesis.

532.6

DISTRIBUTION OF CYTOSKELETAL PROTEINS IN GROWTH CONES IS INFLUENCED BY CONTACT WITH SUBSTRATE-BOUND L1, N-CADHERIN AND LAMININ. S.M. Burden* and V. Lemmon, Dept. of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

We have previously shown that retinal ganglion cell (RGC) growth cones exhibit characteristic morphologies dependent upon the substrate on which they are grown (Payne, et al. (1992) *Cell Motil. Cytoskel.* 21:65-73). Upon contact with a sharp border between two substrates, the growth cones display dynamic changes in morphology (Burden, et al. (1992) *Soc. Neurosci. Abst.* 614.10) that may be due to extensive restructuring of the cytoskeleton. In this study, we have used immunocytochemistry to examine the distribution of three cytoskeletal elements in RGC growth cones growing on L1, N-cadherin or laminin individually, as well as on dishes coated with alternating lanes of these substrates.

We have observed distinct staining patterns for f-actin, microtubules (MTs) and neurofilaments (NFs) in growth cones growing on individual substrates. At border regions between two substrates, growth cones that established lamellipodial contact with the new substrate were observed to have f-actin and MT staining patterns appropriate for the newly encountered substrate. Contact via filopodia alone did not evoke this change. Once the majority of the growth cone had crossed onto the second substrate, redistribution of NFs was also observed. These results suggest that the major cytoskeletal elements of growth cones are rapidly restructured in direct response to substrate contact. Such changes are likely to be crucial for growth cone guidance.
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532.7

LOCALIZED RE-EXPRESSION OF A DEVELOPMENTALLY-REGULATED FORM OF MAP 1B DURING AXONAL REGENERATION *IN VITRO*. D.A. Tonge, J.P. Golding and P.R. Gordon-Weeks*. Biomedical Sciences Division, King's College, Strand, London WC2R 2LS, U.K.

Microtubule organization in growth cones is known to be essential for neurite extension. Phosphorylation of microtubule-associated proteins (MAPs) modifies their affinities for microtubules and this might affect neurite growth. Monoclonal antibody 150 (mab 150) recognizes a novel phosphorylation epitope on MAP 1B which is transiently expressed during development of the nervous system (Eur. J. Neurosci. 5, 1302-1311). In the present study, expression of this form of MAP 1B was studied during axonal regeneration *in vitro*.

Short lengths of spinal nerves with their attached dorsal root ganglia were removed from adult mice, explanted into Matrigel and maintained in serum-free medium for up to 8 days. Profuse growth of naked axons occurred within one day from the cut ends of both the peripheral nerve and the dorsal root, and continued throughout the observation period. Some axons were entirely smooth whilst others showed prominent varicosities. Immunohistochemical staining using mab 150 was observed along the whole length of the axons growing out of the explants, but not within the nerve itself. The staining extended to the growth cones which had elaborate morphology. Other antibodies (e.g. to GAP-43) labeled axons within the nerve as well as those growing in Matrigel. In preparations where the peripheral nerve had been crushed half way along its length, mab 150 staining was absent from the nerve proximal to the crush site, but present in axons which had regenerated within the nerve distal to the crush. These results indicate that re-expression, during axonal regeneration of the form of MAP 1B recognized by mab 150, is restricted to the newly formed segments of axons. The correlation between its expression and axonal growth during development and regeneration suggests that may play a role in axonal extension. (Supported by MRC).

532.8

REORGANIZATION OF MICROTUBULES IN CORTICAL GROWTH CONES DURING CELL-CELL INTERACTIONS. M. Lu* and K. Kalil. Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

Growth cone behaviors such as advance, retraction, fasciculation, turning, and branching are accompanied by reorganization of the cytoskeleton. To understand how microtubules contribute to behaviors of CNS neuronal growth cones, we have studied the disposition of microtubules in growth cones of embryonic cortical neurons as they interacted with other neurons and glia in dissociated cultures. We have used high resolution video enhanced Nomarski optics to visualize morphologies of fixed growth cone captured during various behaviors. Matching fluorescent images of the same growth cones stained immunocytochemically with anti beta tubulin were used to localize the positions of microtubules during these behaviors. We found that microtubules were symmetrically distributed in the proximal region of growth cones advancing in straight trajectories. In contrast, growth cones turning away from straight trajectories had microtubules oriented in the direction of turning. When growth cones fasciculated with other neurites the orientation of their microtubules became aligned with those of the neurite. Microtubules also invaded the branches along neurites as well as growth cone bifurcations. In video microscopy, we have previously observed that when growth cones collapse and withdraw they often leave behind a long filopodial strand upon which the growth cone reextends. Immunocytochemistry revealed that these strands were usually invaded by microtubules. Preliminary results in which microtubules were stained with antibodies to detyrosinated tubulin suggest that the microtubules observed during dynamic growth cone behaviors are relatively labile. These results suggest that the invasion of labile microtubules may underlie growth cone behaviors that require changes in direction.
Supported by NIH Grant NS 14428 (K.K.)

532.9

SUPPRESSION BY ANTISENSE mRNA DEMONSTRATES A REQUIREMENT FOR DREBRIN IN THE FORMATION OF NEURITE PROCESSES. M. Toda[1,2], T. Shirao[3], H. Asou[1*], S. Toya[2], and K. Uyemura[1]. Depts. of [1]Physiol. and [2]Neurosurg., Keio Univ. Sch. of Med., Tokyo 160, [3]Dept. of Neurobiol. and Behavior, Gunma Univ. Sch. of Med., Maebashi 371, Japan.

Drebrin are developmentally-regulated actin-binding proteins. Although neurite outgrowth is the first step in neuronal network formation, the intracellular mechanism of neurite outgrowth is not yet clear. To determine the role of drebrin in these phenomena, we have studied the rat neuroblastoma cell line, B104, which constitutively expresses drebrin. Deprivation of serum or retinoic acid treatment induces a characteristic change in morphology; cells produce drebrin-positive processes. Then, in an effort to interfere with the expression of drebrin in B104 cells, we transfected them with an antisense construct of human drebrin E cDNA (Biochem. Biophys. Res. Commun. 196: 468-472, 1993), driven by a β-actin promoter. Two stable antisense cell lines from separate transfections were isolated and were shown to be drebrin negative by Western blot analysis and immunofluorescence studies. The antisense cell lines were inhibited in their ability to extend significant neurite processes in response to serum deprivation or retinoic acid treatment. These data support the conclusion that the actin-binding protein, drebrin, is required for the formation of stable neurite processes.

532.10

MECHANISMS REGULATING THE STABILITY OF CYTOSKELETAL mRNAS IN REGENERATING RAT SENSORY NEURONS. P.F. Moskowitz* and M.M. Oblinger Dept. Cell Biol. and Anat., Chicago Med. School, North Chicago, IL 60064

In eukaryotes, the steady-state mRNA level for a given gene is dictated by the balance of transcriptional and post-transcriptional controls. For cytoskeletal genes, mRNA stability is believed to play a major role in the specification of steady-state mRNA levels, but little is actually known about this issue in mammalian neurons. The model system we have used to study this is the axotomized, regenerating dorsal root ganglion (DRG) neuron wherein it is well documented that neurofilament (NF) mRNA levels are rapidly depressed while those of certain tubulin isotypes are rapidly increased following axonal injury. We have examined whether these mRNA level changes are controlled at the level of cytoplasmic mRNA degradation/stabilization and have examined the role of protein synthesis in the post-axotomy mRNA changes. Half-lives of NF and tubulin mRNAs were assessed in normal and 7 d post-axotomy DRG using Northern blotting of RNA isolated at various intervals after transcriptional blockade was induced by in vivo microinjection of actinomycin D. The half-lives of NF mRNA are normally quite long, but a more rapid decay of NF-L, NF-M and NF-H mRNAs was found in axotomized neurons. Interestingly, β-tubulin mRNA decay was also found to be more rapid in axotomized DRG neurons. Protein synthesis was found to be essential for some, but not all, of the post-axotomy mRNA changes since synthesis blockade induced with cycloheximide 4 h prior to axotomy prevented the NF-M mRNA loss, but not the NF-L or NF-H mRNA downregulation that occurred in DRG neurons by 1 d post-axotomy.

MECHANISMS OF AXON GUIDANCE

533.1

LAZARILLO: A GPI-LINKED GLYCOPROTEIN INVOLVED IN AXON GUIDANCE IN THE GRASSHOPPER EMBRYO IS A MEMBER OF THE LIPOCALIN FAMILY. D. Sánchez, M.D. Ganfornina, and M.J. Bastiani*. Department of Biology, University of Utah, Salt Lake City, Utah 84112, U.S.A.

Our work is focused on the signaling events that ensure neurites find their appropriate pathway to target cells during neurogenesis. We are studying a new cell surface molecule called **Lazarillo** which is expressed by a subset of neurons and neuronal precursors in the grasshopper nervous system. This molecule is linked to the plasma membrane by a glycosyl-phosphatidyl-inositol (GPI) group and possesses abundant N-linked oligosaccharides (native Mr=45 kD, deglycosylated Mr=28 kD). The monoclonal antibody against **Lazarillo** (10E6 MAb) is able to perturb the direction of several growth cones in cultured embryos. In particular, a pair of commissural pioneer neurons are disrupted; their growth cones are delayed or abruptly change their direction of growth.

We have identified and characterized a cDNA clone coding for the **Lazarillo** protein. The predicted protein sequence has the signal peptides necessary to target the protein to the endoplasmic reticulum and anchor it to the membrane by the GPI tail. It possesses seven N-linked glycosylation sites and four cysteine residues. The cDNA has a long (2.3 kb) 3' untranslated region. *In situ* hybridization using the whole cDNA sequence as probe shows that **Lazarillo** mRNA co-localizes with the 10E6 MAb. Sequence comparisons reveal that **Lazarillo** belongs to the lipocalins family; extracellular carriers of small hydrophobic ligands in a wide variety of systems. A phylogenetic analysis of the family sets **Lazarillo** in a cluster containing the mammalian Apolipoprotein D, the insect bilin binding proteins, and all the serum retinol binding proteins from vertebrates. **Lazarillo** is, to the best of our knowledge, the first lipocalin (1) GPI-anchored to the plasma membrane of cells, (2) restricted to a subset of neurons and neuroblasts in a developing nervous system, and (3) involved in growth cone pathfinding. Supported by NIH (NS 25387), Fulbright (M.D.G.) and Fogarty (D.S.) fellowships. M.D.G. and D.S. contributed equally to this work.

533.2

CALCIUM ENTRY AND CALCIUM STORES CONTROL NERVE GROWTH IN AN APPLIED ELECTRIC FIELD. **R. Stewart & C.D. McCaig*. Department of Biomedical Sciences, University of Aberdeen, Aberdeen AB9 1AS, Scotland.**

Cultured nerves show striking growth responses to a small dc applied electric field of physiological magnitude. Calcium entry has been implicated. Neurites from Stage 20 *Xenopus* neural tubes grown on tissue culture plastic in a dc field of 150mV/mm, 1. grow faster than in the absence of the field; 2. grow faster cathodally than anodally; and 3. turn to grow cathodally. Using a pharmacological approach, we have studied both the role of selective voltage dependent calcium channels (VDCCs) and of calcium release from intracellular stores in the control of these three responses. We report 1. that faster growth in an applied electric field is lost in the presence of the P-type VDCC inhibitor omega-agatoxin IVA and in the presence of thapsigargin, which disrupts calcium release from inositoltrisphosphate sensitive intracellular stores; 2. that ryanodine, which disrupts calcium-induced calcium release from intracellular stores, abolishes the growth rate differential between cathode and anode facing neurites and 3. that the P- and N-type VDCC inhibitors omega-agatoxin IVA and omega-conotoxin GVIA respectively, as well as ryanodine and thapsigargin each partially inhibit cathodal orientation. We conclude that calcium entry through specific VDCCs in the growth cone, linked to calcium release from specific intracellular stores controls different aspects of electric field-induced growth and orientation.

533.3

ISOLATION OF PROTEOGLYCANS FROM THE DEVELOPING SUPERIOR COLLICULUS OF THE SYRIAN HAMSTER. Diane Hoffman*, Arthur D. Lander, and Sonal Jhaveri. Dept. of Brain & Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA.

Recent experiments suggest important roles for sulfated proteoglycans (PGs) in influencing axonal growth. In addition, chondroitin sulfate and keratan sulfate PG (CSPG, KSPG) expression patterns correlate at certain times with regions where axons preferentially travel, such as the cortical subplate, and at other times with regions which axons distinctly avoid, such as the midline of the superior colliculus. We have examined the expression of PGs in the superior colliculus of the developing hamster, around the time of retinal axon ingrowth and arborization in this target. PGs were isolated from tectal homogenates by anion exchange chromatography, radiolabeled, and digested with enzymes to remove specific glycosaminoglycan (GAG) chains. Resulting protein cores were separated by electrophoresis. During the first postnatal week, we have identified PGs which, on the basis of molecular weight, solubility properties, and GAG type, bear distinct resemblance to developmentally regulated PGs previously isolated from rat brain. Early postnatal tectum contained at least four distinct HSPGs, with molecular weights corresponding to glypican, cerebroglycan, M7 and M10 (see Herndon and Lander, Neuron 4, 1990). CSPGs of sizes consistent with neurocan, S2 and M8 were also expressed. In addition, two novel bands appeared in response to digestion with keratanase, although the possibility of proteolytic contamination in the keratanase has not yet been ruled out. Experiments are currently under way to isolate PGs at other ages, and to localize their sites of expression within the superior colliculus, with emphasis on gaining potential insights from comparing expression along the midline and in retinorecipient zones of lateral regions. Supported by NIH grants EY05504, NS26862, and EY02621.

533.4

TWO CHONDROITIN SULFATE PROTEOGLYCANS EXPRESSED DURING THE SEGMENTATION OF THE PERIPHERAL NERVOUS SYSTEM OF THE CHICK. C. Ring* and W. Halfter, Univ. of Pittsburgh School of Medicine, Dept. of Neurobiology, 15261.

Chondroitin sulfate proteoglycans (CSPGs) purified from cartilage have been shown to be inhibitory to neurite outgrowth *in vitro*. Based on these findings, it has been hypothesized that CSPGs function as barrier molecules to neurite outgrowth *in vivo*. We wanted to examine if CSPGs derived from non-cartilagenous embryonic tissues would have similar effects. We have isolated two monoclonal antibodies (mAbs), 2B9 and 9BA12, which recognize two CSPGs that are developmentally expressed in the trunk region of the chick embryo. The 2B9 mAb has been shown to recognize collagen type IX proteoglycan (CIXPG). It is expressed in the posterior sclerotome of the somites at a time when motor axons project, and neural crest cells migrate through the anterior sclerotome. Dorsal root ganglion (DRG) explants can adhere to, and extend short neurites on, nitrocellulose-bound CIXPG. Neurites from DRG explants grown on a combination of fibronectin/CIXPG appear shorter and more fasciculated than control explants grown on fibronectin alone.

The 9BA12 mAb recognizes an as yet unidentified CSPG in neural tissue. It is expressed throughout the trunk region of the developing chick embryo, in the neural tube, throughout the somite, and is absent only from the dermamyotome. As with the CIXPG, DRG explants can adhere to and extend short neurites on, nitrocellulose-bound 9BA12 antigen. Alternatively, in combination with fibronectin, the explants were able to extend neurites on the 9BA12 antigen that appeared identical to control explants grown on fibronectin alone. Our study demonstrates that CIXPG in the posterior sclerotome may contribute to the avoidance of this tissue by developing neurites and migrating neural crest cells. In addition, we conclude that not all species of proteoglycan may be inhibitory to axon outgrowth, and that careful attention be paid to the source and type of proteoglycan used in neurite outgrowth assays.

533.5

LAMININ AND FIBRONECTIN GUIDEPOSTS SIGNAL SUSTAINED BUT OPPOSITE EFFECTS ON PASSING GROWTH CONES. T. B. Kuhn, M. F. Schmidt$ and S. B. Kater*. Dep. of Anatomy and Neurobiology, Colorado State University, CO 80523; $ Division of Biology, Caltech, Pasadena, CA 91125.

Guidepost cells affect behavior and navigation of growth cones *in vivo*. Yet, the nature of communication and the type of signals employed between growth cones and guideposts are largely undefined. Here we report that in a *in vitro* assay system both navigation and behavior of advancing DRG growth cones of the chick were significantly altered during transient encounters with monomolecular model-guideposts; 4.5μm polystyrene beads covalently coated with potential guidance molecules. Evoked growth cone behavior, a series of stereotypic responses, depended on the molecular nature of the model guideposts. Growth cones routinely paused and displayed filopodial sampling at guideposts. Laminin-model guideposts caused a sustained, two-fold increase of growth cone advance on a fibronectin substrate ($p<0.01$; n=17) while fibronectin-model guideposts led to a sustained, two-fold decrease of the rate of advance on a laminin substrate ($p<0.001$; n=13). Notably, these effects lasted substantially beyond the time of physical contact between growth cone and model guidepost. Arrays of laminin-model guideposts produced a saltatory growth cone advance and provided unambiguous, directional guidance. Interestingly, an increased overall growth rate (above that on the poly-L-lysine substrate) was observed equivalent to a homogeneous laminin substrate, despite periodic pausing occurred at each guidepost. Taken together, theses results demonstrate that 1) as little as an individual molecular species is required to designate a point in space as a functional guidepost, 2) transient growth cone-guidepost contacts appear to initiate signalling pathways rather than just influence adhesion, and 3) the enforced pausing and sampling may represent a unique feature of guidepost-mediated pathfinding resulting in a periodic reassessment of the environment by the growth cones.

533.6

A NOVEL Ca^{++}-BINDING PROTEIN IS EXPRESSED BY A SUBSET OF PERIPHERAL NEURONS WHICH SELECTIVELY FASCICULATE IN A SINGLE AXON TRACT IN LEECH CNS. K.K. Briggs, K.M.Johansen, & J. Johansen*. Dept. of Zoology & Genetics, Iowa State University, Ames, IA, 50011.

The lan 3-6 antigen is expressed in a subpopulation of peripheral neurons whose afferents segregate into a single axon fascicle in the developing leech CNS. By screening an expression vector library with the antibody we obtained a clone which suggests that the antigen is a novel protein possessing two EF-hand Ca^{++}-binding domains. The Ca^{++}-binding domains are likely to be functional since fusion protein made from the clone and expressed *in vitro* binds $^{45}Ca^{++}$ after SDS-PAGE and transfer to nitrocellose paper. That the clone corresponds to the lan 3-6 antigen was verified by *in situ* hybridizations with the clone to embryos, the labeling of which exactly matches the lan 3-6 antibody staining pattern. Northern analysis suggests that translation of the lan 3-6 message would result in a protein with a molecular weight of about 18 kD. Immunoaffinity purification with the lan 3-6 antibody followed by SDS-PAGE and analysis of the gels by silver staining yields a protein band of this predicted size. However, a prominent protein band of approximately 200 kD is being selectively co-purified with the lan 3-6 antigen by this procedure. Broad protein bands of this nature are frequently charateristic of glycoproteins. Thus these results suggest that the lan 3-6 antigen is a small Ca^{++}-binding protein which may be associated with and regulating a larger 200 kD glycosylated protein. The very restricted expression of the lan 3-6 antigen to a subset of peripheral neurons which specifically fasciculate together into a single tract during development raises the possibility that this putative protein complex may play a role in this process. Supported by NIH grant NS 28857 and NSF grant DIR 9113595.

533.7

THE CLONING OF A CHICK BRAIN cDNA ENCODING A PROTEIN SEQUENCE RELATED TO COLLAPSIN. Y. Luo*, M. Renzi and J. A. Raper, Department of Neuroscience, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Collapsin is a recently identified protein (Luo et al. 1993, Cell 75 217-227) that is likely to serve as a repulsive guidance cue during growth cone navigation since it induces the collapse of specific growth cones *in vitro*, is homologous to the axon guidance molecule fasciclin IV (semaphorin I), and is expressed in discrete regions of the developing brain. Partially purified brain-derived collapsin induces the collapse of both DRG and retinal growth cones, yet recombinant collapsin induces DRG growth cone collapse without affecting retinal growth cones. We therefore hypothesize that there is a family of collapsin-related molecules in brain that exhibit distinct neuronal target specificities. To identify possible collapsin relatives, we screened a λgt10 chick brain cDNA library with collapsin probe under low stringency conditions. One cDNA clone was identified that hybridizes with the collapsin probe at low stringency (45 °C) but not at high stringency (65 °C). The 1.6 kb cDNA insert of this clone encodes a portion of a novel protein that is homologous to a part of the fasciclin IV-like and the Ig-like domains in collapsin. In the fasciclin IV-like domain over 70% of the predicted amino acid sequence is identical to that of the corresponding domain in collapsin. We are now in the process of obtaining the full coding sequence for this collapsin related protein. We then hope to express this recombinant version and test it for collapsing activity on a variety of growth cones *in vitro*.

533.8

THE PATTERN OF COLLAPSIN mRNA EXPRESSION IN THE DEVELOPING CHICK CNS. S. Chang, Y. Luo, and J. A. Raper*, Department of Neuroscience, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Collapsin is a recently identified protein that induces the collapse and paralysis of specific growth cones *in vitro* (Luo et al. 1993, Cell 75 217-227). Here we examine the distribution of collapsin mRNA in the developing chick brain.

Wholemount *in situ* hybridizations were performed using a full length digoxigenin derivatized collapsin probe that was later reacted with an alkaline phosphatase conjugated anti-digoxigenin antibody. In the chick brain at E2 (HH stage 11), collapsin message was localized to rhombomeres 3 and 5 of the hindbrain. By E3, the rhombomere expression declines and collapsin message is strongly expressed in specific regions of the diencephalon. It is localized dorsally in an annulus around the developing pineal and ventrally as two stripes along either side of the stalk of the pituitary. On E6, collapsin message is also found in a stripe on the lateral wall of the forebrain in a region that may correspond to the archaestriatum. A pair of rostro-caudally oriented stripes on either side of the midline of the hindbrain also express collapsin message. The expressing cells in these stripes are near the ventricular surface These two pairs of collapsin expressing stripes extend caudally into the ventral portion of the spinal cord.

This highly specific and localized pattern of expression at developmental times when extensive axon outgrowth is occurring in the developing CNS is consistent with collapsin playing a role in growth cone guidance.

533.9

DETERMINANTS OF AXON BRANCHING AND GUIDANCE IN THE ACCESSORY OLFACTORY SYSTEM. K. Yoshida, G.A. Schwarting*, and J.E. Crandall. The Shriver Ctr., Waltham, MA 02254 & Prog. in Neuroscience, Harvard Med. Sch.

CC1 and CC6 are monoclonal antibodies that recognize subsets of vomeronasal (VNO) neurons. Axons from $CC1^+$ neurons terminate in the rostral accessory olfactory bulb (AOB), whereas axons from $CC6^+$ neurons terminate predominantly in caudal glomeruli of the AOB. In addition, subsets of $CC6^+$ axons branch from the main vomeronasal nerve and grow caudally toward the forebrain. Many LHRH neurons utilize these $CC6^+$ axon branches as guides to migrate along the medial surface of the olfactory bulb (OB). Occasionally $CC1^+$ axons also grow caudally toward the forebrain, but turn at the OB/forebrain border and grow back into the rostral AOB. These data suggest that molecules associated with the pathway determine the trajectories of chemically distinct VNO axons. Whole mount immunohistochemical studies indicate that both attractive and repulsive activities may operate in the developing AOB. Most $CC1^+$ axons appear to stop abruptly at the border of the caudal AOB. However, a small number of $CC1^+$ axons enter the caudal AOB but alter their direction by turning sharply and entering the rostral AOB. Tissue culture studies are underway to examine factors that affect axon growth and branching. E18 VNO neurons are grown on coverslips coated with alternating stripes of protein homogenates from neonatal rostral and caudal AOBs. Preliminary results suggest that subsets of VNO axons turn and grow on rostral AOB homogenates but appear to be unaffected by caudal AOB homogenates. Supported by DC00953, NS24386 and HD05515.

533.10

IDENTIFICATION OF MYELIN-ASSOCIATED GLYCOPROTEIN AS A MAJOR MYELIN-DERIVED INHIBITOR OF NEURITE GROWTH. L. McKerracher, S. David, D.L. Jackson, V. Kottis, R.J. Dunn, P.E. Braun*, Centre for Res. in Neuroscience, McGill Univ. and Montreal General Hosp. Res. Inst., 1650 Cedar Ave., Montreal, P.Q. Can., H3G 1A4.

Potent contact-dependent axon growth inhibitory activity is present in CNS myelin. To identify inhibitory proteins present in myelin we used non-denaturing chromatography and tested the fractions for growth inhibition by bioassay. NG108-15 cells extend neurites in response to cAMP. Purified bovine CNS myelin, used as a tissue culture substrate, permitted cell attachment but inhibited cAMP-induced neurite outgrowth. When myelin was extracted with octylglucoside and the extract chromatographed on a DEAE anion exchange column, several peaks of inhibitory activity were eluted with a salt gradient. Analysis of the fractionated proteins revealed that myelin-associated glycoprotein (MAG) co-chromatographed with bioactivity. To determine if MAG contributes to growth inhibition, MAG was removed from these fractions by immunodepletion. Removal of MAG reduced neurite growth inhibitory activity. Direct evidence for the inhibitory effect of MAG on neurite outgrowth was obtained by testing the extracellular domain of recombinant MAG produced in cultured insect cells. This recombinant MAG was a potent inhibitor of neurite outgrowth. These results, which establish an inhibitory activity for MAG, suggest that the growth inhibitory properties of MAG may be critically important after nerve injury in the CNS where myelin debris is not removed very quickly after injury.

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533.11

NEURITE GROWTH INHIBITORY ACTIVITY IN THE MAMMALIAN PNS AND CNS AND ITS MODULATION BY LAMININ. S. David*, L. McKerracher, D. Jackson, V. Kottis, and P. Braun. Centre for Research in Neuroscience, Montreal General Hospital Research Institute, and Dept. Biochemistry, McGill University, Montreal, Canada.

In addition to the axon growth inhibitor in CNS myelin identified by Caroni and Schwab (J. Cell Biol.106:1281), we have identified another inhibitor in myelin (see Abst. McKerracher et al.,). We now provide evidence that peripheral nerve (PN) myelin also possesses neurite growth inhibitory activity. Bovine PN myelin was prepared by homogenization with either a Polytron (PN-p), or frozen in liquid N_2, pulverized and homogenized with a Dounce homogenizer (PN-d). These 2 preparations were then subjected to sucrose density gradient centrifugation to purify myelin. PN myelin was plated on to polylysine-coated 96 well plates (8μg/well), and neurite growth assessed with cAMP treated NG108-15 cells. On PN-p myelin 81% of the cells extended neurites, as compared to 1.5% on PN-d myelin. Western blots of the 2 myelin preparations with anti-laminin antibody indicate substantial amounts of laminin in the myelin preparation that was permissive for neurite growth (PN-p). Preincubation of PN-p myelin with anti-laminin antibody reduced neurite growth. In a similar neurite growth assay, the inhibitory activity in bovine CNS myelin (4%) was overridden (80%) by coating the wells with a mixture of myelin (8μg) and laminin (1.5μg). These results suggest that neurite growth inhibitory activity is present in PN and CNS myelin, and can be modulated by laminin.

533.12

AN INSECT PIONEER NEURON USES MESODERMAL CELLS AS A SUBSTRATE FOR NORMAL PATHFINDING. I.Rajan*., J.L.Denburg, Dept. of Biological Sciences, University of Iowa, Iowa City, Iowa 52242.

Previous studies of axon growth of insect pioneer neurons by Bentley's lab have indicated that mesodermal cells are not required for elongation and pathfinding. We report that the formation of the stereotypical axon projection by the Fe_2 pioneer neurons in the limbs of cockroach embryos requires mesodermal cells. Using immunocytochemical and confocal microscopy techniques we show that Fe_2 neurons use the internal core of mesodermal cells as a substrate for growth. Surgical removal of the leg mesoderm and subsequent culturing of the embryos resulted in axonal growth on the epidermal epithelium but with an altered trajectory. Similar changes in path and substrate choice are observed in axons growing in embryos cultured *in vitro* in the presence of the enzyme phosphatidyl inositol phospholipase-C or certain glycosaminoglycans like heparan sulphate, heparan and dermatan sulphate. These results indicate that *in vivo* the Fe_2 axons prefer to grow on the surface of certain mesodermal cells. However after surgical manipulation or addition of chemical perturbants they will grow on ectodermal epithelial cells but with an altered path. There appears to be a myriad of guidance mechanisms of pioneer axon growth in the legs of insect embryos.
Supported by NIH grant NS 14295.

533.13

IDENTIFICATION OF AN EXTRACELLULAR MATRIX GUIDANCE CUE FOR THE PROXIMAL GROWTH OF INSECT LEG PIONEER AXONS. J.K. Nyhus*, B.A. Norbeck and J.L. Denburg. Biological Sciences Dept. University of Iowa, Iowa City, Iowa 52242.

Molecular gradients of environmental cues have been proposed to be important directional signals for the proximal growth of pioneer axons in embryonic insect legs. We have produced a monoclonal antibody (MAb) that binds to an extracellular matrix protein that is non-uniformly distributed along the proximal-distal axis of embryonic cockroach leg at the time of pioneer axon growth. This mAb was named PROD-2 because it labels the proximal parts of the leg more intensely than the distal end. The spatial and temporal distributions of the PROD-2 antigen make it a good candidate for an environmental guidance cue for Til pioneer axon growth. To demonstrate a role for the antigen in axon guidance, PROD-2 MAb is added to the medium in which whole embryos are cultured for 48 hours. Under control conditions, the Til axons grow and follow their normal path. Treatment with PROD-2 MAb has no effect on the rate of elongation of the Til axon, but alters the direction in which they grow. The perturbation was found to be dose dependent; with increasing concentration of the mAb there was an increase in the total number of pathfinding mistakes as well as an increase in the severity of the mistakes. These results indicate that the PROD-2 antigen is a guidance cue responsible for the formation of the stereotypical projection of the Til pioneer axons on their normal substrate.

533.14

LAMININ OLIGOPEPTIDE DERIVATIZED AGAROSE GELS FACILITATE THREE-DIMENSIONAL NEURITE EXTENSION IN VITRO AND ENHANCE PERIPHERAL NERVE REGENERATION IN VIVO. R. Bellamkonda*, J. Ranieri, M. Borkenhagen and P. Aebischer. Surgical Research Division, CHUV, Lausanne University Medical School, Switzerland.

The phenotypic expression of various cells is influenced by extracellular matrix (ECM) molecules. A three-dimensional (3-D), hydrogel based ECM equivalent tailored to support neurite extension from neural cells and promote nerve regeneration was developed. Oligopeptide domains GRGDSP, CDPGYIGSR, and IKVAV which are partly responsible for the cell attachment and neurite promoting properties of laminin (LN), were covalently immobilized to agarose gels. Embryonic day 9 (E9) chick dorsal root ganglia (DRG) and PC12 cells were suspended in three dimensions in derivatized agarose gels and their neurite extension was evaluated. Agarose gels derivatized with CDPGYIGSR and a cocktail of all three peptides, enhanced neurite extension from E9 DRGs while IKVAV derivatized gels inhibited neurite extension. However, IKVAV derivatized gels enhanced neurite extension from PC12 cells compared to underivatized agarose gels and gels derivatized with other LN oligopeptides. The effect of derivatized agarose gels on the regeneration of transected rat spinal dorsal roots was evaluated by using 6 mm long polymer guidance channels filled with CDPGYIGSR-agarose to bridge a 4 mm gap in a transected dorsal root model. After 4 weeks, significantly greater numbers of myelinated axons were observed in the channels filled with CDPGYIGSR-agarose gels compared to channels filled with underivatized agarose gels. Thus 3-D matrices carrying appropriate neuroactive peptides can be tailored to evoke specific responses from cells and tissues exposed to them. They can be used for 3-D neuronal culture and may serve to enhance regeneration by optimally presenting neurite promoting molecules to the regeneration environment.

533.15

AXONIN-1 PLAYS A ROLE IN GUIDANCE OF COMMISSURAL NEURONS IN THE CHICK SPINAL CORD. E.T. Stoeckli* and L.T. Landmesser. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

In chicken embryos the cell adhesion molecule axonin-1 has been shown to exist in both a secreted and a GPI-linked form. Its role in growth cone guidance and pathfinding of commissural neurons, which express it during initial axonogenesis, was tested by in-vivo injections of axonin-1 into the spinal cord central canal in early embryos (Stage 18-24). Dorsally located commissural neurons normally extend axons toward the floor plate in response to a diffusible factor. Upon reaching the floor plate they make a sharp turn to cross the midline, then turn rostrally along the contralateral border of the floor plate. In axonin-1 injected embryos, a substantial proportion of commissural axons did not cross the midline, but turned rostrally on the ipsilateral side; in addition they grew in a more defasciculated manner. Since Ng-CAM has been identified as a receptor for axonin-1 on DRG neurons, we tested whether injection of Fab fragments of anti-NgCAM would result in similar pathfinding errors. Although commissural axons grew in a more defasciculated pattern following anti-NgCAM injection, pathfinding errors did not occur. This suggests the existence of at least one other receptor for axonin-1 within the floor plate; saturation of this "floor plate" receptor by injected axonin-1 could lead to the observed misguidance of commissural neurons. The nature of this receptor is currently being investigated.

533.16

SUBSTRATES PATTERNED WITH A DOMAIN OF THE B2 CHAIN OF LAMININ GUIDE AXONS OF EMBRYONIC HIPPOCAMPAL NEURONS IN VITRO. M. Matsuzawa[1], R. S. Potember[1], F. F. Weight[2] and P. Liesi[2]*
1. Applied Physics Lab, The Johns Hopkins University, Laurel, MD 20723.
2. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Substrates patterned with synthetic peptides derived from the B1 and B2 chains of laminin were used to guide axons of embryonic hippocampal neurons in vitro. These substrates were formed by attaching the peptides to chemically patterned surfaces fabricated via deep UV lithographic procedures. Neurons grown on the patterned substrates (parallel lines of 5 μm width) developed a bipolar morphology, whereas neurons on uniform (non-patterned) substrates had randomly growing multiple neurites. Substrates patterned with peptide P20, derived from a neurite outgrowth domain of the B2 chain of laminin (Liesi et al, 1989), promoted directional neurite outgrowth and morphological maturation of neurons. Neurons on patterned P20 substrates expressed the 200 kDa neurofilament protein in the longer "axonal" process within a 24 hour cultivation. Substrates patterned with peptide P31, derived from a cell attachment domain of the B1 chain of laminin (Graf et al, 1987), did not support directional neurite outgrowth or axonal differentiation.

The present study provides direct evidence for a rôle of a neurite outgrowth domain of the B2 chain of laminin as an axonal guidance and differentiation factor for embryonic hippocampal neurons in vitro.

Liesi et al., (1989) FEBS Lett 244: 141-148.
Graf et al., (1987) Cell 48: 989-996.

533.17

Substance P Modulates Chemotropic Influences of Floor Plate on Developing Neurites in the Central Nervous System. C De Felipe, R.D.Pinnock° and S. P Hunt. (*Spon. BRA) MRC Laboratory of Molecular Biology, Division of Neurobiology, Hills Road, Cambridge, CB2 2QH, °Parke-Davis Neuroscience Research Unit, Robinson Way, Cambridge, UK CB2 2QB.

The floor plate, a neuro-epithelial structure found at the ventral midline of the developing central nervous system, has been shown to function in dorso-ventral patterning in the spinal cord and at later embryonic stages, in guidance of commissural axons by release of chemoattractants. We have found that embryonic rat floor plate cells express the neurokinin 1 receptor and that there is a transiently appearing subpopulation of commissural axons containing Substance P, the neuropeptide ligand for this receptor. Receptor coupling to an intracellular effector system in dissociated floor plate cells was established by monitoring the intracellular calcium levels of dissociated floor plate cells using the Ca^{+2} indicator fura-2. Bath application of 10 to 1000nM substance P or the selective NK1 receptor agonist $[Sar^9,Met(O_2)^{11}]$substance P^{12} for 120s produced a rapid and reversible increase in internal calcium levels which was concentration dependent. Prolonged application of sub-threshold concentrations (0.1-3nM) of substance P applied for up to 10min produced rhythmic oscillations in internal calcium levels. The NK2 and NK3 receptor agonists $[\beta\text{-}Ala^8]$neurokinin A-(4-10) and senktide (100nm) were inactive. 50nM concentrations of the rat selective NK1 receptor antagonist RP67580 blocked the response to substance P. Finally, Substance P analogues increased the amount of axon outgrowth from dorsal horn explants when co-cultured with floor plate in collagen gels. These results suggest that SP released from pioneering neuronal pathways can subtly change the extracellular environment and influence the growth and differentiation of developing neuronal systems by regulating the release of chemoattractants from floor plate cells.

533.18

THE FLOOR PLATE INHIBITS TROCHLEAR MOTOR AXON OUTGROWTH AT A DISTANCE IN VITRO. S.A. Colamarino*, and M. Tessier-Lavigne. Dept. of Anatomy, University of California, San Francisco, CA 94143-0452.

Floor plate cells at the ventral midline of the neural tube secrete a diffusible chemoattractant for some ventrally-directed axons. Netrin-1 and netrin-2, two recently identified vertebrate homologues of *C. elegans* UNC-6, possess this chemotropic activity. UNC-6 is required for accurate circumferential migrations in both the ventral and dorsal directions in the nematode, and may function by attracting axons that migrate ventrally and repelling those that migrate dorsally. This raises the question whether in vertebrates the floor plate (and netrins) repels dorsally migrating axons.

To test this hypothesis we have focused on the formation of the trochlear (IVth) nerve in the region of the junction between the midbrain and hindbrain. The cell bodies of neurons in the trochlear nucleus lie ventrally, but their axons extend circumferentially away from the floor plate to exit the brain dorsally. When explants of the ventral portion of the midbrain/hindbrain junction containing the trochlear nucleus are isolated from E11 rat embryos and cultured in collagen gels, a distinctive bundle of axons exits the dorsal cut-edge of the explants and projects into the collagen. Visualization of the cell bodies and axons with the motoneuron marker F84.1 (Prince et al, 1992) reveals that the axons appear to grow in a narrow corridor within the explant. When a floor plate explant is placed directly opposite the trochlear explant at a distance, the F84.1+ axon bundle grows through the neuroepithelium as usual but now stops when it reaches the cut edge and does not grow into the collagen. This inhibitory effect of the brainstem floor plate is mimicked by spinal cord floor plate. The possible involvement of netrins in mediating this long-range inhibition is under investigation.

533.19

IDENTIFICATION AND CHARACTERIZATION OF A NETRIN SYNERGIZING ACTIVITY (NSA). M. J. Galko*, T. Serafini, and M. Tessier-Lavigne. Dept. of Anatomy, Univ. of California at San Francisco, San Francisco, CA 94143-0452.

Floor plate cells at the ventral midline of the spinal cord secrete a factor(s) that promotes outgrowth of spinal commissural axons and reorients these axons *in vitro*. We recently identified two proteins, netrin-1, (which is highly expressed in floor plate) and netrin-2 (expressed at lower levels in ventral spinal cord) which possess the outgrowth-promoting and reorienting activities of the floor plate. Here we report the characterization of a biochemically distinct activity that potentiates the effects of the netrins. This activity, termed NSA (for netrin synergizing activity), was found in high salt extracts of embryonic brain membranes that had been depleted of netrins by passage over a heparin column in high salt. On young (E11) rat dorsal spinal cord, addition of NSA increased the potency of the netrins in promoting commissural axon outgrowth by one order of magnitude. Surprisingly, commissural axons in older (E13) spinal cord were more sensitive to the netrins, and, concomitantly, NSA had relatively little effect at this age. The reasons for the differences in responsiveness of commissural axons of different ages is not known but could be explained if E13 dorsal spinal cord produces NSA.

To determine whether the potentiation observed with E11 dorsal spinal cord is a true synergy, we identified a concentration of the netrins that, alone, evoked relatively little outgrowth from E11 explants, just as the standard concentration of NSA evoked little or no outgrowth. When E11 explants were cultured with the netrins and NSA at only half these defined concentrations, robust outgrowth was observed which cannot be accounted for by a simple additivity of their effects.

Initial experiments revealed that NSA is protease sensitive and has a molecular weight in excess of 100kD. Purification and identification of NSA will make it possible to determine the mechanism of the synergy and to determine whether NSA modulates netrin function and contributes to commissural axon guidance *in vivo*.

533.20

EXPRESSION PATTERN OF NETRINS SUGGESTS MULTIPLE ROLES IN AXON GUIDANCE AND CELL MIGRATION. J. R. de la Torre*, T. E. Kennedy, T. Serafini, E. D. Leonardo and M. Tessier-Lavigne. Dept. of Anatomy, Univ. of California, San Francisco, CA 94143-0452.

During the development of the spinal cord, commissural axons extend ventrally toward the floor plate. Previous studies have identified two related proteins, netrin-1 and netrin-2, homologues of the *C. elegans* protein UNC-6, which are capable of orienting commissural axons and promoting their outgrowth *in vitro*. Here we report the patterns of expression of the *netrin* genes during development. RNA *in situ* hybridization revealed that both *netrin* genes are expressed in the developing spinal cord during the period of commissural axon growth. *Netrin-1* is expressed in the floor plate from the earliest stages of floor plate development, extending from the caudal diencephalon to the spinal cord. *Netrin-2* is expressed at lower levels and more diffusely in the ventral neural tube at similar axial levels. Expression of both genes persists throughout the period of commissural axon growth, and well after commissural axons have reached the floor plate and projected to different axial levels. The *netrin* genes are also expressed in the developing forebrain. Northern analysis indicated that expression of both genes in the brain and spinal cord persists throughout development and into adulthood. Both are also expressed outside the nervous system. At early stages, *netrin-1* is expressed in the notochord as soon as it forms. *Netrin-1* and *netrin-2* are present in the developing paraxial mesoderm, and are significantly enriched in the developing dermamyotome. In addition, *netrin-1* is expressed in heart, and at lower levels in lung. *Netrin-2* is also expressed at high levels in the developing gastro-intestinal tract and lung, and at lower levels in heart. These patterns persist into the adult, where *netrin-1* expression is also detected in muscle and the ovary, and *netrin-2* expression is found in the spleen, ovary and testes. These diverse patterns of expression suggest that the netrins may function as chemotropic factors for multiple neuronal or migratory cell types.

FORMATION AND SPECIFICITY OF SYNAPSES IV

534.1

NEUROTROPHINS POTENTIATE NEURONAL EXCITABILITY BY INHIBITING GABAERGIC SYNAPTIC TRANSMISSION IN CORTICAL NEURONS. Han G. Kim*, Ti Wang and Bai Lu. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Neurotrophins (including NGF, BDNF, NT-3, NT-4/5) have traditionally been regarded as slowly acting signals essential for neuronal survival and differentiation. A recent study showed that BDNF and NT-3 can rapidly potentiate synaptic activity in developing neuromuscular synapses. However, little is known about the role of neurotrophins on functional synapses in the central nervous system. Here we report that NT-3 and NGF rapidly increased the frequency of spontaneous action potentials, and synchronized synaptic activities in developing cortical neurons. In addition, the inhibitory synaptic transmission mediated by $GABA_A$ receptors was reduced by NT-3. Thus, the excitatory effects of neurotrophins on spontaneous action potentials were attributable to a reduction of GABAergic transmission. Our finding, together with previous report of the rapid regulation of CNS neurotrophin expression by neuronal activity, suggest a new mechanism for modulation of synaptic transmission and activity-dependent synaptic plasticity.

534.2

NGP, a Synaptically Released Protein with Homology to Acute Phase Proteins of the Immune System.
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Division of Neuroscience, Dept. of Otorhinolaryngology, Dept. of Pathology, Baylor College of Medicine; Houston, Texas 77030.

We have identified, by affinity chromatography, a binding protein for the snake venom toxin, Taipoxin. The sequence of this 47 kD protein is unique, is suggestive of a secreted protein and has homology to the acute phase proteins, serum amyloid P protein and C-reactive protein, of the pentraxin family. Northern analysis reveals a brain specific message. In situ hybridization shows high NGP message levels in neurons of the cerebellum, CA3 and dentate gyrus of the hippocampus, layer 6 of the cortex with no message in glia. Antibody to this protein stains synapses on cerebellar Purkinje cell somas and glomeruli and a subset of glia. Reflecting this, we have named this protein, Neuronal-Glial Pentraxin or NGP. Because NGP appears to be released synaptically and has homology to immune proteins potentially involved in uptake of lipidic, toxic or other antigenic material, we suggest that NGP may be involved in the uptake of synaptic molecules during synaptic remodeling.

534.3

EFFECT OF UNILATERAL SENSORY DEPRIVATION ON SYNAPTIC VESICLE PROTEIN'S EXPRESSION IN RAT OLFACTORY BULB. B. Tavitian, I. Poignon, Kenneth L. Moya, O. Stettler*. INSERM U334, CEA, SFHJ, Orsay, France.

Little is known about the regulation of expression of the growing number of proteins identified on synaptic vesicles. One possible regulatory factor could be the level of neuronal activity, which in sensory systems is dependant on the level of afferent stimulation. We examined the effects of unilateral suppression of odor-mediated stimulation to the olfactory bulb (OB) on 3 synaptic vesicle proteins (SVPs): synaptophysin, SV2, and rab3a, a small GTP-binding protein of the ras superfamily.

The right nostrils of six newborn rats were cauterized under anaesthesia. The animals were sacrificed 6 weeks later, their brains removed and the left and right OBs immunostained using specific antibodies against the 3 SVPs. Tyrosine hydroxylase (TH) immunohistochemistry served as a control for the efficiency of the sensory deprivation. Sections were digitized and densitometry analysis performed in the glomerular (GLOM), the internal plexiform (PLEX) and the granular (GRAN) layers. The level of staining in the striatum was used as an internal standard to normalize between animals.

TH staining was reduced in the GLOM (-30%, $p<.0001$) but not in the other layers. Among the SVPs, only rab3a showed a reduction, both in the GLOM (-15%, $p<.0001$) and in the PLEX (-21%, $p<.002$). In contrast, staining for synaptophysin and SV2 was similar in all layers in the left and right OBs.

These results demonstrate that the level of expression of rab3a, but not of synaptophysin or SV2, are influenced by the afferent input to the OB. One possible interpretation is that rab3a would act as a regulatory element of SV trafficking, while synaptophysin and SV2 could be regarded more as "constitutive" components.

534.4

LONG TERM MAINTENANCE OF NERVE TERMINAL FUNCTION IN THE ABSENCE OF MUSCLE FIBERS IN THE FROG. A. Dunaevsky and E. A. Connor* Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003.

Although some of the signals involved in forming and maintaining the synaptic specializations of a target cell are now identified, little is known about the processes that lead to the differentiation and maintenance of the presynaptic nerve terminal. Here the role of the muscle fiber in the maintenance of nerve terminal function has been investigated. We demonstrate that motor nerve terminals permanently deprived of targets maintain the ability to release and recycle synaptic vesicles in response to stimulation. We assayed nerve terminal activity in preparations of innervated muscle basal lamina sheaths using a fluorescent dye FM1-43 that stains nerve terminals in an activity-dependent fashion. Innervated basal lamina sheaths were prepared by excising cutaneous pectoris muscle fibers without damaging nerve terminals. X-irradiation prevented regeneration of the muscle fibers. One to five months after muscle fiber excision, the function of target-deprived nerve terminals was tested by exposure to FM1-43 dye in high K^+ Ringer's solution. Synaptic sites were identified with rhodamine-labeled peanut agglutinin which stains terminal Schwann cell and synaptic basal laminae. Like intact preparations, nerve terminals deprived of target for 1-5 months incorporated FM1-43 when stimulated. Further, the same terminals were destained by either nerve stimulation or depolarization with high K^+ Ringer. The ability of these nerve terminals to release and recycle synaptic vesicles indicates that the molecular machinery required for vesicular release is maintained in a functional state for long periods without muscle.

534.5

NEURONAL SURFACE PHOSPHOPROTEINS ASSOCIATED WITH NEURITOGENESIS AND SYNAPTOGENESIS. Z. Pawlowska, W. Chen, M. V. Hogan, H-Ai Yang and Y.H. Ehrlich*. CSI/IBR Ctr. Dev. Neurosci., CUNY at Staten Island, NY 10314

Ecto-protein kinases provide the powerful regulatory machinery of protein phosphorylation to the extracellular environment of the nervous system, where delicate interactions among cells, and between surface proteins and components of the extracellular matrix, play significant roles in neuritogenesis and synaptogenesis. The present study identified proteins whose surface phosphorylation coincides with these developmental events. Cultured, embryonic chick-brain neurons possess proteins of MW 12K and 13K whose surface phosphorylation was maximal during the onset of neuritogenesis. Surface phosphorylation of such a low MW protein duplex was detected early in the development of pyramidal neurons cultured from embryonic mouse hippocampus, and this activity decreased after neuritogenesis was completed. Upon maturation and during synaptogenesis, the phosphorylation of another protein duplex (MW of 48K and 50K) apeared. As anticipated from the results listed above, we found that a hybrid, pyramidal-neurons cell line (HN9) derived from embryonic cells has high phosphorylation of the low MW protein duplex associated with neuritogenessis, and a more mature line (HN33) displayed the high surface phosphorylation of the 48K-50K protein duplex, associated with synaptogenesis. The functions of these surface protein phosphorylation systems can now be studied in homogenous populations of cloned neural cells.

534.6

PHOSPHORYLATION OF EXTRACELLULAR DOMAINS OF MICROTUBULE-ASSOCIATED PROTEIN (MAP) 1B MAY BE INVOLVED IN SYNAPSE FORMATION BETWEEN CORTICAL NEURONS. K.Muramoto*, M.Kawahara, K.Kobayashi, H.Taniguchi# & Y.Kuroda. *Department of Molecular & Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu-shi, Tokyo 183, Japan. and #Division of Biomedical Polymer Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake-shi, Aichi 470-11, Japan.*

Synapse formation between cultured rat cortical neurons is inhibited by the continuous application of K-252b, an ecto-protein kinase inhibitor, which can not permeate the cell membrane (Muramoto, *et al.*, Proc.Jpn.Acad., **64**, 319, 1988; Kuroda, *et al.*, Neurosci.Lett., **135**, 255, 1992). In order to identify membrane proteins which may be involved in synapse formation, [γ-^{32}P]ATP was applied to the cultured cells for brief periods to phosphorylate their extracellular domains. The phosphorylated proteins were separated by SDS polyacrylamide gel electrophoresis and detected by autoradiography. Some of these bands were immediately phosphorylated and this phosphorylation was suppressed by the addition of K-252b to the medium. We examined the partial amino acid sequence of these substrates. Phosphorylated bands were cut from the gel and digested with lysyl endopeptidase. Peptide fragments were separated by capillary HPLC and analyzed by mass spectrometry. The band with the highest molecular weight, whose phosphorylation was strongly inhibited by K-252b, was identified as microtubule-associated protein (MAP) 1b. These results suggest the possibility that the phosphorylation of extracellular domains of MAP1b in involved in synapse formation between cortical neurons.

534.7

THE NITRIC OXIDE/ CYCLIC GMP SYSTEM IS INVOLVED IN THE MATURATION OF INSECT NEURONS J.W.Truman & E.E.Ball*. Res. Sch. Bio. Sci., Australian Nat. Uni., Canberra, ACT, 2601 Australia

We have used immunocytochemical techniques (De Vente et al., 1987, Neuroscience **22**:361) to monitor cGMP changes in identified neurons in Locusta migratoria in response to agents such as sodium nitroprusside (SNP) that generate nitric oxide (NO). Although relatively few neurons respond to SNP during postembryonic life, most motor, sensory and interneurons show a window of NO sensitivity during embryogenesis. Observations on identified neurons such as RP2, ACC and the H cell show that NO responsiveness begins after the completion of axon navigation, is strong throughout axonal branching, and ends after synaptogenesis is apparently completed. During this embryonic window, the main site of cGMP accumulation is the nucleus, possibly providing a direct link between cell-cell signaling and transcription. Using NADPH-diaphorase staining to detect possible sites of NO synthase, we found diaphorase staining first associated with nerve fibers at 40-45% of development and it increased in intensity in the neuropil through the remainder of development. These observations suggest that the maturational phase of neurons is a period uniquely characterized by the presence of the NO/cGMP signaling system and of nuclear targets for cGMP. NO-synthase inhibitor experiments are being used to define the exact role of this system in neuronal maturation.

534.8

BLOCKADE OF SPONTANEOUS NEURAL ACTIVITY IMPAIRS THE DEVELOPMENT OF THE DENDRITIC SPINES IN CULTURE. Teng Wu*, Mark J. Maguire, Ford F. Ebner. Institute for Developmental Neuroscience, John F. Kennedy Center, Vanderbilt University, Nashville, TN 37203

Blocking patterned neural activity has been shown to prevent the normal development of dendritic spines *in vivo*. We have investigated whether spontaneous neural activity is necessary for induction of spine formation *in vitro*. Neurons from rat somatic sensory cortex were enzymatically dissociated on postnatal day 1 and plated on a glial monolayer. Pyramidal neurons were injected with Lucifer yellow at the end of 2, 3 or 4 weeks in culture to label dendritic spines. Injected neurons were fixed with 4% paraformaldehyde, dehydrated and mounted in krystalon before imaging them with a Leica confocal microscope. Confocal images of control cultures showed only a few dendritic spines after two weeks in culture, whereas pyramidal neurons were densely covered with spines by the end of weeks 3 and 4. Mushroom, stubby, long-thin and branched dendritic spines were clearly discernible in the confocal images. Tetrodotoxin (1 μM, TTX) added to the culture medium interfered with the development of dendritic spines depending on the onset and duration of exposure. The presence of TTX throughout weeks 1,2,3 or only weeks 2,3 dramatically decreased the number of spines when examined at the end of week 3. TTX exposure only during week 2 blocked the development of dendritic spines at the end of week 3; however, high densities of dendritic spines did develop by the end of week 4. This delay in spine initiation suggests that induction of dendritic spine formation is critically dependent upon spontaneous neural activity. (Supported by NS-13031)

534.9

DEVELOPMENTAL ELECTRICAL ACTIVITY CHANGES IN CULTURED CORTICAL NEURAL NETWORKS P. J. Charlety, Y. Jimbo, E. Maeda, H. Kamioka, and A. Kawana* NTT Basic Res. Labs., Atsugi, Kanagawa, 243-01 Japan

Long term multi-site monitoring of electrical activity in neural networks should yield insight into the mechanisms of network development. Here we describe the long term profile of electrical activity in both dissociated and organotypically cultured cortical neural networks of rat, recorded using planar electrode arrays (PEA).

The methods for fabricating the PEA and culturing the dissociated cortical neurons were described before (Y. Jimbo et al, IEEE Trans BME). In the case of organotypic cultures, P0 cortical slices were fixed on the PEA using coagulated chicken plasma and cultured in the same way as dissociated cultures. The electrical activities were measured under culture conditions. At 4 or 5 days *in vitro* (DIV), periodic bursting appeared in the dissociated culture, with a period of more than 20 sec and large delays between burst initiation between the electrodes. The period and the delay became shorter and shorter with further days of culture. As these burstings were reversibly inhibited by TTX and APV, they are accompanied by transient Ca^{2+} increases as described before (Robinson et al, J. Neurophys.). After 21 DIV, the electrical activity changed into a more complex profile consisting of burstings and spiking which appeared to be inherent or stable activity profile of the networks. In the case of organotypic culture, no signals was observed through electrodes until 7 DIV. Periodic bursting appeared from 8 DIV and changed into a more complex profile from 11 DIV. Taking into the consideration the data on the development of NMDA receptors and inhibitory synapses in the cortex, these results suggest that the periodic bursting effects a change in the networks from a developmental stage to the inherent one.

534.10

LAMININ AND NEUROPEPTIDE Y ARE INCREASED BY SYNAPSIN TRANSFECTION IN CULTURED NG108 NEUROBLASTOMA/GLIOMA HYBRID CELLS. G. Fried*, H-Q. Han, B. Meister, T. Hökfelt and P. Greengard. Dept. of Molecular and Cellular Neuroscience Rockefeller University, New York, Dept. of Physiology, Pharmacology and Neuroscience, Karolinska Institute, Stockholm, Sweden

We have investigated the presence and expression of some neuronal markers in several NG108 cell lines transfected with synapsin Ib, IIa or IIb and differentiated with prostaglandin E_1 plus IBMX. Laminin, a basal membrane glycoprotein that promotes adhesion and induces neurite outgrowth and neuronal differentiation, was increased in all transfected cell lines examined. Neuropeptide Y (NPY) was also increased in all transfected cell lines examined. Immunohistochemical analysis combined with confocal laser microscopy showed that NPY staining was granular and very often enriched in neuritic varicosities. The distribution and the staining pattern of NPY was consistent with storage of NPY in large dense cored vesicles. The catecholamine-synthesizing enzymes tyrosine hydroxylase (TH) and dopamine -B-hydroxylase (DBH) were more frequently expressed in transfected cell lines, although both were also present in mock-transfected cell lines. The results indicate that, in differentiated neurons, the synapsins increase the levels of a biochemical marker for large dense cored vesicles and of an extracellular matrix protein associated with neuronal maturation.

NEUROTRANSMITTER SYSTEMS AND CHANNELS: OTHER TRANSMITTERS

535.1

Embryonic Epinephrine Synthesis: Association with Cardiogenesis S.N. Ebert*, M. Fujinaga, J.M. Baden, B. Siddall and D.L.Wong, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305

Phenylethanolamine N-methyltransferase (PNMT) is the final enzyme in the pathway for epinephrine synthesis; hence, PNMT serves as a marker for the cells which produce this neurohormone/neurotransmitter. While much is known about the developmental expression of PNMT in the adrenal gland and the brain, very little is known about its developmental expression elsewhere. We demonstrate that PNMT mRNA can be detected in rat embryos as early as gestational day 9.5. By gestational day 11, PNMT mRNA and active enzyme can be specifically loclalized to the heart. Immunocytochemical analysis further reveals that PNMT protein is predominantly contained within the embryonic cardiac cells. Our results therefore suggest that the embryonic heart is the primary site of PNMT expression early in development. The presence of PNMT mRNA, enzyme activity and protein well before the appearance of nerve cells around the heart suggests that the embryonic heart may be capable of synthesizing epinephrine during early cardiogenesis. Since cardiac cells are already beating and can respond to epinephrine at this stage of development, our findings indicate a potential role for epinephrine in early cardiac function.

535.2

EVIDENCE FOR DOPAMINE RECEPTOR PRUNING IN CORPUS STRIATUM BUT NOT IN NUCLEUS ACCUMBENS M.H. Teicher*, J. C. Hostetter, & S.L. Andersen. Mailman Research Center, McLean Hospital, Harvard Med. Sch., Belmont, MA 02178.

The most important developmental change to take place in the brain immediately before adulthood, is the pruning of overproduced synapses or receptors. Previously, we demonstrated that dopamine (DA) D_1 and D_2 receptors density in rat striatum peaked at approximately 40 days of age (oset of puberty), and declined by 58-75% by adulthood (120 days). In this experiment, we sought to more specifically establish the time course of synaptic elimination in the striatum and to compare the time course in the striatum with the nucleus accumbens.

D_1 and D_2 receptor density were determined in 9 male rats at 40, 60, 80, 100, and 120 days of age (n=45) with quantitative autoradiography. D_1 receptors were assessed using [^{3}H]-SCH-23390, while D_2 receptors were quantified using [^{3}H]-YM-09151-2. Binding data were corrected for protein content. We found that pruning occurred predominately between 40 and 60 days of age. For the D_1 receptor system, 91% of DA receptors lost between 40 and 120 days, were lost by day 60. Bmax decreased from 1583 ± 53 at day 40 to 790 ± 22 by day 120. In the D_2 system, the decline in receptor number appeared to take place more gradually (Bmax at day 40 was 941 ± 39 , declined to 672 ± 23 by day 60, and 535 ± 21 by day 120). In contrast, we found no evidence for either D_1 or D_2 receptor elimination in the nucleus accumbens (Bmax increased from 890 - 962 for D_1, and 380 - 445 for D_2 between 40 and 120 days).

This finding suggests that synaptic elimination may not be a universal phenomenon. An alternative possibility is that elimination occurs at an earlier age. We are in the process of investigating this possibility.

535.3

NEONATAL 6-OHDA LESIONS ALTER THE DEVELOPMENTAL EXPRESSION OF STRIATAL DA D1 RECEPTORS AND D1 mRNA. P.A. Frohna*, B.S. Neal-Beliveau, and J.N. Joyce. Depts. Psychiatry and Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA, 19104.

The postnatal developmental profile of striatal dopamine innervation and dopamine receptor expression suggest that the two may be related. Our previous studies have shown that adult rats that received postnatal day 1 (P1) 6-OHDA lesions have a significant loss of D1 receptors and a significant increase in D1 mRNA in the dorsolateral (DL), dorsomedial (DM) and ventromedial (VM) caudate (CPu), regions with the greatest loss of TH-positive fibers. Thus, normal DA innervation during postnatal development may be important for the appropriate control of DA D1 mRNA and receptor expression. To determine the temporal relationship between the loss of DA innervation and the changes in DA receptors/mRNAs we have examined the developmental profile of D1 receptors and D1 and D2 mRNA in rats with P1 6-OHDA lesions.

Lesion-induced changes in D1 receptor binding were found within a week of the lesion (P7) with significant ($p<.05$) losses in the DM (~11%) and VM (~15%) CPu that coincided with a significant loss of D1 mRNA (~10% & ~18% respectively). D1 receptors remained significantly decreased in the DL (~9-16%), DM (~10-16%), and VM(~9-15%) CPu at P14, P35, and P90 . However, in the medial CPu there was a rebound by P14 when lesioned animals showed a significant increase(+20-29%; $p<.05$) in D1 mRNA. This increase in D1 mRNA was also seen at P35 in the DL (+15%), DM (+19%), and VM (+20%) CPu. This suggests that the earliest postnatal DA innervation may be necessary for the initial expression of D1 receptors and the appropriate coupling between D1 mRNA and D1 receptor expression. 6-OHDA lesions initially decreased D2 mRNA (~10%; $p<.05$) in the DL CPu of the P7 group, but was significantly increased by P14 in the DL (+10%) and DM (+8%) CPu. D2 mRNA levels returned to control levels by P35, similar to P90. Therefore, P1 6-OHDA lesions cause only a transient upregulation of D2 receptor mRNA. These results further suggest that the developmental expression of striatal D1 and D2 receptors/mRNAs are differentially regulated, and that D1 receptors require normal DA innervation for proper expression. This work supported by MH 48813.

535.4

PERINATAL INSULTS TO DEVELOPING DOPAMINE SYSTEMS IN RAT. K.C. Langley* and C.C. Quimet. Program in Neuroscience, Florida State University, Tallahassee, FL 32306.

Perturbation of developing dopamine systems in neonatal rat brain may lead to cytoarchitectural restructuring that persists into adulthood. Such disarrangements allow a functional compensation for the animal and yet provide an excellent opportunity for investigation of pathology proposed for neurological disease states. In these studies day-old Sprague-Dawley rat pups received injections of the neurotoxin 6-hydroxydopamine (6-OHDA) into striatum or prefrontal cortex (PFC). These brain regions reflect differentially organized dopamine systems: the striatum is a dopaminoceptive target of the substantia nigra (SN) while prefrontal cortical regions receive dopamine from the ventral tegmental area. Immunohistochemistry for tyrosine hydroxylase was employed to assess effects of experimental manipulations. As expected, cell body staining in the SN was absent from those rats who had received 6-OHDA injections in striatum. Apparent axonal sprouting was observed in prefrontal cortical areas of rats who had been subjected to 6-OHDA in PFC. Such sprouting was not seen in control animals nor in rats who had received striatal 6-OHDA lesions. These results suggest that an early insult to developing dopamine systems can lead to viable aberant neuronal circuitry in PFC. Supported by Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A. and the Bryan W. Robinson Neurological Foundation, Inc.

535.5

D_1 DOPAMINERGIC AGONISTS INCREASE THE EXPRESSION OF DYNORPHIN AND C-FOS IN PRIMARY STRIATAL CULTURE OF RAT.

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The effects of dopamine receptor agonists on the expression of dynorphin (DYN) and Fos or Fos related antigens (FRA) were studies in primary striatal cultures from 7-day old rat pups. Levels of DYN, c-Fos or FRA immunoreactivity and their mRNAs were investigated by using immunocytochemistry, *in situ* hybridization and radio immunoassay techniques. Activation of DA receptors by (±)-chloro-APB hydrobromide (APB), a specific D_1 dopamine receptor agonist, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal culture and increased DYN level in the media 3 to 4 fold compared to control or quinpirole, a specific D2 agonist. Apomorphine, a mixed D1 and D2 agonist, also increase the level of DYN in the media to 2 fold. *In situ* hybridization experiments showed that both DYN and c-fos mRNA were increased by APB treatment but not by quinpirole. In order to further understand the cellular mechanisms underlying the regulation of DYN expression by the dopamine system, the effects of forskolin, an adenylate cyclase activator, were studied. Forskolin caused an increase in the number of both DYN and c-Fos positive cells indicating that cAMP may be involved in the regulation of DYN expression.

535.6

DEVELOPMENT OF SEROTONIN UPTAKE SITES IN BRAIN REGIONS OF THE OFFSPRING OF CONTROL AND ETHANOL-FED RATS.

J.-A. Kim* and M.J. Druse-Manteuffel. Neuroscience Program and Molec. & Cell. Biochem. Loyola U. Med. School, Maywood, IL 60153.

This laboratory previously demonstrated that in utero ethanol exposure markedly impairs the development of the serotonergic system. As an extension of these studies we examined the effects of in utero ethanol exposure on the development of serotonin (5-HT) uptake sites in multiple brain areas. [^{3}H]-Citalopram was used to radiolabel 5-HT uptake sites on twenty micron brain sections. Brains were obtained from 19- and 35-day-old offspring of pair-fed control, pair-fed ethanol or ad libitum control dams that consumed a liquid diet on a chronic basis prior to parturition. The relative density of [^{3}H]-citalopram binding in each brain area was determined using the NIH Image program.

In all brain areas examined [^{3}H]-citalopram binding was comparable in the age-matched offspring of pair-fed and ad libitum control rats. In addition, the offspring of ethanol-fed dams demonstrated a normal concentration of 5-HT uptake sites in the dorsal and median raphe at 19 and 35 days of age. However, [^{3}H]-citalopram binding appears to be decreased in the hippocampal CA3 region and in the substantia nigra of the ethanol-exposed offspring.

This research was supported by the USPHS - AA03490.

535.7

CENTRAL ADMINISTRATION OF rhGDNF CAUSES AUGMENTATION OF DOPAMINERGIC ACTIVITY IN VIVO.

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Glial-derived neurotrophic factor (GDNF) a member of the TGF-β superfamily was recently cloned, expressed, and shown to manifest potent trophic activity for embryonic midbrain ventral mesencephalic dopaminergic neurons in vitro (Lin et al., 1993).

The present studies were designed to investigate the behavioral and neurochemical effects of rhGDNF (0.1-100μg/μl) or vehicle (2μl) after administration into either the striatum, substantia nigra or intraventricular (icv) in F344 male rats. The injection of rhGDNF into either of the three sites produced dose-dependent increases in motor activity when compared to vehicle controls. Motor activity returned to baseline within 7-10 days for striatal or icv administered rhGDNF. This effect was extended (14-21 days) when rhGDNF was administered into the substantia nigra.

Neurochemical analysis was performed on the substantia nigra at the two week time point, dopamine levels were elevated in the substantia nigra compared to the contralateral non injected side. The increase in dopamine levels by rhGDNF was dose-dependent and was not dependent upon whether rhGDNF was given into the striatum or substantia nigra. Eight weeks post rhGDNF administration into the substantia nigra, dopamine levels were back to control levels.

Taken together, these results suggest that rhGDNF elicits an augmentation of the substantia nigra dopaminergic system in vivo. Therefore, rhGDNF may be worth investigating in restoring the compromised dopaminergic system in Parkinson's disease a neurodegenerative disorder.

535.8

DEVELOPMENTAL PROFILE OF VARIOUS CHOLINERGIC MARKERS IN THE RAT MAIN OLFACTORY BULB USING QUANTITATIVE AUTORADIOGRAPHY.

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The main olfactory bulb (OB) is considered to be a good model of centrifugal cholinergic innervation in a cortical region. Accordingly, we investigated the development of various cholinergic markers in the OB of Wistar rats at post-natal days 2, 9, 14, 20, 30, and in 3 month-old adults. Using in vitro receptor autoradiography, the comparative profiles of the high affinity choline uptake ([^{3}H]hemicholinium-3, HC-3), acetylcholine vesicular transporter ([^{3}H]vesamicol, Ves), muscarinic M1 and M2 ([^{3}H]pirenzepine and [^{3}H]AF-DX 384, respectively) and nicotinic ([^{3}H]cytisine) receptors were determined. Well established pre-synaptic cholinergic markers ([^{3}H]Ves and [^{3}H]HC-3) were not necessarily in close register with either muscarinic M1 and M2, and nicotinic receptors in both immature and adult OB. For example, a subset of atypical olfactory glomeruli known to receive strong and precocious cholinergic centrifugal inputs was most enriched with HC-3 and Ves labelling but only contained low amounts of M1 and M2 sites. Developmentally, most markers exhibited their lowest levels of expression at birth increasing gradually during the first 4-5 post-natal weeks to reach their adult level. Interestingly, both [^{3}H]Ves and [^{3}H]HC-3 bindings were relatively abundant early after the birth (day 2) preceding the clear appearance of acetylcholinesterase (AChE) staining in the atypical glomeruli (Le Jeune and Jourdan, 1991, J. Comp. Neurol. 314). A transient over-expression of [^{3}H]Ves sites was noted around day 20th, corresponding to the increase in AChE staining in intrinsic neurons. The ontogenetic profiles of M1 and M2 receptors were rather similar in all laminae of the OB, except for a somewhat earlier appearance of the M1 sub-type. Thus, presynaptic cholinergic markers appear to develop earlier than muscarinic M1 and M2 receptors during the post-natal ontogeny of the OB.

535.9

EXPRESSION OF SOLUBLE AND MEMBRANE CHOLINE ACETYLTRANSFERASE DURING DEVELOPMENT.

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Choline acetyltransferase (ChAT) exists as soluble and membrane forms. However, the developmental changes of these isoforms during fetal and postnatal life are currently unknown. In this study, the brains of rat pups at various ages were obtained to assess the developmental changes of ChAT activities in these brains. Rats were sacrificed by decapitation at 7, 20, and 30 days after birth and their brains were removed. Brains were homogenized in a glass-teflon motor driven homogenizer, and soluble and membrane ChAT fractions were prepared by differential centrifugation. ChAT activity was assayed radiochemically. At 7 days of age, each brain contained soluble ChAT of 2.7 units (U: nmol ACh formed/min) and membrane of 1.1 U with specific activity (s.a.: U per mg protein) of 0.22 and 0.15, respectively. ChAT activity was drastically increased with increasing age. At 20 and 30 days of age, soluble ChAT increased 12-fold to 33 UGW (units/gram wet weight) and 16-fold to 43 UGW with s.a. of 1.1 and 1.3, respectively, while membrane ChAT increased 12-fold to 13 UGW and 14-fold to 15 UGW with s.a. of 0.51 and 0.62, respectively. Furthermore, there was a higher percentage of membrane ChAT at a younger age (7 days) as compared to soluble ChAT at an older age (20 and 30 days).

535.10

DEVELOPMENT OF BUTYRYLCHOLINESTERASE-POSITIVE CELL POPULATIONS IN THE MESENCEPHALON OF THE RAT.

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The enzyme butyrylcholinesterase (BuChE, E.C. 3.1.1.8) is found in a distinct population of midline mesencephalic neurons which project to the spinal cord. BuChE has been implicated in neuronal development and neurotransmission, and may function in development prior to the appearance of acetylcholinesterase. Therefore, we have investigated changes in the spatiotemporal distribution of this enzyme during late prenatal and early postnatal development in the midline mesencephalic area which includes the region containing cilary preganglionic neurons. Brain sections from rats at ages E14, E16, E19, P0, P7 and P14 were stained for BuChE using a modification of the histochemical method of Karnovsky and Roots, 1964. Adjacent sections were stained with AChE, ChAT and NADPH-d histochemistry, and their distributions were compared to that of BuChE. The intensity of BuChE staining within neurons located in the region of the mesencephalon increased with age. At days E14 and E16 somata within the region of the medial tegmental neuroepithelium, ventrolateral to the cerebral aqueduct, exhibited a light BuChE stain. At P0 the corresponding mesencephalic region showed a much more intense BuChE stain than seen in the embryo but the distinct pattern of distribution seen in the adult had not yet emerged. By P7 the staining pattern of these neurons approximated the adult BuChE distribution. P14 showed the same spatial distribution of BuChE that is seen in the adult, with comparable BuChE cell populations in the midline mesencephalon, anterior median nucleus, nucleus Darkschewitsch and the nucleus raphe dorsalis. The results show that BuChE can be used as a developmental marker for populations of neurons in the mesencephalon, and changes in the distribution of BuChE may reflect the maturation of neural structures within this region. Supported by the MRC and the Scottish Rite Charitable Foundation of Canada.

535.11

BRAIN AND ENDOTHELIAL NITRIC OXIDE SYNTHASE EXPRESSION IS REGIONALLY AND TEMPORALLY REGULATED IN FETAL SHEEP BRAIN. F.J.Northington, R.C.Koehler, R.J. Traystman*, and L. J. Martin. Dept. of Pediatrics, Anesthesiology/Critical Care Medicine, Pathology, and Neuroscience. The Johns Hopkins Medical Institutions, Baltimore, MD 21287.

Using immunohistochemistry, we tested the hypothesis that brain NOS (bNOS) and endothelial NOS (ECNOS) expression is regulated developmentally in fetal sheep brain at 60, 71, 93, 110 and 136 days (d) of gestation (term=145d). No nonspecific immunoreactivity (IR) was found if the 1^0 antibody was omitted; no cross reaction between bNOS and ECNOS was seen at any gestational age; and neither isoform was localized to glial cells. At 60d, bNOS has a laminar localization in neuropil of the lower cortical plate of the telencephalic vessicle which becomes less distinct as the cortical plate matures, dissipating by 136d. A subset of nonpyramidal cortical neurons first shows discrete bNOS IR at 71d. By 136d, two subsets of interneurons with somatodendritic IR for bNOS are dispersed throughout cerebral cortex. Regional differences in bNOS IR within cortex occur at 71 and 93d but not at 110 and 136d. IR for bNOS in striatum increases from first appearance at 71d to peak density of bNOS-positive medium sized aspiny neurons at 110d. Labeling of cerebellar basket cells peaks at 136 days. The vascular bed expresses ECNOS throughout gestation. The radial vascular architecture evoles into a complex branching pattern between 60 and 110d. A dense capillary bed with ECNOS IR is found within white matter in contrast to the relative absence of bNOS IR in this area. By 136d, ECNOS in vascular beds of brainstem, diencephalon, basal ganglia, and cortex is homogeneously distributed, in contrast to the absence of ECNOS IR in the ventricular zone. We conclude that bNOS and ECNOS are present prior to midgestation in the fetal sheep, and the developmental regulation of NOS expression, particularly bNOS, parallels regional brain maturation.

535.12

EFFECTS OF SERA ON INDUCTION OF NADPH DIAPHORASE ACTIVITY (NADPH-DA) IN CULTURED PRINCIPAL NEURONS OF THE SUPERIOR CERVICAL GANGLION (SCG) OF THE POSTNATAL RAT. J.A. Garcia, M.D. Johnson and D.D. Potter*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Induction of NADPH-DA was reported in dissociated and cultured SCG neurons of the postnatal Long Evans rat by Senatus et al., 1992. Characterization of the time course and level of induced NADPH-DA in cultured SCG using growth medium with 5% normal rat serum (NRS; Sigma) demonstrated NADPH-DA in less than 1% of SCG neurons at time of plating and in about 80% of neurons after 1 week in culture. The inductive effects of 5% NRS have now been compared to growth medium supplemented with 5% dialyzed fetal calf serum (dFCS; 10,000 MW cut-off; Sigma). SCG neurons cultured in 5% dFCS also displayed a positive inductive effect on NADPH-DA, but the percentage of neurons expressing NADPH-DA was significantly lower (<1% at time of plating and about 30-40% after 1 week in culture). Culture in a serum-free medium resulted in 15-20% of neurons expressing NADPH-DA after 1 week, and culture in growth medium supplemented with 5% heat inactivated serum resulted in 20-25% of neurons expressing NADPH-DA after 1 week.

To characterize further the time course and nature of these positive inductive effects, a serum reversal experiment was performed. Neurons cultured in 5% NRS were switched to 5% dFCS and vice versa. Serum reversal on culture Day 19 had no subsequent effect on the percentage of NADPH-DA positive neurons. Serum reversal on culture Day 6-7 had the following effects: neurons cultured first in 5% NRS and later switched to 5% dFCS maintained an 80% level of NADPH-DA (no change); however, neurons cultured first in 5% dFCS and later switched to 5% NRS went from a 30-40% NADPH-DA level to an 80-85% level of NADPH-DA. These data suggest that the factor(s) in serum that promotes induction of NADPH-DA is heat labile and is effective under these conditions in only a limited period in culture. Supported by PO1 NS 02253 and the Freudenberger Fund.

535.13

REGULATION OF EXPRESSION OF NADPH-DIAPHORASE ACTIVITY (NADPH-DA) AND NEURONAL NITRIC OXIDE SYNTHASE (NOS) IN PRINCIPAL NEURONS OF RAT AND MOUSE SUPERIOR CERVICAL GANGLIA. **MD Johnson*, JA Garcia,PB Senatus,DG Green, and DD Potter, Department of Neurobiology,Harvard Medical School, Boston, MA 02115**

Preganglionic sympathetic axons of adult rats and mice express NADPH-DA and NOS immunoreactivity *in vivo*; however, principal neurons of the superior cervical ganglion (SCG) express these markers for NOS lightly or not at all (e.g. Grozdanovich et al, 1992, Neuroscience 48:225; Dun et al 1993, Neurosci. Lett. 158:51; Johnson et al, unpublished). When postnatal rat and mouse SCG neurons (and some adult SCG neurons) were cultured for 1 week in medium containing 5% rat serum, however, NADPH-DA was visibly induced in about 80% of such cells; the staining varied from barely detectable to intense (Senatus et al, 1992, Abstracts 18:772). In many neurons, NADPH-DA was co-localized with immunoreactivity for the neuronal isoform of NOS (antibody provided by Dr. Bernd Mayer). In mice homozygous for deletion of the neuronal NOS gene (provided by Dr. Paul Huang; cf Huang et al, 1993, Cell 75:1273), and in which there was no NADPH-DA in the preganglionic sympathetic fibers *in vivo*, clear light NADPH-DA was present in principal neurons in adult ganglia, and in cultured postnatal neurons; the staining of the cultured neurons was significantly lower than in neurons from wild-type mice. The presence of numerous ganglionic non-neuronal cells in the cultures reduced the proportion of NADPH-DA stained cells. Supported by NS02253 and the Freudenberger Fund. We thank Kara Dunn for technical assistance.

535.14

MEASUREMENT OF NEUROTENSIN (NT), NT1-11 AND NT1-8 IN THE DEVELOPING RAT BRAIN S. H. Moss, G. W. Bennett and C. A. Marsden (SPON: Brain Research Association) Dept. Physiology and Pharmacology, Medical School, Queens Medical Centre, Nottingham, NG7 2UH, UK.

Neurotensin (NT) appears to function as a neurotransmitter or neuromodulator in the adult rat brain, whilst in the developing rat brain, an early transient appearance of NT precursor mRNA in some brain regions (Sato et al. (1990) Dev. Brain Res. 54:249-255), suggests a developmental role for NT, perhaps in the establishment of neuronal organisation.

In the present study NT-LI has been quantified in the diencephalon, telencephalon and ventral mesencephalon of Wistar rat brains aged from embryonic day 15 (E15), through birth (22nd day of gestation), to postnatal day 2 (P2), using a sensitive radioimmunoassay technique (0.9 fmoles/tube).

NT-like immunoreactivity (NT-LI) appeared during the prenatal period in all three regions studied and exhibited two general ontogenic patterns. In the diencephalon and telencephalon NT-LI levels peaked at birth and E20, respectively, then declined, whilst, in the ventral mesencephalon, where levels were approximately 25% of those in the diencephalon and telencephalon, NT-LI after an initial increase reached a plateau around E18, which was maintained through to P2.

The polyclonal NT antibody used was N-terminally directed and thus recognised and measured the NT metabolites, NT1-8 and NT1-11, in addition to NT itself. Therefore, a High Pressure Liquid Chromatography (HPLC) method, utilising a methanol gradient from 30% to 60% over 30 minutes, was developed to separate these three peptides prior to measurement by a RIA of increased sensitivity (0.3 fmoles/tube). The latter two techniques are thus presently being used to quantify the proportions of NT, NT1-11 and NT1-8 in foetal rat brain regions.

535.15

BRAIN DEVELOPMENT DURING SMOLT TRANSFORMATION IN SALMON MAY BE AFFECTED BY ENVIRONMENTAL CONDITIONS. L.O.E. Ebbesson, K. Drew and S.O.E. Ebbesson*, Institute of Marine Science and Institute of Arctic Biology, Univ. of Alaska, Fairbanks, AK 99775.

Smolt transformation (ST), which occurs midlife, is associated with olfactory imprinting and dramatic physiological adaptations. Our studies have also revealed that it is a critical period of neural development characterized by new neural connections, sequential surges of neurotransmitters, and receptors of various types. These events probably reflect orderly changes in the brain necessary for subsequent survival and return to the natal stream.

It is well known that ST is affected by environmental factors such as light and water temperature. Significantly different return rates have been observed for chinook salmon reared under normal conditions as compared to those whose growth and maturation has been accelerated by rearing in warm water. We have begun studies to determine if brain development necessary for olfactory imprinting and survival is the same in different rearing conditions. We report here that during ST, there are significant differences in the surges of select brain monoamine and amino acid neurotransmitters under the two rearing strategies. The plasma thyroxine levels are also different. The significance of these findings in relation to normal versus inadequate brain development are, however, not known and require further investigations. Supported by the Alaska Sea Grant Program and the NIH.

536.1

COORDINATED REGULATION OF *TRKA* AND *CHAT* EXPRESSION IN DEVELOPING RAT BASAL FOREBRAIN: EVIDENCE THAT ENDOGENOUS NGF REGULATES CHOLINERGIC DIFFERENTIATION THROUGH TRKA. Y. Li, D.M. Holtzman, L.F. Kromer, D.R. Kaplan, J. Chua-Couzens, D.O. Clary, B. Knusel, W.C. Mobley*. Dept. of Neurology & Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143; Dept. of Cell Biology, Georgetown Univ. Med. Ctr. Washington, DC 20007; Eukaryotic Signal Transduction Group, ABL-Basic Research Program, NCI-Frederick Cancer Research & Dev. Ctr., Frederick, MD 21702; & Div. of Neurogerontology, Andrus Gerontology Ctr. & Dept. of Biological Sciences, USC, Los Angeles, CA 90089.

To understand the role of NGF in the differentiation and maturation of basal forebrain cholinergic neurons (BFCNs), we examined the expression of trkA in rat BFCNs during postnatal development. Using in situ hybridization, we have previously shown that trkA and ChAT are colocalized to the same group of cells throughout development. In the present work, we further characterized the regulation of trkA expression in BFCNs during development. Northern analysis showed that expression of trkA was highly regulated and the temporal pattern was very similar to that for ChAT, suggesting that expression of both genes was coordinately regulated. Immunocytochemical and Western analysis confirmed that trkA protein was present in the basal forebrain during the postnatal period. Intraventricular (ICV) injection of NGF induced tyrosine phosphorylation of trkA protein by postnatal day 7. Furthermore, in vivo infusion of NGF resulted in increases of both trkA and ChAT mRNA in BFCNs, while ICV injection of an anti-NGF antibody inhibited expression of *trkA* and *ChAT* as well as suppressed the normal developmental increase in BFCN cell size. Our findings indicate that NGF is a neurotrophic factor for developing BFCNs and regulates *trkA* and *ChAT* expression in BFCNs during development.

536.2

MULTIPLE LEVELS REGULATION OF TRKA IN PC12 CELLS BY NGF. J. Zhou, J.S. Valletta, M.L. Grimes*, W.C. Mobley, Dept. of Neurology and the Neuroscience Program, UCSF, San Francisco, CA 94143.

Induction of TrkA gene expression is a feature of NGF responses in both PNS and CNS neurons. To discover the significance, we asked whether increased TrkA gene expression resulted in an increase in NGF receptors and NGF signaling. TrkA mRNA was first increased 48 h after NGF exposure, and by 7 days it was induced 5.3-fold. Using Western blot analysis, we found that gp140^{trk} was down-regulated within the first 24 hours of NGF treatment. Thereafter, protein synthesis increased in parallel with the increase in TrkA mRNA. ^{125}I-NGF binding and chemical cross-linking analysis showed that there was an increase in gp140^{trk} at the surface of cells treated with NGF for 7 days. NGF primed cells bound and internalized more NGF than unprimed cells. Significantly, in response to NGF the level of tyrosine phosphorylation of gp140^{trk} and PLC-γ1 was increased in primed cells. Our results suggest that after an initial down-regulation of gp140^{trk} in response to NGF, induction of gene expression eventually leads to an enhanced ability for PC12 cells to respond to NGF.

536.3

NERVE GROWTH FACTOR ADMINISTRATION ALTERS THE EXPRESSION OF OTHER NEUROTROPHINS IN ADULT SENSORY GANGLIA. S. C. Apfel*, C. Dormia, M. E. Newell and J. A. Kessler. Depts. of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

During neural development, specific neuronal subpopulations in the dorsal root ganglion are dependent upon different members of the neurotrophin family, including nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin 3 (NT3). However, regulatory mechanisms in adult sensory ganglia may differ because of paracrine/autocrine interactions. To address this issue, the effects of exogenously administered recombinant NGF on expression of BDNF mRNA, in the dorsal root ganglia of adult (150-200 gm) female rats, was examined. Each animal was injected subcutaneously with 1 mg/kg NGF and the cervical dorsal root ganglia were removed 24 hours later for measurement of BDNF mRNA, using a solution hybridization/nuclease protection assay. Administration of NGF significantly elevated levels of BDNF mRNA within 24 hours. We are currently investigating this type of regulation with other neurotrophins, and in other peripheral nervous system tissues. The ability of one neurotrophin to regulate the expression of another may have important implications for choosing neurotrophins for clinical use.

536.4

STEROID REGULATION OF NEUROTROPHIC FACTORS IN THE RAT HIPPOCAMPUS. H.M. Chao*, R.L. Spencer, M. Frankfurt and B.S. McEwen, Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021

In the rat hippocampus, glucocorticoids modulate neuronal morphology and plasticity, and may play a role in the neuronal loss associated with aging. Neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and basic fibroblast growth factor (bFGF), can affect the plasticity, integrity and function of hippocampal neurons, both in vitro and in vivo. In the hippocampus, the colocalization of these neurotrophins with their receptors, suggests that the factors may act locally through autocrine mechanisms to protect against damage and promote neuronal survival. We have examined the adrenal steroid regulation of the genes encoding these neurotrophins, their receptors and the neuronal growth-associated protein GAP-43. Adrenalectomy resulted in an increase in GAP-43 mRNA expression in CA1 and CA3 and a decrease in bFGF mRNA levels in the CA2 subfield; these effects were reversed by corticosterone replacement. In CA3 and the dentate gyrus, there was evidence for glucocorticoid modulation of the BDNF and NT3 mRNAs, but not of the bFGF mRNA or of the mRNAs encoding the receptors for these neurotrophic factors.

ß-amyloid peptides, which accumulate in neuritic plaques and are derived from the amyloid precursor protein (APP), can be either trophic or toxic to hippocampal neurons. We have examined the steroid regulation of the most abundant APP mRNA isoform in rat brain, APP695. In aged female rats we observed a decrease in the level of APP695 mRNA relative to young female rats, while no such age difference was evident in male rats. Physiological, surgical and pharmacological manipulation of glucocorticoids appeared to have no effect on APP695 levels in the hippocampus. Ovariectomized rats treated with estrogen and progesterone exhibited higher levels of APP695 mRNA than ovariectomized controls.

536.5

INDUCTION OF NGF AND bFGF mRNA BY GLUCOCORTICOIDS IN RAT BRAIN. A. Colangelo[1], I. Mocchetti[1], V. Y. Hayes and P. J. Isackson*. [1]Dept. of Cell Biology, Georgetown Univ. Sch. of Med., Washington DC 20007, and Mayo Clin. Jacksonville, FL 32224.

Nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) exert trophic activity in selective neuronal populations. Glucocorticoid hormones have been shown to possess neurotrophic activity in the adult brain. In view of the massive release of adrenal steroids during stress and their potential role in neuronal survival and function, we deemed of importance to verify whether glucocorticoids modify the expression of NGF and bFGF mRNAs. Dexamethasone (DEX), administered to rats at a concentration (0.5 mg/kg, s.c.) that mimics the blood-born steroid levels following stress, elicited, within 3 hr, a three-fold increase in the content of NGF mRNA in the cerebral cortex only. By 6 hr the content of NGF mRNA declined to the control levels. In contrast, DEX elicited a two-three fold increase in bFGF mRNA in the hippocampus, hypothalamus and cerebral cortex. In all of these areas the DEX-increased bFGF mRNA was rapid (within 3 hr) and lasted up to 24 hr. Aldosterone failed to change either NGF or bFGF mRNA at any time points. Accumulation of bFGF mRNA following DEX occurred also in the hippocampus and cerebral cortex of 8 day old and 2 year old Fisher 244 rats, suggesting that glucocorticoids regulate trophic factor expression throughout life. *In situ* hybridization studies were attempted to characterize the anatomical specificity and cell types wherein DEX increases bFGF and NGF mRNAs. The increase in NGF mRNA was localized in the superficial layers of the neo- and entorhinal cortex and to a less extend to the rostral portion of the hippocampus dentate granule cell layer, suggesting that NGF expression is induced in selected neuronal populations. bFGF mRNA follows a different pattern of expression. Indeed, DEX increased bFGF mRNA throughout the brain, suggesting that DEX increases bFGF mRNA in glial cells. Our data suggest that glucocorticoids by inducing NGF and bFGF mRNA expression in the brain could potentially possess a neurotrophic activity.

536.6

GLUCOCORTICOID REGULATION OF BASIC FIBROBLAST GROWTH FACTOR GENE EXPRESSION IN THE RAT BRAIN. M.A. Riva* [1,2], J.M.C. Blom[2], F.Fumagalli[2], E. Donati[2], E. Lovati[2] and G. Racagni[2], [1] DIBIT, San Raffaele Hospital, and [2]Center for Neuropharmacology, Institute of Pharmacological Sciences, University of Milan, Italy.

Several trophic factors have been identified which are important for CNS neurons not only during development but also in the repair and maintenance of neuronal integrity after injury. Basic fibroblast growth factor (bFGF), one of such factors, is expressed in astrocytes and in specific neuronal populations. We have previously demonstrated that bFGF gene expression can be modulated with a specific spatio-temporal pattern by limbic seizures produced by focal application of bicuculline or systemic injection of kainate. We investigated the mechanisms regulating bFGF gene expression during limbic motor seizure examining the role of glucocorticoid hormones in kainate-induced bFGF induction. Bilateral adrenalectomy produced a significant reduction of bFGF mRNA levels in hippocampus and striatum at 3 and 7 days following surgery; no effect was observed in hypothalamus. In situ hybridization showed a general decrease of bFGF expression with a marked reduction of labelling in CA2 pyramidal layer of hippocampus. The regulatory influence of glucocorticoid hormones was confirmed by in vitro experiments showing a robust induction of bFGF gene expression in rat cortical astrocytes following dexamethasone exposure. Despite adrenalectomy, kainate was still able to produce a significant elevation of bFGF mRNA levels in striatum and hippocampus when measured 6 hr following convulsant injection. In conclusion, although bFGF gene expression in the rat brain appears to be under the control of glucocorticoid hormones, removal of this 'tonic' influence does not influence acute modulation of bFGF gene expression by neuronal activity. As bFGF and other neurotrophic factors exert trophic actions on different neuronal populations, these mechanisms can be important for the regulation of neuronal plasticity in physiological as well as pathological situations.

536.7

CATECHOLAMINERGIC MEDIATION OF COCAINE-INDUCED INCREASE IN REGIONAL LEVELS OF bFGF mRNA. D. Masco*, I. Mocchetti[1] and K. Gale. Departments of Pharmacology and [1]Cell Biology, Georgetown Univ. Medical Center, Wash., DC., 20007.

The behavioral stimulant response produced by cocaine is known to show a long-lasting increase with repeated administrations. Such long term plastic changes are likely to involve trophic factors. We have previously shown that the mRNA encoding basic FGF (bFGF) is increased in selected brain areas after a single, prolonged cocaine exposure. In this study, we examined the neurotransmitter receptors that mediate this effect. Rats (450g) received 40 mg of cocaine over 8 hr, after which they were sacrificed and bFGF mRNA was measured. Cocaine induced a significant increase (2-3 fold) in bFGF mRNA in striatum, substantia nigra and frontal cortex. Pretreatment with haloperidol (3.5mg/kg) or chlorpromazine (20mg/kg) prevented the cocaine-induced increases in bFGF mRNA. However, pretreatment with either pimozide (2mg/kg), prazosin (1mg/kg), or a low dose of chlorpromazine (2mg/kg) did not attenuate the cocaine-induced effect on bFGF; pretreatment with propranolol (20mg/kg) was likewise ineffective. It therefore appears that concurrent blockade of dopamine receptors and noradrenergic alpha receptors is necessary for preventing the induction of bFGF mRNA expression in response to cocaine. This suggests that cocaine may influence neurotrophic factor expression via either dopaminergic or alpha adrenergic mediated processes. Studies in progress with selective alpha antagonists + selective dopaminergic receptor antagonists will more precisely identify the relative contribution of these receptors to the changes in bFGF mRNA levels that follow cocaine exposure.

536.8

ALTERED STRIATAL NEUROTROPHIC mRNA LEVELS IN HYPOTHYROID *WEAVER* MUTANT MICE Mariann Blum*, Emilee Carrasco, Nancy Goodman and Cynthia Shannon Weickert Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

The degeneration of nigrostriatal dopamine neurons that occurs in the *weaver* mutant mouse can be viewed as failed postnatal development. For example, nigrostriatal neurons do not make the normal transition from a primarily patchy innervation to a more diffuse innervation of the striatum during the first weeks of postnatal life. It is possible that insufficient levels of target-derived neurotrophic support is responsible for the abnormal dopaminergic neuronal development and subsequent degeneration. Therefore, we measured the levels of the mRNAs encoding two putative dopaminergic trophic factors using a quantitative nuclease protection assay. Unexpectedly, we observed that at P22 the levels of BDNF and GDNF mRNA levels are elevated in the *weaver* mutant striatum relative to controls ($p<.05$ and $p\leq.01$, respectively).

Not only does the *weaver* brain appear to develop abnormally, but also their body growth is stunted and their eyelid opening is delayed. Since body growth rate and timing of eyelid opening are regulated by thyroid hormone, we hypothesize that *weaver* mice are hypothyroid. Using radioimmunoassay, we determined that the mutant mice have significantly lower thyroid hormone (T4) levels during the third postnatal week of life ($p\leq.001$). This corresponds to the time when the development of mutant dopaminergic neurons is arrested. We speculate that the reduction in thyroid hormone, which is a potent regulator of brain development, could account for altered trophic factor mRNA levels and/or altered dopaminergic neuronal development.

536.9

INDUCTION OF NGF AND BDNF BY THYROID HORMONE IN ADULT RAT BRAIN. S. Carswell*, E.R. Brown, E. Robbins, K.C. Hartpence, and M.S. Saporito. Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

There is evidence that thyroid hormone (TH) is required for the differentiation and maintenance of septal cholinergic neurons. It is also well-documented that NGF prevents the degeneration of basal forebrain cholinergic neurons. In previous studies, chronic treatment with TH (0.5 mg/kg) induced NGF in the hippocampus, but not the cortex, of aged rats (Giordano et al., 1992). Our interest was to determine whether the effects of TH on cholinergic cells are mediated through induced NGF. RNase protection analyses of NGF mRNA after acute systemic administration of various doses of either T_3 or T_4 revealed induction of NGF in the hippocampus and cortex, but not in the septal region of adult Sprague Dawley rats. Peak increases (approximately 1.5-fold) occurred at 0.5 and 5mg/kg with T_3 and T_4, respectively. The effect of each hormone on NGF synthesis was shown to be stereospecific. TH induced BDNF as well as NGF in the cortex, and the induction of both neurotrophins was sustained for at least 20 h. Evaluation of TH in the fimbria fornix lesion model of basal forebrain cholinergic neuronal degeneration will also be discussed.

536.10

NGF RECEPTOR low affinity IMMUNOSTAINING IN CHOLINERGIC NEURONS DURING ADULT HYPOTHYROIDISM. L. Calzà*, §L. Aloe, °L. Giardino, #M. Zanni, §R. Levi Montalcini*. Inst. Of Human Physiology, University of Cagliari; §Inst. of Neurobiology, CNR, Rome, Italy; Inst. Otolaryngol., University of °Milano and #Modena, Italy.

Adult hypothyroidism is characterized by neurochemical abnormalities involving neurotransmitters, neuropeptides, neuronal proteins and also trophic factors. Several clinical and experimental data suggest that the cholinergic system of the basal forebrain is sensitive to the peripheral levels of thyroid hormones. In this paper we investigated the NGF receptor low affinity (LNFGR)-like immunostaining in the horizontal nucleus of the diagonal band of adult hypo- and hyperthyroid male rats, in basal conditions and after intracerebral injection of colchicine (75µg/µl/animal, survival time 24 hours). Hypothyroidism was induced by thyroidectomy after 60 days from birth, hyperthyroidism by treatment with T4 (.18mg day, added to the food pellets). The LNGFR was detected by means of indirect immunofluorescence using monoclonal antibody (clone IgG192, *generously supplied by E.M. Johnson*). The staining intensity was measured by means of computerized microdensitometry. In colchicine untreated rats, we found a significant increase of LNGRF-like immunostaining in the basal forebrain neurons of hypothyroid rats. The colchicine treatment induces an increase of LNGFR expression in these brain area which is not different in hypo-, eu- and hyperthyroid rats. These data confirm an effect of thyroid hormone on neurons during adulthood also in extrahypothalamic areas and they support a possible role of thyroid hormone in aging brain quality and cognitive performance.

536.11

REGULATION AND POLARITY OF NEUROTROPHIN SECRETION. Laurie J. Goodman and Franz Hefti*. Department of Neuroscience, Genentech Inc. South San Francisco, CA 94080.

The neurotrophins, BDNF and NT4/5 are factors that regulate the growth and survival of selected populations of peripheral and central nervous system neurons. In this study the regulation and polarity of neurotrophic factor secretion was investigated. Analysis of expression of either BDNF or NT4/5 following expression in the pheochromocytoma-derived cell line, PC12, resulted in the constitutive secretion of immunoreactive proteins migrating at a molecular weight identical to mature BDNF or NT4/5. In the presence of KCl (50mM), secretion of NT4/5 from PC12-transfected cells increased 10-fold as determined by radiolabeling/immunoprecipitation and western blot analysis. This indicates that the protein is primarily released by a regulated pathway of secretion. As an initial approach to understanding the polarity of neurotrophin secretion, the release of BDNF and NT4/5 was investigated in the polarized epithielial cell line, MDCK. Previously studies have determined that many of the same protein sorting mechanisms exist in MDCK cells as do in polarized hippocampal neurons *in vitro*. Cells expressing BDNF or NT4/5 were grown on transwell filters so as to establish a tight monolayer displaying high electrical resistence. Under these conditions, 80-90% of the BDNF secreted from transfected MDCK cells was released from the apical domain, whereas NT4/5 was secreted equally from both the apical and basolateral domains. This suggests that BDNF may be released primarily from the axonal domain in polarized hippocampal neurons. We are currently testing our hypothesis in transfected hippocampal neurons grown *in vitro*.

536.12

Identification of a potential estrogen response element in the gene coding for brain derived neurotrophic factor (BDNF). F. Sohrabji*, R. C. Miranda and C. D. Toran-Allerand. Dept. Anatomy and Cell Biology, Columbia University CPS, New York, N.Y. 10032.

We are studying the role of estrogen in the survival and differentiation of neurons in the basal forebrain, and its targets in the cerebral cortex, hippocampus and olfactory bulb. We have shown previously that estrogen-target neurons in these regions widely co-express the mRNAs for the neurotrophin peptide growth factors, NGF, BDNF, and NT-3, suggesting a potential substrate for estrogen-neurotrophin interactions. Recent evidence suggests that estrogen may regulate BDNF mRNA in regions of the cortex and hippocampus (M. Singh, personal communication). Since the estrogen receptor is a ligand-activated transcription factor, we have begun to address whether estrogen can regulate gene transcription of members of the neurotrophin family. We have identified a sequence in the gene coding for BDNF that appears to bind the estrogen receptor. Using a band shift assay, we found that nuclear protein extract, harvested from diethylstilbestrol (DES, a synthetic estrogen)-treated MCF-7 mammary tumor cells, resulted in a shift in the migration of a 47 bp DNA fragment from the BDNF gene. Competition studies and receptor antibody recognition assays indicate that the shift results from the binding of the estrogen receptor-ligand complex to this DNA fragment, suggesting that estrogen may potentially regulate transcription of the BDNF gene. We are currently examining the regulation of this gene by estrogen in *in vitro* models. *Supported by grants from NIH, NIMH, NSF, AHAF and an ADAMHA-RSA to C.D.T-A.)*

536.13

BDNFmRNA LEVELS IN THE CHICK OPTIC TECTUM ARE REGULATED BY NEUROTRANSMITTERS DURING SYNAPTOGENESIS. K.-H. HERZOG* AND Y.-A. BARDE. Dept. of Neurobiochemistry, Max Planck Inst. for Psychiatry, Am Klopferspitz 18a, 82152 Martinsried, Germany.

We previously reported that BDNFmRNA is expressed in the visual system of the chick embryo. During the earliest time of target encounter, BDNFmRNA levels are regulated in the tectum by electrical activity of the innervating retinal ganglion cells (RGC). When applied onto the chorioallantoic membrane, nicotinic acetylcholine receptor antagonists do not cause a reduction in BDNFmRNA expression in the optic tectum at E7.5, whereas muscarinic blockers (atropine or pirenzepine) do. Similar results were obtained following optic stalk transection, intraocular TTX application, and of the $GABA_A$-receptor agonist muscimol. Whether the cholinergic contribution in the regulation of the BDNF gene is due to RGC or derives from the tectum is currently under investigation.

BDNFmRNA is not only detected in the tectum, but also in the retina, and at E11, comparable levels are observed. Using BDNF-peptide antibodies, we observed a BDNF-like immunoreactivity in the retina, but not in the tectum. The most prominent expression was in the ganglion cell layer, but not all ganglion cells were labelled. Suprisingly, following optic stalk transection, the levels of this BDNF-like immunoreactivity did not seem to be dramatically reduced when compared to the control retina. We are currently investigating whether BDNF is actually synthesized in RGC or taken up from their surrounding cells in the retina.

536.14

CO-LOCALIZATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND PARVALBUMIN IN THE RAT CEREBRAL CORTEX M.P.Berzaghi[1], T.Freund[2], E.Papp[2], E.Castren[1], U.Zirrgiebel[1], H.Thoenen[1] and D.Lindholm[1]* [1]Department of Neurochemistry, Max-Planck-Institute for Psychiatry, Martinsried, Germany & [2]Department of Functional Neuroanatomy, Institute of Experimental Medicine, Hungarian Academy of Science, Budapest, Hungary

The expression of BDNF mRNA (brain-derived neurotrophic factor) in neurons has been shown to be regulated by neuronal activity. The cholinergic system is essentially involved in the regulation of BDNF mRNAs in the developing brain. The aim of the present study was to analyse to which extend the cholinergic system is also involved in the regulation of BDNF protein in the cortex. We have used a polyclonal rabbit antiserum directed against specific peptide from the N-terminus of mouse BDNF to study the distribution of BDNF protein in the rat cerebral cortex . BDNF was localized to a subset of neurons in different cortical layers . Administration of pilocarpine, a muscarinic agonist, up-regulates the BDNF- positive cells in cortex of adult rats. The BDNF immunoreactive cells are also positive for the calcium binding protein parvalbumin, as demonstrated with the mirror technique analysis. The possibility of crossreactivity of the BDNF antiserum with parvalbumin was excluded by means of Western blotting analysis. The results suggest that BDNF protein is upregulated in an activity-dependent manner and accumulated in parvalbumin containing neurons in the rat cerebral cortex.

536.15

SIGNALS THAT INDUCE EXPRESSION OF LEUKEMIA INHIBITORY FACTOR (LIF) IN RAT SUPERIOR CERVICAL GANGLIA (SCG) AND THEIR NERVES AFTER AXONAL TRANSECTION. Y. Sun*, S. C. Landis and R. E. Zigmond. Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

We previously have shown that LIF contributes to the axotomy induced neuropeptide expression in rat and mouse superior cervical ganglia (SCG). The expression of LIF itself is also induced in SCG within 2h after axotomy or explantation. The initial time course of LIF mRNA induction (from 30min to 6h after nerve transection) are similar *in vivo* and *in vitro*, suggesting that the two systems possess common early LIF-inducing signals after axonal transection. However, there is a difference in the duration of the increase in LIF mRNA levels between the two systems. LIF mRNA levels start to decline 12h after axotomy *in vivo*, while they remain elevated until 48h in culture. To examine the early signals responsible for the rapid induction of LIF mRNA after axon transection, we used short term explant cultures. Defined medium conditioned for 30min by freshly dissected adult rat SCG (SCM) contained LIF mRNA inducing activity when tested in dissociated neonatal SCG cultures with non-neuronal cells. Like SCM, defined medium conditioned by portions of the three major nerve truncks of the SCG for 3h (NCM) also increased LIF mRNA levels in dissociated SCG cultures. Further, LIF mRNA induction was observed in those nerve pieces themselves, suggesting that Schwann cells along the axons are one of the sources of LIF production after nerve lesion. The LIF-inducing activity of both SCM and NCM was present in a >10kd fraction and its appearance did not require new protein synthesis. SCM and NCM induced peptide expression in SCG of normal, but not LIF-deficient, mice, suggesting that the effect of SCM and NCM on peptide expression is mediated by LIF. Based on these observations, we hypothesize that when SCG are axotomized, an existing molecule is activated and/or released by transected axons and possibly by axotomized neuronal cell bodies as well. This molecule in turn causes local Schwann/satellite cells to produce LIF which participates in regulating neuropeptide expression in SCG neurons.

536.16

INCREASES IN VASOACTIVE INTESTINAL PEPTIDE (VIP) LEVELS IN CULTURED RAT SUPERIOR CERVICAL GANGLION (SCG) CAN BE REDUCED BY AN ADENYLATE CYCLASE INHIBITOR. R. P. Mohney* and R. E. Zigmond. Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

The expression of a number of neurotransmitters changes after axonal injury, possibly reflecting a shift in the priorities of these neurons from synaptic transmission to maintenance and regeneration. For example, VIP is normally present in extremely low quantities in sympathetic neurons of the adult rat SCG, but levels of this peptide increase dramatically after SCG are placed into explant culture. The gene that encodes for VIP contains a cAMP-response element that is required for cAMP-regulated transcription. Ganglionic cAMP levels are relatively low under normal conditions and do not significantly increase in short-term cultures. Previously, we have shown that agents which elevate intracellular cAMP levels (forskolin and secretin) are also capable of increasing VIP-like immunoreactivity. We now demonstrate that the increases in VIP levels produced by the addition of cAMP-elevating agents to cultured SCG can be prevented by the addition of an adenylate cyclase inhibitor, 2',5'-dideoxyadenosine (DDA). DDA (1 mM) reduced elevations in both cAMP and VIP levels by greater than 60% in SCG cultured in medium containing cAMP-elevating agents or in control medium alone. Histological examination was performed to determine if DDA adversely affected the health of the neurons. No apparent differences in neuronal morphology were found between SCG cultured in control medium or in medium containing DDA for 48 h. An increase in the concentration of DDA to 2 or 6 mM was effective in reducing VIP levels measured in the presence of cAMP-elevating agents by 85% or 95%, respectively. These data support the observations that VIP levels in sympathetic neurons can be regulated by alterations in cAMP levels. Furthermore, they suggest that the increase in VIP that occurs by placement of the SCG into culture with control medium is dependent on the maintenance of basal cAMP levels.

536.17

CHANGES IN GPA LEVELS IN CILIARY GANGLION TARGETS DURING AND AFTER THE CELL DEATH PHASE. T.P. Finn and R. Nishi*. Dept. of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201

During normal development of the avian ciliary ganglion, the neurons become dependent upon their targets in the eye for survival. The process begins about E8 and continues until about E14, by which time half of the original neurons present have died off. Production by the targets of specific neurotrophic factors may mediate the level of neurons which ultimately survive. Growth promoting activity (GPA), a 21.5 Kd protein originally purified from adult chicken sciatic nerves and E15 chick eyes, is one such candidate trophic factor. To help determine what role GPA plays in ciliary ganglion survival, the cellular location and timing of GPA expression in ciliary targets was investigated by immunocytochemical, bioassay, and western blot analysis. Antibodies prepared to a purified and highly active form of recombinant GPA detected a 21.6 Kd protein in choroid-iris extract on western blots. The intensity of the band increased with embryonic age. Immunocytochemical staining of isolated choroid layers revealed that GPA immunoreactivity was localized to smooth muscle cells, and in the anterior portion of the eye, GPA-like immunoreactivity was localized to muscle fibers of the ciliary body. Both of these cell populations are normal targets of CG neurons. GPA expression was first detectable at E10, and increased through the end of the cell death phase at E14. GPA expression continued to intensify after the cell death phase, possibly indicating a continued dependence of the neurons for GPA. To test this possibility we are performing neuronal survival assays on various age ganglia to determine the age at which CG are no longer dependent upon GPA. We are also comparing GPA-like biological activity before, during, and after the cell death phase in an effort to correlate GPA levels with neurotrophic activity in target extracts. Supported by NS25767

536.18

OKADEIC ACID POTENTIATES AND GENISTEIN INHIBITS THE PLATELET-ACTIVATING FACTOR (PAF) ENHANCEMENT OF INDUCIBLE PROSTAGLANDIN SYNTHASE

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PAF accumulates in brain during ischemia or convulsions. TIS10/PGS-2 is a primary response gene encoding the inducible form of prostaglandin synthase. In the presence of retinoic acid, a PAF-dependent (1-50 nM) activation of luciferase reporter constructs driven by regulatory regions of the TIS 10/PGS-2 gene transfected in the neuroblastoma cell, NG 108-15 was observed (N. Bazan et al., Proc. Natl. Acad. Sci., in press). Here we report that when DOTAP is used for transfection of TIS10/PGS-2 promoter luciferase constructs, PAF elicits a 5- to 10-fold activation in neuroblastoma cells and CV-1 cells. Deletion studies restrict the major PAF *cis*-acting response element of the TIS10/PGS-2 gene to a 70-nucleotide sequence as an intracellular inducer of TIS10/PGS-2 expression. Okadeic acid (10 ng/ml) elicits a 3-fold potentiation of the PAF inductive effect. Genistein (100 μg/ml), on the other hand, inhibits the PAF-induced effect. These results strongly suggest that, in the intracellular pathway of signal transduction between the membrane-derived lipid mediator PAF and the modulation of gene expression of TIS10/PGS-2, there is a necessary step that comprises protein tyrosine kinase-protein tyrosine phosphatase. These regulatory events may play a role in neural plasticity responses such as long-term potentiation and epileptogenesis (NINDS NS23002).

537.1

OPTIC NERVE GLIA EXPRESS NEUROTROPHINS AND FULL-LENGTH TRK RECEPTORS: AN IMMUNOCYTOCHEMICAL STUDY DURING DEVELOPMENT. S. Elkabes*, W. J. Friedman and I B. Black. Neurosci. & Cell Biol., Robert W. Johnson Med. Sch., UMDNJ, Piscataway, NJ, 08854.

We have previously reported that neurotrophin (NGF, BDNF, NT-3 and NT-4/5) mRNAs are expressed in the developing and adult optic nerve. Developmental decreases in trk B and especially in trk C expression were also shown by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers that selectively amplified the extracellular domains (Elkabes et al. 1993, Abstr. Soc. Neurosci., 19, 251). To investigate whether mRNA levels detected by RT-PCR are sufficient for the synthesis of neurotrophin and trk proteins, and to determine whether glia express full-length trk receptors, we have performed immunocytochemical studies utilizing antibodies specific for neurotrophins and for the extracellular or intracellular domains of trk A, B and C (kindly provided by Dr. D. R. Kaplan, NCI, Frederick, MD).

BDNF, NT-3 and NT-4/5-like immunoreactivities were detected in glia of developing and adult optic nerve. Antibodies to the extracellular and intracellular domains of the trks also labeled cells in the optic nerve, suggesting that glia express full-length forms of the receptors. These findings were confirmed by RT-PCR, using primers that amplified the intracellular domains. The number of cells expressing trk B and trk C in the postnatal day 10 optic nerve appeared to be higher than in the adult, supporting our previous findings. Our studies suggest that neurotrophins are elaborated by optic nerve glia and that responsiveness in glial cells may be mediated through the full-length receptor. Further studies are in progress to determine which glial subtypes express neurotrophins and trks (This work was supported by NIH grant POI HD 23315-06A1).

537.2

DIFFERENTIAL GENE EXPRESSION OF TROPHIC FACTORS BETWEEN EMBRYO AND EXTRAEMBRIONIC MEMBRANES IN CULTURED E9 MICE G. W. Glazner*, D. E. Brenneman, N. Jain, and J. M. Hill Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD, 20892

Whole mouse embryo cultures have been used to examine the effects of trophic factors on embryonic growth and development in the absence of maternal influences. Exogenous application of vasoactive intestinal peptide has been shown to increase relative size, protein and DNA content, and anatomical stage of development (Gressens et al., Nature, 1993, 362:155-158). In an effort to characterize the underlying trophic milieu in which these embryos grow, RT-PCR techniques were used to determine the presence and relative amounts of mRNA for known neurotrophic and mitogenic factors. Mouse embryos (20-24 somites), with intact extraembryonic membranes, were cultured in 75% human serum and 25% rat serum for 6 hours. At the end of this time, embryos were detached from the membranes, and total RNA was purified from each embryo and corresponding membranes separately. Equal amounts of RNA were reverse transcribed, and these DNA products used as templates in PCR reactions. Specific PCR primers were designed to detect the following mouse mRNAs: β-actin, brain-derived growth factor (BDNF), leukemia inhibitory factor (LIF), transforming growth factor β (TGFβ), basic fibroblast growth factor (bFGF) and insulin-like growth factor 2 (IGF-2). Each primer pair gave a single band of the expected size. There was no difference in actin relative to total RNA between embryos and membranes. Although present in both, there was no significant difference in IGF-2 gene expression relative to actin or total RNA between embryos and membranes. TGFβ was also present in both, and tended to be more abundant in membranes, but the difference was not significant. bFGF and BDNF were both significantly higher (> 50 fold) in embryos than in membranes relative to actin and total RNA. LIF was significantly higher (> 20 fold) in membranes than in embryos relative to actin and total RNA. These data show that not only are these messages present in cultured mouse embryos, but also there is a differential level of expression between embryo and extraembryonic membranes.

537.3

NERVE GROWTH FACTOR RECEPTOR GENE EXPRESSION IN HUMAN AGING. S.D. Kittur*, L.Song, H.Endo, W.H.Adler.National Institute of Aging, 4940 Eastern Avenue,Baltimore,MD 21224 (*current address, Perry Point VA Medical Center, Perry Point, MD 21902).

Nerve growth factor modulates the immune function as a consequence of binding to the nerve growth receptor (NGF-R). Therefore we hypothesized that NGF-R will be made by the immune system in the peripheral blood lymphocytes. Northern Blot analysis of mRNA from human peripheral blood lymphocytes was performed using cDNA probe for low affinity - NGF-R (p75 NGF-R). We show that p75 NGF-R mRNA is present in human peripheral lymphocytes and expressed maximal levels at 14 hours after stimulation with phytohemaglutinin. Also, we shows that p75 NGF-R did not change with the aging process in peripheral blood lymphocytes in human volunteers.

537.4

SUBCELLULAR LOCALIZATION OF NGF IMMUNOREACTIVITY IN BASAL FOREBRAIN CHOLINERGIC NEURONS. AN ULTRASTRUCTURAL DOUBLE-LABELLING STUDY IN THE RAT. Linsen Hu, A. Ribeiro-da-Silva, L. Garofalo* and A.C. Cuello. Dept. Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada H3G 1Y6

Nerve growth factor (NGF) is produced in target areas (e.g, cerebral cortex) and retrogradely transported to basal forebrain neurons where it exerts biological effects. However, it is not clear yet whether basal forebrain neurons themselves have the capacity to produce NGF. To address the above issue, we developed an improved protocol allowing the ultrastructural demonstration of NGF immunoreactivity with good preservation of the ultrastructure. NGF-immunoreactive (IR) sites were demonstrated by means of the ABC method, using a polyclonal anti-NGF antibody (Conner et al., J. Comp. Neurol. 319:454-462, 1992), and choline acetyltransferase (ChAT)-IR sites by means of a monoclonal antibody (Boehringer Mannheim) revealed by a pre-embedding immunogold protocol. In nucleus basalis magnocellularis (NBM) ChAT-IR neurons, NGF immunoreactivity occurred predominantly in multivesicular bodies, which were shown in a previous study to be immunoreactive for the low affinity NGF receptor (Pioro et al., Brain Res. 527: 109-115, 1990) and probably represent the final form of the internalized ligand-receptor complex , within secondary lysosomes. Some NGF immunoreactivity was also detected in association with ribosomes and in the Golgi apparatus. These results would indicate that most of the NGF in NBM cholinergic neurons originates from the accumulation of retrogradely transported neurotrophin. However, ultrastructural evidence for limited NGF synthesis by the cholinergic neurons themselves was found. These observations may suggest that the NGF acting on the cholinergic NBM neurons is not entirely target-derived. Some of it may originate from the cholinergic cells and act autotrophically.

(Research supported by the Canadian MRC)

537.5

HIGH MOLECULAR WEIGHT NGF-LIKE ACTIVITY IN THE RAT PITUITARY. S. Soinila* and J. Lakshmanan. Dept. Anatomy, PO Box 9, 00014 University of Helsinki, Finland.

We previously reported evidence for NGF immunoreactivity and biological activity in the rat pituitary gland (PG). Since the biological properties of the PG NGF differed from those reported for the rat iris muscle, we examined the molecular properties of the rat PG NGF-like material and compared it with those found in the mouse submaxillary gland (SMG). Homogenized PG tissue was subjected to immunoblotting using antiserum against mouse ß-NGF. For bioassay studies, PG homogenate supernatants were subjected to ultrafiltration and fractions with M_r > 30 kD were tested for neurite outgrowth response from newborn superior cervical ganglion explants. Mouse ß-NGF antiserum identified two proteins with M_r 53 kD and 73 kD. The 13 kD band was absent in the PG. PG ultrafiltrates containing proteins of M_r > 30 kD produced neurite outgrowth from newborn ganglia. Based on our data, we suggest that rat PG expresses 53 kD and 73 kD NGF prohormones and that they represent the post-translationally modified products of the short and long NGF prohormone transcripts, respectively.

537.6

BDNF mRNA in the human locus ceruleus. S Borson*, M Miller, J Leverenz, A Unis, M Bothwell. Depts of Psychiatry, Neurology, and Physiology & Biophysics, Univ Washington Sch Med, Seattle, WA 98195.

The brainstem locus ceruleus (loC) participates in learning, memory, and other behavioral functions through its regulatory effects on input responsivity of its targets, gating of stimuli for further processing, and interactions with other neurotransmitter systems including the septohippocampal cholinergic system. In the rat, some loC neurons express BDNF mRNA, but its function in this site is not known; embryonic cell cultures of rat loC respond to BDNF with increased numbers of TH+ neurons. In humans, loss of loC neurons occurs during aging and is exaggerated in Alzheimer's disease (AD). We examined BDNF mRNA expression in noradrenergic neurons of the human loC using *in situ* hybridization with an S^{35}-labeled riboprobe synthesized from a 477 bp EcoRI/PstI fragment subcloned from a human BDNF cDNA (gift of K. Jones and L. Reichardt). Fresh frozen brainstems were obtained at autopsy from 3 adult human brains (1 AD, 1 dementia with gliosis, 1 control) and from a 20-week fetal human specimen following elective abortion. *In situ* hybridization was performed on 20µm cryosections using 2 pmol/ml of labeled antisense or sense riboprobe and tissues examined by film and emulsion autoradiography. Expression of BDNF mRNA was found in noradrenergic neurons of all 3 adult nuclei, and in cells lining the 4th ventricle and in the region of the developing loC in the fetus. These results suggest that BDNF in the human loC may have a functional autocrine or anterograde role during development and into late adult life. Supported by grants from the Alzheimer's Association [S Borson PI] and NIA (UW ADRC [G Martin PI]), and by the UW Fetal Tissue Program [A Fantel PI].

537.7

TRANSIENT EXPRESSION OF BDNF mRNA IN RAT LOCUS COERULEUS FOLLOWING ACUTE RESERPINE ADMINISTRATION. K.B. Seroogy*. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

In vitro studies indicate that certain members of the neurotrophin family of trophic factors can support the survival of, and are neuroprotective for, populations of catecholaminergic neurons. In the present study, the expression of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) mRNAs in neurons of the locus coeruleus was examined *in vivo* following acute reserpine administration. Adult rats received a single injection of reserpine (10 mg/kg; i.p.) or vehicle followed by survival times of 4hr, 8hr, 16hr, 24hr, 3 days, 4 days and 8 days. Cryostat-prepared tissue sections through the locus coeruleus were subsequently processed for the *in situ* hybridization localization of BDNF, NT-3 and TH mRNAs using ^{35}S-labeled cRNA probes. BDNF mRNA was barely detectable in locus coeruleus in normal, untreated rats and in vehicle-injected rats. After reserpine treatment, a substantial increase in hybridization density for BDNF mRNA was observed in locus coeruleus which peaked at 24 hrs and gradually returned to control levels by 8 days post-injection. Expression of TH mRNA in the nucleus exhibited a similar transient elevation, in agreement with previous studies. In contrast, no change in expression of NT-3 mRNA was detected. Administration of the tricyclic antidepressant desipramine (25 mg/kg; i.p.), 30 min prior to reserpine treatment, partially attenuated the elevation in BDNF mRNA. These data indicate that depletion of catecholamines induces a transient increase in BDNF mRNA expression in locus coeruleus neurons and raises the possibility of BDNF involement in neurological disorders associated with the locus coeruleus, such as depression. Supported by the National Parkinson Foundation and the Scottish Rite Schizophrenia Research Program.

537.8

INCREASED EXPRESSION OF BDNF mRNA IN ADULT VENTRAL MIDBRAIN FOLLOWING ACUTE 6-OHDA LESIONS OF THE NIGROSTRIATAL PATHWAY. S. Numan* and K.B. Seroogy. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

In vitro studies have demonstrated that BDNF and NT-3 support the survival of fetal rat mesencephalic dopamine neurons. Furthermore, several studies have provided evidence that BDNF may be neuroprotective for cultured dopaminergic neurons undergoing various toxic insults. *In vivo*, adult dopaminergic neurons, which express BDNF and NT-3 mRNAs, may initially respond to various toxic insults by altering the expression of neurotrophin mRNAs. To examine this possibility, adult rats received a unilateral injection of 6-OHDA or vehicle into the ascending medial forebrain bundle. Following various survival periods (8, 16, 48, and 120 hrs), *in situ* hybridization of ^{35}S-labeled cRNA probes for BDNF, NT-3,and TH mRNAs was performed in midbrain sections. After 8, 16 and 48 hours, an increase in the hybridization density for BDNF mRNA was observed in the substantia nigra and ventral tegmental area ipsilateral to the 6-OHDA injection, as compared to the uninjected control side. In contrast, no alterations in the hybridization density for NT-3 or TH mRNAs were observed at these time points. After a 120 hour survival period, there was a decreased hybridization density for TH, BDNF, and NT-3 mRNAs in the ventral midbrain ipsilateral to the 6-OHDA injection, as compared to the control side. No alterations in the hybridization density for BDNF, NT-3, or TH mRNAs were observed in the ventral midbrain of rats that had received unilateral injections of vehicle. These data indicate that there is an early transient response by BDNF mRNA in the ventral midbrain to the neurotoxin 6-OHDA, which may suggest a role *in vivo* for this neurotrophin in neuronal protection. Supported by the National Parkinson Foundation and the Scottish Rite Schizophrenia Research Program.

537.9

IMMUNOHISTOCHEMICAL STUDY OF THE EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN ADULT RAT BRAIN. Q. Yan*, C.R. Matheson, O.T. Lopez, N. Hawkins, R.D. Rosenfeld, and L. Bennett Amgen, Inc, Amgen center, Thousand Oaks, CA 91320

We examined the expression of endogenous BDNF in adult rat brains using a BDNF specific antibody by immunohistochemistry. We found distinct BDNF immunoreactivity (IR) in many structures which are known to be BDNF mRNA positive from previous studies. These structures included neocortex, piriform cortex, amygdaloid complex, hippocampal formation, claustrum, supramammillary region and other thalamic nuclei, some hypothalamic nuclei, substantia nigra, and neurons in many brain stem structures. In the hippocampal formation, very heavy BDNF IR was found in the granular layer of dentate gyrus and pyramidal and radiatum layers of CA3 and CA4, but not in CA1 and CA2 regions of hippocampus. However, with kainic acid treatment (i.p), which is known to upregulate BDNF mRNA, BDNF IR was readily detected in CA1 and CA2 regions. Staining was also seen in areas not previously reported as BDNF mRNA positive. These included the bed nucleus of stria teminalis, dorsal endopiriform nucleus, lateral septum, medial preoptic nucleus, olivery pretectal nucleus, lateral paragigantocelluar nucleus, and nucleus ambiguus. The results of this study suggest BDNF may play a broad role in the adult central nervous system.

537.10

BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA IS EXPRESSED IN THE DEVELOPING TASTE BUD-BEARING TONGUE PAPILLAE OF RAT. C. A. Nosrat, S. H. Sun*[1] L. Olson. Dept of Neuroscience, Karolinska Institute, S-171 77, Stockholm, Sweden; [1]Institute of Neuroscience, National Yang-Ming Medical College, Taipei, Taiwan.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is expressed in many areas of the central nervous system. Its possible roles in the peripheral nervous system have been less studied. Using in situ hybridization we now have investigated the possible presence of BDNF mRNA in the developing fungiform and circumvallate papillae of the rat tongue. BDNF mRNA is present in the epithilium of the developing fungiform papillae in E15, E16 and E17 rat embryos. BDNF mRNA was also located in the epithilium of the developing circumvallate papillae on the dorsum of the tongue in E16 and E17 rat embryos. The expression of BDNF mRNA in areas where taste buds are to be formed suggests that BDNF may be one crucial factor in the formation of the papillary and/or taste bud innervation apparatus. Further studies are needed to clarify which nerve fiber types within the two main sources of innervation of the taste bud-bearing papillae, the chorda tympani (n. VII) and the lingual branch of the mandibular nerve (n. V), that may respond to BDNF.

537.11

STRUCTURE, EXPRESSION AND FUNCTION OF RAT GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF). M. Trupp[1], M. Rydén[1], E. Arenas[1], T. Timmusk[1], N. Lindefors[2]* and C. F. Ibáñez[1]. [1]Laboratory of Molecular Neurobiology, MBB and [2]Department of Clinical Neurobiology, Karolinska Institute, S-17177 Stockholm, Sweden.

A cDNA encoding rat GDNF was cloned by RT-PCR from P1 rat brain RNA. An alternatively spliced variant (termed GDNFβ), similar to that described by Schaar et al. (1993), with a 78 bp deletion in the prepro region was also cloned. Both mRNAs were expressed in developing striatum, cerebellum and some peripheral organs as assessed by RNAse protection analysis. GDNF mRNA expression was developmentally regulated and appeared to be altered by neuronal lesions. The cellular localization of GDNF mRNA expression during rat development is being studied by in situ hybridization.

Western blotting and immunoprecipitation analysis with anti GDNF antibodies generated against predicted loop regions of the molecule showed that the deletion decreased the level of GDNF protein produced by transiently transfected COS cells. Recombinant GDNF produced in baculovirus-infected insect cells was correctly processed and glycosylated. GDNF protein purified to homogeneity promoted survival and neurite outgrowth from embyonic sympathetic ganglia with a time-course and morphology clearly different from nerve growth factor, suggesting activation of distinct signalling pathways. Genetically engineered fibroblast cell lines producing both GDNF mRNA forms were generated and are currently being used to investigate the function of GDNF *in vivo*.

537.12

DEVELOPMENTAL EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) mRNA IN RAT CNS AND PERIPHERY. D. Choi-Lundberg and M. C. Bohn*. Dept. of Neurobio. & Anat., Univ. of Rochester Med. Ctr., Roch., NY 14642.

GDNF is a newly described member of the TGF-b family isolated from the rat glial tumor cell line, B49. GDNF promotes survival, dopamine uptake, and neurite extension of embryonic dopaminergic (DA) neurons *in vitro*.[1] We have used a semi-quantitative RT-PCR with primers specific to GDNF to study the development of GDNF expression in CNS and peripheral tissues of embryonic rat on gestational days E13.5 and E18, neonatal rat on postnatal days P0 and P10 and adult rat. GDNF mRNA is expressed in all major regions of the CNS; however, the level of expression is both region and age dependent. The highest levels of GDNF mRNA are expressed in P0 spinal cord and in P0 and P10 striatum. Lower levels are expressed in brainstem (including ventral mesencephalon, which contains the substantia nigra), cerebellum, diencephalon and telencephalon. GDNF mRNA is also expressed in many peripheral tissues. In some tissues, GDNF expression is higher than in brain, particularly in embryonic limb bud, kidney and gut; neonatal kidney, gut, lung and testis; and adult lung, liver and ovary. Interestingly, GNDF mRNA is also present in the head, torso and chorion/amnion of the E11.5 embryo.

These observations suggest that GDNF may be a target derived and/or locally acting neurotrophic factor for DA neurons of the substantia nigra, as well as for other classes of CNS neurons. Moreover, GDNF may play an early role as a neurotrophic factor in the PNS or serve as a growth factor for non-neuronal cells. (Supported by the Markey Charitable Trust & NIH grant ES01247). [1] L.-F. H. Lin *et al.*, *Science* **260**, 1130-2 (1993).

537.13

ISOLATION OF MULTIPLE GDNF TRANSCRIPTS FROM THE C_6 GLIOMA CELL LINE. M. D. Swope*, A. Y. Deutch, and E. Lolis. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Glial cell line-derived neurotrophic factor (GDNF) is a newly discovered member of the transforming growth factor β superfamily which exhibits neurotrophic effects on midbrain dopamine neurons both *in vitro* and *in vivo*. GDNF was originally isolated from the B49 glial cell line. We have analyzed two other glial cell lines, the rat C_6 glioma and the human Hs683 glioma, for expression of GDNF. Polyadenylated mRNA was isolated from these cell lines and subjected to RT-PCR for first strand cDNA synthesis. Polymerase chain reaction was used with primers against regions near the initiating methionine and terminal stop codon to amplify the sequence. Two bands were seen from C_6 cells when the PCR reaction products were analyzed by agarose gel electrophoresis and visualized with ethidium bromide. In contrast, no bands were present in the Hs683 PCR reaction.

These results indicate that expression of GDNF is not limited to B49 cells but also occurs in C_6 cells. In addition, the C_6 cells express two GDNF transcripts, which has not been previously reported. Further characterization of these transcripts is in progress.

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537.14

Developmental Expression in the CNS of LERK-2, a Ligand for the Receptor-Tyrosine Kinase ELK M.K. Carpenter*, H. Shilling, T. Van den Bos, M.P. Beckmann, B. L. Davison, J.N. Kott†, L.E. Westrum†, F.A. Fletcher. Dept. of Molecular Immunology, Immunex Corporation, 51 University St., Seattle, WA 98101, † Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Elk is a member of the *eph* family of receptor-like tyrosine kinases and is expressed only in brain and testes in adult animals. The function of this receptor is unknown although it has been postulated to play a role in the development of the nervous system. Using Northern analysis, we have found that RNA coding for elk is expressed at high levels in many areas in the embryonic and postnatal rat brain. Expression decreases with age in some areas, but shows moderate expression in the adult diencephalon, brain stem and cerebellum. LERK-2 (elk ligand) RNA is expressed at high levels in all areas of the developing rat brain we examined. Expression decreases dramatically with age in all but one of these areas. In the adult rat brain, the olfactory bulb has a moderate level of LERK-2 expression. A unique feature of the adult olfactory bulb is that it is continuously innervated by the olfactory epithelium. These data are consistent with the idea that elk and LERK-2 play a role in development of the nervous system.

† Supported in part by NIH grant NS09678.

537.15

EXPRESSION OF STEM CELL FACTOR AND ITS RECEPTOR, c-kit PRODUCT, IN NEURAL CELLS. Su-Chun Zhang* and Sergey Fedoroff, Dept. of Anatomy, Univ. of Saskatchewan, Saskatoon, SK, S7N 0W0, Canada.

The protooncogene, c-kit, encodes the tyrosine kinase receptor (c-kitR) for stem cell factor (SCF). This receptor/ligand plays a central role in hematopoiesis, gametogenesis and melanogenesis. It is found in other tissues including brain, yet its function in the nervous system is unknown. By immunostaining the isolated mouse cortical neurons (E15), astroglia and microglia (newborn) in culture with the monoclonal antibodies against c-kitR and stem cell factor (generously provided by Immunex Corp.), we analysed the cellular localization of the ligand and receptor. In 2~3 day-old cultures, only some of the neurons expressed c-kitR and SCF. Subsequently, most neurons became strongly positive for SCF and few expressed c-kitR. In contrast, their expression in astroglia appeared mainly in the first 5~7 days in culture and became weak thereafter. Microglia strongly expressed c-kitR and SCF throughout the culturing period. These observations indicate that the expression of c-kitR and SCF is developmentally regulated and that both paracrine and autocrine signalling may be involved in neuron-glial and glia-glial interactions. Recombinant SCF (Immunex Corp.) by itself had no observable effects on neural cells. However, SCF, in combination with other cytokines such as CSF-1, IL-1β, TNFα, affect cytokine production by glial cells. Apparently, the function of c-kitR/SCF is to modulate the production of trophic factors (cytokines) by glia.

Supported by a grant from Canadian NCE NeuroScience Network.

537.16

BONE MORPHOMETRIC PROTEINS ARE EXPRESSED IN DEVELOPING MOUSE BRAIN AND ARE NEUROTROPHIC IN VITRO. Z. Zang*, M. F. Mehler, R. Marmur , J. M. Wozney[1] and J. A. Kessler, Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York, 10461 and [1]Genetics Institute, Cambridge, MA, 02140

The bone morphometric proteins (BMPs) are a group of proteins within the TGFβ superfamily. To investigate the potential role of these trophic factors in CNS development, the expression of BMP mRNAs in the developing mouse brain (striatum, hippocampus, cerebral cortex, cerebellum and brain stem) was examined and effects of BMPs on cultured embryonic neurons were defined. Nuclease protection assays for BMP-2, 3, 4, 5, 6, 7 and 8 demonstrated distinct regional and temporal patterns of expression for each mRNA in the developing mouse brain. BMP-2, 6 and 7 mRNAs were expressed as early as E12. By contrast, BMP-3, 4 and 5 mRNAs were present in higher levels in later developmental stages and remained high in adult brain. Regional studies indicated that BMP-2 and 4 had simmilar patterns of expression, whereas BMP-6 and 7 were expressed in other areas in a parallel fashion. Treatment of dissociated cultures of embryonic brain demonstrated enhanced neuronal survival and process outgrowth in response to the BMPs (0.1-10 ng/ml). There was region-specific neurite outgrowth effects of BMP-2 on cerebral cortex, BMP-5 and 6 on cerebellum, and BMP-4 on the other brain regions. These findings suggest that the BMPs may play significant roles in regulating brain development.

537.17

IMMUNOLOGICAL LOCALIZATION OF A POSSIBLE NEUROTROPHIC FACTOR, ACTIVIN A, IN RAT BRAIN RELATING TO PROTEIN MALNUTRITION. H.Fujimura[1], T. Murata[1], M.Funaba[1], A.Niijima[2*] and K. Torii[1,3]. [1]ERATO, R&D Corp. of Japan, Yokohama,221 Japan.; [2]Dept. Physiol., Sch. of Med., Niigata Univ., Niigata, 951 Japan.;[3]Ajinomoto Co., Inc., Central Research Lab., Yokohama, 244 Japan.

In serum of rats fed a diet with or without proper protein, an increase of inhibin, a heterodimer of α-βA subunits, or activin A, a homodimer of βA subunits was detected respectively by an ultra-sensitive bioassay of *Hyrda Japonica* for peptide hormones and growth factors (Torii et al. 1993).

Activin A has been known to be implicated in several brain functions. We recently demonstrated the presence of immunoreactive activin βA subunit in some regions of the brain relating to control of food intake and homeostasis of nutrients,such as the arcuate N.(ARN), supraoptic N. (SON) and PVN. In this study, the localization of βA subunit was precisely examined with a laser scanning confocal microscope by indirect immunofluorescence assay using affinity-purified antisera against synthetic βA(1-11). The immunoreactivity was localized in the neuronal cell bodies of the NTS, ARN, SON and PVN. Fluorescence was detected in the peri-nuclear cytoplasm as scattered granular spots. These results suggest that activin may modulate the functions of neurosecretory cells in PVN, SON or ARN in an autocrine or paracrine manner in recognition and adaptation to protein malnutrition.

537.18

PREPROINSULIN I AND II mRNA ARE PRESENT IN FETAL RAT CENTRAL AND PERIPHERAL NERVOUS SYSTEM. R. Schechter*, T. Gaffney and J. Whitmire. William K. Warren Med. Res. Inst., Univ. of Okla. Health Sci. Ctr., and Dept. of Neonatology, Saint Francis Hosp., Tulsa, OK 74136.

We demostrated the presence of insulin-like substance (ILS) within the central nervous system (Brain Res 582:27-37, 1992). The present study was undertaken to demonstrate the presence of insulin mRNA within the nervous system. Total RNA purified from 19 day gestation fetal rat brain, spinal cord and dorsal root ganglia (DRG) was subjected to reverse transcription (RT) and polymerase chain reaction (PCR) or reverse transcriptase template specific PCR (RS-PCR) to minimize nonspecific hybridization during PCR. All samples were digested with DNAse before the RT or RS-PCR reaction. Pancreatic RNA served as positive control. Negative controls were performed without the presence of pancreatic or brain RNA. PCR was performed using a primer corresponding to the prepro-region and a primer corresponding to the A chain. The PCR products of 330 bases from the fetal brain were further identified by restriction enzyme digestion (RE) to demonstrate their identities as preproinsulin (PP) I and II. RsaI cleaved PP I into two fragments 118 and 212, but did not cut PP II. HinfI cleaved PP II into two fragments 140 and 190, but did not cut PP I. The predicted fragments were obtained by RE in the brain, spinal cord, DRG and pancreatic RNA products. In solution hybridization using a 32 P rat PP I and II cRNA probe demonstrated the presence of PP I and II mRNA within the nervous system. The sequence of the PCR products showed a 100% homology with rat pancreatic PP I and II. Radioimmunoassay showed an ILS within the brain. Thus, 1) preproinsulin I and II mRNA are present within the rat fetal central and peripheral nervous system, 2) these data are highly suggestive of the local synthesis of insulin within the the nervous system.

538.1

THE NERVOUS SYSTEM OF BASIC FGF OVER-EXPRESSING TRANSGENIC MICE. E. Eckenstein*, C. Hicks
Dept. of Cell Biology and Anatomy
Oregon Health Sciences University, Portland, OR 97201

Fibroblast growth factors (FGFs) *in vitro* exhibit a wide variety of neurotrophic and mitogenic effects. Nine members of the FGF multigene family are currently known. The adult central nervous system contains very high levels of acidic and basic FGF (aFGF and bFGF), molecules that lack hydrophobic signal peptide sequences and are not efficiently released from normal healthy cells. Consistent with these observations is our hypothesis that aFGF and bFGF function as initiators of injury responses in CNS.

In order to test this hypothesis we have obtained (a generous gift from Drs. D. Coffin and R. Florkiewicz) transgenic mice that overexpress human bFGF. Initial characterization of these animals showed a highly significant increase in bFGF levels in the CNS of transgenic animals. No gross morphological abnormalities were detected in the brains of these animals, and highly preliminary experiments did not show marked changes in the immuno-histochemical distribution of neuropeptides and neurotransmitter synthesizing enzymes.

538.2

REGULATION OF RAT BDNF GENE PROMOTERS IN TRANSGENIC MICE. T. Timmusk, U. Lendahl*, H. Funakoshi, E. Arenas and M. Metsis*
Lab. of Molecular Neurobiology and *Dept. of Developmental Biology, Karolinska Institute, 17177 Stockholm, Sweden.

The rat brain-derived neutrotrophic factor (BDNF) gene consists of four 5′ exons linked to separate promoters and one 3′ exon encoding the prepro-BDNF protein. Transgenic mice have been generated, that express bacterial chloramphenicol-acetyl transferase (CAT) under the control of six different promoter constructs of the BDNF gene. When fused separately to bacterial CAT gene the upstream regions of the 5′ exons of rat BDNF gene were able to direct the transgene expression in tissues that partially overlapped with the endogenous sites of particular BDNF promoter activities. High-level expression of reporter gene in correct neuronal populations of mouse brain was achieved by using the combinations of longer promoter regions that lie close to each other in the genome (promoters I+ II and promoters III+IV respectively) and by including sequences of BDNF intron/exon splice junctions and 3′ UTR in the constructs. The genomic regions responsible for the *in vivo* upregulation of BDNF expression after kainic acid-induced seizures and KCl-induced spreading depression were mapped. We also report that 5.5 kb of BDNF promoter IV sequence directs axotomy-induced expression of reporter gene in the sciatic nerve of transgenic mice in a manner similar to the regulation of endogeneous BDNF mRNA in the same paradigm. These BDNF promoter constructs provide useful tools for targeted expression of neurotrophic factors to study their function and regulation in central and peripheral nervous system.

538.3

A CALCIUM RESPONSIVE PROMOTER IN THE RAT BDNF GENE. J. F. Bishop*, G. P. Mueller and M. M. Mouradian, Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, and Department of Physiology, USUHS, Bethesda, MD 20892.

Transcription of the rat brain-derived neurotrophic factor (BDNF) gene results in multiple transcripts containing at least five alternate first exons that are separately spliced to a common protein coding exon. Since Ca^{2+} is a prominent second messenger involved in the regulation of BDNF expression, rat C6 glioma cells, known to express BDNF, were exposed to the Ca^{2+} ionophore A23187 and BDNF mRNA levels were measured by reverse transcription (RT) followed by quantitative PCR (QPCR). The cells were incubated with A23187 (5×10^{-6} M) or vehicle for 1 hour, rinsed, incubated another 3 hours in culture medium and total RNA was extracted. QPCR results indicated that BDNF coding exon containing transcripts were elevated approximately 9-fold compared with vehicle treated controls. Inclusion of the RNA polymerase inhibitor actinomycin D (10 μg/ml) in culture medium led to significant decreases in both basal and A23187-stimulated BDNF mRNA concentrations indicating that, in C6 cells, BDNF expression is regulated primarily at the level of transcription. To identify which of the multiple promoters in the BDNF gene is transactivated by increased calcium, the effects of A23187 on the expression of each of the alternate 5' exons were investigated. Of the three alternate 5' exons expressed in C6 cells, only one (exon 1e) increased significantly (approximately 4-fold) which is less than the response of the common coding exon measured in the same samples. Additional calcium-responsive promoters directing the expression of the BDNF gene not detected in the present investigation may account for this observation.

538.4

Exon III of the rat BDNF gene can be induced in the presence of protein synthesis inhibitors: evidence for an immediate-early gene. J. C. Lauterborn*[1], C. T. Stinis[1], V. Y. Hayes[2], P. J. Isackson[2], and C. M. Gall[1].
[1]Dept. of Anatomy and Neurobiology, University of California, Irvine, CA, 92717 & [2]Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville, Fl 32224.

The rat brain-derived neurotrophic factor (BDNF) gene has been shown to contain four short 5' exons (Exons I-IV), associated with distinct promoters, and one 3' exon (Exon V) that encodes the mature BDNF protein. BDNF mRNA transcripts containing the different 5' exons can be differentially induced in brain following seizure although the mechanisms by which this occurs have yet to be elucidated. In the present study, the effect of anisomycin, a protein synthesis inhibitor, on the expression of Exon I and III mRNAs following a stimulated epileptiform afterdischarge of hippocampal neurons was analyzed in adult rat brain. Treatment groups were: 1) single hippocampal afterdischarge (AD) via perforant path stimulation (10 Hz, 4-6 sec) and sacrifice 1.5 hrs post-AD; 2) anisomycin (25mg/kg, s.c.), injected 30 min prior to and 30 min following AD, and sacrifice 1.5 hrs post-AD; 3) 2 injections of anisomycin 1 hr apart and sacrifice 1 hr after last injection; 4) naive controls. Changes in BDNF exon mRNA content in the granule cells were assessed by in situ hybridization using ^{35}S-labeled cRNAs. Following AD, Exon I mRNA was increased 10 to 30-fold above control levels in the granule cells. This effect was reduced by 82% with anisomycin. Like Exon I, Exon III mRNA was markedly increased following AD to approximately 7-fold above control levels. However, the effect of AD on Exon III expression was not reduced by anisomycin. Anisomycin alone had no effect on either Exon I or III. The present results show that Exon III expression can be increased in the presence of protein synthesis inhibitors suggesting that this exon, as opposed to Exon I, is directly induced by physiological activity without intervening protein synthesis (i.e., as an immediate-early gene). The presence of distinct mechanisms regulating the transcription of the 5' exons within one cell type suggests each may be induced under different circumstances. *Supported by NS26748 to C. G., EFA & Abbott Labs award to J. L., and Mayo Foundation funds to P. I.*

538.5

Identification of an Inducible Promoter in the Nerve Growth Factor Gene that Initiates Transcription at Exon 3. MM Racke, PJ Mason, MP Johnson, BW Siegel*, MD Linnik, Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

NGF has been demonstrated to facilitate neurite outgrowth, rescue neurons from injury, and prevent programmed cell death. However, the therapeutic potential of NGF is limited by pharmaceutical difficulties common to many multisubunit proteins. These limitations might be circumvented by identifying compounds which facilitate endogenous transcription of NGF in the brain. Thus, we sought to determine the site of all pharmacologically inducible promoters in the NGF gene using a differential analysis based on semi-quantitative reverse transcription PCR. L929 cells were serum deprived and NGF was induced by treatment with PMA and calcitriol (1,25-dihydroxy- vitamin D3). Of the 4 major transcripts previously identified in mouse (Mol Cell Biol, 7:3057,87), a 2.5 - 4 fold increase in transcripts initiated at exon 1 was noted in cDNA from cells induced with PMA and calcitriol, confirming the previous demonstration of a promoter region near exon 1 (Mol Brain Res, 15: 67,92). In addition, we also noted a 3 - fold increase in cDNA transcripts initiated at exon 3, suggesting a second, previously unidentified inducible promoter in this region. Sequence analysis revealed a consensus HRE in the 3' region of exon 3 which might be responsible for this increased expression. In conclusion, these studies demonstrate a second inducible promoter in the NGF gene near exon 3 that could be targeted for therapeutic intervention.

538.6

PRODUCTION AND PROCESSING OF THE NGF FAMILY OF NEUROTROPHIC FACTORS. **D.P. Lamb*, W. Zhu, O. Lu, J. Stanisz and M. Fahnestock. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.**

The neurotrophic factors NGF, BDNF and NT-3 are translated as pre-pro peptides which require proteolytic processing at dibasic amino acid residues to attain their mature, biologically active forms. Little is known about the processing of the pre-pro forms of the neurotrophins. The ability of furin to process NGF, and the presence of related enzymes in human brain, suggest these serine proteases may play a role in neurotrophic factor processing. The study of neurotrophic factor processing has been hindered by the lack of sufficient substrate for analysis. We report here the production of quantities of pre-pro and mature neurotrophic factors sufficient for processing studies.

Baculoviruses were constructed expressing cDNAs for NGF and NT-3 as well as PC2, a member of the prohormone convertase family of proteases related to furin. The resultant recombinant viruses were used to infect Sf9 insect cells,and both mature and pre-pro trophic factors were identified on SDS-PAGE gels and analyzed by Western Blotting. The biological activities of the neurotrophins were confirmed in neurite outgrowth assays using neonatal mouse SCG and DRG neuronal cultures. Expression of PC2 was confirmed by Western blotting and protease assays using synthetic substrates.

Coinfections were carried out using NGF and NT-3 recombinant virus along with PC2 recombinant virus to determine if PC2 could enhance neurotrophic factor processing in insect cells. The extent to which PC2 could enhance processing was determined by photoimage analysis of the bands from the Western Blots. The NGF+PC2 coinfected supernatants showed approximately seven fold more mature trophic factor than the control infection, whereas NT-3+PC2 coinfected supernatants showed about a three fold increase in mature NT-3. *(Supported by NIH and MRC*

538.7

Beta- Nerve Growth Factor (NGF) is an artifact. J. Lakshmanan[1*], S. Soinila[2], K. Pesonen[2], T. Lahtinen[2], A. Baker[1], J. Perheentupa[2] and D. A. Fisher[1]. Harbor UCLA Medical Center, CA, USA[1] and University of Helsinki, Helsinki, Finland[2].

We previously reported the presence of beta NGF in normal adult mouse sera of both sexes (1-2 ng/ml) and in sera of adult male aggressive mice (115-490 ng/ml) [Am. J. Physiol. 250: E386-E392 1986]. We now have examined the molecular species of serum NGF both by immunoblotting and column chromatographic analysis. Aliquots (250 µl) of mouse sera were immunoprecipitated with beta-NGF antiserum and the precipitates analyzed by immunoblotting. Similar volumes of sera were subjected to Sephadex G-100 chromatography; column fractions were analyzed by beta-NGF RIA. Immunoblotting analysis revealed the presence of a 53 kDa NGF protein but no 13 kDa NGF immunoreactive protein in sera of either normal or aggressive male mice. The radioactive intensity of 53 kDa NGF protein was several fold greater in aggressive mouse sera compared to normal mouse sera. By chromatographic analysis, the NGF-immunoreactivity of normal male mouse sera eluted as a single peak that appeared in the void volume while that of aggressive mice eluted in two distinctive peaks. The first appeared in the void volume (as in normal mice sera) and the second corresponded to the elution profile of 13 kDa NGF. The latter peak accounted for more than 92% of total immunoreactivity. Both aggressive mouse serum and SMG-53 kDa NGF stimulated neurite outgrowth from chick embryo dorsal root ganglion explants. The absence of 13 kDa NGF in neat mouse sera immunoprecipitates and its presence in aggressive serum samples subjected to Sephadex-chromatography suggest that the 13 kDa NGF is an artifactual proteolytic fragment of 53 kDa NGF generated in vitro during chromatography.

538.8

IDENTIFICATION OF RNA BINDING PROTEINS WHICH MAY CONTROL NERVE GROWTH FACTOR mRNA STABILITY. B. Tang*, S.P. Pshenichkin, and B.C. Wise. Fidia-Georgetown Institute for the Neurosciences and Department of Pharmacology, Georgetown University, Washington, D.C. 20007

Our previous studies have shown that interleukin-1 and okadaic acid, a phosphoprotein phosphatase inhibitor, increase the half-life ($t_{1/2}$) of the nerve growth factor (mRNA) in primary cultures of cortical astrocytes. The 3'-untranslated region (UTR) of the NGF mRNA contains an AU nucleotide rich region which, similar to other short-lived mRNAs, might modulate its rate of degradation. Using Northwestern blotting analysis, we have now identified a protein with a MW of about 140 kDa which binds to a radiolabeled RNA probe containing the AU-rich domain in the 3'UTR of the rat NGF mRNA. RNA binding activity was observed using a full-length NGF RNA probe, but was not seen using a probe lacking the 3'-UTR or using a β-actin RNA probe. The binding activity or amount of this protein was increased in astrocytes treated with okadaic acid (5-20 nM). Maximal induction of binding activity was seen following 9 h of treatment at the maximal okadaic acid concentration tested. Under these conditions NGF mRNA content was also elevated by about 15-fold compared to untreated astrocytes. The RNA binding activity was abolished by treating astroglial cell extracts with trypsin, and the induction of the RNA binding protein by okadaic acid was prevented by cotreatment with cycloheximide (10 µg/ml) indicating that okadaic stimulates the synthesis of this putative modulator of NGF mRNA degradation. Finally, the induction of this binding activity by okadaic acid is associated with an increase (4-fold) in the $t_{1/2}$ of the NGF mRNA. Transfection of cell lines with expression vectors containing the full-length NGF cDNA or NGF cDNA lacking the 3'UTR are underway to study the role of the 3'-UTR and the putative modulator in the control of NGF mRNA degradation.

538.9

PROCESSING OF NGF, BDNF, AND NT-3 PRECURSORS BY PROHORMONE CONVERTASES. N. G. Seidah#, S. Pareek, S. Benjannet#, J. Laliberté, M. Chrétien#, and R. A. Murphy*. Montreal Neurological Institute, McGill University and the #Clinical Research Institute of Montreal, Montreal, Quebec, CANADA

Conversion of prohormones and precursor proteins into biologically active molecules involves the concerted action of a number of convertases. Furin can process the precursor of mouse ß-NGF (Bresnahan *et al., J. Cell Biol.*, 111, 2851-2859, 1990) but no information is yet available to explain how other members of the neurotophin family are generated. In this study, we have compared the processing of NGF, BDNF and NT-3 by prohormone convertases. Recombinant vaccinia virus vectors were used to co-express the convertases PC1, PC2, and furin together with NGF, BDNF or NT-3 in human colon carcinoma LoVo cells, lacking endogenous furin. Virally infected cells were metabolically labeled with ^{35}S-methionine and conditioned medium was treated with antibodies to NGF which immunoprecipitate NGF and NT-3. BDNF was identified on Western blots using a BDNF-specific peptide polyclonal antibody. Results show that NGF is synthesized in LoVo cells as a 48 kDa precursor (probably glycosylated) and processed into 14.5 (probably glycosylated) and 13.2 kDa products. NGF processing was effectively carried out by furin and no other convertase. NT-3 was processed efficiently by furin with a small amount of processing carried out by PC1 and PC2 as well. BDNF was effectively processed by furin, with some processing by PC1. These studies suggest that the neurotrophins are most effectively processed by furin, and that other convertases have limited activity as well.

538.10

DETECTION OF PROHORMONE CONVERTASES IN DEVELOPING AND ADULT MOUSE SUBMANDIBULAR GLANDS. H. Farhadi, S. Pareek*, M. Marcinkiewicz#, N. G. Seidah#, M. Chrétien#, and R. A. Murphy. Montreal Neurological Institute, McGill University and the #Clinical Research Institute of Montreal, Montreal, Quebec, CANADA

The processing enzyme(s) responsible for generating mature ß-NGF from its precursor at the amino terminal end have not been identified. Prohormone processing enzymes, called convertases, have been implicated in the processing of precursor proteins at pairs of basic amino acids and a companion study from our laboratories presented at this meeting (Seidah *et al.*, 1994) have shown that at least one member of this family can cleave NGF, BDNF, and NT-3. The aim of this study was to investigate the relationship of NGF and convertases in the mouse submandibular gland. Northern blot analysis and *in situ* hybridization revealed high levels of mRNA coding for PC1, PC2, PC5 and furin in the salivary glands of neonatal and pubescent male mice; levels in NGF-containing granular tubule cells of adults are significant but lower than during pubescence. These results were confirmed by immunocytochemistry. Levels of PC1 mRNA and protein were signficantly lower than those of the other processing enzymes. Adult female salivary glands expressed only low levels of NGF and processing enzymes. Our results suggest that one or more prohormone convertases may be involved in processing the precursor of NGF in mouse submandibular glands.

538.11

RETROGRADE TRANSPORT AND SIGNALING MECHANISMS BY NERVE GROWTH FACTOR AND LEUKEMIA INHIBITORY FACTOR IN SYMPATHETIC NEURONS. D. R. Ure* and R. B. Campenot. Department of Anatomy and Cell Biology, University of Alberta, Edmonton, AB T6G 2H7.

Nerve growth factor (NGF) and leukemia inhibitory factor (LIF) are both retrogradely transported by rat sympathetic neurons in a compartmented culture system. We have further characterized the retrograde transport of ^{125}I-NGF and ^{125}I-LIF. The level of NGF transport appears to be unaffected by the acute presence of LIF. Reciprocally, LIF transport is equivalent whether neurons are supplied with 10 or 200 ng/ml NGF. In contrast, pretreating neurons for 6 days with LIF leads to the reduction of both NGF and LIF transport by at least 50%, suggesting that LIF-differentiated neurons may display attenuated NGF-dependent responses. NGF transport levels are not affected by brain derived neurotrophic factor or neurotrophin-3 applied concurrently and at concentrations in 100-fold excess of ^{125}I-NGF. Kinetic analysis of NGF transport/processing revealed that following a 5-hr pulse of ^{125}I-NGF to peripheral neurites, most of the radiolabel is transported and released within the first 24 hr. However, cell-associated radiolabel is still present several days following the pulse. Experiments are now being performed to address whether the retrograde transport of NGF or LIF is necessary for transcriptional changes induced by these signaling ligands.

538.12

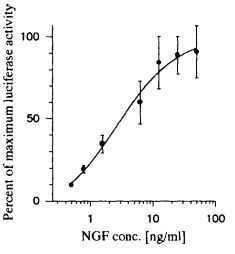
ASSAY-DEPENDENT VARIABILITY IN THE CROSS-REACTIVITY OF ß-NGF ANTIBODIES WITH RELATED NEUROTROPHINS S. Varon* and J.M. Conner UCSD, Department of Biology, La Jolla, CA 92093

Previous studies have indicated that antibodies raised against purified ß-NGF may cross-react with other neurotrophin family members sharing similar sequence homology, such as BDNF, NT-3 and NT-4/5. New antibodies generated against any one neurotrophin must, therefore, be examined for potential cross-reactivity with the others. The assay system used to evaluate cross-reactivity is frequently chosen for its convenience. While characterizing our ß-NGF antibodies, we have found that the choice of the assay system markedly affects cross-reactivity results. In an immunoblot assay (following SDS-PAGE), mouse and human NGF were robustly detected by the antibodies while NT-3 and BDNF were virtually undetectable. In a slot-blot assay, the antibodies recognized NGF and NT-3 but BDNF and NT-4/5 were only detected after fixative treatment of the blot. In a one-site ELISA (antigen coupled to the solid phase) the antibodies recognized both BDNF and NT-3, but more weakly than ß-NGF. Using a two-site ELISA (with the same antibody for capture and detection), no cross-reactivity with BDNF, NT-3 or NT-4/5 occurred. When injected into normal rats (intrastriatal injection; 600ng each), all four neurotrophins were immunohistochemically detected to about the same extent. In vitro assays with dissociated E8 chick sensory neurons showed that BDNF, NGF, and NT-3 all supported cell survival (to varying degrees), but the antibodies could only block the biological activity of NGF. These findings clearly demonstrate that cross-reactivity between NGF antibodies and related molecules is a function of the assay used for the evaluation and caution against transferring antibody specificity data across assay systems. Supported by NINCDS NS-16349.

538.13

A RAPID ASSAY FOR NGF-ACTIVITY IN NEURONS BASED ON INDUCTION OF LUCIFERASE UNDER CONTROL OF THE NGFI-A PROMOTER. S.M. Elsas, F.F. Eide* and W.C. Mobley. Dept. of Neurology, Univ. of California, San Francisco, CA 94143.

Detailed characterization of neurotrophin structural features important for activation of receptors of the *trk* gene family would benefit by the availability of a rapid and versatile screening assay in neurons. We designed an assay for NGF activity based on rapid induction of the NGFI-A gene by NGF.



PC12 cells were transiently transfected with a plasmid containing the luciferase gene under control of the NGFI-A promoter. Two days after transfection the cells were exposed to NGF or other compounds and were then lysed. After addition of luciferin substrate, light output was measured.

NGF induced a specific, dose-dependent increase in luciferase activity. The dose-dependency (EC_{50} = 2.7 ng/ml) correlated well with that for neurite outgrowth and for autophosphorylation of the *trk* receptor. At 50 ng/ml the signal to noise ratio was about 1:100. An exposure of 30 min. was sufficient to induce a maximum activation after a delay of 4 hrs. Ligand specificity was evaluated by comparing the NGF response to that for bFGF and EGF. While all elicited a response, only that for NGF was inhibited by K252b. In that this assay can be carried out in 96-well plates, it represents a useful new tool for defining NGF - NGF receptor recognition determinants.

The authors thank J. Milbrandt for the NGFI-A/luc construct.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION IV

539.1

INCREASED INSULIN-LIKE GROWTH FACTOR II GENE EXPRESSION IN DIABETIC RAT LUMBAR DORSAL ROOT GANGLIA AND MOTONEURONS. F.J. Liuzzi*, A.S. Depto and A.I. Vinik. The Diabetes Institutes, Eastern Virginia Medical School, Norfolk, VA 23501.

Neuropathy is a major complication of diabetes mellitus and can involve sensory as well as motoneurons. While no single cause of diabetic neuropathy has been identified, the contributions of a number of pathogenic mechanisms have been extensively studied. The insulin-like growth factors (IGFs) promote neurite outgrowth *in vitro* and axonal regeneration *in vivo*. These data coupled with reports of reduced IGF serum levels suggest the possibility that a decline in IGFs available to neurons contributes to the pathogenesis of diabetic neuropathy. To address this possibility, we examined IGF-II gene expression in lumbar dorsal root ganglia (DRGs) and spinal cords of rats made diabetic by a single dose (55mg/kg, i.p.) of streptozotocin. Age-matched, sham-injected rats served as controls.

After nine weeks of diabetes (blood glucose levels maintained between 200-400mg/dl) the diabetic rats and the age-matched controls were sacrificed and tissues harvested for *in situ* hybridization with a cRNA probe to IGF-II. Contrary to our expectations, preliminary quantification revealed a three to four-fold increase in IGF-II mRNA levels in lumbar DRGs of diabetic rats as compared to those of normal, non-diabetic rats. Similar changes were observed in lumbar spinal motoneurons.

These observations seem enigmatic in light of the diminished regenerative capacity of sensory and motoneuron axons in diabetic rats. It is possible, however, that a compensatory up-regulation of local IGF-II synthesis by neurons, in response to low serum levels or reduced uptake in diabetes, inhibits axonal regeneration by switching-off the regenerative program normally exhibited by axotomized sensory and motoneurons.

539.2

DEVELOPMENTAL EXPRESSION OF NEUROTROPHINS IN THE SPINAL CORD AND LIMB BUDS OF THE RAT EMBRYO. L.A. Scarisbrick*[1] P.J. Isackson[2] and E.G. Jones[1]. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA, 92717[1] and Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville Fl, 32224[2].

The expression patterns of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 5 (NT-5) were examined within the spinal cord and limb bud from E13 to E19, a period corresponding to the main period of naturally occurring spinal motoneuron degeneration in the rat. The domains of gene expression were compared with the appearance of NCAM and HNK-1 immunoreactive cells and nerve fibers in parallel sections. From the onset of expression of multiple neurotrophins on E14, a partially overlapping pattern of expression of each was observed in the ventral horn of the spinal cord and in the mesenchymal and epithelial components of the limb bud. The peak expression of each neurotrophin, at each site, occurred from E14 to E16. Over this period, NT-3 transcripts are the most abundant in the spinal cord and developing limb bud muscle masses. Significantly lower levels of each neurotrophin are detected at each site by E19. Only NT-5 transcripts are detected at significant levels in the limb bud on E13, when NCAM immunostained myoblast condensations, in some cases already associated with NCAM immunostained nerve fibers, are observed. The transient, dense expression of multiple members of the neurotrophin gene family in select regions of the limb bud and spinal cord implicates the neurotrophins in early critical events at several levels of the developing spinal motoneuron-muscle cell axis. Peak levels of expression coincide with the early phases of naturally occurring spinal motoneuron cell death in the spinal cord and with the early stages of arrival of NCAM immunostained nerve fibers and the differentiation of myoblasts in the limb bud. The development of the spinal cord and limb bud are therefore linked by the co-ordinate expression of members of the neurotrophin gene family. Supported by NIH grant NS 30109-02.

539.3

SELECTIVE EXPRESSION OF NEUROTROPHIN-3 mRNA IN MUSCLE SPINDLES OF THE RAT. J.C.V.M. Copray* and N. Brouwer. Department of Medical Physiology, University of Groningen, 9712 KZ Groningen, The Netherlands.

To evaluate the possible involvement of NT-3 in the formation and maintenance of muscle spindles, an in situ hybridization study was set up to analyze the expression of neurotrophin-3 (NT-3) mRNA in rat muscle spindles from the first embryonic stages of their formation unto their mature appearance in adult animals. Serial, sagittal cryosections of 25 µm were made of 4% paraformaldehyde-fixed intact hindlimbs or dissected hindlimb muscles derived from embryonic (E15-E21), neonatal (P1,P6,P17 and P30) and adult Wistar rats. In situ hybridization for NT-3 mRNA was done in combination with the immunohistochemical detection of desmin, calretinin and slow tonic MHC. Starting from the appearance of the firstly formed intrafusal fiber, i.e. the nuclear bag2 fiber, at E19, the intramuscular expression of NT-3 mRNA was confined to the intrafusal fibers of the muscle spindles. High levels of NT-3 mRNA were found in the equatorial region of the nuclear bag type intrafusal fibers and remained present in these intrafusal fibers throughout life. During the entire period of muscle formation, examined from E15 on, as well as in mature muscles, no NT-3 mRNA could be detected in extrafusal fibers. The exclusive intramuscular expression of NT-3 mRNA within intrafusal fibers substantiates the involvement of NT-3 in the formation as well as the maintenance of muscle spindles.

539.4

REGULATION OF BDNF, NT-3 AND NT-4 mRNA EXPRESSION IN RAT MUSCLE DURING DEVELOPMENT AND AFTER SCIATIC NERVE LESION. A.Sh.Parsadanian, O.Griesbeck, P.Carroll, R.A.Hughes, M.Sendtner, H.Thoenen*. Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, 82152 Martinsried, Germany.

Expression of BDNF, NT-3 and NT-4, was examined in rat skeletal muscle during development (E15-adult) and in adult rat gastrocnemius muscle at different times after transection of the sciatic nerve using quantitative Northern blot analysis of poly A^+ RNA and in situ hybridization. At E15, the highest level of expression was found for NT-3 (63pg/µg poly A^+ RNA), approximately 20 times greater than that of either BDNF or NT-4. During later development, the levels of all neurotrophin mRNAs fell. BDNF and NT-3 mRNA remained low in adulthood, whereas NT-4 mRNA levels increased postnatally, such that adult muscle contained similar quantities of NT-3 and NT-4 mRNA, but very little BDNF message. In situ hybridization of E15 limb bud showed a clear signal for NT-4 only in epidermis, for BDNF mostly in mesenchyme and in some muscle cells, while NT-3 was abundantly expressed in muscle. 24 hours after sciatic nerve lesion, the levels of BDNF and NT-4 mRNA in muscle were reduced considerably. While the amount of NT-4 mRNA remained low, BDNF mRNA levels increased with time after lesion, reaching a level 2.6-fold greater than control 14 days after lesion. In contrast, NT-3 mRNA was not affected after nerve injury. Hybridization of transverse sections of denervated muscle with a BDNF riboprobe gave a signal over peripheral nerve. No significant labeling was found in the surrounding muscle tissue. The same riboprobe also labels cells in a distal segment of the sciatic nerve 4 weeks after lesion, suggesting that the source of increased BDNF mRNA production in denervated muscle is Schwann cells in peripheral nerves leading through the muscle.

539.5

DISTINCT REGULATION OF NT-4 mRNA IN THE SKELETAL MUSCLE SUGGESTS A ROLE FOR NT-4 IN THE MAINTENANCE OF THE NEUROMUSCULAR JUNCTION.
H. Funakoshi*[1], N. Belluardo[1,3], E. Arenas[1], Y. Yamamoto[2], H. Otsuka[2], H. Persson[1] and C. F. Ibáñez[1]. [1]Laboratory of Molecular Neurobiology, MBB and [2]Department of Nobel Neurophysiology, Karolinska Institute, S-17177 Stockholm, Sweden and [3]Institute of Human Physiology, University of Catania, Catania, Italy.

We have previously shown that the levels of NT-4 mRNA in skeletal muscle, in contrast to those of the other neurotrophins, decreased 1 day after sciatic nerve transection (Funakoshi et al., J. Cell Biol. 123:455, 1993). We now show that, as opposed to the other neurotrophins, NT-4 mRNA levels in muscle increase during postnatal development. The differential regulation of NT-4 mRNA expression suggested that its level may be regulated by motorneuron activity. In agreement of this, electrical stimulation of the sciatic nerve increased NT-4 mRNA levels in gastrocnemius and soleus muscles in a dose-dependent manner. NT-4 mRNA levels peaked 12 hrs after stimulation and decreased thereafter, reaching basal levels 24 hrs after stimulation. Elevated NT-4 mRNA levels could be sustained after repeated stimulations. A similar effect could be obtained after direct stimulation of the muscle. In contrast, the mRNA levels of the other neurotrophins are not affected by electrical stimulation. These observations suggest NT-4 may play a local role in the maintenance of the neuromuscular junction in an activity-dependent manner. This possibility is currently being explored using purified recombinant NT-4 protein and grafts of genetically engineered fibroblasts producing NT-4.

539.6

AN RNA SPLICING MUTATION THAT DISRUPTS THE HUMAN CILIARY NEUROTROPHIC FACTOR (CNTF) GENE.
R. Takahashi[1], H. Misawa[1], H. Yokoji[2], M. Hayashi[2], T. Komori[2], A. Inaba[2], T. Ohtake*[2], Jianguo Hu[3] and T. Deguchi[1,3] Department of Neurology (1), Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu City, Tokyo, Department of Neurology (2), Tokyo Metropolitan Neurological Hospital, Tokyo, and Reseach and Development Center (3), BML, Inc., Saitama, Japan

Ciliary neurotrophic factor (CNTF) promotes the survival of a variety of neurons. A recent report showed that disruption of the CNTF gene in mice caused motor neuron degeneration, suggesting an essential role of CNTF in the survival of motor neurons (Masu et al, 1993). We report a null mutation in the human CNTF gene. The mutated allele shows a G to A transition producing a new splice acceptor site and the resulting mRNA species codes for an aberrant protein. Analysis of tissue samples from various genotype subjects and transfection of CNTF minigenes into cultured cells demonstrates that the mutated allele expresses only the mutated mRNA species. Mutated CNTF protein is not detected either by immunoblot or immunohistochemical analyses of human tissue samples. In 391 Japanese people tested, 242 (61.9%) were normal homozygotes, 140 (35.8%) heterozygotes, and 9 (2.3%) mutant homozygotes; the mutated allele frequency was 20%. The distribution of the three genotypes is similar in healthy and neurological disease subjects. Detailed electrophysiological tests on 30- and 64-year-old mutant homozygotes reveals no apparent abnormalities. In contrast to the report on mice, our findings indicate that human CNTF deficiency does not induce obvious neurologic dysfunction.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION V

540.1

GLIAL-DERIVED NEUROTROPHIC FACTOR cDNA SEQUENCE AND mRNA LOCALIZATION AND REGULATION.
J.E. Springer*, J.L. Seeburger, L.W. Bergman, and J.Q. Trojanowski. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA 19102, and Dept. of Neuropathology, University Pennsylvania, Philadelphia, PA. 19104.

The use of GDNF and other neurotrophic factors as potential therapeutic agents in the treatment of Parkinson's disease has been proposed. However, recent studies provide evidence that the distribution, and thus the actions of GDNF may be more widespread than previously suggested. In the present study, we report that GDNF mRNA is expressed in a Schwann cell line previously shown to exhibit GDNF-like activity, in adult rat skeletal muscle, a tissue known to contain several neurotrophic factors, and in a number of rat and human CNS structures. Two GDNF transcripts were amplified by RT-PCR from Schwann cell total RNA. The predicted ($GDNF_{633}$) and a smaller ($GDNF_{555}$) product were found in these samples, with $GDNF_{555}$ being consistently more abundant than $GDNF_{633}$. The sequence of $GDNF_{555}$ was identical to $GDNF_{633}$ with the exception of a missing 78 base pair segment containing a cryptic splice site. It is likely, given our Southern blot data, that GDNF mRNA is transcribed from a single gene, and $GDNF_{555}$ mRNA is a splice variant of $GDNF_{633}$. The expression of some neurotrophic factors in skeletal muscle is modified following peripheral nerve damage, possibly as a compensatory response to denervation. In our experiments, the medial gastrocnemius muscle was denervated, and the treated animals allowed to survive for 1, 7, 14, or 42 days. The expression of $GDNF_{633}$ in denervated muscle was found to be elevated at 7 to 14 days compared to controls. In conclusion, while GDNF has been reported to be a selective neurotrophic factor for embryonic mesencephalic dopamine neurons, the results of this and other studies provide evidence that GDNF mRNA is expressed and regulated in different tissues. It is possible that this neurotrophic factor may be of biological significance for other neuronal populations, including those ensheathed by Schwann cells and innervating skeletal muscle.

540.2

SEIZURES INCREASE GDNF mRNA IN RAT HIPPOCAMPUS.
C. M. Gall*[1] and J.E. Springer[2]. [1]Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717 and [2]Dept. of Neurology, Hahnemann Univ. School of Med., Philadelphia, PA. 19102

Glial-derived neurotrophic factor (GDNF) is a recently described neurotrophic factor with trophic activity for mesencephalic dopamine neurons. In rat brain GDNF mRNA expression decreases during postnatal development to very low levels in the adult. In the present, study the influence of lesion- and kainic acid-induced seizures on GDNF mRNA content in adult rat brain was examined. In untreated rats, in situ hybridization to GDNF mRNA labeled cells in the olfactory tubercle and anterior thalamus but was not detected in hippocampus. In association with hilus lesion-induced limbic seizures (which recur from 2 to 10 hrs after surgery), hybridization was markedly increased in the dentate gyrus granule cells. GDNF mRNA reached half-maximal levels by 4 hrs and maximal levels by 10 hrs after seizure onset and then declined to near control levels by 24 hrs. In these rats hybridization was not increased in other brain areas. RT-PCR analysis of total mRNA isolated from the dentate gyrus/CA1 of hilus lesion rats demonstrated a preferential increase in GDNF633 compared to GDNF555. Intraventricular injection of 0.5 ug kainic acid stimulated a similar bilateral increase in GDNF mRNA in the granule cells. However, at 10 hrs post-injection, hybridization to GDNF mRNA was also elevated in fields of neuronal damage in the ipsilateral hemisphere including stratum pyramidale of hippocampal region CA3, medial thalamus, and perirhinal cortex. These results indicate that the intense physiological activity of seizure is sufficient to induce GDNF mRNA expression in hippocampal granule cells but not in other populations of neurons engaged in the seizure episode (i.e., cortical pyramidal cells). The additional fields of hybridization in kainic acid-treated rats indicate that GDNF expression is also increased in some populations of neurons following neurotoxic insult. Supported by NS26748.

540.3

SEIZURES CAUSE ELEVATIONS OF BRAIN DERIVED NEUROTROPHIC FACTOR mRNA IN WIDESPREAD AREAS OF DEVELOPING RAT BRAIN.
H. I. Kornblum*[1,2], R. Sankar [2,3], D. Shin[3], C. G.Wasterlain[3], K.Tatsukawa [1,2] and C. M. Gall[4], Departments of Pharmacology[1], Pediatrics[2], and Neurology[3], UCLA School of Medicine, Los Angeles, CA 90024, and Department of Anatomy and Neurobiology[4], UC Irvine, School of Medicine, Irvine, CA, 92717.

Prior studies have demonstrated that seizures induce an elevation in messenger RNAs for nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) within adult rat brain, but that seizures prior to 2 weeks of life do not produce similar changes. In the present study, we demonstrate that limbic seizures, using two different experimental models, dramatically enhance hybridization densities for BDNF mRNA in developing rat brain. Rats from 7 to 60 days of age were pretreated with lithium chloride followed 16hr later by 100 mg/kg pilocarpine. Some animals were treated with kainic acid (3 mg/kg). Controls were treated with vehicle. Additional animals were pretreated with diazepam to prevent seizures. Animals were killed 1,2,4, or 8 hrs post injection. Brains were then processed for *in situ* hybridization using ^{35}S-labeled cRNA probes. Both treatments induced electrographic and clinical seizures at all ages studied. At P12 and older, pilocarpine-induced seizures resulted in dramatic elevations of hybridization densities in multiple brain areas including neocortex; hippocampus; piriform, cingulate and entorhinal cortices; and amygdala. Maximal effects were observed between 2 and 4 hrs post injection. Elevations in hybridization were prevented by pretreatment with diazepam. On P7 there were significantly elevated hybridization densitieswithin cingulate, piriform and and entorhinal cortex. Kainate-induced seizures led to markedly enhanced hybridization only within the CA3 subfield of the hippocampus of 9 day old rats. The present study demonstrates that neurotrophin expression can be dramatically altered by seizures within the developing brain, at ages when significant maturational events are taking place. Furthermore, different models of limbic seizures produce different effects on neurotrophin expression.

540.4

REGULATION OF BDNF PROTEIN AND NEUROPEPTIDES AFTER LIMBIC SEIZURES.
H. Nawa*[1], J. Carnahan[2], and C. M. Gall[3], 1;Beckman Neuroscience Ctr, Cold Spring Harbor Lab. Cold Spring Harbor, NY 11724, 2;Amgen Ctr, Thousand Oaks,CA 91320, and 3;Dept. of Anatomy & Neurobiology, Univ. of Calif., Irvine, CA 92717.

Messenger RNA for brain-derived neurotrophic factor (BDNF) is distributed in many brain regions and regulated by excitatory neuronal activity. Despite numerous studies of BDNF mRNA, the production of BDNF protein is poorly understood because of a lack of its quantitative measurement. Recently, we have established a two-site enzyme immunoassay (ELISA) for BDNF protein. The combination of high-affinity antibodies and fluorometric detection has increased sensitivity to 1pg/well, which enables us to measure trace amounts of BDNF protein in tissues. In normal rat brain, BDNF content was highest in hippocampus, followed by hypothalamus, neocortex, thalamus, cerebellum and striatum. This resembles the distribution of BDNF mRNA, suggesting accuracy of the ELISA. To test the hypothesis that seizure-induced BDNF mediates later changes in neuropeptide expression, we evaluated the temporal and spatial correlation between changes in BDNF protein and neuropeptide levels after limbic seizures. In hippocampus BDNF was rapidly increased about 2-fold by 4 hr, reached maximal levels at 16 hr, and remained elevated through 96 hr after seizure onset. In contrast, the BDNF increase was delayed in cortex and returned to the basal level by 24 hr. After an initial decline, neuropeptide Y content increased to control levels by 16 hr and to well above control levels at 24-96 hr in all regions examined. Enkephalin content was also clearly increased by 16 hr after seizure onset in all areas. In contrast, hippocampal dynorphin levels were markedly below control values from 2 through 96 hr after seizure onset. These data demonstrate increases in BDNF protein precede the major change in enkephalin and neuropeptide Y content and are thus consistent with the hypothesis that BDNF may play an important role in neuropeptide regulation after limbic seizures.

540.5

INDUCTION OF BDNF AND trkB BY ELECTROCONVULSIVE SEIZURE (ECS): REGIONAL REGULATION AND ROLE OF CREB. M. Nibuya*, L. Rydelek-Fitzgerald, S. Morinobu, D.S. Russell, E.J. Nestler, and R.S. Duman. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT

Recent studies from our laboratory demonstrate that acute ECS increases the expression of BDNF (brain derived neurotrophic factor), and that chronic ECS enhances the induction of BDNF in rat frontal cortex. This work has been extended to studies of the regional regulation of BDNF and trkB, the protein tyrosine kinase receptor for BDNF, and to studies of CREB (cAMP response element binding protein) in mediating the induction of BDNF by ECS treatment. In situ hybridization demonstrates that acute ECS (2 h) increases the expression of BDNF mRNA in several regions of hippocampus (dentate gyrus, CA1, and CA3) and cerebral cortex (piriform, occipital, and frontal cortex). Acute ECS (2 h) also increases levels of trkB mRNA and immunoreactivity, determined by northern and western blot, respectively, whereas chronic ECS down-regulates trkB immunoreactivity in frontal cortex. Unilateral, local infusion of CREB antisense oligonucleotide, which we have shown to decrease levels of CREB immunoreactivity, into the hippocampus blocks the induction of BDNF mRNA in the area surrounding the infusion site. In contrast, local infusion of CREB sense strand oligonucleotide, which does not alter levels of CREB, into the contralateral hippocampus does not influence the induction of BDNF by ECS. These studies provide a preliminary characterization of the regional regulation of BDNF and trkB expression by ECS, and demonstrate that CREB may be necessary for the induction of BDNF mRNA.

540.6

Regional Increase in Brain-Derived Neurotrophic Factor and Nerve Growth Factor mRNA, but not in Acidic and Basic Fibroblast Growth Factor mRNA in Kindling. K. Sato[1,2], K. Kashihara[3], K. Morimoto*[4], K. Otsuki[1], Y. Fujiwara[1], K. Akiyama[1], T. Hayabara[2] and S. Kuroda[1].
[1]Department of Neuropsychiatry and [3]Department of of Neurology, Okayama University Medical School, Okayama 700, Japan. [2]Clinical Research Institute, National Sanatorium Minamiokayama Hospital, Okayama 701-03, Japan. [4]Department of Neuropsychiatry, Kagawa Medical School, Kagawa 761-07, Japan.

The levels of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), acidic and basic fibroblast growth factor (FGF) have been studied in the kindling model of epilepsy. Using in situ hybridization and ^{35}S-labeled oligonucleotide probes, the induction of mRNAs was evaluated in the rat brain 1h after generalized seizures induced by daily electrical stimulations from the amygdala. BDNF mRNA levels were markedly elevated bilaterally in the granule cell layer of the dentate gyrus and the pyramidal cell layer of CA1, CA2, CA3 and CA4. The magnitude of increase in the dentate gyrus was 4- to 5-fold of that of the sham-operated controls, whereas moderate increase was observed in other regions. The significant increases in NGF mRNA were observed in the granule cell layer of dentate gyrus, CA1, CA2, CA3, CA4 and amygdala. Acidic FGF mRNA slightly increased in the granule cell layer of the dentate gyrus. No detectable changes, however, were observed in basic FGF mRNA in response to kindled seizures in the regions examined.

These results indicate that the mRNA inductions of neurotrophic factors, especially BDNF mRNA expression in the dentate gyrus, corresponded to increases in metabolic and electrical activity associated with seizures or neuronal vulnerability coinsident with the kindling-induced seizures. The observed differences in the spatial pattern of the neurotrophic factor mRNAs suggest that the mechanisms undelying expression of individual neurotrophic factors in kindling are heterogeneous.

540.7

UNILATERAL NEONATAL BUT NOT ADULT HIPPOCAMPAL LESIONS DECREASE BDNF mRNA IN THE CONTRALATERAL HIPPOCAMPUS. H. van Praag*, R.C.Elliott, P. Qu , C.F. Dreyfus and I.B. Black, Dept. Neuroscience & Cell Biology, UMDNJ/Robert Wood Johnson Med School, Piscataway, NJ08854.

Unilateral neonatal hippocampal lesions cause a longlasting impairment in spatial memory, whereas unilateral lesions made in the adult rat do not. The discrepancy between the adult and the neonatal lesions may be due to a deficit in the remaining hippocampus. We hypothesized that hippocampal trophic factors may play a role since we found previously that the projecting septum and locus coeruleus are dysfunctional after the neonatal hippocampal lesion. Moreover, previous work had suggested that trophic molecules are involved in learning and memory.

Unilateral electrolytic hippocampal lesions were made on postnatal day 1 or in 3 month old adult rats. Using a solution hybridization assay we measured NGF and BDNF mRNA in the remaining hippocampus and adjacent cortex at 4 weeks after the lesion. BDNF but not NGF mRNA was significantly decreased by 30% in the remaining hippocampus 4 weeks after a neonatal but not an adult unilateral lesion. Neither BDNF nor NGF mRNA was changed in the overlying cortex. These findings raise the possibility that a decrease in the level of BDNF mRNA in the remaining hippocampus may contribute to the spatial memory deficit observed after a unilateral neonatal hippocampal lesion. Supp. NIH HD23315.

540.8

CONSTITUTIVE EXPRESSION OF BDNF AND TRKB mRNAs IN RAT BRAIN WITH AGE AND LEARNING IMPAIRMENT S.D. Croll*, N.Y. Ip , R.M. Lindsay, & S.J. Wiegand, Regeneron Pharmaceuticals, Tarrytown, NY 10591.

mRNAs for the neurotrophin BDNF and its receptor, TrkB, have been localized in most regions of the adult rat brain. These messages reach peak expression late in development, and remain at high levels into adulthood. We examined the constitutive expression of mRNA for BDNF and TrkB in rat brain from young adulthood into old age. The levels of many neuroactive chemicals in old age have been found to depend on the functional state of the animal. To determine if the constitutive expression of BDNF and TrkB mRNAs depend on the cognitive status of aged rats, animals were pre-screened on the Morris water maze task, and were subsequently divided into aged learning-impaired and aged learning-unimpaired groups. Northern blot analyses were completed for olfactory bulb, neocortex, hippocampus, diencephalon, striatum, midbrain, hindbrain, and cerebellum of 4 mos, 13 mos, 24-26 mos (aged) learning-unimpaired, and aged learning-impaired rats. Results demonstrate that BDNF and TrkB mRNA levels do change with age in some, but not all, brain regions studied. Both BDNF and TrkB mRNAs were decreased with normal aging in olfactory bulb, cortex, and hippocampus, but were increased with age in the diencephalon. mRNA levels for both BDNF and TrkB in olfactory bulb and cortex, and for TrkB in striatum, were higher in aged learning-impaired animals compared to aged learning-unimpaired animals. Northern analysis was performed for CNTF to test the specificity of these impairment-related mRNA differences. CNTF mRNA did not show the same impairment-related changes as BDNF mRNA. An *in situ* hybridization study of BDNF and TrkB mRNA expression with age and learning impairment is currently being conducted.

540.9

CHANGES IN NGF AND BDNF mRNA EXPRESSION INDUCED BY ENTORHINAL CORTEX LESIONS. M.I. SHIFMAN* and D.G. STEIN. Brain Research Laboratory, I.A.B., Rutgers University, Newark, NJ, 07102.

Injury to the mammalian brain induces plasticity processes(e.g. axonal sprouting) that may contribute to behavioral recovery of function after injury. Unilateral ablation of the entorhinal cortex(EC) in adult rats leads to robust sprouting responses by remaining intact afferents in the hippocampus(HC). Axons from the contralateral EC and C/A hippocampal projections sprout collateral terminals that reinnervate the dentate granule neurons. Axons from the septal nucleus also reinnervate portions of hippocampus. We were interested in examining expression of injury-induced neurotrophic factors and induction of axonal sprouting in the hippocampus caused by unilateral damage to the rat's EC.

The effects of unilateral, electrolytic EC lesions on NGF and BDNF mRNAs expression were determined by non-radioactive Northern blot analyses. Seven days after lesion, the expression of NGF mRNA in the hippocampus ipsilateral to the injury, was increased by 20%, but did not change on the contralateral side compared to intact animals. An 40% increase of NGF mRNA was also detected in the ipsilateral and contralateral HC at 14 dπ after lesion. The expression of BDNF mRNA in the hippocampus ipsilateral to the injury was elevated by 40% one week after EC lesion, but returned to control level by 14 days. The expression of BDNF mRNA on the contralateral side did not change at any of the time points in comparison to intact animals.

These results suggest that the evaluated expression of NGF and BDNF may contribute to the induction or maintenance of sprouting in the hippocampus after EC lesions.

Supported by Ipsen Foundation.

540.10

REGULATION OF FGF-2 AND RECEPTOR IN BRAIN INJURY. F. Gómez-Pinilla*, L. Vu and C. Cotman. Dept. Neurology and IRU Brain Aging, Univ. of California, Irvine, CA 92717.

We focused our studies in an action of FGF-2 on astrocytosis, and its possible modulation by FGF receptors and extracellular matrix components. A bilateral lesion in the motor-sensory cortex was performed, and either FGF-2 alone, FGF-2 plus heparan sulfate proteoglycan (HSPG), or saline contained in a piece of gelfoam was applied unilaterally within the wound cavity. Rats that received treatment with recombinant FGF-2 showed an induction of: astrocytes, FGF-2 cells, and FGF receptor-1 (FGFR-1) cells by the area surrounding the lesion. The group of rats that received FGF-2 combined with HSPG showed a larger increase than FGF-2 on the same cellular parameters. Application of HSPG without FGF-2 also triggered an induction of FGFR-1 cells, although, to a much lesser extent compared to FGF-2 alone. Our results indicate that the FGF-2/FGFR system is involved in the regulation of astrocyte proliferation *in vivo* , and its action is potentiated by HSPG. The co-induction of FGF-2 and receptor suggests that FGFR-1 plays a critical role in astrocytosis.

540.11

DIFFERENTIAL EFFECTS OF THE ABSENCE OF RETINAL CONNECTIONS ON BDNF GENE EXPRESSION IN THE MOUSE VISUAL SYSTEM. M.H. Hankin* and J.S. Reese. Department of Anatomy, Medical College of Ohio, Toledo, OH 43614.

In the present study we examine how BDNF mRNA levels in visual target regions (superior colliculus, **SC**; visual cortex, **vCtx**) is affected by the absence of retinal connections. To determine the effect of eye removal before innervation of the primary visual nuclei, we have taken advantage of congenitally blind *ocular retardation* (***orJ***) mice in which the optic nerve fails to form. We have also studied normal mice with bilateral eye removal either at birth (5d after initial retinocollicular contact) or from adults. Preliminary data from RNAse protection assays indicate that BDNF RNA levels in the vCtx are normally about 30% greater than in the SC. While BDNF message levels in the *orJ* SC were comparable to normal, levels in *orJ* vCtx were approximately 3 times greater than in normal vCtx. Eye removal at birth had differential effects in the SC and vCtx (examined up to 5m): while BDNF RNA in the SC was reduced to approx. 30% of normal, it appeared to be increased to nearly 3 times normal in vCtx (similar to the *orJ* vCtx). Eye removal from adult mice resulted in downregulation of BDNF RNA in both SC and vCtx by 7d - an observation consistent with work showing a similar effect of dark-rearing and TTX on BDNF gene expression in vCtx of postnatal and adult rats (Castrén et al. *PNAS* **89**:9444). Our observations suggest that different brain regions involved with visual input (1° & 2°) respond differently to eye removal during the perinatal period: BDNF gene expression was downregulated in the SC, while the vCtx showed upregulation. Continuing work will determine (1) how the normal postnatal increase in BDNF RNA in the vCtx (which is attenuated by blocking light input to the brain) compares in the complete absence of retinal connections, and (2) whether BDNF is expressed in retinorecipient layers of the SC. Supported by NIH Grant NS26777.

540.12

THE EFFECT OF OVARIECTOMY AND ESTRADIOL-REPLACEMENT ON BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA EXPRESSION IN BRAIN REGIONS OF FEMALE SPRAGUE-DAWLEY RATS. M. Singh*, EM Meyer and JW Simpkins. Center for the Neurobiol. of Aging. Univ. of Fla., Gainesville, Fl. 32610.

In Alzheimer's disease (AD), the most consistent and widespread deficit seen is that of the basal forebrain cholinergic system. We have previously demonstrated that estradiol (E2) modulates the function of these neurons and maintains them by preventing the ovariectomy (OVX)-induced decrease in ChAT activity. As such, we hypothesized that E2 affects cholinergic function by modulating the levels of certain neurotrophic factors. We previously showed that 3 month OVX leads to a significant reduction in cerebral cortical NGF mRNA levels. In the present study, we extended our original hypothesis to include the effects of OVX and E2-replacement on BDNF mRNA levels using *in situ* hybridization. BDNF mRNA levels were evaluated in 3 groups of animals: ovary-intact, OVX (9 weeks and 28 weeks), and E2-replaced animals. After 9 weeks of OVX, significant decreases in BDNF mRNA in the frontal (28%) and temporal cortex (38%) were observed. E2 replacement to OVX animals normalized BDNF mRNA levels in both these regions. 28 week OVX led to significant reductions in the frontal (34%), parietal (29%) and temporal (34%) cortices although E2 replacement at this longer time point was without effect. In the hippocampus, 9 weeks of OVX resulted in significant reductions in the pyramidal cell layer of CA 1 (23%) and the granule cell layer of the dentate gyrus (DG; 12%) while E2 replacement normalized BDNF signal only in CA 1. 28 week OVX resulted in significant reductions in the CA1 by 20%, CA 2 by 31%, CA 3 by 39%, CA 4 by 36% and DG by 31% while E2 replacement increased BDNF mRNA levels in all subregions except the CA 1 region. Collectively, the data demonstrate that E2 deprivation leads to a reduction in BDNF mRNA which becomes more widespread as the length of OVX is increased. Our data support the potential importance and therapeutic merit for E2 replacement in such disorders like AD. Supported by Grants AG 10485 and IT32AG00196.

540.13

INDUCTION OF GLIA-DERIVED GROWTH FACTOR (S100β) IN RATS FOLLOWING CARDIAC ARREST. S.P. Jaw*, R.R. Matsumoto and D.D. Truong. Dept. of Neurology, Univ. of California Irvine, Irvine, CA 92717.

S100β, a small, dimeric, acidic calcium-binding protein, is known to stimulate neurite outgrowth and promote survival of serotonergic neurons. After resuscitation from 8 min of cardiac arrest (CA), Sprague-Dawley rats developed motor impairments, e.g. post-hypoxic myoclonus. Biosynthesis and metabolism of 5-HT were also altered in post-CA rats; cortical 5-HT_{2A} receptors were reduced; and treatment with the 5-HT_{2A} agonist, (±)-1-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), significantly attenuated myoclonus. These results indicate alteration of functions of serotonergic neurons may occur in post-hypoxic rats. In contrast, by 45 days following CA, motor functions of rats recovered and cortical 5-HT_{2A} receptors of these animals were increased from low levels at early timepoints. In the present study, we, therefore, investigated involvement of S100β in the recovery process. Brain levels of S100β were examined with quantitative immunoblot analysis. Significantly increased levels of S100β were found in rats 14 days and >45 days post-CA ($P < 0.05$). The results indicate that reactive astrocytosis and elevated levels of S100β may participate in the recovery processes following hypoxic-ischemic insults to the brain.

540.14

NERVE GROWTH FACTOR (NGF) IS LOWER IN SEMEN FROM SPINAL CORD INJURED (SCI) MEN COMPARED TO NORMAL MEN. O.F. Padron, N.L. Brackett, C.M. Lynne*, J.A. Weingartner and K.A. Crutcher. The Miami Project to Cure Paralysis and the Dept. of Urology, Univ. of Miami Sch Med, Miami, FL 33136; Dept. of Neurosurgery, Univ of Cincinnati Med Ctr, Cincinnati, OH 45267

In many cell types, NGF is required for normal cellular function in man. Sperm cells from SCI men are lower in motility and viability than sperm cells from normal men. The present study tested the hypothesis that levels of NGF in the semen of SCI men are lower than in the semen of normal men.

SCI men were subjects in The Male Fertility Research Program of The Miami Project to Cure Paralysis. We analyzed the antegrade semen of 8 SCI men (mean age 29.3 ± 2.4 SEM, mean duration of injury 8.4 ± 2.5 years) and 9 healthy controls (mean age 31.2 ± 1.3 years). Complete semen analysis was performed on each sample. Semen was obtained in SCI men via electroejaculation (n=3), vibratory stimulation (n=4) or masturbation (n=1). Controls produced samples via masturbation. Whole semen samples were analyzed via a 2-site enzyme-linked immunosorbent assay (ELISA) for NGF.

There was a ten-fold decrease in NGF levels in the semen of SCI men (0.03 ± 0.02 ng/ml) compared to controls (0.29 ± 0.07 ng/ml). This difference was statistically significant ($p<0.01$, ANOVA).

Our data indicate that the semen of SCI men has significantly lower levels of NGF than the semen of normal men. This condition may contribute to the asthenospermia and necrospermia seen in SCI men.

Supported by The Miami Project to Cure Paralysis and by NIH grant NS31410.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION VI

541.1

Expression of estrogen and androgen receptor mRNAs in immortalized oligodendrocyte cell lines. R. Stoika, L. Foster, A. Campagnoni, and P. E Micevych*. Mental Retardation Research Center and the Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles CA 90024-1763

Oligodendrocytes may mediate developmental effects of sex steroids on the CNS including the production of neurotrophins. The effect of the sex steroids, estrogen and androgen, in the development and regulation of oligodendrocytes has not been studied. To begin addressing the questions of the effect of sex steroids on oligodendrocyte growth, proliferation and the expression of neurotrophins during development, we used a series of cell lines immortalized at specific stages in the oligodendrocyte lineage (Foster et al., Dev Neurosci 1993 15:100-109). All of the cell lines stained positively for markers of the oligodendrocyte lineage, A_2B_5 and 2'3'-cyclic nucleotide 3'-phosphodiesterase (CNP); none of the cell lines, however, expressed myelin basic protein which suggests that these were immature oligodendrocytes. Poly A+ RNA was isolated from 10 oligodendrocyte cell lines. Northern blot analysis indicated that several of these lines expressed mRNA coding for the estrogen receptor (N7, N9, N11, N19) while mRNA for the androgen receptor was expressed in the N2, and the N19 cells. All the cell lines expressed mRNAs coding for both brain derived neurotrophic factor and nerve growth factor. Cell lines identified to have specific steroid receptors as well as the N16 line, which does not express either estrogen or androgen receptor mRNA, were grown at the permissive temperature (34°) in a defined (serum and phenol red free) medium with and without sex steroids. These results suggest that sex steroids support the proliferation and growth of these cell lines and may also regulate the expression of neurotrophins that influence the development and survival of other cells in the nervous system. Supported by NS21220 and HDO4612.

541.2

β-INTERFERON INCREASES ASTROCYTE PRODUCTION OF NEUROTROPHIC FACTORS. T. Boutros and V. W. Yong*. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Recent clinical evidence has suggested that β-interferon is efficacious in the treatment of multiple sclerosis although the mechanism of action remains unclear. Given that astrocytes can provide trophic support to neurons, and since ciliary neurotrophic factor (CNTF) has been reported to protect oligodendrocytes against apoptotic-damage, we examined whether β-interferon could increase the production of specific neurotrophic factors by astrocytes. Mouse β-interferon (1 to 1000 U/ml) was added to confluent or non-confluent neonatal mouse astrocyte cultures for varying periods of time. Total RNA was extracted and subjected to Northern blot analyses for levels of mRNA for CNTF, nerve growth factor (NGF) and the astrocyte filament protein, glial fibrillary acidic protein (GFAP). Doses of β-interferon from 100 U/ml significantly increased the mRNA for CNTF and NGF, but not for GFAP. Increase in mRNA for both CNTF and NGF (2-fold) was evident by 24 hours following β-interferon treatment. The β-interferon-enhanced trophic factor production was not reproduced by interleukin (IL)-2, IL-6 or γ-interferon. Western blot analysis is currently in progress to evaluate the corresponding protein levels. We conclude that the astrocyte production of neurotrophic factors by β-interferon may be relevant to its clinical efficacy.

541.3

SYNERGISM OF PHORBOL ESTERS WITH IL-1β ON IL-6 RELEASE AND GENE EXPRESSION IN ASTROCYTES.

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Dip. Neuroscienze, Sec. Pharmacology; *CEINGE; Univ. of Napoli, Via S. Pansini 5, I-80131; #IRBM, Via Pontina Km 30,400, Pomezia Roma

Cultured Astrocytes produce and release interleukin 6 (IL-6) in basal and following stimulation with various agents. We have already demonstrated that Phorbol esters (PMA) can increase the release of IL-6. Moreover, it has been reported that interleukin 1β(IL-1) released IL-6 by astrocytes. PMA concentration-dependently stimulated IL-6 release with an EC_{50} of about 50 nM. The effect of PMA lineraly increased from a minimal stimulation of secretion after 3 h. of exposure to a maximal stimulation at 24 h. At 24 h the concentration-related curve showed saturation at 300 nM of PMA, indicating that PMA actions required at least 24 h to be fully expleted. PMA effect upon IL-6 release by cortical astrocytes was inhibited by the treatment with PKC blockers. Similarly, PKC-desensitization, by means of PMA pretreatment, caused a complete block of IL-6 release stimulated by PMA. The basal release of IL-6 resulted set at a higher level following PKC desensitization. IL-1 stimulated IL-6 release concentration-dependently and its effect was potentiated by the blockade or by the desensitization of PKC. Conversely, calphostin C, over 24 h lasting incubation, did not affect IL-1 stumulation. Both IL-1 and PMA increased IL-6 mRNA expression, but, IL-1 stimulated more consistently IL-6 mRNA expression than PMA. Finally, the costimulation of astrocytes with either IL-1 and PMA resulted in a synergistic stimulation of IL-6 release that was not affected by blocking PKC. This synergism, was also found at level of mRNA expression. (Supported by MPI 40%, CNR 9100612 and TP on Aging,, AIRC 92-93)

541.4

INCREASED ^{125}I-IGF-2 BINDING IN THE COLCHICINE LESIONED HIPPOCAMPUS: FUNCTIONAL mRNA EXPRESSION FOLLOWING NEURONAL CELL DEATH AND GLIOSIS.

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Insulin-like growth factors (IGFs) are a family of small molecular weight peptide hormones which may act as neurotrophic factors in the central nervous system. We have previously examined ^{125}I-IGF-1 and ^{125}I-IGF-2 binding in the rat brain following cytotoxic colchicine lesions to the dentate gyrus of the hippocampus at time points up to 10 days post-lesion. These results demonstrated a time dependent increase in ^{125}I-IGF-2 binding in the lesioned region and overlying cortex (Neuroscience Abstracts 26.9, 1991). Nissl stained sections showed an ablation of the granular cell layer of the dentate gyrus, and extensive glial ingrowth into the lesioned and damaged areas. *In situ* hybridization revealed no observable mRNA for type-2 receptors in the lesioned area, while demonstrating normal expression to the ipsilateral CA1/CA3 and the contralateral hippocampus. Western ligand blotting for IGF-binding proteins (IGF-BP) has suggested that the increase in ^{125}I-IGF-2 binding may be due to expression of IGF-binding proteins. Our laboratory is currently examining colchicine lesioned animals for IGF-BP mRNA expression, as well as the expression of IGF-1, IGF-2, and other neurotrophic factors. Such expression would suggest that astrocytes produce IGF-BPs in response to neuronal injury, possibly to attract or maintain levels of the IGF peptides, which may in turn influence astrocytic proliferation and protein expression following neuronal cell death.

541.5

BASIC FIBROBLAST GROWTH FACTOR (bFGF) EXPRESSION IN ASTROCYTES AND GLIOMA CELLS.

M.K. Stachowiak, J. Moffett, K. Neary, J.R.-Shapiro, W. Shapiro*, E.K. Stachowiak, Barrow Neurological Institute, Phoenix, AZ 85013

Expression of bFGF is transiently induced during a reversible transition of quiescent to proliferating reactive astrocytes. In constantly proliferating glioma tumors expression of bFGF is constitutively elevated. To model the molecular mechanisms underlying induction of bFGF we developed cultures of human astrocytes and glioma cells. Confluent cultures of astrocytes were quiescent and did not express bFGF. By reducing cell density, events occurring *in vivo* in reactive astrocytes were mimicked: induction of bFGF proteins, their accumulation in the nuclei, cell hypertrophy and proliferation that lasted until a new confluent state was reached. Changes in bFGF protein content reflected changes in the levels of bFGF mRNA. We used bFGF-luciferase reporter plasmids to show that the depletion of bFGF mRNA in confluent astrocytes results from repressed transcription and is mediated by sequences upstream from the transcription start site of the bFGF gene. In U251 and SF767 glioma cells bFGF proteins, mRNA, and bFGF-luciferase constructs were expressed at constitutively high levels and were not susceptible to the inhibition at confluence. The growth of the SF767 glioma cells was stimulated by either the 18kd or the 22, 23, and 24 kd isoforms of bFGF expressed intracellularly from the CMV promoter, but not by exogenously added bFGF. This suggests an intracrine mechanism of action. The growth of astrocytes was stimulated by exogenous bFGF. Our results suggest that: (1) bFGF participates in the regulation of the cell cycle in human astrocytes, and (2) deregulated expression of bFGF gene may underlie abnormal growth of glioma cells, and (3) deregulation of bFGF reflects altered trans-regulation of the bFGF gene promoter. Supported by APDA, AHA, and NIH.

541.6

REACTIVE ASTROCYTES IN THE IMMUNOLESIONED BASAL FOREBRAIN PRODUCE NERVE GROWTH FACTOR-LIKE MOLECULES.

J. Yu*, R. Wiley, and R. Perez-polo. Dept. of Human Biological Chemistry and Genetics, U. of Texas Medical Branch, Galveston, Texas 77555-0652.

It is currently believed that under normal conditions hippocampal neurons synthesize nerve growth factor (NGF) which provides trophic support to cholinergic neurons projecting from the basal forebrain. The hypothesis that reactive glial cells may produce NGF under lesioned conditions was examined in this study. The immunotoxin 192-saporin is a covalently linked complex made up of the monoclonal antibody to the low affinity NGF receptor and the ribosome inactivating protein saporin. We injected 192-saporin into the left lateral ventricle of rats. The cellular location of NGF-like immunoreactivity (NGF-LIR) was studied in the septum and hippocampus at 5, 11, and 29 days after treatment. A double immunostaining technique, which allowed a simultaneous localization of NGF-LIR and of the astroglial marker glial fibrillary acidic protein, was used. Our data showed that cholinergic neurons of the basal forebrain were lost as early as 5 days but more completely by 11 days after lesion. The NGF-LIR content of the cholinergic neurons was also reduced while many reactive glial cells in the basal forebrain became NGF-like immunoreactive. Also, NGF-LIR levels were increased in hippocampus. These results support the hypothesis that a characteristic response of central cholinergic neurons to injury might be to provide signals that stimulate the production of endogenous trophic agents, such as NGF in neurons and reactive glial cells. Our findings may be of importance in considerations concerning trophic support to the cholinergic neurons of the basal forebrain whose impaired function is a component of cognitive deficits in neurodegenrative disease such as Alzheimer's dementia. This is publication 21A from USPHS grant P01 AG10514 awarded by NIA. Also supported by NINDS NS 18708.

541.7

EXTRACELLULAR GUANOSINE AND GTP STIMULATE NGF AND FGF-2 SYNTHESIS BY ASTROCYTES.

J.W. Gysbers*, P.J. Middlemiss, S. Hindley and M.P. Rathbone. Dept. of Biomed. Sci. McMaster Univ. Hamilton, ON, Canada, L8N-3Z5.

Extracellular guanosine (Guo) and GTP affect both nerve cells (Gysbers and Rathbone (1992) NeuroReport 3:997-1000, 1992) and astrocytes (Kim et. al. (1991) J. Neurosci. Res. 28:442). Guo or GTP also stimulate neurite outgrowth and branching from mouse hippocampal neurons co-cultured with astrocytes (Soc. Neurosci. Abs. (1993), 19:24.7). Astrocytes in culture synthesize and secrete a number of neurotrophins, *e.g.* NGF, BDNF, FGF-2 and NT-3. We determined whether the neuritogenic effects of Guo and GTP might be due to stimulation of trophic factor production by the astrocytes. Guo or GTP (10μM) was added to cultures of proliferating mouse astrocytes. After 24 hours total mRNA was extracted and analyzed by Northern blotting. Cultures treated with Guo or GTP increased both FGF-2 and NGF mRNA over that in untreated cultures. The release of NGF into the astrocyte culture medium, as determined by ELISA, was also increased under these conditions. Since Guo and GTP stimulate neurite outgrowth from PC12 cells in the absence of astrocytes we questioned whether Guo and GTP stimulated the production of neurotrophins in PC12 cells. Both Guo and GTP induced c-*fos* as determined by Northern and Western blotting. This may lead to activation of other genes involved in neuritogenesis. Moreover FGF-2 neutralizing antibody added to culture medium inhibited the enhancement of NGF-induced neuritogenesis by Guo. This raises the possibility that Guo induces FGF-2 synthesis in PC12 cells that contributes to their ability to enhance neurite outgrowth. Support: Hospital for Sick Children Foundation of Toronto and George M. Calder.

541.8

THE REGULATION OF ASTROCYTE FUNCTION BY DEPOLARIZING SIGNALS.

H. Wu*, P. Ou, I. B. Black, C. F. Dreyfus, Dept. Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Previous studies from our laboratory and others have indicated that astrocytes produce neurotrophins. To understand regulatory mechanisms and the potential interaction of neuronal signals, we have evaluated effects of depolarizing signals on astrocyte NGF and BDNF mRNAs.

Purified astrocyte cultures were established from P1 rat basal forebrain using published techniques (McCarthy and DeVellis; O'Malley et al). The cultures contained > 95% astrocytes, revealed by GFAP immunocytochemistry. Solution hybridization was used to define levels of neurotrophin mRNA in the cultures. NGF mRNA was expressed at a higher level (0.48pg/ug of total RNA) than BDNF mRNA (0.32pg/ug of total RNA). To determine whether depolarizing signals influence neurotrophin expression, cultures were exposed continuously to KCl (25 mM) for 4 hours or 48 hours. Both 4 and 48 hours of exposure resulted in a significant increase in BDNF mRNA. Effects were greatest at 4 hours (3-fold increase) and decreased at 48 hours (50% increase). This increase in BDNF mRNA was relatively specific, since NGF mRNA was not similarly affected. Increases in neurotrophin expression at 48 hours were accompanied by significant decreases in GFAP expression and the alteration of astrocyte morphology from mature process-bearing cells to more immature flat cells.

Our data suggest that depolarizing signals influence astrocyte function by modulating neurotrophin expression and astrocyte morphology. We are presently exploring the roles of neurotransmitters and mechanisms underlying these events. (Supp: NICHD:HD23315 and the UMDNJ Foundation)

541.9

5-HT SELECTIVELY PROMOTES THE SURVIVAL OF O-2A PROGENITOR CELLS PREPARED FROM THE STRIATUM OF THE E16 RAT: INTERACTION WITH bFGF. J.W. Commissiong,* T. Takeshima,[1] Jane M. Johnston and K. Shimoda.[2] NTU-LMCN-NINDS-NIH, Bldg.10/5N214, Bethesda, MD 20892. [1]Div. Neurol., Tottori Univ. Sch. Med., 86 Nishimachi, Yonago 683, Japan. [2]Div. Neurol., Natl. NishiTottori Hosp., 876 Mitsu, Tottori 689-02, Japan

We showed recently that oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells prepared from the striatum of the E16 rat, produce a potent neurotrophic factor that is secreted, and causes a marked increased survival of dopaminergic neurons in culture (Takeshima et al., Neurosci. Letts. 166: 178-182, 1994). A similar, target-derived factor that is transported retrogradely to the dopaminergic neurons in the substantia nigra, might be produced in the striatum, in the adult, by an O-2A-like cell, in response to neuroeffectors released in the striatum. Since dense serotonergic and dopaminergic innervations are present in the striatum, we tested the effect of dopamine (DA), and 5-hydroxytryptamine (5-HT), (and norepinephrine as a control) at 1.0×10^{-6} M, for their ability to promote the survival of O-2A progenitors in culture, after plating at $5.0 \times 10^4/cm^2$. The cells were primed with serum (5.0%) for the first 24 hr, and then grown in a serum-free medium. DA was severely toxic. Only the small number of type-1 astrocytes in the DA-treated cultures survived beyond the second day in culture (DIV2). NE had no significant effect. 5-HT caused a significant protection ($P<0.05$). bFGF (10 ng/ml) caused a significant ($P<0.05$) inhibition of the toxic effect of DA. 5-HT + bFGF caused a robust effect that was significantly greater ($P<0.01$) than that produced by bFGF alone at DIV5. Experiments to: 1) determine if 5-HT is mitotic; 2) purify the putative dopaminergic neurotrophic factor (DNTF) produced by primary O-2A progenitor cells; 3) screen the striatum for A2B5-positive cells during critical stages of development; 4) determine the dose-response effects of 5-HT and the 5-HT receptor(s) involved are in progress. One function of the serotonergic innervation of the striatum might be to provide neurotrophic support of an A2B5-like cell.

MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT

542.1

MODULATION OF GAP-43 mRNA EXPRESSION IN CULTURES OF RAT CEREBELLAR GRANULE CELLS.

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In situ hybridization studies from our lab have localized GAP-43 mRNA in granule cells of the developing and adult rat cerebellum (Soc. Neurosci. Abst., 16:813, 1990). The level of GAP-43 mRNA expression was found to be developmentally regulated since higher levels of GAP-43 mRNA were present in the neonatal cerebellum as compared to adult cerebellum. The objective of this present study was to determine if putative cerebellar neurotransmitters (e.g. GABA and excitatory amino acids), acting at granule cell synapses, are involved in the modulation of GAP-43 mRNA expression. Both GABA and glutamate have been implicated in granule cell trophic activity and their relative levels appear critical for granule cell maturation. The model system chosen for our study was dissociated cerebellar cultures enriched for granule neurons. Cells were exposed to agonists and antagonists of GABA and EAAs in serum-free media for 7 days in tissue culture chamber slides. Cultures were hybridized with a ^{33}P-labeled oligonucleotide to GAP-43 mRNA and the amount of hybridization within process-bearing cells was quantitated *via* an image analysis system. Results from these studies indicates that addition of the inhibitory amino acid GABA, (25 and 50uM), the $GABA_B$ agonist, baclofen (25 and 50uM), as well as the glutamate receptor antagonists, MK-801 (25 and 50uM) and CNQX (25uM), decrease GAP-43 mRNA expression. This study shows that GAP-43 mRNA expression in granule cell bodies could be modulated by synaptic input from both excitatory glutamatergic mossy fibers and inhibitory GABAergic Golgi interneurons. Thus, GAP-43 modulation by these neurotransmitters may contribute to the growth of parallel fibers and neuroplasticity at the parallel-Purkinje cell synapse.

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542.2

Tα1 PROMOTER:NLACZ CONSTRUCT IS INDUCED DURING BOTH REGENERATION AND SPROUTING OF MATURE NEURONS IN TRANSGENIC MICE. **W. Wu*, A. Gloster and F. D. Miller. Center for Neuronal Survival, Montreal Neurological Institute, Montreal, PQ H3A 2B4**

We have previously demonstrated that one member of the α-tubulin multigene family, termed Tα1 in rats, is regulated as a function of growth in both developing and mature neurons. Furthermore, we have demonstrated that 1.1 kb of the 5' flanking sequence of this gene confers a similar pattern of expression in transgenic mice, and more specifically, i) specifies gene expression as a function of neurogenesis, and ii) regulates levels of gene expression as a function of neuronal growth. Expression of Tα1 mRNA is upregulated during the regenerative growth of axotomized motor and sympathetic neurons. We report here that the expression of Tα1:nlacZ transgene is induced during the regeneration of facial motoneurons (by X-gal staining) and sympathetic neurons of the superior cervical ganglia (by Western blot). The induction is seen at 5 days following injury, when the endogenous Ta1 mRNA is increased to maximal level, and remains at increased levels for up to 49 days if regeneration is prevented. These observations enable us to define the axotomy-induced upregulation of Tα1 gene expression as transcriptional, thereby providing the basis for attempting to define the sequence elements in this promoter that are responsible for modulating Tα1 α-tubulin gene expression as a function of axonal injury. We have also previously demonstrated that Tα1 α-tubulin mRNA levels are increased during collateral sprouting of mature sympathetic neurons and by systemic or locally applied NGF in sympathetic neurons in vivo and in culture. We report here that the Tα1 promoter fragment also confers NGF-inducibility in sympathetic neurons of transgenic mice in vivo, as detected by Western blot analysis. This upregulation occurs both during collateral sprouting and following systemic administration of NGF to newborn litters. Thus, the Tα1 promoter also contains the sequences necessary to mediate NGF-responsiveness in both developing and mature neurons.

542.3

STRAIN AND AGE-DEPENDENT ATYPICAL CREATINE KINASE ISOENZYME TRANSITIONS AND SPECIFIC ACTIVITY OF CHICK NEUROMUSCULAR TISSUES. O.C. Ramírez* and E.Jiménez. Departamento de Bioquímica, Centro de Investigación y de Estudios Avanzados del I.P.N., México, D.F. 07000.

It is currently believed that only isoenzyme BB of creatine kinase (BB-CK) initiates in all vertebrate neuromuscular organs during ontogeny. In White Leghorn chick and rat skeletal muscle, BB-CK is replaced by MB and finally by MM-CK. It is thought that BB-CK remains the sole type in brain. Here, we found that Rhode Island chick brain and heart cytosolic(c)CK specific activities showed the lowest and relatively constant values from stage 30 up to month 8, when an abrupt increase occurred. cCK values from breast and thigh muscles increased 223% and 421%, at hatching. At month 8, they returned to basal levels. On the contrary, cCK breast and thigh values increased 2100% and 1260% at year 1.5. Brain isoforms followed a transition pattern opposite to the accepted one. With creatine in heart, typical and atypical MB isoforms were present from stage 30 until hatching, and varieties of MM and MB-CK were found in thigh and breast muscles. BB-CK and varieties of MM and MB-CK were found from hatching up to month 8 in heart, thigh and breast muscles. In senile animals varieties of BB, MB and MM-CK isoenzymes were present in heart and thigh muscles, whereas no MB varieties were found in brain and breast muscle, coinciding with the most anaerobic period of ATP synthesis, when transphosphorylation is indispensable.

542.4

DIFFERENTIAL EXPRESSION OF GLYCINE RECEPTOR ISOFORMS BY EMBRYONIC RAT SPINAL CORD NEURONS DURING DEVELOPMENT *IN VITRO*. M. D. Withers* and P. A. St. John. Program in Neuroscience, Dept. of Anatomy, University of Arizona, Tucson, AZ 85724.

It is well established that acetylcholine receptors undergo a change in subunit composition during development at the neuromuscular junction. By comparison, relatively little is known about developmental changes in neurotransmitter receptors in the central nervous system. We are studying the developmental regulation of glycine receptors expressed by rat spinal cord neurons. Previous results show that embryonic rat spinal cord neurons undergo a change in strychnine-sensitivity of responses to glycine during the first week in culture. To rule out the possibility that strychnine-insensitive responses to glycine are a product of cross-activation of GABA receptors, we have used a monoclonal antibody, mAb 4a, raised against the alpha subunit of glycine receptor to provide independent evidence for the expression of glycine receptors by these neurons. This antibody recognizes an epitope common to all glycine receptor alpha subunits cloned to date, but is not found in any GABA receptors cloned thus far. Initial results demonstrate that embryonic rat spinal cord neurons express glycine receptor alpha subunit immunoreactive material by the first day in culture. This is at a time when these neurons exhibit strychnine-insensitive responses to glycine but before they bind strychnine in radio-ligand binding assays. This suggests that these neurons are expressing different isoforms of the glycine receptor at different times during development *in vitro*.

(Supported by NIH and The Robert S. Flinn Foundation).

542.5

POSTNATAL DEVELOPMENT OF SERINE/THREONINE PROTEIN PHOSPHATASES IN THE RAT BRAIN: RELATIONSHIP WITH MICROTUBULES. G.V.W. Johnson and S.M. Dudek*. Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294-0017.

Serine/threonine protein phosphatases regulate a wide variety of processes within neurons, and apparently play crucial roles in mediating appropriate structural and functional changes during development. Since alterations in the phosphorylation of specific structural proteins apparently modulates their function and can result in a rearrangement of the cytoskeleton, the levels and activities of specific phosphatases and their association with microtubules during postnatal development were measured. Although expression and activity were low, calcineurin (phosphatase 2B) was detected as early as postnatal day 1 (P1) in both the microtubule preparation and whole homogenate. It appears that calcineurin levels increase with age until P20, afterwhich there is no further increase. These results differ from previous studies which showed no detectable calcineurin before postnatal day 4 (Polli *et. al.*, 1991). Interestingly, calcineurin has been shown to exist in growth cones, and apparently depends on an intact cytoskeleton for this localization (Ferreira *et. al*, 1993).

In contrast to calcineurin, activity of phosphatase 2A is highest during the first postnatal week and declines thereafter. It is unknown at this time whether this increased activity early in postnatal life is due to increased levels of an isoform of the phosphatase 2A catalytic subunit, or from lower levels of the 2A regulatory subunits. Phosphatase 1 levels do not appear to change during this this time. Studies are underway to determine the relationship between these changes in phosphatases which occur during postnatal development, and the age-related alterations in the phosphorylation state of specific microtubule-associated proteins. Supported by NIH grants EY06509, NS27538, and AG06569.

542.6

Oligodeoxynucleotides Antisense to Calmodulin mRNAs Inhibit Proliferation and NGF-Induced Differentiation of PC12 Cells. S.-P. Zhang* R.A. Nichols, and B. Weiss, Dept. of Pharmacol., Medical College of PA, Phila., PA 19129.

Calmodulin (CaM) is involved in numerous biological processes, including regulation of the cell cycle and cell differentiation. Using the PC12 pheochromocytoma cell line as a model for studying neuronal growth and differentiation, we have previously shown that nerve growth factor (NGF)-induced neurite outgrowth from PC12 cells was associated with increased levels of CaM and CaM mRNA. Our recent immunocytchemical and in situ hybridization analyses have demonstrated that CaM and the CaM mRNAs are present not only in cell bodies but also in neurites of PC12 cells differentiated with NGF. In order to address the role of CaM in PC12 cells differentiation, we investigated the effects of antisense phosphorothioate oligodeoxynucleotides (ODNs) targeted to the initiation sites of the CaM mRNAs transcribed from the three CaM genes in PC12 cells. We first assessed the uptake of the ODNs into PC12 cells by incubating an FITC-labeled oligodeoxynucleotide antisense to the mRNAs from CaM gene I which was then incubated with the cells. Confocal microscopy revealed that the FITC-labeled ODN was taken up into the cytoplasm and nuclei. The addition of all three ODNs antisense to the mRNAs encoded by the three CaM genes produced a dose-dependent inhibition of the proliferation of the PC12 cells. The three CaM antisense ODNs also inhibited NGF-induced neurite outgrowth when present at concentrations of 16 μM each. Similar concentrations of an ODN having a randomized sequence did not significantly inhibit neurite outgrowth. Immunocytochemical analyses showed that the CaM antisense ODNs also reduced the levels of CaM in PC12 cells treated with NGF, supporting the view that the inhibitory effect of the CaM antisense ODNs on cell differentiation was due to their ability to block the expression of the CaM genes. Our results suggest that CaM is involved in the proliferation of PC12 cells as well as in their differentiation cells induced by NGF. (Supported by NS30724).

542.7

CHARACTERIZATION OF A CONSERVED LEARNING-ASSOCIATED PROTEIN IN SEA URCHIN OOCYTE:BIOCHEMICAL LOCALIZATION AND PHYSIOLOGICAL EFFECTS. T.J.Nelson*, J. L.Olds, A. Hutter, L. Cameron and D.L. Alkon, Laboratory of Adaptive Systems, NINDS, National Institutes of Health, Bethesda, MD 20892, USA.

It has been shown that protein kinase C (PKC) is translocated from cytosol to membrane fractions following memory formation in several species. Cp20 is an ARF-related GTP-binding protein associated with memory, and is a high-affinity PKC substrate that undergoes changes of phosphorylation during associative learning (Nelson et al. Science 1990). Both cp20 and PKC were identified in S. purpuratus oocytes by immuno-staining and fluorescent BODIPY-phorbol acetate binding, respectively. The identity of cp20 was confirmed by dot blot analysis of HPLC.

Treatment of oocytes with Brefeldin A, a fungal metabolite known to interfere with ARF-related proteins, resulted in a transient increase in free-intracellular Ca^{++} as assessed by Fura-2/AM imaging. Subcellular fractionation indicated that cp20 was primarily associated with free ribosomes and rough ER, while only small amounts were found in smooth ER, Golgi, or cytosol. Additionally, approximately 20% of the cp20 was also found in the nucleus. In smooth ER, approx. 50% of the cp20 was in the phosphorylated state, while in rough ER, >90% of the cp20 was found to be phosphorylated. Treatment of oocytes with BFA (200 nM) caused a marked reduction in cp20 recovered from ER, and resulted in a small increase in amount recovered from the nucleus. Previous results (Nelson et al., 1992) indicated that purified cp20 can markedly enhance the incorporation of P-32 uridine into mRNA. Thus, cp20 may play a role in conjunction with PKC in inducing the increases in mRNA and protein previously demonstrated with associative learning.

542.8

MODULATION OF TAU PHOSPHORYLATION IN DEVELOPING RAT FOREBRAIN. Michelle A. Freeman* and Shelley Halpain. Department of Neuroscience, University of Virginia, Charlottesville, VA 22908

The microtubule associated protein tau promotes microtubule bundling and polymerization and is regulated by phosphorylation. Modulating tau's phosphorylation state may be a means by which neurons regulate the cytoskeleton during neuritogenesis. We have examined tau phosphorylation in developing rat brain using quantitative immunoblot analysis with the monoclonal antibody AT8, which selectively binds tau when serine 193 is phosphorylated. AT8 immunoreactivity changed dramatically during development: it was undetectable at embryonic day 18, was present at birth and during the first posnatal week, then declined to undetectable levels by the end of the second postnatal week. In homogenates from an *in vitro* slice preparation, we observed that immunoreactivity is reduced to approximately 5% of initial levels after incubation for 30 minutes in ACSF, suggesting that tau is highly vulnerable to endogenous protein phosphatase activity. This loss of immunoreactivity was prevented by the addition to the incubation buffer of inhibitors of protein phosphatases 1 and 2A, okadaic acid or calyculin A (1μM), but not the selective PP2B inhibitors FK506 (1μM), FK520 (20μM) or deltamethrin (50μM). These data suggest that PP1 and/or 2A regulate phosphorylation at ser-193 but that PP2B plays little or no role during this period. Neither phorbol esters nor forskolin induced an increase in phosphorylation at this site; however, incubation for 30 minutes with basic fibroblast growth factor increased tau phosphorylation in a dose dependent manner. This is consistent with a role for proline-directed kinases such as MAP kinase in the regulation of this site.

542.9

ONTOGENY OF [^{3}H]MK-801 BINDING TO THE NMDA-GATED ION CHANNEL. C-S. Li, J. He, J. Haracz*, R. Sircar, Laboratory for Developmental Neuroscience, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, New York.

The NMDA receptor plays an important role in developmental plasticity. Phencyclidine (PCP) and PCP-like drugs (MK-801, ketamine) act as non-competitive antagonists at the *N*-methyl-D-aspartate (NMDA) receptor. We have earlier shown that postnatal PCP treatment in rats produced long-term changes in seizure susceptibility. Here we report the developmental profile of interactions of L-glutamate, glycine and spermidine with [^{3}H]MK-801 binding in the immature rat brain. Rat pups were sacrificed on specific days after birth. Forebrain crude synaptosomal membranes (CSMs) were prepared. Aliquots of CSM were incubated with [^{3}H]MK-801 in the absence or presence of L-glutamate, glycine and spermidine. Non-specific binding was determined in the presence of excess unlabelled MK-801. For autoradiographic determinations, fresh frozen brain sections from postnatal days 1, 5, 7, 10, 14, 18 and 21 were incubated with 10 nM [^{3}H]MK-801 in the absence and presence of excess nonradiolabeled MK-801, washed, dried and juxtaposed against tritium-sensitive film. [^{3}H]MK-801 binding in postnatal rat brain increased with age. Modulations of [^{3}H]MK-801 binding by L-glutamate, glycine and spermidine were age-specific. These results may clarify the long-term neurobehavioral effects of postnatal exposure to noncompetitive antagonists.

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542.10

EXPRESSION OF INTERMEDIATE FILAMENTS IN ZEBRAFISH. A.Canger, M.Passini, M.Hagel, E.M.Levine, C.Fuchs, R.Druger, A. Francis and Nisson Schechter. Dept of Biochemistry and Cell Biology; Dept. of Psychiatry and Behavioral Sciences. SUNY at Stony Brook, NY 11794.

The visual pathway of fish is an important model system to study molecular events that regulate and support optic nerve growth and regeneration. In addition, zebrafish embryos are amenable to direct observation and manipulation and are thus an excellent model to study molecular events that occur during embryogenesis. Our interest is in the role of intermediate filaments (IFs) in these processes. Although a precise physiologic function for IFs has not been determined, their distinctive cellular distribution and molecular diversity suggest that, in addition to a structural role, they may function to support cell differentiation and growth during embryogenesis. We have previously isolated and characterized several intermediate filaments from the goldfish visual pathway. Goldfish retinal ganglion cells express two novel neurofilament proteins designated plasticin and gefiltin. In addition, goldfish optic nerve glial cells express keratins as their predominant intermediate filament protein. Currently we are characterizing the expression of these intermediate filament proteins in the zebrafish. (NIH EY05212 to N.S.)

542.11

DISTRIBUTION OF CAM KINASE II AND α-INTERNEXIN IN THE RAT DRG AND DORSAL HORN: COMPARISON WITH PERIPHERIN. D.C. Molliver* and W.D. Snider. Dept. of Neurology, CSNSI, Washington University, St. Louis, MO 63110.

Calcium-calmodulin dependent protein kinase (CaMK) plays an important role in the Ca^{++} second messenger cascade and is capable of phosphorylating neurofilaments. We have used immunocytochemistry to examine the distribution of CaMK-α and the type IV intermediate filament protein (IFP) α-internexin in the rat dorsal root ganglion (DRG) and spinal cord. Labelling for the type III IFP peripherin was used as a comparison, because it has been shown to preferentially label small dark DRG neurons. CaMK-like immunoreactivity (CaMK-LI) in the DRG was restricted to a subset of small-diameter neurons. Virtually all cells positive for CaMK also showed heavy labelling for α–internexin, while the remaining neurons contained faint α-internexin-LI. However, a few small, brightly α–internexin-positive cells were not stained for CaMK. Both CaMK and α–internexin antisera labelled a subset of peripherin-positive neurons. In the spinal cord, robust CaMK-LI was seen in the dorsal horn, corticospinal tract and tract of Lissauer but not in the dorsal columns, with the strongest staining in lamina II, somewhat less in I and faint labelling of III-IV. α–internexin labelled processes throughout the cord, but clearly showed afferents from the dorsal root entering both the dorsal columns and lamina I. Little staining was evident in lamina II. Strikingly, peripherin labelling of the dorsal horn was very similar to α–internexin, with extensive labelling of the dorsal columns, lamina I and III-IV, but little staining in lamina II. Despite the fact that both IFP antibodies labelled large cell bodies only faintly, both stained myelinated fiber bundles entering the dorsal horn medially and turning into lamina III, characteristic of Aβ low-threshhold mechanoreceptors. Thus, it appears that the extent of DRG soma IFP-LI is not a good indicator of axonal IFP content.

542.12

EXPRESSION OF AN *engrailed*-LIKE PROTEIN IN *APLYSIA*. R. Marois*, P. Hofstadter, D. Zaas & T.J. Carew. Interdepart. Neurosci. Progr., Depts. of Biol. and Psychol., Yale Univ., New Haven, CT 06520-8205.

The homeobox gene *engrailed* is found in invertebrates and vertebrates, where it is thought to play a role in segmentation and neurogenesis. The function or even the presence of homeobox genes in gastropod nervous systems is as yet unknown. We have used the MAb 4D9 (gift of Nipam Patel) to examine the expression of *engrailed*-like gene products in whole animals and in the CNS of developing and adult *Aplysia*.

engrailed-like immunoreactivity (ELI) was found in the nucleus of a specific subset of cells in the CNS of both larval, juvenile and adult *Aplysia*. In juveniles, there is only one ELI cell in the left cerebral ganglion (none on the right), a bilaterally symmetrical cell in the buccal ganglia, and a few scattered cells in the pedal ganglia. By contrast, the abdominal and especially the pleural ganglia contain several ELI cells. In the pleural ganglia, the left pleural giant (LP1) cell is immunopositive, and so are cells of the anterior, medial, and posterior clusters. In the abdominal ganglia, in addition to a few unidentified clusters of cells, the giant cell R2 is immunopositive, as well as cells that we have tentatively identified as R10-R12 and L13. That both R2 and LP1 are immunopositive is consistent with the idea that these cells are bilateral homologues arising from the same developmental program but merging with different ganglia (Hughes & Tauc, 1963).

A cell count performed in the pleural ganglia indicates that there is a significant increase in immunopositive cells during juvenile development ($p<0.01$). Cells may be fated to express the *engrailed*-like protein before they migrate into the CNS, since preliminary evidence suggests that ELI cells are found in the body wall, a site of neurogenesis in *Aplysia*.

Our results suggest that an *engrailed*-like protein is expressed in the nucleus of a specific subset of neurons in the CNS of *Aplysia*, where its expression pattern is consistent with it having a role in determination of cell fate.

542.13

POSTNATAL HANDLING ALTERS AP-2, NGFI-A, NGFI-B mRNA EXPRESSION IN RAT HIPPOCAMPUS. J. Diorio, L. Donaldson, J. Yau, S. Sharma, J.R. Seckl, & M.J. Meaney*. Douglas Hosp Res Ctr, McGill Univ., Montreal H4H 1R3 Canada & Dept. Medicine, Univ. Edinburgh, Western Gen Hosp, Edinburgh, Scotland EH4 2XU, UK

Postnatal handling alters glucocorticoid receptor (GR) gene expression and HPA function in the rat. The effect on GR expression appears to involve activation of intracellular cAMP levels. cAMP is known to increase the expression of a number of transcription factors, including activator protein-2 (AP-2) as well as NGFI-A and NGFI-B. In these studies we examined the acute effects of handling on the expression of mRNA levels for these factors in Day 7 pups that were handled and sacrificed 0, 30, 60, 90 or 120 minutes following handling. Levels of mRNA expression were determined using in situ hybridization on brain sections containing dorsal hippocampus.

Constituitive expression for all three transcripts was generally low, but detectable on Day 7 of life. Handling resulted in a modest, but reliable increase in AP-2 mRNA levels throughout the hippocampus. NGFI-A mRNA levels were increased dramatically throughout the hippocampus. In both instances, the increase was observed for at least 120 min following handling. In contrast, handling reduced NGFI-B mRNA levels to below detectable limits, and again the effect persisted for at least 120 min following handling.

The promoter region for the rat GR gene (Jacobson & Yamamoto, unpublished) contains putative binding regions for both AP-2 and NGFI-A. Thus, handling could alter GR expression by increasing cAMP formation and increasing AP-2 and/or NGFI-A expression. Interestingly, environmental enrichment, which results in increased GR expression in peripubertal rats, also increases NGFI-A and decreases NGFI-B expression in rat hippocampus.

542.14

EFFECTS OF CONGENITAL HYDROCEPHALUS ON HEAT SHOCK PROTEIN, IMMEDIATE EARLY GENES, AND NEUROTRANSMITTERS. J.P. McAllister II*, R.W. Connelly, N.G. Harris and H.C. Jones. Dept. of Neurosurgery, Cleveland Clinic, Cleveland, OH 44195 and Dept. of Pharm. and Therap, Univ. of Florida, Gainesville, FL 32610.

In an attempt to determine the onset of the first neuronal response, and possibly of irreversible neuronal pathology, during infantile hydrocephalus, the present study has examined the expression of heat shock protein (hsp70), immediate early cell genes (v-fos, jun-B), glutamic acid decarboxylase (GAD) and neuron specific enolase (NSE) in the H-Tx rat model of aqueductal stenosis. Affected H-Tx rats develop ventriculomegaly prenatally which progresses rapidly during the first postnatal month. In this study, fresh frozen tissue from rostral neocortex, caudal neocortex, and combined neostriatum and basal forebrain was obtained from 5 hydrocephalic and 5 normal animals at 6, 12 and 21 days of age, corresponding to mild, moderate and severe stages of ventriculomegaly. Northern blot analyses indicate that v-fos, hsp70 and jun-B expression is unchanged at all time points. These findings do not rule out the possibility that changes in hsp70 and IEGs require more severe ventriculomegaly or have occurred transiently at other time points. Ongoing analysis of GAD expression suggests that reductions occur in the hydrocephalic brain at 21 days. These changes are consistent with decreases in neurotransmitters reported in other models, and which appear to be irreversible. Further results for NSE and other time points will be presented and discussed.

542.15

POU-DOMAIN GENES AND FEMALE PUBERTY: OCT-2 GENE EXPRESSION AFTER HYPOTHALAMIC LESIONS THAT INDUCE SEXUAL PRECOCITY. J.K. Hill, S.R. Nagalla, R.P. Searles* and S.R. Ojeda, Div. Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Homeobox genes are transcriptional regulators considered to be upstream components of the regulatory hierarchy of genes controlling development. Among these, the POU-domain gene family is expressed in the forebrain during embryonic development. Since brain injury recapitulates early developmental events, we employed hypothalamic lesions that induce precocious puberty to begin examining the hypothesis that POU-domain genes may be involved in the early events that lead to the initiation of puberty. Using deoxyoligonucleotides complementary to the amino and carboxy terminal of the POU domain, we isolated by RT-PCR POU-domain sequences from the anterior hypothalamus of immature rats subjected to puberty-inducing electrolytic lesions. Half of the almost 100 clones analyzed corresponded to Oct-2, a family II POU-domain gene originally discovered in B lymphocytes but now known to be also expressed in brain. A predominance of Oct-2 sequences was evident as early as 24h after the lesion. Hybridization histochemistry revealed an increased prevalence of Oct-2 transcripts in reactive astrocytes surrounding the lesion site. Electrophoresis mobility shift assays employing the DNA octamer motif to which POU proteins bind to initiate their actions demonstrated that hypothalamic nuclear extracts contain proteins with mobilities corresponding to at least two of the known Oct-2 protein variants. One of these proteins also formed a prominent protein-DNA complex with a portion of the 5' flanking region of the transforming growth factor alpha (TGFα) gene harboring a presumptive POU binding site. Since TGFα has being implicated in the initiation of puberty and is also expressed in reactive astrocytes, the results suggest that the Oct-2 gene may participate in controlling the onset of puberty via transregulation of TGFα gene expression. Supported by NSF SGER Grant #IBN-9305583 and NIH grants HD18185 and RR00163.

542.16

ONTOGENY OF THE MOUSE SEROTONIN TRANSPORTER. S. Schroeter*^, J.M. Lauder^, and R.D. Blakely^. Dept of Anatomy and Cell Biology, Emory Univ., Atlanta, GA 30322^ and Dept of Cell Biology and Anatomy, Univ. of N. Carolina, Chapel Hill, NC 27599^.

Serotonin (5-HT) is specifically accumulated by embryonic tissues in a manner analogous to the molecule's sequestration by the 5-HT tranporter (SERT) at adult synapses. Notably, 5-HT transport antagonists block embryonic 5-HT accumulation and lead to craniofacial and heart abnormalities. These results suggest that transport per se may regulate a morphogenetic action of 5-HT in the embryo. A basic prerequisite to understanding the mechanism of this action is to describe the appearance and sequence of features that lead to morphogenetic changes. We have used immunohistochemistry in concert with *in situ* hybridization to document both SERT protein and gene expression in early mouse development. A fusion-protein antibody (CT-2) directed against the carboxy-terminus of SERT and ^{33}P-labeled synthetic RNA probes were used correlate the location of SERT gene and protein expression with fluoxetine- and sertraline-sensitive [^{3}H]5-HT uptake in whole embryo culture. Our results reveal a precocious and heterogeneous distribution of SERT protein and mRNA at several sites throughout the embryo including craniofacial epithelia and mesenchyme, midbrain, lung, and heart. Correlation of morphogenetic events with the spatial and temporal patterns of SERT expression will more clearly delineate the embryonic involvement of 5-HT in development and provide basic information necessary for interpretation of pharmacological and genetic disruptions of the transporter.

542.17

REGULATION OF THE PKC SUBSTRATE MARCKS IN RETROVIRUS TRANSDUCED HIPPOCAMPAL CELLS: EFFECT OF LITHIUM AND TEMPERATURE INDUCED DIFFERENTIATION. D.G. Watson[1,3], J.K. Malhotra[1], E.M.Eves[2], and R.H. Lenox[3]*. Dept Psychiatry, University of Vermont[1], Burlington, VT; Ben May Inst., University of Chicago[2], Chicago, Il; and Dept Psychiatry, University of Florida[3], Gainesville, FL.

Previous studies in our laboratory and others have provided evidence for a role of PKC in mediating the effects of chronic lithium in the brain (see review, Manji and Lenox, 1994). Our data have demonstrated that lithium alters the long-term regulation of a major PKC substrate, MARCKS, in both rat hippocampus (Lenox *et al*, 1992), and in immortalized hippocampal cells in culture (Lenox *et al*, 1993). In these current studies we have examined the effects of morphological differentiation and lithium exposure on the regulation of MARCKS in the hippocampal cell line H19-7, which was immortalized using retroviral transduction of a temperature-sensitive SV40 T antigen (Eves *et al.*, 1992). Morphological differentiation at the non-permissive temperature (39°C) in the presence of 1% FBS resulted in a significant increase in MARCKS expression as determined by Western blot analysis. However, in the presence of either the phorbol ester TPA (1μM), or lithium ion (10mM) there was a significant reduction in MARCKS expression following the shift to 39°C. Regulation of MARCKS expression in neuronal cells may be a shared molecular target in the action of lithium and the process of differentiation in the brain. (Supported by NIMH grant MH50105).

542.18

EFFECT OF NITRIC OXIDE SYNTHASE GENE KNOCKOUT OR BLOCKADE ON THE DEVELOPMENT OF RETINAL PROJECTIONS. D.O. Frost*[1], X.-F. Zhang[1], P.L. Huang[2] and M.C. Fishman[2]. [1]Pharm. & Exp. Ther., U. of MD School of Med., Baltimore, MD 21201 and [2]Cardiovascular Res. Center, Mass. General Hospital, Charlestown, MA 02129.

Nitric oxide (NO) may be part of the mechanism that determines whether immature neural connections are stabilized or eliminated during development. In developing rodents, retinofugal axons from the two eyes initially overlap in some visual nuclei, then segregate; the axons also project transiently to non-visual targets. Here we examine if elimination of nitric oxide synthase (NOS) activity during the time when transient retinal connections are normally eliminated, affects the final pattern of connections.

Using anterograde transport of HRP, we traced the retinal projections of adult C57Bl6/129 mice in which the gene for the neuronal form of NOS, the synthesizing enzyme for NO, is knocked out. We also traced retinal projections in 14-day-old hamsters that were given daily IP injections of N(ω)-nitro-L-arginine (NA; 5, 25, 50 or 100 mg/kg) from postnatal day 0 or 1 to postnatal day 14 (saline-injected hamsters were controls). NA blocks NO formation by competing with arginine for both the neuronal and endothelial forms of NOS. In adult knockout mice and 14-day-old NA-treated hamsters, retinal projections are normal with respect to their nuclei of termination. In visual centers, the segregation of projections from the two eyes also appears normal as judged by the tangential spread of the projections from each eye, their laminar distribution and the formation of discrete clusters by some crossed and uncrossed projections. We have not excluded small quantitative anomalies in the retinal projections. Thus, lack of neuronal NOS activity may not affect the partial elimination of immature retinal projections. Support: NIMH-MH49568, NIH-HL02792, Bristol Myers Squibb.

542.19

TRANSIENT EXPRESSION OF NITRIC OXIDE SYNTHASE IN DEVELOPING RAT CEREBELLUM. J. Leo[1], J.R. West[2], N.J. Pantazis*[1], 1. Anatomy, Univ. of Iowa, Iowa City, IA 52242; 2. Human Anatomy & Medical Neurobiology, Texas A&M, College Station, TX 77843

Nitric oxide (NO) may play a key role in neuronal development. Although nitric oxide synthase (NOS), the enzyme which produces NO, is present in granule cell and basket cell neurons in mature, adult rat cerebellum, the localization of NOS in developing cerebellum is less precisely known. The goal of this study was to examine NOS expression over time in developing rat cerebellum and compare to mature cerebellum.

Sprague-Dawley rats were sacrificed at various ages between PD1 and PD18, a time during which cerebellar development is occurring in the rat. NOS was detected by two methods in cerebellar sections (40 μm) taken from the vermis: 1) NADPH-diaphorase histochemical staining; 2) Immunochemical staining. On PD1, Purkinje cell neurons in the anterior lobe of the cerebellum contain NOS. Purkinje cells in the posterior lobe did not express NOS until PD5. Peak staining intensity for NOS occurred on PD10-11 with dense labeling of Purkinje cell soma and apical dendrites. Subsequently, NOS staining in Purkinje cells declined and was no longer detected in these cells at PD16. At the earliest time period studied, PD1, granule cells were NOS positive and retained NOS labeling into adulthood.

In conclusion, developing cerebellum has a different pattern of NOS expression compared to mature cerebellum. Purkinje cells transiently express NOS during development, but not in adulthood. There is a dichotomy of NOS expression in the developing cerebellar vermis, with the anterior lobe preceding the posterior lobe. The functional role of NO in developing cerebellum is unknown, but the possibility that this molecule may help shape cerebellar cytoarchitecture is intriguing. (Support: NIAAA Grant AA05523)

542.20

TEMPORAL REGULATION OF CELL ADHESION MOLECULES (CAMs) DURING MYOGENESIS OF CULTURED SECONDARY MYOTUBES. V.F. Rafuse* and L.T.Landmesser. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

At the onset of spontaneous contractile activity *in-vivo*, secondary myoblasts align along the surfaces of primary myotubes, later fusing to form independently contracting myofibers. During this process N-cadherin and the structural forms of NCAM are temporally and spatially regulated. To determine whether similar expression patterns occurred in culture, myoblasts were harvested from St 37-38 chick pectoral muscles, plated on collagen coated coverslips, and incubated in F-10 medium. Myoblasts began to fuse by 48 hrs, formed multinucleated myotubes by 72 hrs, and began to spontaneously contract by 4-6 days. CAMs were temporally expressed on these cultured myotubes in a pattern similar to that observed *in-vivo*. Biochemical analysis showed that the 145 kD isoform of NCAM was down regulated with time while the 130 kD GPI-linked form was up-regulated, as occurs *in-vivo*. Immunohistochemistry showed that, as *in-vivo*, N-cadherin was high on myoblasts and newly formed myotubes and was then down regulated, and that overall NCAM remained high throughout myogenesis. In contrast, polysialylated NCAM was not expressed until 6-8 days when myotubes began to contract. Blockade of these contractions by high K^+ or culturing the myotubes in low Ca^{++} prevented the expression of polysialytated NCAM . The mechanisms regulating such CAM expression, including the role of electrical activity, are presently being examined. *Supported by NSF grant IBN-9496177. VFR is supported by the Rick Hansen Man in Motion Foundation.*

542.21

DISCRETE EXPRESSION OF THE RXRγ GENE IN THE CHICKEN HINDBRAIN AND RETINA. F. HOOVER*, A. KIELLAND, and J.C. GLOVER, Institute of Physiology, University of Oslo, 0317 Blindern, Oslo, Norway.

We are interested in identifying regulatory mechanisms underlying regional patterns of neuronal differentiation in the hindbrain of the chicken embryo. The vitamin A derivative, retinoic acid (RA) has been implicated as a signaling molecule involved in patterning this region of the CNS. Retinoic acid exerts its effect by binding to a family of nuclear receptors which upon activation elicit diverse transcriptional cascades. To date six members of the RA-receptor family have been identified, RAR (α,β,γ) and RXR (α,β,γ), but their expression patterns remain poorly characterized in the CNS of the chicken embryo.

We have used non-radioactive in situ hybridization to localize chicken RXRγ receptor transcripts in embryos. Previous studies have demonstrated RXRγ transcripts in neural crest derivatives in the periphery (Rowe et al., Development 111: 771). Here we report on their presence in two regions of the CNS: the hindbrain and the retina. At the earliest time point thus far examined (stage 19) we find RXRγ expression along the midline of the optic tectum, in an intermittent ventrolateral cell column in the hindbrain, and in scattered cells in the central region of the retina. By stage 25, when many neuron populations have differentiated in the hindbrain, we find expression in a ventrolateral swath of cells extending from about the level of the trigeminal nerve to the isthmic region. The location of the RXRγ-positive cells suggests that they are postmitotic, and that they may include but are not restricted to some of the central components of the trigeminal system. The expression pattern in the retina is similar to that observed earlier, but the RXRγ-positive cells in the central retina are more numerous. Since the retina develops in a central to peripheral gradient, this suggests that RXRγ expression is highest in developmentally more advanced retinal cells.

542.22

CLONING GENES INVOLVED IN INDUCTION OF CENTRAL NEURONS BY PERIPHERAL TISSUE. T.R. Gershon* & E.R. Macagno Dept of Biological Sciences, Columbia University, New York, NY 10027

The two midbody ganglia of the leech ventral nerve cord that normally innervate the male organs (MO), the sex ganglia, have many obvious specializations that differentiate them from other midbody ganglia. One such specialization, a unique population of several hundred small neurons, the Peripherally Induced Central (PIC) neurons, is produced by a transient increase in mitogenesis in the sex ganglia late in development. This late round of cell division occurs only after efferents from the sex ganglia contact the MO, and can be prevented from occuring in an individual sex ganglion by disrupting its neural connections with the MO. We have previously determined that an inductive signal travels independently to each of the sex ganglia from the MO in retrograde fashion via efferent fibers. In an effort to understand the molecular mechanisms by which this signalling occurs, we are screening for genes expressed differentially in the sex ganglia as opposed to other midbody ganglia, as well as genes that are expressed in the sex ganglia only if the MO is innervated.

To identify differentially expressed genes, we have developed a PCR technique for amplifying cDNA from pools of a few ganglia that incorporates innovations from Belyavsky et al. and Korneev et al. that maximize amplification while minimizing artifactual noise. From the amplified cDNA, we have constructed tissue- and state-specific libraries that we are screening for differentially-represented clones. Identified clones will then be selected for further study by in situ hybridization on embryonic wholemounts. The resulting expression patterns, considered in light of our observations on the cellular processes of induction, will provide information critical to a molecular analysis of the mechanisms by which the MO induces the specialization of the sex ganglia.

A Belyavsky, T Vinogradova, and K Rajewsky 89 Nuc Ac Research (17) 2919-2932
S Korneev, S Blackshaw, and J Davies 94 Progress in Neurobiology (42) 339-346

542.23

REGULATION OF NEURONAL GENE EXPRESSION BY TARGET INTERACTIONS IN VIVO. L.R. Wolszon* and E.R. Macagno, Dept. of Biological Sciences, Columbia University, New York, NY 10027.

Developing neurons and their synaptic partners exchange information that can affect their subsequent expression of particular genes. For neurons that can innervate either of two very different targets, their resultant patterns of gene expression may be significantly different from each other. We are studying this interactive process in the Retzius (Rz) cell, an identified neuron that is found as a pair in each segmental ganglion of the leech nerve cord and innervates one of two targets, either body wall or the sex organs. The Rz neurons that innervate the sex organs in body segments 5 and 6 have different morphology, physiology and function than their homologues in other body segments, but will attain the same characteristics if the sex organs are ablated early in embryogenesis. Comparing the patterns of gene expression between Rz neurons with different targets should allow us to find the genes that account for the two Rz neuronal phenotypes. To this end, we have amplified mRNA from individual adult Rz neurons in sex and non-sex segments, made cDNA from these mRNAs, and then used a differential display technique to compare expression profiles. We have thus far identified in these profiles a population of genes that are expressed exclusively in either sex- or non-sex Rz cells, and are in the process of obtaining information about their sequences and developmental patterns of expression in the nervous system. This information will help us to understand how neuronal fate is determined by early interactions with potential synaptic partners.

542.24

ROLE OF AP-1 PROTEINS IN TRANSCRIPTIONAL CROSS-TALK AT SOMATOSTATIN CYCLIC AMP RESPONSE ELEMENT (CRE). C.L. Szymeczek-Seay* and D.E. Millhorn. *The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 and The University of Cincinnati, Cincinnati, OH 45267.*

The mechanism by which specific gene regulation, such as developmental and tissue-specific regulation, occurs is largely unknown. Given the ubiquitous nature of transcription factors and the large number of genes containing regulatory DNA binding consensus elements, how subsets of genes are transcribed in particular cells at certain stages of development remains an open question. The finding that cross-talk occurs among the bZIP protein families, the Fos/Jun family and the CREB family, at the TRE (12-*O*-tetradecanoylphorbol-13-acetate response element) and CRE provides a possible mechanism for the specificity of developmental- and tissue-specific gene regulatory events. Our finding that Jun is involved in complexes of cerebellar and diencephalic nuclear protein extracts which bind to the CRE led us to speculate that Jun levels and/or transcriptional activity could mediate CRE-driven transcriptional responses, especially CRE responses which are temporospatially regulated. We tested this hypothesis by phorbol-12-myristate-13-acetate (PMA) and/or forskolin treatment of pheochromocytoma (PC12) cells transfected with the CRE-driven promoter of the somatostatin gene, which is known to be developmentally and tissue-specifically regulated. PMA (50 nM) increased transcription of the somatostatin promoter, presumably through the CRE, as did 25 μM forskolin. Treatment with both agents resulted in synergistic stimulation of transcription. In addition, gel shift studies showed that treatment of PC12 cells with PMA and forskolin increased binding of nuclear proteins from these cells to the CRE. These data suggest that the ratio of transcription elements, such as Fos/Jun and CREB, which are capable of interaction at each other's cognate DNA sequence, is crucial to determining the specificity of transcriptional events.

542.25

Nova-2: A New Neuron-Specific RNA-Binding Protein Associated With Paraneoplastic Opsoclonus-Myoclonus-Ataxia. Yolanda YL Yang, Ronald J Buckanovich, Robert B Darnell*. Laboratory of Molecular Neuro-Oncology, Rockefeller University, New York, NY 10021 USA.

The Nova proteins were originally identified as target antigens in the neurodegenerative disorder paraneoplastic opsoclonus-myoclonus-ataxia (POMA). Analysis of the first cloned family member, Nova-1, revealed that it was a neuron-specific RNA-binding protein with developmentally regulated expression. Using antiserum from a patient with POMA, we have now cloned a second Nova family member, Nova-2.

Nova-2 shares extensive amino acid sequence homology with Nova-1. Both have homology with the KH RNA-binding domains (RBD's) found in FMR-1 (the familial mental retardation gene), hnRNP-K, and the yeast splicing factor MER-1. Comparison of Nova-2 to Nova-1 reveals 100% amino acid identity in the three RBD's, indicating a possible shared RNA target. Between RBD2 and RBD3, Nova-2 contains a unique domain with several stretches of alanines and glycines and one purine-rich region, suggesting that functional differences between Nova family members may map to this region.

In situ hybridization (ISH) and mRNA analysis indicate that Nova-2 expression is limited to neurons in discrete regions of the developing and adult mouse nervous system, but with a different pattern of expression than Nova-1. We are further defining the expression of Nova-2 by Northern, RT-PCR, and ISH to distinguish between the Nova family members. In summary, we expect that the Nova family of RNA-binding proteins regulates neuronal development and function through their action on RNA metabolism, by such means as alternative splicing, stability, or translational regulation.

CEREBRAL CORTEX AND LIMBIC SYSTEM II

543.1

CONNECTIVITY OF FETAL LIMBIC CORTEX TRANSPLANTED INTO NON-LIMBIC CORTEX AFTER LONG TERM SURVIVAL OF HOSTS. M.F. Barbe*, Physical Therapy Dept, Temple Univ, Philadelphia, PA, 19140.

Previous studies using a molecular marker of limbic phenotype a well as DiI tracing techniques, in combination with brain tissue transplantation, have examined the developmental specification of regional cortical differentiation. The present study was undertaken to determine if connection phenotypes observed in earlier short term survival studies were maintained after long term survival of hosts receiving grafts. Limbic perirhinal (prh) or sensorimotor (Sm) fetal tissue from rats aged embryonic (E) 12-14 was transplanted into neonatal rat Sm cortex. Animals were sacrificed 65 days later and DiI crystals were inserted into the graft. After a 12-14 week incubation period, the location of DiI-labeled neurons and axons were mapped in cortex and thalamus. Homotopic Sm transplants had no limbic thalamic or contralateral cortical connections, and only 1 of 7 had a connection with limbic ipsilateral anterior cingulate cortex (ACg). Most E14 prh grafts in host Sm region had hybrid connections: out of 12 total animals analyzed in this group, 11 had both limbic and sensorimotor connections when one considers contralateral and ipsilateral cortices as well as the thalamus, 9 had hybrid ipsilateral cortical connections, while 7 had hybrid thalamic connections (ventral posterior and lateral dorsal nuclei), the remaining having either just limbic or sensorimotor connections only. None of the E13 or E12 prh into Sm cortex grafts had any connections with limbic thalamic nuclei or limbic contralateral cortices, 2 were connected to ipsilateral ACg. The data suggest that projection pattern phenotypes are subject to complex environmental influences. Supported by NSF grant 9209459.

543.2

REGION SPECIFIC CHANGES IN PARVALBUMIN EXPRESSION IN RABBIT NEOCORTEX FOLLOWING PRENATAL COCAINE EXPOSURE. X.H.WANG*, A.FINLEY and E.H.MURPHY. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

We previously reported that the number of GABA-immunoreactive (ir) neurons was increased in the anterior cingulate cortex (ACC), but not in the primary visual cortex (VC), in the rabbits prenatally exposed to cocaine (Wang and Murphy, Soc.Neurosc. Abst. 1993, 19:50). In the present study, we investigated the development of the subgroup of GABAergic neurons which are immunoreactive for the calcium binding protein parvalbumin. Since the major effect of cocaine is believed to be on the uptake of dopamine, we compared an area of cortex rich in dopaminergic input (ACC) with an area lacking dopaminergic input (VC) following prenatal cocaine exposure. Pregnant rabbits received intravenous injections of cocaine (4mg/kg, twice daily) from day 8 to day 29 of gestation. The same volume of saline was injected in control animals. The brains of rabbit pups were examined at P10, P20 and P60. Immunocytochemical methods were used to visualize parvalbuminergic neurons. Quantitative analysis revealed a significant increase in the number of secondary and tertiary dendrites per neuron which were parvalbumin-ir in ACC in cocaine exposed brains in all age groups examined compared to controls. In VC, however, there is no difference between cocaine and saline animals in the number of secondary and tertiary dendrites per neuron which were parvalbumin-ir. In both ACC and VC, there were no significant difference between cocaine and saline in the number of primary dendrites per neuron which were parvalbumin-ir nor in the number of parvalbumin-ir neurons. Expression of parvalbumin in dendrites is characteristic of immature neurons, and has been associated with kindling and epileptic activity. Our data suggest that the increased distribution of parvalbumin in ACC in cocaine-exposed animals may reflect a disturbance in the normal balance of excitation and inhibition in this dopamine rich neocortical area. Supported by NIDA-DA06871.

543.3

NEONATAL TREATMENT WITH 192 IgG-SAPORIN PRODUCES DEFICITS IN FOREBRAIN CHOLINERGIC SYSTEMS. R.T. Robertson*, B.P. Yu, G.H. Kageyama, K.J. Claytor, J.C. Lauterborn, C.M. Gall and R.G. Wiley, Department of Anatomy and Neurobiology, Univ. California, Irvine, CA 92717, and Laboratory of Experimental Neurology, DVAMC, Nashville, TN 98 37212.

Previous work demonstrated that an immunotoxin, consisting of the 192 IgG antibody to the low affinity NGF receptor coupled to the ribosome inactivating protein saporin, destroys basal forebrain cholinergic neurons in adults (Wiley et al., *Brain Res.*, **562**, 1991). The present studies examined the specificity of this toxin when administered to neonatal animals. Injections of 100-200 ng of 192 IgG-saporin were made intraventricularly in postnatal day 0 (P0) to P2 Sprague-Dawley rat pups. Following survival periods of 3 days to 6 months, animals were sacrificed and forebrains processed for histochemistry, immunocytochemistry, and *in situ* hybridization. AChE histochemical and ChAT immunocytochemical studies indicate that early treatment with 192 IgG-saporin results in long term reduction in number of basal forebrain cholinergic neurons and marked reductions in their axonal projections to cerebral cortex. Cholinergic neurons of the striatum also show a decrease in numbers. *In situ* hybridization studies reveal a decrease in ChAT mRNA positive neurons in basal forebrain and striatum, but no apparent decrease in GAD mRNA positive neurons in basal forebrain. Cortical lamination, subplate neurons, and AChE-positive, but non-cholinergic, neurons of dorsal thalamus, appear not to be affected by the toxin. These data indicate that 192 IgG-saporin has a selective effect on forebrain cholinergic neurons and results in a marked cholinergic deafferentation of developing cerebral cortex. *Supported by NIH grant NS 30109 and the Department of Veterans Affairs.*

543.4

ENTORHINAL-HIPPOCAMPAL CONNECTIONS ESTABLISHED EARLY IN HUMAN FETAL DEVELOPMENT. R. F. Hevner* and H. C. Kinney. Dept. of Pathol., Harvard Med. Sch., Brigham & Women's Hosp., Boston, MA 02115.

Previous studies have shown that the entorhinal cortex (EC), a transitional area of "periallocortex," matures histologically and neurochemically earlier than neocortical areas. To determine if connections between the EC and hippocampus (HC) also develop early (by mid-gestation), we injected the lipophilic tracer dil into the EC and HC of paraformaldehyde-fixed human fetal brains at 17-20 weeks post-conceptional age (CA) ($n = 9$). After 8-40 weeks diffusion, sections were cut on the freezing sliding microtome, counterstained with DAPI, and examined by fluorescence microscopy. Injections into the EC labeled the following: (1) radial glial fibers extending to the temporal horn of the lateral ventricle; (2) heavy anterograde projections to the HC, mainly to stratum radiatum of CA1, but also to dentate gyrus, CA3, subiculum, and presubiculum; (3) retrograde projections to pyramidal neurons in CA1 and subiculum; and (4) retrograde projections to a few neurons in temporal neocortex. Injections into CA1-CA3 labeled neurons in EC layers 2-3 retrogradely, and projections to superficial and deep EC layers anterogradely. Injections into superficial parietal and occipital neocortex labeled radial glial fibers and Cajal-Retzius cells only. Injections of the optic nerve and tract labeled the lateral geniculate nucleus (LGN) specifically; evidence of eye-specific laminar segregation of projections to the LGN was seen with the optic nerve injections. The above findings demonstrate that cortico-cortical connections between EC and HC develop in the first half of human gestation, well before those in other areas, e.g. visual cortex.

To illustrate the potential of this method for studying HC/EC malformations, we injected dil into the medial temporal cortex (presumptive EC) of a 16 wks CA thanatophoric dwarf (in which HC and EC develop abnormally). Labeling of radial glial fibers and a few Cajal-Retzius cells, appropriate for neocortex at that CA, was observed; EC-appropriate connections were absent.

543.5

EARLY PRESENCE OF GAD-CONTAINING INTERNEURONS IN THE DEVELOPING GRANULE CELL LAYER OF THE RAT DENTATE GYRUS. S.T. Dupuy*, B.A. Havens and C.R. Houser. Dept. of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90024.

Postnatal (PN) development studies of GAD67 mRNA-containing neurons within the rat hippocampal formation using non-radioactive *in situ* hybridization methods have revealed a prominent population of GAD67 mRNA-labeled neurons in the dentate gyrus at early ages (PN0-PN5). These neurons were highly concentrated both within and slightly above the outer border of the developing granule cell layer, creating a labeling pattern different from that of the adult. In order to determine whether the GAD67 mRNA-labeled neurons were GAD-containing interneurons or granule cells transiently expressing GAD67 mRNA, immunohistochemistry for GAD67 and birthdating studies were performed. At these early PN ages, GAD67 protein was evident in neurons occupying similar positions to the GAD67 mRNA-labeled cells, and the morphology of these neurons suggested that they were not granule cells. Birthdating studies of neurons labeled with bromodeoxyuridine at E14, the birthdate of many GABA neurons in the dentate gyrus, revealed labeled neurons in positions corresponding closely to those of the GAD67 mRNA and protein-containing neurons at the early PN ages. This suggests that the neurons with early birthdates arrive in the granule cell layer at early ages and are GAD67-containing interneurons. Double labeling studies are being conducted to confirm this. These GAD-containing interneurons, due to their position and early appearance, could play a key role in the cytoarchitectural development of the dentate gyrus.

543.6

B-HYDROXYBUTYRATE FUELS SYNAPTIC FUNCTION DURING DEVELOPMENT: HISTOLOGICAL AND PHYSIOLOGICAL EVIDENCE IN RAT HIPPOCAMPAL SLICES Y. Izumi*, AM Benz, CF Zorumski & JW Olney, Dept of Psychiatry, Washington Univ. Medical Sch., St. Louis MO 63110.

In non-precocial species, including rats and humans, β-hydroxybutyrate (βHB), a ketone body, is thought to be able to substitute for glucose as an energy substrate during the suckling period. Although the relatively excellent prognosis of primary ketotic hypoglycemia suggests that ketone bodies may be able to support vital brain functions, there is no direct experimental evidence at a cellular level establishing that ketone bodies can substitute for glucose to maintain bioelectric functions of the neuron or protect the neuron against degeneration under hypoglycemic conditions. To explore this issue, we have directly applied βHB to glucose-deprived hippocampal slices from rats at various ages (PND15, 30, 120) and determined its ability to preserve synaptic function and morphological integrity in the absence of glucose.

Glucose deprivation lasting 1 hour abolished excitatory postsynaptic potentials (EPSPs) in the CA1 dendritic region. Subsequent perfusion of the slice with 10 mM βHB for 30 min restored EPSPs at PND 15 but not at PND 30 or 120. Glucose deprivation for 3 hours induced distinctive pathomorphological changes which were prevented at PND 15 and 30 and partially at PND 120 by administration of 10 mM D-βHB throughout the period of glucose deprivation. L-βHB was ineffective at all ages in preserving morphological integrity. These observations suggest that D-βHB may act at the cellular level in the mammalian CNS during hypoglycemia to maintain brain function, including bioelectric activity, and to preserve morphological integrity. These neuroprotective actions of βHB may be more effective in the immature than mature CNS. Supported by the Diabetes Research and Training Center (YI), MH 45493 (CFZ) and RSA MH 38894 (JWO).

543.7

SURVIVAL AND TRANSMITTER SYNTHESIS IN RAT SEPTAL NEURONS FOLLOWING EXCITOTOXIC HIPPOCAMPAL LESION IN EARLY POSTNATAL DEVELOPMENT

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Target cells are thought to regulate the survival and transmitter expression of basal forebrain cholinergic neurons by supplying neurotrophic factors (Thoenen and Barde, 1980, Physiol. Rev. **60:** 1284-1335).

It has been shown in adult rats that septohippocampal cholinergic neurons survived after excitotoxic ablation of their target region and retained their immunoreactivity for the transmitter-synthetizing enzyme choline acetyltransferase (ChAT; see Sofroniew et al., 1990, Science **247:** 338-342).

To investigate whether the hippocampus is essential for survival and transmitter expression of developing septohippocampal neurons we have destroyed the hippocampus by unilateral injections of the excitotoxic amino acid NMDA in 5 and 10 day old rats. Three or ten weeks postoperatively, serial sections of the septal region were immunostained using antibodies against ChAT or the calcium-binding protein parvalbumin (PARV). The latter antibody was used to stain GABAergic neurons (Freund, 1989, Brain Res. **478:** 375-381).

We have found that a large number of septal neurons retained their immunoreactivity for ChAT and PARV, respectively, at both survival times despite the early loss of their target region.

We conclude from our experiments that the target region has only a limited influence on survival and transmitter expression of developing septohippocampal neurons.

(Supported by the DFG: Leibniz Program and Fr 620/4-1).

543.8

ANALYSIS OF THE DISTRIBUTIONS OF DMAPS 45 AND 55 DURING NEURONAL DIFFERENTIATION. S.L. Rogers[1], S. Srinivasan[3], J.A. Kiehm[2], C.Q. Doe[1]*, W.T. Greenough[1,2] and T.L. Karr[1,3]. Depts. of Cell & Struct. Biol[1]., Psych[2]., Biochem[3]., and Beckman Inst. Univ. of Illinois, Urbana, IL 61801.

DMAPs 45 and 55 were originally identified as 45 and 55 kD proteins that co-sedimented with microtubules purified from *Drosophila* embryos (Srinivasan et al, SN Abstr., 1993). DMAP45 is present in the cortical cytoplasms of all cell-types throughout early embryonic development and also accumulates in the developing nervous system to levels above those seen for surrounding tissues. DMAP55 has a cytoplasmic distribution similar to tubulin and is associated with mitotic spindles.

These proteins have also been examined in several neoplastic cell lines. In undifferentiated N1E-115 neuroblastoma cells, DMAP45 is present in an actin-rich girdle of lamellipodia and microspikes protruding from the cell membrane, but is absent from the cytoplasm. Upon differentiation with DMSO, the protein is enriched in the distal tips of advancing growth cones and in microspikes protruding from the neurite shaft. In the fibroblastic NIH-3T3 cells line, DMAP-45 stains the endoplasmic reticulum.

DMAP55 is present along the lengths of the N1E neurite shafts in differentiated cells, and co-localizes with cytoplasmic microtubules in both undifferentiated N1Es and 3T3 cells.

543.9

POSTNATAL DEVELOPMENTAL EXPRESSION OF A NOVEL MICROTUBULE-ASSOCIATED PROTEIN IN THE RAT HIPPOCAMPUS. M.K. Johnson, T.A. Comery, A.K. Johnson*, T.L. Karr and W.T. Greenough. Depts. of Psych., Biochem., Cell and Struct. Bio., Neurosc. Prog., and Beckman Inst., Univ. Illinois, Urbana, IL 61801 and Depts. of Psych. and Pharmacol., Univ. Iowa, Iowa City, IA 52242.

A set of novel microtubule-associated proteins (termed DMAPs [previously termed FLAT-MAPs]) have been identified in *Drosophila* and are present in the purified microtubule protein fraction of the rat brain. Developmental regulation of DMAPs has been observed in the rat cerebral cortex and cerebellum (Comery et al., *Soc. Neur. Abst., 1993*). The late developing nature of the dentate gyrus and the stereotyped pattern of connectivity within the hippocampal formation provide an ideal system to investigate the potential involvement of these proteins in process extension, differentiation and synapse formation. Expression of DMAP45R in somata and dendrites of the pyramidal cells of the CA regions and the granule cells of the dentate gyrus correlates with process extension and cell differentiation. Expression of DMAP45R in the CA3 pyramidal cells correlates with the innervation of these cells by mossy fibers from the dentate gyrus. DMAP45R is also expressed in the inhibitory interneurons of the hilus and CA3 region with peak expression occurring at P59. DMAP55 immunoreactivity in axons correlates with the development of the mossy fiber projection from the granule cells of the dentate gyrus to CA3. The level of expression of DMAP55R increases during the growth of the mossy fiber system, reaching a peak level at P20, following which the level of expression gradually declines. Similar patterns of immunoreactivity during the development of other fiber systems suggests a possible role for DMAP55R in axonal extension. Supported by NIMH 35321 and R01 GM47962-01.

543.10

ENHANCED EXPRESSION OF A NOVEL MICROTUBULE-ASSOCIATED PROTEIN IN THE DENERVATED DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESIONS. K.E. Armstrong*, T.A. Comery, T.A. Jones, K.E. Bates, T.L. Karr and W.T. Greenough. Depts. of Psych., Biochem., Cell and Struct. Bio., Neurosc. Prog., and Beckman Inst., Univ. Illinois, Urbana, IL 61801.

Current work suggests that the DMAP proteins may play a role in process outgrowth and cell differentiation during development and in experience dependent structural plasticity in the visual cortex (Johnson et al., and Werner et al., *Soc. Neur. Abst., 1994*). In the present studies, the expression of a novel microtubule-associated proteins (DMAP45R) was investigated using a well characterized model of post-lesion reorganization in the hippocampus (Steward, Cotman & Lynch, 1974). Unilateral lesions of the entorhinal cortex result in denervation of the ipsilateral dentate gyrus, granule cell dendritic reorganization and perforant path afferent reorganization. Following denervation, DMAP45R expression in the granule cell somata and dendrites increases both in the level of expression as well as the apparent number of cells and processes being labelled. Changes in DMAP45R expression appear greatest in the outer two-thirds of the molecular layer of the ipsilateral dentate gyrus, which is consistent with documented patterns of axon loss, dendritic reorganization and reactive synaptogenesis.
Supported by NIMH40631, NIMH35321, MH10422 and R01 GM47962-01.

543.11

EXPRESSION OF A NOVEL MICROTUBULE PROTEIN IN RAT VISUAL CORTEX IS MODULATED BY VISUAL EXPERIENCE. D. Werner, N. Hawrylak, T. A. Comery, T.L. Karr*, W.T. Greenough Depts of Biol., Psych. and Biochem., Neuro. Prog., Beckman Inst., U. of Illinois, Urbana IL 61801

The possible role of cytoskeletal elements in experience-dependent plasticity was investigated. The antibody DMAP-45 raised against a novel microtubule-associated protein isolated from *Drosophila* cross reacts with a developmentally regulated antigen (DMAP45R) in rat brain (Comery et al. *Soc. Neuro. Abst.*, 1993). We examined the immunoreactivity of DMAP45R in layer 5 in the visual cortex of monocularly deprived rats.

One eye of rat pups was sutured closed at P12. A nonsutured control group was used for comparisons. Coronal sections of the visual cortex were stained with DMAP-45. Pyramidal cells were classified on the basis of immunoprecipitate in the soma and associated dendrites (soma/dendrite-positive cell) or in the soma only (soma-positive cell). The number of these two types of cells per unit area (NA) was determined in the visual cortical regions Oc1M (monocular) and Oc1B (binocular) contralateral to the deprived eye.

Deprivation until day 80 resulted in a reduction of DMAP-45 soma/dendrite-positive cells in Oc1M. Removing the sutures at day 75, and allowing 5 days of exposure to light, restored the staining to control levels in the soma/dendrite-positive cells in Oc1M. The density of soma-positive cells was not changed in Oc1M in either condition. No differences were observed in the densities of either soma/dendrite-positive or soma-positive cells in the binocular region. The modulation of DMAP45R by visual experience suggests that MAPs may play a role in experience-dependent structural plasticity. Supported by NIMH 40631, NSF BNS88 21219, GM47962-01.

543.12

3D ANALYSIS OF THE ORGANIZATION OF NEURONS AND DENDRITIC BUNDLES IN CEREBRAL CORTEX OF THE RAT T.Skoglund*, R.Pascher, C-H.Berthold, T.Jansson, C.Malmeström Department of Anatomy and Cell Biology and MEDNET laboratory, University of Göteborg, Medicinaregatan 3, S-413 90 Göteborg, SWEDEN.

Light microscopical images of consecutive semithin (0.7 μm) serial sections of rat cerebral cortex (par 1,2) were digitized into an image analysis system (Teragon) via a CCD-camera attached to a microscope. The CCD-camera was connected to a video-mixer which made it possible to prealign the image to the previous one.

Sequences comprising hundreds of images were then automatically realigned by AUTOCENT, a program we have developed, that is based on a succession of pair-wise image thresholding and binary comparison.

A data base was then built up manually in the image-system using the center coordinates of neurons. The apical dendrites located in dendritic bundles were followed to their soma and also followed towards layer I. The data was transferred to a 3-D work-station (MEGATEK) which generated a transformable representation on scale of the defined space and its objects. A stereotyped shape and a color was given to objects of the same class. The dendrites were visualized as wires and were shown together with the neurons in the cortical prism. Our interconnection of the two systems allowed bidirectional on-line transfer of data. In this way, objects in the reconstruction could be identified in the image-handler and vice versa, rearranged, renamed, reshaped, deleted and new ones added. Parameters as celldensity and lamellar organization were computed.

This work is supported by Medical Faculty in Göteborg, by the Swedish MRC proj. no. 3157, by Lundbergs Foundation and Anna Ahrenbergs Foundation.

543.13

BRAIN MICRO-EEG FREQUENCY OSCILLATIONS. C.C. Turbes* Creighton University School of Medicine, Division of Anatomy, Omaha, NE 68178

In these studies recordings macro and micro electrode recordings from the cat brain, micro-EEG, are considered. Combinations of ion currents give rise to complex patterns of neuronal electrical activity in the brain cell microenvironment, the extracellular space. The flow of ions through populations of ion channels in the neuronal plasma membrane and give rise to transmembrane ion currents. It is the sum of various currents flowing at any point in time that determines the neurons membrane potential. The multiple ion channels with their diverse and interacting regulatory mechanisms allow the neuron to modulate its electrical properties in complex ways of high frequency oscillations and electrical fields.

Some of the cortical and hippocampal neurons have ionic conductances organized to endow them with auto rhythmicity. In many neurons the kinetics of these ionic voltage dependent conductances are such that the cells may respond preferentially to inputs at a certain frequency or frequencies acting as a resonators.

There are interactive processes between the components of the dorsal hippocampus micro-EEG and the evoked potentials off the cortical-EEG and the micro-EEG.

These findings reflect the interaction between the dorsal hippocampus and cerebral cortical regions in the processes of ordering, sequencing the succession patterned sensory information as it relates to memory and conscious states.

544.1

POSTNATAL NEUROGENESIS IN THE DEVELOPING RETINA OF THE BRAZILIAN OPOSSUM. E. Stone*[1,2], C. Jensen[1], J. Iqbal[3], J. Elmquist[4], C.D. Jacobson[2,3] and D.S. Sakaguchi[1,2]. [1]Dept. of Zool. and Genetics, Signal Transduction Training Group, [2]Neuroscience Program and [3]Dept. of Veterinary Anatomy, Iowa State Univ. Ames, IA 50011, [4]Dept. of Neurology, Harvard Medical School, Boston, MA 02115.

We are investigating cellular genesis in the retina of the Brazilian opossum, *Monodelphis domestica*, using bromodeoxyuridine (Brdu) labeling of newly generated cells. Typical of marsupials, morphogenesis and neurogenesis in the opossum extends well into the postnatal period. Cell genesis in the retina begins centrally and proceeds towards the periphery. Despite extensive overlap in the time of postnatal neurogenesis, ganglion cells are produced slightly earlier than other retinal cells. To correlate neurogenesis with cellular differentiation, we have used immunohistochemical procedures to characterize the developmental patterns of neurofilament protein (NF), vimentin (VIM), and glial fibrillary acidic protein (GFAP) in the retina. NF-like immunoreactivity (IR), associated with the axons of the retinal ganglion cells, was especially prominent in the central retina in the developing optic fiber layer (OFL) and optic nerve (ON). VIM-IR was present throughout the early postnatal period (postnatal (PN) 1-25). VIM-IR was observed in two distinct structures, in radially oriented processes extending through the entire width of the inner retina and cellular processes situated in the OFL and ON. It is likely that the majority of the radially oriented structures represent Muller cells, however, in the developing retina it is difficult to distinguish Muller cells from the radially oriented neuroepithelial cells. In contrast, GFAP-IR increased with age and was not observed until PN11 along the inner retina and in the ON. Taken together, these results reveal that a great deal of neurogenesis and differentiation occurs postnatally during the development of the visual system in *Monodelphis*.

544.2

SPATIAL AND TEMPORAL DISTRIBUTION OF SYNAPSE ASSOCIATED PROTEINS IN THE DEVELOPING RETINA OF *MONODELPHIS DOMESTICA*. D.S. Sakaguchi*[1,2], J.J. Swanson[2,3], and C.D. Jacobson[2,3]. [1]Dept. of Zool. and Genetics, Signal Transd. Training Group, [2]Neurosci. Prog. and [3]Dept. of Vet. Anatomy, Iowa State Univ., Ames, IA 50011.

In the present study, we have examined the early postnatal development of the retina in the Brazilian opossum, *Monodelphis domestica*. *Monodelphis* is a small, pouchless marsupial whose young have a protracted period of postnatal neurogenesis and differentiation. Antibodies directed against the synaptic terminal related proteins synaptotagmin (p65), Rab 3a, synaptosomal-associated protein 25 (SNAP-25) and synaptophysin (p38) were used to characterize the distribution of these proteins during the early postnatal period. At birth, postnatal day (PN) 1, the retina is relatively undifferentiated morphologically. Staining for p65 and Rab 3a was observed at PN1 primarily in the axons of retinal ganglion cells (RGCs) in the optic nerve and optic fiber layer (OFL) of the central retina. In addition, occasional presumptive RGC somata were also labeled with the Rab 3a antibody. At PN5/6 the retina is organized into two cellular layers: an inner region of postmitotic cells and an outer region of neuroepithelial, as well as, postmitotic cells. Staining for SNAP-25 and synaptophysin was first detected around PN5/6. SNAP-25 labeling was restricted primarily to the OFL in the central retina, while p38 labeling was observed in the inner portion of the retina. By PN25 the retina is well differentiated, exhibiting p65, Rab 3a, SNAP-25 and p38 reactivities localized in processes of the inner plexiform and outer plexiform layers but showing distinct patterns of distribution. While p65, Rab 3a and SNAP-25 were abundantly expressed in the RGC axons, p38 was not. From these results it is clear that these proteins exhibit developmentally regulated changes in their cellular localization. Since a great deal of the development of the retina occurs postnatally in *Monodelphis*, this species is likely to provide an excellent system for *in vivo* experimental manipulations and analysis.

544.3

DETERMINANTS OF RETINAL SPECIALIZATIONS: A NEW APPROACH USING FINITE ELEMENT ANALYSIS. Alan D. Springer* and Haagen Diener. Department of Cell Biology and Anatomy, New York Medical College, Valhalla, New York 10595.

Neither differential cell death nor differential retinal ganglion cell histogenesis adequately account for the progressive increase in the central/peripheral (C/P) retinal ganglion cell (RGC) density ratio during the course of ocular development in the cat. Differential retinal stretch remains the only mechanism that could account for the final RGC topography. However, this hypothesis cannot be tested directly. This model assumes that the central retina stretches less than does the peripheral retina. Differential stretch of the RGC layer could occur if central retina were thicker than peripheral retina. Measurements made in E35 cat retina indicate that the RGC layer of central retina is 2.5 times thicker than that of peripheral retina. The thick spot is located about 0.8mm from the optic disc. This thickness difference is unrelated to differences in the size or density of RGCs in central vs. peripheral retina. By E62 the C/P ratio becomes 18.3:1 and the distance between the optic disc and the area centralis is about 2.5mm (Lia et. al., '87).

The shape of the E35 cat RGC layer, from the optic disc to the retinal margin, was digitized. The RGC layer was modeled as having uniform material properties. A finite element analysis model was used to stretch the E35 RGC layer from 2 to 10mm in length. The model allowed making measurements of RGC density, as well as distance of the area centralis from the optic disc, over the entire duration of the stretching process. In a retina stretched from 2 mm to 8 mm in length, the obtained C/P ratio was 19.3 and the optic disc to area centralis distance was 2.5 mm. These values are very similar to the *in vivo* results reported by Lia et. al. ('87) . Therefore, the adult RGC layer topography can be predicted accurately by the non-uniform geometry of the RGC layer and by retinal stretch.

544.4

OPSIN GENE EXPRESSION IN ZEBRAFISH (*BRACHYDANIO RERIO*) EMBRYOS. J. Robinson, G.B. Grant* and J.E. Dowling. Cellular and Molecular Biology, Harvard University, Cambridge, MA 02138.

Adult zebrafish (*Brachydanio rerio*) retinas contain 4 types of photoreceptors: rods and three classes of cone (2 single and 1 double cone). Using light microscopy, the first class of photoreceptor to become morphologically distinct (~4 days) and mature (~7 days) is the UV-sensitive short single cone. However, some photoreceptors become post-mitotic as early as 48 hours post-fertilisation (pf). We have used whole mount *in situ* hybridisation to determine the developmental patterns of opsin gene expression in the retina of zebrafish. Zebrafish opsin cDNAs were linearised and used as templates for the production of digoxygenin (DIG)-labelled riboprobes. Whole mount *in situ* hybridisation's were performed at intervals between 18 and 96 hours post-fertilisation. Embryos were hybridised at 65°C overnight with probes generated antisense to each of the cloned zebrafish visual pigment genes (ultraviolet, blue, red and rhodopsin). Using this technique we have determined that the ultraviolet, blue and rhodopsin genes are expressed in a small ventral patch as early as 48 hours post-fertilisation, but the red pigment is only expressed in the pineal region of the brain at this age. These data are in general agreement both with an earlier morphological study (Kljavin, J Comp. Neurol. 260: 461, 1987) and with Raymond and Barthel (Invest Ophthal Vis Sci 35, 1727, 1994) who studied rhodopsin expression. Interestingly, preliminary data suggest that the UV-sensitive opsin is the first cone opsin to be expressed in the retina. We postulate that this means that the short-single cone may be the lead cone in the development of the zebrafish retina.
Supported by NIH grant EY 00811 and by the Wellcome Trust

544.5

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) STIMULATES PROLIFERATION OF NEURONAL PROGENITORS IN THE ADULT GOLDFISH RETINA *IN VITRO*. S.E.M. Boucher* and P.F. Hitchcock. The University of Michigan, Dept. of Ophthalmology, Anatomy and Cell Biology, and Neuroscience Program, Ann Arbor, MI.

The retina of the goldfish grows throughout the life of the animal and regenerates when injured. We developed an organ culture system to test peptide growth factors for their ability to modulate the proliferation of neuronal progenitors in both normal and injured/regenerating retinas. Normal eyes were enucleated and the anterior structures removed. Eyecups were incubated for three days in Media 199, with or without a peptide growth factor. Cultures were pulsed overnight with bromodeoxyuridine (BUdr) before incubation was halted. Eyecups were fixed, frozen, sectioned and processed for BUdr immunocytochemistry. The number of BUdr-labeled pluripotent neuronal progenitors (in the circumferential marginal zone) and rod precursors (in the outer nuclear layer) were counted and averaged. For retinas incubated in media alone, the average number of BUdr-labeled neuronal progenitors ranged from 5 to 15, and BUdr-labeled rod precursors ranged from 1 to 8. For retinas incubated in IGF-I at concentrations of 20, 100, and 200 ng/ml, the average number of BUdr-labeled neuronal progenitors were 56, 113, and 154, respectively. In contrast, there was no significant increase in the number of BUdr-labeled rod precursors. Insulin gave a qualitatively similar result, but only at higher concentrations. Neither fibroblast growth factor nor epidermal growth factor stimulated proliferation of neuronal progenitors or rod precursors. These data suggest that: 1) IGF-I receptors are expressed by pluripotent neuronal progenitors, but not rod precursors, 2) IGF-I is likely to be synthesized in the retina, and 3) IGF-I may play a role in regulating cell proliferation in both the developing and regenerating retina. Supported by NIH (NEI) grants EY07060 and EY07003 (CORE).

544.6

Pax 6 GENE EXPRESSION IN NORMAL AND REGENERATING RETINA OF THE GOLDFISH. P. Hitchcock*ξ, R. E. Macdonaldψ, S. W. Wilsonψ, J. T. VanDeRytξ. ξOphthalmology and Anatomy and Cell Biology, University of Michigan; ψDev. Biol. Res. Cntr., Kings College London.

The retina of the goldfish grows throughout the animal's life and will regenerate when injured. We investigated the expression of the transcription factor encoding gene *pax6* in normal and regenerating retina. Regeneration was induced by surgically excising a small patch of retina, which is replaced by neuronal regeneration. Rod precursors, which normally make rod photoreceptors only, are the presumptive source of the regenerated neurons. Retinas were exposed to bromodeoxyuridine (BUdr) to label dividing cells at various post-lesion time points. Sections were double-immunostained with antibodies against BUdr and the zebrafish pax6 fusion protein (I. Mikola, unpublished). In normal retina the pax6 antibodies stain: 1) nuclei of mature amacrine and some ganglion cells and 2) neuronal progenitors in the circumferential germinal zone. Rod precursors are not stained. This staining pattern is qualitatively identical to that seen in the developing zebrafish retina (Macdonald et al. 1994, Invest. Ophthalmol. Vis. Sci., *35*:1404). In the injured retinas, progenitors of regenerated neurons are also stained by the pax6 antibodies. We make the following conclusions: 1) the pax6 antibodies recognize the same protein in both zebrafish and goldfish retinas; 2) in both normal and regenerating retina, *pax6* is expressed in pluripotent neuronal progenitors; and 3) rod precursors are normally committed to a rod cell fate and nearby injury causes them to become pluripotent. Supported by grants EY07060, EY07003 and a NSF training grant (PH) and a SERC grant (SWW).

544.7

A START AT MAPPING GENES THAT CONTROL RETINAL GANGLION CELL NUMBER: ANALYSIS OF INBRED, F1 HYBRID, AND RECOMBINANT INBRED STRAINS OF MICE. R. W. Williams*, D. S. Rice, and D. Goldowitz. Department of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163

Genes that control neuron number play a critical role in the function and evolution of the vertebrate CNS. We have identified one, or possibly two gene loci that are responsible for normal strain variation in the number of ganglion cells in the mouse retina.

Ganglion cell axons were counted on electron micrographs of optic nerves taken from more than 140 animals belonging to 33 different inbred strains of mice, including strains 129, A, AKR, BALB/c, C3H/He, C57BL/6, C57BL/Ks, CAST/Ei, CBA, CD-1, CE, DBA/2, LP, NZB, NZW, PL, SJL, and SPRET/Ei. Cell number varies substantially among strains, from a low of 49,000 in CAST/Ei to a high of 67,000 in NZW. Errors of the mean are typically ±3,400. Strain averages are distributed bimodally, and most strains have populations that cluster close to 52,000 or 64,000. There is no correlation between cell number and body size, brain weight, or retinal area. Phenotypes of F1 offspring from crosses between high and low strains (BALB/c by C57BL/6, BALB/c by A, and PL by SJL) are consistent with a model involving two gene loci. However, we cannot yet formally rule out a model involving a single locus with three or more alleles. Ongoing analysis of recombinant inbred BXD lines of mice suggests that one of these loci controlling neuron number in the mouse retina maps to chromosome 1, 2, or 6.

Supported by NEI R01-8868.

544.8

THE MARKED NASAL-TEMPORAL ASYMMETRY IN GANGLION CELL SIZE IS AN INTRINSIC PROPERTY OF CANINE RETINAS. Dale Hogan* and Robert W. Williams Dept. Anatomy & Neurobiology, Univ. of Tennessee, 855 Monroe Ave., Memphis TN 38163

The peripheral temporal retina of adult dogs is devoid of alpha ganglion cells (Peichl, '92; Hogan and Williams '94). We are interested in determining how this unusual distribution develops. We measured the sizes of ganglion cells in wholemounted retinas of neonatal dogs prior to eye-opening.

Like other well-studied carnivores, i.e., cats and ferrets, ganglion cells are much smaller in the neonate than in the adult. The average area of over 2,000 ganglion cells measured is 110.5 ± 1.2 μm^2 compared to 490 μm^2 in adults (n >3,000). The largest ganglion cells in temporal retina are 300 μm^2 whereas the largest ganglion cells in nasal retina are 400 μm^2. Although the very large alpha cells are found only in nasal retina, average cell size in temporal retina is substantially larger: 124.0 ± 2.6 μm^2 in temporal retina vs. 106.2 ± 1.3 μm^2 in nasal retina. Temporal retina contains a much higher percentage of cells with areas between 175 and 225 μm^2: 21.0% compared to merely 6.9% in nasal retina. There are also twice as many cells with areas between 225 and 275 μm^2 in temporal retina: 4.1% vs. 1.9%. Nasal cells are more common in the range from 75 to 125 μm^2; 27.8% in nasal vs. 17.8% in temporal.

This substantial nasal/temporal asymmetry in the size of ganglion cells in the neonatal dog retina is unlike that of other carnivores. It is especially intriguing given the absence of alpha ganglion cells in the temporal retina of adult dogs. This raises the possibility of a unique class of large beta-like cells which may subserve some functions of the missing alpha cells.

Supported by NEI R01-6627.

544.9

ASYMMETRIC RETINAL GROWTH IN ADULT GREEN SUNFISH D.A. Cameron* and S.S. Easter. Dept. Biology, University of Michigan, Ann Arbor, MI 48109-1048.

Teleost fish retinas grow throughout life by a balloon-like expansion and by the appositional addition of neurons and glia at the retinal margin. An earlier report (Cameron & Easter, *Vis. Neurosci.* 10:375, 1993) presented the hypothesis that fish retinas with a curved, non-fused embryonic fissure grow asymmetrically along the retinal margin. We have tested this hypothesis in adult green sunfish (*Lepomis cyanellus*), which have a curved, non-fused embryonic fissure, concave towards temporal retina. The thymidine analogue BrdU was intraocularly injected into adult fish (n = 6) and retinal whole-mounts processed for fluorescence anti-BrdU immunohistochemistry 20-377 days later. In each retina an annulus of BrdU-positive nuclei was observed central to the retinal margin (but not along the embryonic fissure). The absolute rates of retinal growth were roughly exponential functions of fish size--small retinas tended to grow faster than large retinas. However, growth asymmetries were present: the normalized distances of the BrdU annuli to the nearest retinal margin were 1.00, 1.03 ± 0.03, 1.27 ± 0.03, and 1.44 ± 0.09, in temporal, dorsal, nasal, and ventral retina, respectively (means ± SEM). Thus temporal and dorsal quadrants enlarged less than ventral and nasal quadrants. The normalized densities of cone photoreceptors in the same quadrants roughly matched the normalized growth distances (1.00, 1.03 ± 0.09, 1.19 ± 0.05, 1.55 ± 0.14). Growth asymmetry was thus attributable to differential cellular addition rather than differential passive expansion.

Support: NIH grants EY-00168 and EY-06469, and a Sokol award.

544.10

NEUROPEPTIDE RETILINE AS A PROTECTOR OF RETINAL DYSTROPHY IN CAMPBELL RATS. M. Rasumovskij*, N. Balashov, A.R. Grigorian, A. Rasumovskaja. Institute of Occupational Guidance, Lab. of Vision Physiology, 195067, St. Petersburg, Bestugevska, 50, Russia.

Twenty-eight newborn male rats were injected for 40 days (daily dose 1 mg/kg b.w.) with the physiologically active substance neuropeptide-retiline isolated from bovine retina. The action of retiline was estimated by the amplitude electroretinogram (ERG) and reproduction of frequency of rhythmical flashes. The flash parameters were the following: frequency 1-34 Hz, brightness 5-10 nt. Using electroencephalography, ERGs were recorded in control and experimental animals on the 30th, 47th, and 60th days of postnatal life. Electrophysiologically it was shown that control as well as retiline-injected rats began to lose sight from the 15th day of postnatal life and by the 30th day were blind. Unlike the control animals, retiline-injected rats lost sight only on the 30th day of postnatal life. This was accompanied by a gradual reduction of ERG amplitude due to "a" wave and a decrease of reproduction frequency of rhythmical flashes. Thus, the neuropeptide retiline, chronically injected in Campbell rats at a dose of 1 mg/kg b.w., has a protective action against the development of retinal degeneration.

544.11

EXPRESSION OF C-FOS AND C-JUN IN THE RETINA OF RATS FOLLOWING PROTRACTED ILLUMINATION. J. Pecci Saavedra*, G. Mallo, J. Goldstein, J.J. López Costa. Inst. of Cell Biology, Sch. of Med., U of Buenos Aires, Argentina.

Neuronal expression of the early oncogenes c-*fos* and c-*jun* has been reported following stimulation of different regions. 30/35-day-old Wistar rats were continuously illuminated with 12000 lμx during 8 days, followed by different darkness periods. Illumination produces degeneration of photoreceptor outer segments. It is known that this phenomenon is reversible after an adequate period of darkness. Retinas were fixed by perfusion with a 4 % paraformaldehyde solution, in phosphate buffer, after a period of illumination followed by 1, 3, 6, 10 and 18 days of total darkness. Cryostat sections were immunocytochemically stained with antibodies to c-*fos* (Affiniti Res. Prod.) (dil. 1:1000) and c-*jun* (CRB) (dil. 1:1000), biotinilated donkey anti-sheep IgG (Sigma) and the extravidin-peroxidase complex (Sigma). C-*fos* and c-*jun* immunoreactivity was detected in the somata of photoreceptors in all retinas thus observed. It was maximum in the photoreceptor nuclei of rats kept in total darkness for 3 days, and decreased thereafter. Staining stabilization was reached at about 10-18 days. We conclude that the described increases in c-*fos* and c-*jun* products play a role in triggering the molecular events that participate in photoreceptor regeneration.

544.12

EXPRESSION OF D1 AND D2 DOPAMINE RECEPTORS AND DOPAMINE TRANSPORTER mRNA IN THE ADULT AND FETAL MONKEY RETINA. O.K. Ronnekleiv*, L. Chai and W.S. Choi. Oregon Regional Primate Research Center, Beaverton, OR 97006 and Dept. of Physiology, OHSU, Portland, OR 97201-3098

Expression of D1 and D2 dopamine receptors and dopamine transporter(DAT) mRNAs were studied in the adult and fetal monkey retina by Ribonuclease Protection Assay(RPA). Eyes were dissected from adult and a 70 day old monkey fetus. Monkey specific cDNA clones corresponding to the carboxy terminus of the rhesus monkey D1 and D2 receptors and to the IX-XII transmembrane domains of monkey DAT were cloned and used to generate the cRNA probes. The standard curves were constructed using known amounts of synthesized sense RNA. In the adult monkey retina, dopamine D1 and D2 receptor mRNAs were expressed in equal quantities and at relative high levels. DAT mRNA was also expressed in the retina, but at a more moderate level. In the day 70 fetal eye, D1 and D2 receptor mRNAs and DAT mRNA were all present. The level of D1 receptor mRNA was higher than D2 receptor mRNA and D2 receptor mRNA higher than DAT receptor mRNA. Thus, several of the genes of dopaminergic neurotransmission are expressed early in the developing fetal eye of rhesus monkey. (Supported by DA07165)

544.13

IMMATURE NEURONS IN THE RABBIT RETINA ARE PROTECTED AGAINST NITRIC OXIDE MEDIATED GLUTAMATE TOXICITY. M.F. Haberecht, C.K. Mitchell and D.A. Redburn*. Dept. Neurobiology and Anatomy, Univ. Texas Med Sch., Houston, TX,77030.

We have previously demonstrated that extracellular levels of endogenous glutamate are high in the developing rabbit retina. This amount of glutamate would be toxic in the adult retina; however, immature neurons survive and maintain normal developmental processes. We wished to determine whether NO which has been postulated to mediate glutamate toxicity in the adult CNS is present and functional in the developing and adult retina. First we used immunocytochemistry and histochemistry to localize nitrinergic neurons in the adult retina and subsequently determined developmental profiles. Secondly using retinal explants we have analyzed viability of immature and adult neurons in response to NMDA and NOS agonist and antagonists. Results show that prototypic nitrinergic neurons are mature early but do not express detectable levels of NOS until day 10. Furthermore immature neurons at day 1 are insensitive to glutamate toxicity, while in the adult retina dose-sensitive toxicity is induced by NMDA and inhibited more than twofold by methyl arginine. We conclude 1) glutamate toxicity in adult retina may be regulated by NO and 2) the resistance of immature neurons to glutamate toxicity may result from a delay in the maturation of the NO system. Supported by EY01655, EYI0608 and Fight for Sight.

544.14

LONG-TERM EFFECTS ON RETINA OF RHESUS MONKEYS FED TAURINE-FREE HUMAN INFANT FORMULA. H. Imaki*, M.D. Neuringer and J.A. Sturman. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; Oregon Regional Primate Research Center, Beaverton, OR.

We have been studying the effects of postnatal deprivation of taurine by feeding rhesus monkeys a commercially available human infant formula alone or supplemented with taurine from birth. Previous reports have documented reduced tissue taurine concentration and smaller proportions of bile acids conjugated with taurine in taurine-deprived monkeys at 3 months and 6 months, although at 12 months these parameters were no longer different, suggesting the biochemical dependence on dietary taurine persists for at least the first 6 months, but has regressed by 12 months. At the early ages, the taurine-deprived monkeys have deficits in visual acuity and morphological changes in the retina and visual cortex compared with monkeys fed the same diet supplemented with taurine. The retinal changes persisted at 6 and 12 months. The present study addresses the question of whether or not the retinal changes persist for a substantial period beyond the time at which there are no longer reduced tissue taurine concentrations. Rhesus monkeys raised for 4 years were examined, and although there were no differences in tissue taurine concentrations between taurine-deprived and taurine-supplemented monkeys, as might be expected from our previous results, all taurine-deprived monkeys showed retinal changes, indicating that the retinopathy of taurine deficiency persists long after the differences in taurine concentrations have disappeared.

Supported by NIH grants HD-18678 and RR-00163.

INFLUENCES ON AXONAL REGENERATION

545.1

ELECTRIC FIELD EFFECTS ON AXONAL TRANSPORT IN THE CORTICOSPINAL TRACT FOLLOWING SPINAL CORD INJURY. J.A. McLane*, T. Cobb and T. Khan. Rehabilitation Research & Development Center, Edward Hines Jr. VA Hospital, Hines, IL 60141.

Unlike axons of peripheral nerves, injured axons of the adult spinal cord seldom undergo a functional regeneration. Recent studies have shown that a direct current field at the injury site enhances neurite outgrowth, and stimulates directed neurite growth. Spinal cord injury in adult rats temporarily increased the level of slow axonal transport to the higher level normally seen in young rats. The present study was designed to test the hypothesis that electrical stimulation increases neurite regrowth by enhancing this period of increased axonal transport following spinal cord injury.

Adult rats received a dorsal spinal cord hemisection at the T8 level. Miniature stimulators were implanted at the time of surgery with platinum disk electrodes placed extradurally proximal (anode) and distal (cathode) to the injury. The stimulators provided a constant 15 uA of DC current. Axonal transport in the corticospinal tract was labeled with ^{35}S-methionine one week, five weeks, or fourteen weeks later by injecting 200 uCi of radioisotope unilaterally into the sensorimotor cortex. One week (fast transport) or three weeks (slow transport) after the isotope injection the animals were perfused with formalin, the entire spinal cord dissected, and the radioactivity in 5 mm segments of the spinal cord determined by scintillation counting.

We found that the amount of radiolabel delivered by slow axonal transport was consistently (but not statistically) higher in the spinal cords of animals treated with electric fields. The spinal cord segment containing the injury site in the electric field treated animals did contain statistically more (154%) label relative to sham animals (no field) one week after injury. This increased label at the injury site disappeared after five or fourteen weeks. Fast transport was not altered at any time point, and therefore likely did not contribute to the increase. These data suggest that electric fields increase the slow transport of materials within the corticospinal tract, and temporarily prevent axonal die back and/or stimulate neuronal sprouting near the injury site.

Supported by the Rehab. R&D Service of the Department of Veterans Affairs.

545.2

EFFECTS OF ELECTRIC FIELD APPLICATION IN A MODEL OF TRANSECTED MAMMALIAN MYELINATED NERVE. K. Mosallaie, N. Vasey, and J.D. Sweeney *. Bioengineering Program, Arizona State University, Tempe, AZ 85287-6006.

In vitro and in vivo studies have shown that a steady cathode-distal low-intensity electric field (LIEF) can potentially be used to induce, accelerate and/or guide nerve regeneration. Some possible proposed mechanisms are suppression of axonal injury currents and/or facilitation of organelle movement via electrophoretic action. Our objectives have been (i) to develop a mathematical model describing a transected myelinated nerve, (ii) to simulate the effects of applied steady LIEFs at regenerating nerve tips, and (iii) to predict optimal electrode configurations and stimulus current magnitudes for applied LIEFs. The nerve model is a non-homogeneous compartmental implementation based on iterative solution of a set of coupled finite-difference equations describing intracellular electroneutrality and ionic current conservation (for Na^+,K^+,Ca^{2+},Cl^- & large anions). The applied LIEF solutions on the nerve trunk consider tissue inhomogeneity and spatial geometry as well as position of the distal and proximal nerve stumps with respect to nerve guide tube electrodes as used in actual experiments. Modeling predicts that, at the transected nerve tip, intracellular sodium and calcium concentrations are increased and the membrane is depolarized. Application of cathode-distal LIEFs tends to further depolarize the tip membrane and shift ionic concentrations along the nerve depending upon electrode positioning. Modeling also predicts that application of LIEFs has only a small affect on injury current suppression. LIEFs may enhance early regeneration by creating an intracellular potential gradient that (i) facilitates organelle electrophoresis and (ii) decreases intracellular free calcium concentrations - thus reducing the degree of axonal dieback.

This research is supported by a grant from the Whitaker Foundation.

545.3

NEUROMUSCULAR ACTIVITY AND SPROUTING IN RAT HINDLIMB MUSCLES. B. Jassar and T. Gordon* Dept. Pharmacol., Div. Neurosci., U. Alberta, Edmonton, CANADA T6G 2S2.

Although it has been shown that electrical stimulation can accelerate sprouting, it is not clear whether increased neuromuscular activity during sprouting has any effect on the size of motor units (MUs) once connections are made. To address this question, we subjected rats to high levels of activity during sprouting and determined the size of enlarged MUs in partially denervated muscles (PD) once connections were well established. Rats were divided into 2 groups, exercised (E) and sedentary (S) in which rats were housed in large cages with running wheels (E) or in small cages with limited movement (S). After acclimatization and training, right hindlimb muscles were partially denervated by cutting either L5 or L4. Four weeks later, soleus, plantaris (PL), medial gastrocnemius (MG) and tibialis anterior (TA) muscles were prepared for muscle and MU recording in PD and contralateral control (CC) legs for measurement of MU number and size. In all 4 muscles, the size of the enlarged MUs in PD muscles was significantly larger than in CC but not different in the 2 groups, E and S. For an average of 50% PD in soleus muscles mean (± S.E.) MU twitch force (mN) was 16.9 ± 0.91 (E) vs 15.6 ± 0.82 (S) as compared to 10.71 ± 0.9 in CC muscles. For 80% PD in TA muscles, MU forces of 89 ± 11 (E) vs 80 ± 9.6 (S) compare with 21.5 ± 1.55 (CC). Since there was little hypertrophy of the active muscles, the MU force was a reasonable reflection of the number of muscle fibers per motoneuron and therefore of sprouting. These results show that high levels of neuromuscular activity do not influence the capacity of remaining motoneurons to form enlarged MUs. Supported by MDAC.

545.4

DETERIORATION OF DISTAL NERVE SHEATHS IS A MAJOR CONTRIBUTOR TO POOR MOTOR RECOVERY AFTER DELAYED NERVE REPAIR. S. Fu*, T. Gordon. Div. of Neuroscience & Dept. of Pharmacology, U. of Alberta, Edmonton, CANADA T6G 2S2.

Contribution of long-term muscle denervation to poor functional recovery after delayed nerve repair was studied using a nerve cross-suture paradigm in which duration of muscle denervation varied independently of motoneuron axotomy. Tibialis anterior (TA) muscle was denervated by cutting the common peroneal (CP) nerve. Its regeneration was prevented for 0-12 months before a freshly cut tibial (TIB) nerve was sutured either to the old CP distal stump or onto denervated TA muscle surface. Six months later the success of regeneration and reinnervation was quantified. After immediate nerve suture, muscle and motor unit (MU) recordings showed that 137±21 TIB motor nerves reinnervated the TA muscle. Compared to the control, reinnervated muscles and MUs developed similar forces and muscle fibers were of similar size (2702±47 μm^2) indicating that they completely recovered from denervation atrophy. When nerve suture was delayed after prolonged denervation (> 6 months) a drastic reduction was found in the number of MUs (to 15±4) and muscle fiber size (to 1171±84 μm^2). When the TIB nerve was sutured directly to freshly denervated TA muscle, number of reinnervated MUs reduced to 52±11 (37% of the control) suggesting that the surface of denervated muscle is a poor substrate for nerve growth. This number further reduced to only 18±9 (13% of the control) in prolonged denervation (>1 month). Thus a condition in which regenerating nerves grow outside the intramuscular nerve sheath simulates the effects of long-term denervated sheath and muscle in terms of reduced support for regenerating nerves. These findings provide strong evidence for a concept that long-term denervated sheath fails to guide regenerating nerve to denervated muscle. It is also supported by histological findings that regenerating nerve escapes from the sheath and grows on denervated muscle surface in delayed nerve repair (Gutmann & Young 1944 J Anat 78:15-44). [Funded by Canadian MRC and MDAC].

545.5

AXONAL REGENERATION IN THE SPINAL CORD FOLLOWING TREATMENT WITH DC ELECTRICAL FIELDS. M.F. Zanakis*, R. Cebelenski, C. Cook, S. Gelman and B.H. Hallas. Depts. of Neuroscience and Biomechanics, NY Coll. of Osteopathic Med., Old Westbury, NY 11568.

Several previous studies have shown that when the rodent spinal cord is damaged, DC electrical field treatment (DCEFT) can result in partial recovery of function. The objectives of this study were to investigate the conditions which determine whether such recovery involves regeneration or sparing of axons. The methods incorporated a spinal hemisection in rats followed by DCEFT (Traxon®) for 6 weeks, and then sacrifice. Control animals received no DCEFT. Prior to sacrifice, animals were injected with WGA-HRP ipsilateral and caudal to the lesion. After histological processing, WGA-HRP transport to the red nucleus was determined by automated grain counting. The results showed that all DCEFT animals exhibited greater WGA-HRP transport to the contrateral brainstem when compared to the ipsilateral side or to control animals. While previous experiments have shown that sparing and regeneration both occur with DCEFT after contusion injury, these experiments show that DCEFT is at least likely to promote axonal regeneration after axotomy in the spinal cord.

545.6

REPEATED INDUCTION OF ELECTROCONVULSIVE SEIZURES BLOCKS CHOLINERGIC SPROUTING, AN EFFECT THAT MAY BE A RESULT OF A SUPERINDUCTION OF A REACTIVE STATE IN ASTROCYTES. O. Steward*, Departments of Neuroscience and Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Previous studies have demonstrated that molecular and morphological changes that are characteristic of reactive astrocytes, specifically the up-regulation of glial fibrillary acidic protein (GFAP) synthesis and hypertrophy, can be induced by seizures. The present study evaluates whether super-induction of a reactive state in astrocytes alters one form of postlesion synaptic reorganization (the sprouting of cholinergic projections in the dentate gyrus after destruction of the entorhinal cortex).

Unilateral entorhinal cortex lesions were produced in mice and electro-convulsive seizures (ECS) were then induced on a daily basis from the day of surgery until 12 days postlesion. Control animals received EC lesions but did not experience ECS. At 2,4,6,8,10,12, and 30 days postlesion, animals were prepared for acetylcholinesterase (AChE) histochemistry in order to reveal the extent of cholinergic sprouting. The extent of the super-induction of GFAP expression was evaluated by measuring GFAP mRNA levels using *in situ* hybridization techniques.

The *in situ* hybridization analyses revealed that repeated electroconvulsive seizures had the anticipated effect on GFAP expression, dramatically increasing GFAP mRNA levels in comparison to animals with lesions alone. Quantitative evaluation of AChE staining revealed that the cholinergic sprouting response was substantially reduced in the animals that experienced daily ECS. These results indicate that the induction of seizures during the postinjury period disrupts at least one form of postlesion synaptic reorganization that would otherwise occur. This disruption of synaptic reorganization may be a consequence of the superinduction of reactive changes in astrocytes. Supported by NIH grant NS29875.

545.7

CHARACTERIZATION OF TWO ENDOGENOUS TROPHIC FACTORS INVOLVED IN REGENERATION OF THE GOLDFISH OPTIC NERVE. J.M. Schwalb, N.M. Boulis, M.-F. Gu, N. Irwin, and L.I. Benowitz* Dept. Neurosurgery, Children's Hosp., Harvard Med. Sch., Boston, MA 02115

Unlike mammals, lower vertebrates are able to regenerate injured pathways of the CNS throughout life. In the optic nerve of goldfish, a great deal of research has been directed towards the changes in neuronal gene expression that accompany regeneration. However, the trophic factors responsible for initiating this process are unknown. We have shown that molecules secreted by the glial sheath cells and collected into culture medium (regenerative conditioned medium, RCM) induce neurite outgrowth in both embryonic mammalian cortical neurons (Caday et al., Molec Brain Res 5:45-50) and dissociated goldfish retinal cells (Boulis et al., these Abstracts, 1993). Conditioned media derived from other organs of the goldfish showed no similar activity.

Using the retrograde dye 4-di-10-ASP, we have shown that RCM acts specifically on its natural target cells, the retinal ganglion cells, by promoting neurite outgrowth but not by influencing survival. RCM contains two trophic factors that differ in physical properties and patterns of expression. The smaller of the two, which accounts for most of the biological activity, is <1 kD in size, heat-stable, pronase and trypsin-insensitive, and has a hydrophobic domain. It is actively secreted and is expressed even in the intact optic nerve. The larger is a heat-labile, trypsin-sensitive, basic protein, M_r=70-100 kD, and is upregulated after axotomy.

Since these molecules are secreted from the optic nerve and target the retinal ganglion cells, they are likely to be important in inducing optic nerve regeneration *in vivo*. We are currently working to isolate the two factors and to evaluate the involvement of defined trophic factors in this system. *Support: NIH EY 05690, HHMI, Boston Neurosurgical Foundation.*

545.8

APOLIPOPROTEIN E AFFECTS ON THE HEALING PROCESS IN THE INJURED RAT OPTIC NERVE. J. Kaster, E. El Gazeyerli, B. Patel, R. Anderson and G. Handelmann*. Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT 84112.

Apolipoprotein E (apo-E) is a lipid transport protein present in glial cells, whose concentration is greatly increased following injury to most parts of the nervous system. The increase is due to synthesis by glial cells and to secretion by invading macrophages. An exception to this is in the optic nerve, where there is no increase in apo-E synthesis by glial cells following injury and there is a retarded rate of macrophage invasion. In addition, very little recovery or repair of the injured optic nerve occurs spontaneously. This study investigated the possibility that apo-E, administered exogenously, might facilitate the repair or healing of the rat optic nerve following injury.

Crush injuries of the right optic nerve were made in anesthetized adult male rats. Apo-E purified from rat blood was injected into the nerve sheaths at the site of injury at varying doses (0, 1, 10, and 25μg). A retrograde tracing dye (biotinylated dextran conjugated to lysine) was injected into the vitreous humor at 2, 6, or 13 days after injury. At 3, 7, or 14 days after injury, the nerves were harvested and prepared for immunohistochemistry to visualize: a. dye transport, b. macrophages, c. myelin.

Apo-E administration affected several events associated with healing in a dose-related fashion. First, the transport of the dye was enhanced, both in distance past the injury and in the number of axons containing the dye. Second, the recruitment of macrophages to the crush site was significantly increased. Finally, the rate of myelin degeneration appeared to be increased. These data suggest that the presence of apo-E promotes several events known to be beneficial to nerve repair, and may provide an environment conducive to nerve repair or regeneration.

545.9

CHANGES OBSERVED FOLLOWING TREATMENT OF CHRONIC INJURIES OF THE BRAIN AND SPINAL CORD WITH AMP1 AND MATRIX. **J.D. Peduzzi[1]*, F.R. Fischer[1], and E.E. Geisert, Jr.[2] [1]Department of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294; [2]Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN 38163.**

Regeneration is generally not observed after adult mammalian CNS injuries. Our efforts have been directed at manipulating the injury site by disrupting the scar and providing an environment favorable to axonal growth.

Adult Sprague-Dawley rats received a brain lesion with a scalpel blade or contusive spinal cord injury (10 g wt. dropped 5 cm) at the T9 vertebral level. After at least 2 months, animals received an injection at the injury site with one of the following: buffer, AMP1 antibody with EDTA, or AMP1 with EDTA and matrix. AMP1 antigen is a 110 kDa molecule that is upregulated at the scar. In the brain injured animals, AMP1 with EDTA appeared to disrupt the scar and growth cone-like structures were seen extending into the matrix. In spinal cord injured animals that received the AMP1 with EDTA and matrix, a slight but significant improvement was found in the combined behavioral score which is based on motor and sensory tests (modified from Gale, Kerasidis & Wrathall, 1985, Exp. Neur. 88:123). Supported by the Spinal Cord Society.

545.10

THE KS/CS-PG, ABAKAN; ITS ISOLATION, CHARACTERIZATION, AND DISTRIBUTION IN THE ADULT RAT OPTIC NERVE. D.J. Bidanset[1]*, J.R. Baker[2], M. Höök[3], E.E. Geisert, Jr.[4], R.J. Podhajsky[1], A.R. Blight[1], and B. Caterson[1], [1]Dept. of Surgery, UNC-Chapel Hill, Chapel Hill, NC 27599-7055, [2]Dept. of Biochem., UAB-Birmingham, Birmingham, AL 36294, [3]Div. of Matrix Biol., IBT, Houston, TX 77030, [4]Dept. of Anat. and Neurobiol., UT-Memphis, Memphis, TN 38163.

During the development of the central nervous system (CNS), the formation of axonal pathways and appropriate neuronal connections occurs by both adhesive and inhibitory interactions between cells and the extracellular environment. Proteoglycans are one of the many members of the extracellular matrix found to function in both the developing and mature CNS. One such proteoglycan, ABAKAN, was found in previous studies in functional boundaries of the developing rat CNS. In addition, ABAKAN was shown to inhibit both neuronal cell attachment and neurite outgrowth on substrates composed of laminin. In the present study, ABAKAN was isolated to purity from adult rat brain and preliminary biochemical analysis was performed to help determine the structure and possible function of the proteoglycan in the normal CNS. The proteoglycan was found to have an M, of approximately 410 kDa and to be composed of a 170 kDa core protein to which both chondroitin and keratan sulfate chains are attached. A third carbohydrate moiety which was found to have HNK-1 antigenicity was also found attached to ABAKAN. To ascertain its function after injury in the CNS, the distribution of ABAKAN relative to specific cell markers and other proteoglycans was determined both in normal and crushed optic nerve. ABAKAN, along with other proteoglycans, was found to be significantly upregulated after crush injury suggesting that ABAKAN plays a role in the inhibition of nerve regeneration. Supported by the Whitehall Foundation (EEG), NIH Grant # AR32666 (BC), the Canadian Spinal Research Organization (AB), and the NIH/NRSA Trauma Research Fellowship (DB).

545.11

EFFECTS OF IN SITU MICROINJECTION OF ANTI-NEUROFILAMENT MABS INTO REGENERATING GIANT CENTRAL NEURONS IN THE LAMPREY G.F. Hall, D.S. Pijak, E. Lamperti*, and M.E. Selzer Dept. of Neurology, Children's Hospital, Boston MA 02115 and Dept. of Neurology, Univ. of Penn., Philadelphia PA 19104.

Identified giant neurons (anterior bulbar cells or ABCs) in the hindbrain of the larval sea lamprey *Petromyzon marinus* have been shown to be an advantageous preparation for studying the effects of axotomy in situ using microinjection techniques. Here we examined the effects of microinjecting monoclonal antibodies (mAbs LCM3 and LCM40) raised against the lamprey neurofilament protein (NF180) into regenerating ABCs. ABCs were injected with either of these mAbs at 4 mg/ml between 7 and 12 days following axotomy at the level of the 5th gill and were reaxotomized in the hindbrain at this time. Lucifer Yellow-dextran (LY-D, 10 mg/ml) was also injected to reveal the injected cells in wholemount. Control injections consisted of rabbit IgG (5 mg/ml) and LY-D. We found that anti NF180 mAbs induced a pronounced axon stump narrowing in treated neurons by 12 days post injection when compared to controls ($p << 0.005$, chi square test). There was otherwise little difference between anti NF180 mAb injected cells and controls in the shape of the cell body and dendrites. In light of the role played by mammalian neurofilaments in maintaining axonal caliber, we propose that microinjection of anti NF180 mAbs blocks the transport of NF180 into regenerating axons. Supported by NIH grant NS29281 to G.F.H.

545.12

MAG CAN EITHER PROMOTE OR INHIBIT NEURITE OUTGROWTH. M.T. Filbin*, Y. Shen, P. Doherty, F.S. Walsh, and G. Mukhopadhyay Hunter College, New York, NY10021 and Guy's Hospital, London, U.K.

Myelin associated glycoprotein (MAG), a member of the immunoglobulin (Ig) superfamily, has been shown to promote neurite outgrowth from rat 1 day old dorsal root ganglion (DRG) neurons. The effect of MAG on neurite outgrowth of neurons from different regions of the nervous system and at different times during development was examined by seeding isolated neurons onto either MAG-expressing transfected Chinese hamster ovary (CHO) cells or control CHO cells. The neurons were cultured for 16-24 h and the neurites were visualized by immunostaining for GAP-43, a protein expressed in abundance in neurites but not in CHO cells. The length of the longest neurite extended from each neuron was measured for at least 200 cells for each experiment. On the control CHO cells, 1 day old rat DRG neurons extended long neurites which, when grown on MAG-expressing cells, almost doubled. In contrast, adult DRG neurons, while still extending long neurites on the control cells, did not do so on MAG-expressing cells; the mean neurite length was decreased by 40% compared to neurons on control cells. Cerebellar neurons, like the DRG neurons, produced extensive neurites on the control cells but for all the ages examined (1,4 and 7 days) neurite extension was decreased by about 70% on MAG cells compared to controls. The specificity of this inhibition was demonstrated by the ability of MAG antibodies to reverse the inhibition by 50%. These results suggest that at different times in development and in different brain regions MAG can have very different effects on the ability of neurons to extend neurites. These results may be important to both axonal guidance during development and to nerve regeneration after injury.

545.13

OLIGODENDROCYTE INHIBITORY PROTEINS RETARD REGROWTH OF RETINAL AXONS BUT DO NOT CHANGE THE BASIC MODE OF GROWTH. D. F. Chen*, M. Schwab & G. E. Schneider. Dept. Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139; Institut fur Hirnforschung der Universitat Zurich, August Forel-Strasse 1, CH-8029 Zurich, Switzerland.

Oligodendrocyte inhibitory proteins retard axonal growth in mature mammalian CNS. We have found that axons from E14 to P0 retina of hamster can show vigorous growth in the mature tectal environment, despite the presence in this target of differentiated oligodendrocytes. To test whether retinal neurons at these ages have a receptor for the oligodendrocyte proteins, supernatant (30%) from hybridoma cells producing the neutralizing antibody IN-1, or from control cells that produce an antibody against HRP, was added to co-cultures of retina (from animals aged E14 to P0) and adult tectum. Cultures were maintained at 37°C for 5 days, fixed, and crystals of DiI were placed in retinal explants to label the regenerating axons. In the presence of control antibody, the retinal axons were able to grow vigorously into tectum of all ages. In the presence of IN-1 antibody, axonal outgrowth was significantly increased. Thus, E14 - P0 retinal neurons clearly have a receptor for the protein, and their growth can be inhibited by it. In addition, we examined the effects of IN-1 antibody in isochronic co-cultures prepared from animals aged P4 or older, when most retinal axons have lost the ability for axon elongation into the central target. We found that some retinal axons grew into tectal slices in an arborization mode at a rate of up to about 100μm per day. Addition of IN-1 antibody increased the average number of axons innervating tectal slices, but it did not change the basic mode of growth. The results suggest that factors intrinsic to the retina are involved in determining the growth ability of retinal axons, while myelin-associated inhibitory protein can limit the growth of retinal axons once they reach the CNS environment. (Support: NIH grants EY00126, EY09621).

545.14

CORTICAL OLIGODENDROCYTES INHIBIT OUTGROWTH IN A MODEL SYSTEM OF REGENERATING PERIPHERAL NERVE IN RAT. B.E. Pulford* and L.R. Whalen, Dept. of Anatomy & Neurobiology, Program in Neuronal Growth and Development, Colorado State University, Fort Collins, CO 80523

Lack of regeneration in the injured mammalian spinal cord appears to be due, at least in part, to the presence of inhibitory proteins on the cell surface of oligodendrocytes. A peripheral nerve model of CNS regeneration provides the opportunity to investigate oligodendrocyte-associated inhibition under conditions which are intermediate in complexity between the spinal cord and *in vitro* environments. The model consists of an axon-free "gap," 5-6mm in length, produced in the sciatic nerve of mature rats into which cultured oligodendrocytes are injected. The gap is produced by severing the nerve 6mm distal to a point marked by sutures in the epineurium, retracting the epineurium of the proximal nerve segment, severing and removing the exposed nerve fascicles, and reattaching the proximal epineurial sheath to the epineurium of the distal nerve stump using 9-0 suture. Cultured rat oligodendrocytes ($3x10^7$ cells/ml) suspended in a 70:30 (v/v) solution of serum free medium (N1):Matrigel (MG) were injected into the epineurial-enclosed gap using a syringe fitted with a 30ga needle. Control gaps were filled with either a 70:30 solution of N1:MG or rat fibroblasts suspended in a 70:30 solution of N1:MG. Nerves from all 3 groups of rats were removed two weeks after surgery and examined using histochemical, immunocytochemical and ultrastructural techniques. Preliminary results indicate that implanted oligodendrocytes survive within the gap for 2 weeks and that maximal axonal outgrowth and number of myelinated and nonmyelinated fibers are decreased in nerves in which oligodendrocytes are present within the gap compared to controls.

545.15

ALTERATIONS IN GLIAL MORPHOLOGIES AND ANTIGENIC PHENOTYPES FOLLOWING IRRADIATION. T.J. Sims*, D.L. Davies and S.A. Gilmore. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Early postnatal exposure of the lumbosacral spinal cord to X-rays results in dramatic *in vivo* alterations in developing glial populations and in subsequent conversion to a CNS glial environment that is permissive for the regeneration of axons (Sims and Gilmore, Brain Res., 1994). To understand mechanisms involved in this conversion to a permissive environment, the status of the glial populations persisting after x-irradiation (40 Gy) of the 3-day-old spinal cord was further assessed by *in vitro* techniques. Control cultures prepared from non-irradiated spinal cord and experimental cultures established from the cords of irradiated littermates were compared by immunocytochemical and histochemical staining after 8 days in culture. The most conspicuous difference was the profound reduction in numbers of both astrocytes and oligodendrocytes in experimental cultures. In addition to differences in macroglia, the microglial population was severely depleted in experimental cultures (see Gilmore *et al.*, this meeting). Five morphologically distinct astrocyte types were observed in both control and experimental cultures. The latter, however, contained a greater proportion of astrocytes with flat morphologies. Addition of dibutyryl cyclic AMP (1 mM for 30 hrs) to control cultures altered the cellular composition to include greater proportions of process-bearing cell types, but failed to elicit a similar change when added to experimental cultures. Unexpected changes in phenotype were observed, however, such as astrocytes with cytoplasmic staining for galactocerebroside, a marker normally associated with oligodendrocytes. This cell culture study strongly suggests that x-irradiation of the spinal cord not only reduces the glial populations, but also dramatically alters its cell composition and generates cells of novel antigenic phenotypes.
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545.16

PDGF-BB AND IGF-I IN COMBINATION ENHANCE AXONAL REGENERATION IN A GAP MODEL OF PERIPHERAL NERVE INJURY. M.R. Wells*, D.K. Batter, H.N. Antoniades, D.G. Blunt, J. Weremowicz, S.E. Lynch, and H.-A. Hansson[1]. Institute of Molecular Biology, Inc., Worcester, MA 01605, and [1]University of Göteborg, Göteborg, Sweden.

Peripheral nervous system injuries often result in significant functional loss, including painful sequelae, that are often refractory to conventional surgical repair. The objective of the present study was to determine if a single application of the platelet-derived growth factor-BB (PDGF-BB)/insulin-like growth factor-I (IGF-I) combination could enhance the repair of injured peripheral nerve.

Forty-two rats were subjected to unilateral gap (8 mm) injuries of the sciatic nerve, and repaired with silicone tubes containing the growth factors in a 2% methyl cellulose vehicle. Concentrations tested ranged from 15-700 μg/ml PDGF-BB and 30-160 μg/ml IGF-I, with molar ratios from 5:1 to 1:50 of PDGF:IGF (N=6/group). After 4 weeks, animals were evaluated by a 'pinch' test to demonstrate the extent of axonal growth, sacrificed, and biopsies were examined for the number of myelinated axons in the center of the tube and distal to the repair site. By these criteria, the PDGF-BB/IGF-I combination produced significantly greater nerve regeneration than treatment with vehicle alone over a broad range of ratios and concentrations. At the optimum molar ratio (1:4, PDGF:IGF), growth factor-treated animals exhibited 6595±1476 myelinated axons (mean±SE) compared to 1096±415 myelinated axons in animals which received vehicle alone. These data suggest that the combination of PDGF-BB/IGF-I might prove efficacious in the treatment of peripheral nerve injuries.

545.17

EFFECTS OF PRIOR NERVE INJURY ON MORPHOLOGICAL DIFFERENTIATION OF ADULT RAT DRG NEURONS IN CULTURE
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Numerous studies have shown that conditioning lesions can enhance the rate of regeneration of subsequently injured peripheral neurons, but it is not clear whether this enhanced rate of nerve regrowth reflects an increased growth capacity of the neurons or changes in the growth environment. To assess whether conditioning lesions alter the properties of the affected neurons, we dissociated neurons from L4 and L5 DRG of adult female Wistar rats subjected to sciatic nerve crush 4-5 days before sacrifice or sciatic nerve ligation 3 weeks before sacrifice. Neurons were then cultured for 1-3 days, fixed, and stained with neurofilament antibodies to visualize neurites. Rates of neurite initiation, neurite outgrowth, and branching frequency in previously ligated or crushed neurons were compared to neurons from control animals or the unoperated side of the same animal. Neurons extended processes at rates of several millimeters per day with interbranch distance being highly correlated with total outgrowth. Neurons which had previously received a crush showed roughly twice as many neurons with processes after 1 day in culture, greater average total neurite length per neurite bearing neuron, and increased interbranch distance compared with control neurons. Ligated neurons also initiated more neurites after 1 day than controls. These results demonstrate that enhanced regeneration of sensory neurons following a conditioning lesion is maintained in isolated cell bodies in culture.

545.18

ORG 2766 ENHANCES AXONAL REGROWTH ACROSS TRANSECTED RAT SCIATIC NERVE
M. Dauzvardis*, S. Sayers, T. Khan, L. Liu, and C. Trausch. Rehabilitation R&D Center, Hines VA Hospital, Hines, IL 60141.

Melanocortins have been found to exert a trophic influence on both the central and peripheral nervous systems. The purpose of this study was to determine whether Org 2766 (a tri-substituted ACTH 4-9 analog) would enhance recovery after complete transection of the rat sciatic nerve as determined by neuronal tracing techniques and twitch tension measurements.

Rats were anesthetized, the left sciatic nerve was cut in the mid-thigh, and the proximal and distal ends were inserted into a silicone tube and held in place with cyanoacrylate adhesive. The right sciatic nerve was left intact. For animals receiving Org 2766 (N.V. Organon, The Netherlands) a 10 µg subcutaneous injection was given after surgery and 1 µg subcutaneous injections at 48 h intervals for a two-week period. After the eight-week survival period, twitch tension measurements were performed, followed by WGA-HRP injection into each nerve distal to the cut. After 48 h, the animals were perfused and the spinal cords were sectioned. The total number of labeled cells were counted from segments L2-S5 on both sides of the spinal cord and results were expressed as a ratio of labeled cells on the experimental side divided by the control side.

The average of the ratios of labeled cells on the experimental (transected) side divided by the control (unoperated) side was 19.2% for the six animals which received injections of Org 2766; three out of the five animals also showed the presence of twitch tension peaks recorded from the experimental side. The average of the ratios of labeled cells was 1.9% for the seven animals which did not receive any injections of Org 2766; only one out of the six animals showed the presence of a twitch tension peak recorded from the experimental side.

This preliminary study demonstrated that Org 2766 did enhance recovery after complete transection of the sciatic nerve as evaluated by neuronal tracing techniques and also by twitch tension measurements.

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545.19

CONTROL OF GAP-43 EXPRESSION CAN BE ACCESSED THROUGH A G-PROTEIN AND ADENYLATE CYCLASE
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Dorsal root ganglion (DRG) neurons display increased expression of GAP-43 following peripheral (but not central) axotomy *in vivo*. To study the signaling mechanism which links peripheral axotomy to changes in GAP-43 expression, we established a cell-ELISA technique to measured the GAP-43 content of microcultures of adult rat DRG neurons. As they do following peripheral axotomy *in vivo*, DRG cells which have been excised, dissociated, and maintained *in vitro* for one week display increased GAP-43 expression. This GAP-43 increase is partially suppressed by continuous exposure to cholera toxin, an activator of the stimulatory G-protein G_S. The GAP-43 increase is also suppressed by the cyclic AMP analogs dibutyryl-cAMP and 8-bromo-cAMP, the adenylate cyclase activator forskolin, and the phosphodiesterase inhibitor Ro 20 1724. We propose that GAP-43 expression is normally suppressed by an extracellular agent acting through G_S and adenylate cyclase, and that removal of this repressive agent leads to the elevation of expression of GAP-43 which follows peripheral axotomy.

545.20

A MONOCLONAL ANTINEURONAL ANTIBODY PROMOTES PRIMARY NEURON OUTGROWTH and NON-NEURAL CELL SPREADING on CNS MYELIN.
AM LOZANO*, C LI, M LABES and A ROACH. Samuel Lunenfeld Research Institute, University of Toronto, Toronto, M5G 1X5

A component of adult mammalian central nervous system (CNS) myelin inhibits axonal growth, a property that may be responsible in part for the lack of regrowth of axons interrupted in the CNS. The mechanism through which the inhibitory activity acts on the neuron to block growth is not known. To identify the neuronal molecules involved, we have generated and selected antineuronal antibodies based on their ability to promote neurite outgrowth on a CNS myelin substrate. We identified one such antibody, 10D, by its ability to promote the outgrowth of neurites from neural cell lines plated on CNS myelin. To test the effect of 10D on primary neurons and non-neuronal cells, we have grown newborn rat superior cervical sympathetic ganglion neurons (SCG) and 3T3 fibroblasts on CNS myelin. SCG neurons did not extend neurites and 3T3 fibroblasts did not spread on 10 ug/cm^2 of CNS myelin. In contrast, on CNS myelin in the presence of 10D, there was a vigorous outgrowth of neurites from SCG neurons and extensive spreading of fibroblast cell processes. These effects were not seen with control antibodies. The results indicate that 10D influences the process through which myelin substrates exert their inhibitory effects on neuronal and non-neuronal cells.

TRANSPLANTATION V

546.1

DYNAMIC REGULATION OF STRIATAL DOPAMINERGIC GRAFTS DURING LOCOMOTOR ACTIVITY.
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After fetal dopaminergic cell grafts into hemiparkinsonian model rats, extracellular levels of DA increased nearly to normal and methamphetamine-induced rotations were reduced completely. However, more complex motor behaviors such as treadmill running at high speeds recovered to a lesser extent. Thus, besides recovery in tonic release of DA, additional mechanisms such as higher levels of DAergic reinnervation under dynamic regulation in the host brain might be necessary for better motor control. The present experiment was designed to estimate the neurochemical activity of DAergic grafts during locomotion and to examine the functional importance of dynamic regulation of the grafted neurons in the host brain. Grafted rats were trained to run on a straight treadmill at various speeds (300, 660, 1200, 1800 cm/min), and extracellular DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured by in vivo microdialysis during and after running. Grafted rats were devided into two groups depending on the running ability (treadmill running test) and data were compared with those of normal and lesioned controls. Although tonic levels of extracellular DA in grafted rats recovered to 70-80 % of control, those of DOPAC and HVA remained 15-20% of controls. Small numbers of grafted rats showed full recovery on treadmill running tasks and percentage increases of DOPAC and HVA showed similar time courses and magnitudes as those of normal rats. The majority of grafted rats showed partial recovery of locomotor ability and percentage increases in DOPAC and HVA remained at lower levels than in normal rats. However, there were no differences in tonic levels of DA, DOPAC and HVA between the two grafted groups. Data suggest that grafted DAergic cells were dynamically regulated by the host brain and that the integrated release of DA might be involved in recovery of complex motor behaviors.

546.2

TWO NEURONAL CELL LINES WITH DOPAMINERGIC PROPERTIES AS CANDIDATES FOR NEUROTRANSPLANTATION
K.N. Prasad[1], F.S. Adams[23], E.H. Kriek[23]*, S. Kentroti[34], A. Vernadakis[34], E. Carvalho[1], C.R. Freed[23] and J. Edwards-Prasad[1] Depts. of Radiology[1], Medicine[2], Pharmacology[3] and Psychiatry[4] Univ. Colorado Sch. of Medicine., Denver, CO 80262

We investigated the dopaminergic characteristics of two established cell lines. The first, $2N_{27}$, was derived from ED12 rat mesencephalic cells that were immortalized by transfection with a plasmid vector, pSV$_2$*neo*. The second cell line, NBP_2 was murine neuroblastoma. *In vitro*, both lines expressed tyrosine hydroxylase (TH) and dopamine transporter (DAT) immunoreactivity. Terminal differentiation of NBP_2 cells with 200 µg/ml RO20-1724, a phosphodiesterase inhibitor, and 20 µg/ml ß-carotene caused neurite formation and increased TH density. DAT expression appeared unaffected. Compared to the neuroblastoma cells, $2N_{27}$ had faint TH and DAT labelling. Treatment with RO20-1724 and dibutryl-cAMP (1 mM) led to subtle changes in morphology without noticeably affecting TH or DAT. Cells prelabelled with the fluorescent dye, DiI, were transplanted into rat brain and were found to survive one month after transplantation without tumor formation.

546.3

THE EFFECT OF FETAL TISSUE GRAFT PLACEMENT ON BEHAVIORAL RECOVERY IN A RAT MODEL OF PARKINSON'S DISEASE.

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Rats with unilateral striatal dopamine depletion are often used as an animal model of Parkinson's Disease. Fetal ventral mesencephalon grafts into the striatum of these lesioned rats have been used successfully to reduce asymmetries in both drug-induced and spontaneous behavior. We have found previously that grafts into the substantia nigra can also reduce the amphetamine-induced rotational bias. Thus, in the present experiment, adult lesioned rats received fetal tissue grafts into either the striatum, substantia nigra, or into both. They were tested postsurgery for a reduction in bias of both amphetamine-induced rotation, apomorphine-induced rotation, and spontaneous homecage behavior. Fetal tissue was labeled with bromodeoxyuridine (BrdU). At the conclusion of the experiment, biocytin was infused into the substantia nigra to trace the growth of the grafts. Coronal brain sections were stained for tyrosine hydroxylase, BrdU and biocytin immunoreactivity. Trends in the data indicate that grafts into the striatum are the most effective in reducing drug-induced rotational behavior, followed by grafts into both sites simultaneously, and then grafts into the substantia nigra. Full data will be presented at the meeting.

[Supported by NS22157]

546.4

GRAFTS OF EMBRYONIC MESENCEPHALON IN AN ANIMAL MODEL OF PARKINSON'S DISEASE: BEHAVIORAL EFFECT OF VARYING THE AMOUNT OF GRAFTED TISSUE.

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Grafts of embryonic dopaminergic tissue have shown promise as a therapy for Parkinson's disease in humans. One basic parameter under debate is the amount of tissue necessary to achieve optimal behavioral recovery. To address this, the present experiment contrasted the behavioral effects of grafting ED15 ventral mesencephalic tissue (MESEN) from one (4μl) or four (16μl total) donors into the striatum of adult male Sprague-Dawley rats with unilateral destruction of the mesostriatal dopamine system by 6-hydroxydopamine. Controls received equal volumes of ED15 spinal cord. Before grafting, the tissue was extruded into 200 μm strands. Injections of either 1μl or 4μl each were made at four sites into the striatum of each recipient. Circling after methamphetamine (METH; 5.0 mg/kg) was measured prior to grafting as well as one, two and three months afterwards to assess transplant efficacy. Following the third test, the rats were water deprived and trained to run on a circular treadmill for water reward, ipsi- and contralateral to the side of grafting. Only the animals receiving 16μl of MESEN showed a reduction in contraversive METH-induced turning, whereas those with 4μl MESEN or 4 or 16μl spinal cord did not show a reduction. A deficit in contraversive learned-turning was seen; however, none of the four treatments was effective in alleviating this deficit. Paradoxically, rats receiving 16μl of MESEN showed the worst performance. Tyrosine-hydroxylase (TH) immunohistochemistry revealed TH positive cells in the groups that had received MESEN. We conclude that grafting with MESEN from four embryos produces a better behavioral outcome than from a single embryo.

546.5

THE STUDY OF APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR IN PARTIAL LESIONED RAT PARKINSONIAN MODELS WITH 6-HYDROXYDOPAMINE

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An apomorphine-induced rotational test has been used in the evaluation of rat parkinsonian model lesioned with 6-hydroxy-dopamine. We evaluated 50 partial lesioned rat parkinsonian models using the cylindrical rotometer devices with flat bottom (diameter, 30.5cm). Depending on the pattern of the rotation induced by apomorphine(0.5mg/kg i.p.), the rats were grouped into 4 groups(Group 1: contraversive pivotal rotation, 16 cases ; Group 2: contraversive wide rotation, 20 cases: Group 3: ipsiversive rotation including pivotal rotation, 3 cases; Group 4: non-preferred or no rotation, 11 cases). Twelve of 16 rats in group 1 and 14 of 20 rats in group 2 met our criteria of rat parkinsonian model(at least 90 turns/30 min, 10 min after apomorphine injection). Although another 4 rats in group 1 didn't meet our criteria, all of them showed severe losses(more than 95%) of the TH-immunoreactive neurons(SNpc) in the lesioned side. Another interesting finding was that rats in group 2 and 3 showed moderate losses(50-80%) of TH-immunoreactive neurons(SNpc) in the lesioned side even though their rotational responses were different. These results suggest that pattern of rotation(pivotal rotation) is a more reliable index of neuronal losses of SNpc than the total numbers of the rotational response to apomorphine.

The exact cause of the abnormal ipsiversive rotation noted in group 3 is unclear. Further research should be pursued to explain this finding.

546.6

OPTIMAL SURVIVAL OF GRAFTED MESENCEPHALIC DOPAMINE (DA) NEURONS IS ATTAINED FROM TISSUE TAKEN DURING NEUROGENESIS OF THE SUBSTANTIA NIGRA IN NON-HUMAN PRIMATE

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We examined a model of Parkinson's disease to test the effect of embryonic grafts of ventral mesencephalon on motor disability induced by MPTP. DA increases on average 500% near grafts (see Elswoth abst). Although staining revealed 20,000+ DA neurons in grafts derived from a single embryo, we have seen variability in the percentage of DA neuroblasts that survive grafting. This may be related to the age of the donor tissue; neurons that have extended axons would be subject to degeneration while cells taken from ages prior to neurogenesis might lack appropriate developmental clues. Grafts were selected from pregnant monkeys by ultrasonography; 14 donors were chosen from embryonic days 30-47. Solid tissue grafts were placed into the caudate nucleus. Brains were collected at 3-6 months after surgery and were stained for tyrosine hydroxylase (TH). DA neurons ranged from 1,300 to 28,000 per host; 9 of 14 animals contained between 8,200 and 28,400 TH neurons. The numbers of TH neurons in grafts seen at each age examined were: E30 (3,500), E38(13,780), E39(9,288), E41(21,296), E42(n=4; 1,300, 18,000, 11,700, 12,136), E44(n=4; 3104, 4,100, 15,064, 28,400), E47(n=2; 8,200, 13,344). The average of donors (n=11) taken during putative neurogenesis (E36-E43 ± 24hrs.) of the substantia nigra was 12,560±2456. The adult African green monkey has about 80,000 DA neurons in both substantia nigrae. Thus, an average survival of 12,560 neurons is about 16% of the adult population; the best survival obtained (i.e. 28,400) is about 36% which is substantially better than results obtained from studies using xenografted human cells. Supported by NIH grants NS 24032 and RSA MH 00643(DER).

546.7

DOPAMINE IS PRODUCED BY EARLY STAGE FETAL VENTRAL MESENCEPHALON BUT NOT CEREBELLAR TRANSPLANTS IN MPTP MONKEYS

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Occasional studies in parkinsonian animals have suggested that the mechanism of neural transplant-induced beneficial effects may not rely on dopamine (DA) generated from the neural graft. These studies address this issue in MPTP-treated monkeys.

Grafts of fetal ventral mesencephalon (VM) or cerebellum were placed stereotactically in the striatum of MPTP-treated parkinsonian monkeys. Behavior of each animal was rated twice daily. At sacrifice, tissue micropunches were removed from brain slices for biochemical analyses. The remainder of the slice was processed for autoradiography or tyrosine hydroxylase (TH) immunohistochemistry.

Striking elevations (500%) in caudate DA concentration within 2 mm of the graft and improved behavior were associated with transplanted 2.5 - 4 cm crown-rump length (CRL) fetal VM (n = 19). Fetal VM (8 - 11 cm CRL) produced small increases in DA. DA concentration was not increased by grafted fetal cerebellum (n = 12), or 15 - 18 cm CRL fetal VM (n = 11), or after sham implantation (n = 5).

Radiolabeled cocaine derivatives ([^{125}I]β-CIT, [^{125}I]β-isopropylCIT) with extremely high affinity for the DA transporter were used to measure by autoradiography the growth of transplanted 2.5 cm CRL fetal VM cells. High density of binding was seen at the site of the grafts. Graft presence was confirmed by TH immunohistochemistry. At some coronal levels a majority of the caudate was occupied with an increased density of binding to DA uptake sites. Correlative *in vivo* brain imaging by SPECT has been performed in some grafted MPTP-treated monkeys using [^{123}I]β-CIT.

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546.8

HOST STRIATUM PROJECTS FIBERS INTO TRANSPLANTED FETAL MESENCEPHALON IN A PARKINSONIAN PRIMATE MODEL.

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Fetal mesencephalic grafts into the striata of partially dopamine (DA)-depleted African green monkeys become well developed and show numerous TH-positive neurons when examined with immunohistochemical (IHC) staining. These neurons project fibers into the adjacent host striatum. Since control of DA synthesis/release may be influenced by the host brain, the establishment of reciprocal connections is of importance. A double label IHC staining technique was used for simultaneous evaluation of both graft and host fiber projections. TH-containing neurons were stained as before. Endogenous host striatal projecting neurons, which normally communicate with the intact substantia nigra using the neurotransmitters GABA and substance-P, were stained using antibodies against glutamic acid decarboxylase (GAD), a GABA synthetic enzyme, and substance-P. Different chromogens were used to label the different cell types. This staining protocol was performed on 8 grafted animals. Numerous GAD-positive and substance P-positive fibers were identified within the TH-positive grafted fetal mesencephalic tissue. Many of these fibers were found in proximity to TH-positive graft cells. Some of the fibers adjacent to TH-positive cells exhibited enlargements suggestive of potential contact points. These preliminary results provide a morphologic basis for the presence of reciprocal connectivity between the host striatum and the transplanted fetal mesencephalon. Electrophysiological and electron microscopic studies of an analogus experimental paradigm will be needed to verify this suspected connectivity. Supported by a Chicago Medical School Research Fellowship, Axion Research Foundation, and NIH grants NS 24032 and RSA MH 00643 to D.E.R

546.9

FUNCTIONAL RECOVERY IN HEMIPARKINSONIAN MONKEYS FOLLOWING GRAFTS OF ENCAPSULATED PC-12 CELLS

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Following training for 2- months on a hand reaching task, Rhesus monkeys were rendered hemiparkinsonian via an intracarotid injection of n-methyl-4 phenyl 1,2,3,6 tetrahydropyridine (MPTP). Following the unilateral lesion, these animals were greatly impaired or would not perform with their affected limb while function was not affected on the intact side. Three months following the lesion, monkeys received 2 grafts into the caudate nucleus and three grafts into the putamen of polymer capsules containing PC-12 cells (n=3) or empty capsules (n=1). Over the next 6 months, two of the three monkeys receiving the PC-12 cell grafts recovered their use of the affected limb and performed at near-baseline levels. The third PC-12 grafted monkey and the one control monkey remained impaired. The capsules were then removed. The capsules from monkeys which behaviorally recovered contained numerous PC-12 cells which expressed high levels of basal and potassium evoked release of dopamine. The capsules from the PC-12 grafted monkey which did not recover contained very few viable cells and both basal and potassium evoked release of dopamine was undetectable. Following the removal of the capsules, the functional recovery in the two PC-12 grafted monkeys was sustained for up to 2 months. These data suggest that grafts of PC-12 cells can induce functional recovery in nonhuman primates although the mechanism of action remains unclear.

546.10

POLYMER-ENCAPSULATED HUMAN NERVE GROWTH FACTOR-SECRETING FIBROBLASTS PREVENT THE LOSS OF DEGENERATING CHOLINERGIC NEURONS FOLLOWING IMPLANTATION INTO NONHUMAN PRIMATES

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Basal forebrain cholinergic neurons degenerate in several human dementing illnesses including Alzheimer's disease (AD). Nerve growth factor (NGF) has potent target-derived trophic and tropic effects upon cholinergic basal forebrain neurons and may represent a useful treatment strategy for AD and/or other diseases characterized by basal forebrain-mediated cholinergic deficits. We have developed a polymer encapsulation system that allows for a continuous and site-specific delivery of NGF to the CNS. Baby hamster kidney (BHK) cell lines that constitutively express high levels of human NGF (BHK-hNGF) were developed using the dihydrofolate reductase-based vector system. Polymer devices loaded with BHK-control or BHK-hNGF cells were maintained *in vitro* and hNGF release was quantified with ELISA. BHK cell-containing devices were grafted into the lateral ventricle of four cynomolgus monkeys immediately following a unilateral transection of the fornix. Three control monkeys received non-hNGF-secreting cells and one monkey received a fornix transection only. Control monkeys displayed extensive losses of choline acetyltransferase and p75 NGF receptor-immunoreactive neurons within the medial septum (MS; 53% and 54%) and vertical limb of the diagonal band (VLDB; 21% and 30%) ipsilateral to the lesion. In contrast, monkeys receiving implants of BHK-hNGF cells exhibited a modest loss of cholinergic neurons within the MS (19% and 20%) and VLDB (7%) together with a dense sprouting of cholinergic fibers within the septum. Retrieved capsules contained numerous viable cells which continued to produce hNGF. These results support the use of polymer-encapsulated cell therapy in neurodegenerative diseases such as AD where basal forebrain degeneration is a consistent pathological feature.

546.11

THE AGED MONKEY BASAL FOREBRAIN: RESCUE AND SPROUTING OF DEGENERATING CHOLINERGIC NEURONS FOLLOWING GRAFTS OF POLYMER-ENCAPSULATED NGF-SECRETING CELLS.

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Six aged Rhesus monkeys (24-29 years old) received unilateral transections of the fornix. Three monkeys then received intraventricular transplants of polymer-encapsulated baby hamster kidney (BHK) fibroblasts which had been genetically modified to secrete human nerve growth factor (hNGF). The remaining three monkeys received identical grafts except the cells were not modified to secrete hNGF. Monkeys receiving control grafts displayed extensive reductions in the number of ChAT (57-75%) and p75 NGFr-immunoreactive (ir) (53%) medial septal neurons ipsilateral to the lesion/graft. In contrast, monkeys receiving grafts of encapsulated hNGF-secreting cells display only a modest loss of ChAT-(0-36%) and p75 NGFr-(7-22.4%)-ir septal neurons. Additionally, all monkeys receiving the hNGF-secreting implants, but none receiving control implants, displayed robust sprouting of cholinergic fibers within the septum ipsilateral to the transplant. Just prior to sacrifice, the capsules were retrieved and determined to contain viable BHK cells releasing biologically relevant levels of hNGF. These data demonstrate that hNGF can provide trophic and tropic influences to degenerating cholinergic basal forebrain neurons in aged nonhuman primates supporting the contention that hNGF may prevent degeneration of basal forebrain neurons in Alzheimer's disease.

546.12

GRAFTS OF POLYMER ENCAPSULATED HUMAN NGF SECRETING CELLS DO NOT ALTER ß AMYLOID OR APOLIPOPROTEIN E EXPRESSION IN YOUNG OR AGED MONKEYS.

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Grafts of polymer encapsulated cells which have been genetically modified to secrete human nerve growth factor (hNGF) rescue axotomized septal neurons for up to 1 month in young and aged monkeys (see adjacent abstracts). Since NGF has been shown to increase ßAPP production in nonprimates, we examined whether biologically delivered NGF would alter the expression of ß amyloid or apolipoprotein E (ApoE)-containing elements in young and aged nonhuman primates. The following groups of monkeys were studied: a) young adult monkeys with NGF grafts (<15 years old; n=2); b) young adult monkeys with control grafts (<15 years old; n=2); c) aged monkeys with NGF grafts (25-29 years old; n=3); d) aged monkeys with control grafts (24-26 years old; n=3); e) intact aged monkeys (23-31 years old; n=10). None of the young adult monkeys displayed ß amyloid or ApoE regardless of treatment. Aged monkeys displayed numerous ß amyloid containing neuritic plaques within the temporal and parietal neocortex as well as the hippocampus. These animals also displayed numerous ApoE-containing plaques, primarily located within the temporal neocortex. The expression of ß amyloid and APoE was similar in intact aged monkeys and monkeys receiving control grafts or grafts secreting hNGF. Thus, it appears that hNGF can provide trophic support for degenerating cholinergic basal forebrain neurons in young and aged primates without altering some pathological features associated with aging processes.

546.13

HYPERTROPHY OF CHOLINERGIC AND NPY CONTAINING STRIATAL NEURONS FOLLOWING IMPLANTS OF POLYMER ENCAPSULATED CELLS GENETICALLY MODIFIED TO SECRETE HUMAN NGF.

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The present study examined the effects of polymer encapsulated grafts containing baby hamster kidney fibroblasts (BHK) genetically modified to secrete human NGF (hNGF) upon the size of cholinergic and noncholinergic striatal neurons following implantation into the intact rodent striatum. Three groups received BHK-hNGF grafts into the right striatum and were sacrificed 1, 2, or 4 weeks post-implantation. The remaining three control groups were treated identically except that BHK cells were not modified to secrete hNGF. In control BHK grafted rats, the size of ChAT-ir striatal neurons was unchanged (<2%) at all time points. In the BHK-hNGF grafts, there was a hypertrophy of ChAT-ir striatal neurons 1 (29.4%), 2 (34%) and 4 weeks (32%) following grafting relative to the intact striatum. Optical density measurement within ChAT-ir somata were also significantly enhanced in BHK-hNGF grafted rats relative to control grafted animals. These effecsts were lost in rats receiving BHK-hNGF grafts for one week and allowed to survive an additional 3 weeks following graft removal. Control grafts did not alter the size of NPY neurons within the striatum. However, the NGF-hNGF grafts significantly increased the size of NPY neurons 1 (13%), 2 (16%) and 4 (13%) weeks post-implantation. Removal of the capsules just prior to sacrifice revealed healthy BHK cells which secreted sufficient levels of hNGF to differentiate PC12A cells. These data indicate that polymer encapsulated cells which were genetically modified to secrete hNGF can influence both cholinergic and noncholinergic neurons within the striatum.

546.14

CLINICAL RECOVERY AND GRAFT SURVIVAL IN PARKINSONIAN NON-HUMAN PRIMATES AFTER STRIATAL GRAFTING OF FIBROBLASTS GENETICALLY MODIFIED WITH TYROSINE HYDROXYLASE cDNA.

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Somatic gene therapy uses grafts of genetically modified autologous cells to produce and deliver therapeutic substances to specific sites within the body and eliminates the need for immunosuppression or barrier devices. We are utilizing a process that consists of harvesting autologous cells through a biopsy, followed by *ex vivo* genetic modification of these cells to produce specific therapeutically active proteins, and subsequent grafting of these cells to an appropriate site. 7 MPTP-treated overlesioned hemiparkinsonian monkeys were used for this study. Using MRI guided stereotaxic surgery the 3 monkeys were grafted with autologous fibroblast transfected with retroviral vector (MFGS) carring a human tyrosine hydroxylase (TH2) cDNA, 2 monkeys were implanted with non-transfected fibroblasts and 2 animals were left non-implanted. Prior to the implantation all animals were behavioraly characterized for 9-12 months using parkinsonian rating scale, apomorphine-induced turning, arm use scale and activity monitors. TH implanted monkeys showed immediate clinical improvement and increase of activity after the implantation which lasted for 4 months, reduction of apo-rotation and they regained functional arm use. Animals were sacrificed at 4 months. Control implanted animals recovered only from apomorphine-induced rotation, while control non-implanted monkeys remained unchanged. MRI scanning detected grafts in the striatum and closure of the blood brain barrier at 2 weeks post-implantation.
Histological examination of the implanted animals showed surviving, well vascularized and integrated in the host striatum fibroblast grafts representing over 40% of implanted cellular volume. Immunocytochemistry and in-situ hybridization analysis of the brains are in progress. These results validate potential application of ex-vivo gene therapy for neurodegenerative brain disorders.

546.15

NON-HUMAN PRIMATE MODEL OF PARKINSON'S DISEASE FOR STUDY OF TROPHIC AND/OR DOPAMINE REPLACEMENT THERAPY. M.E.Emborg*, W.W.McLaughlin and K.S.Bankiewicz. Somatix Therapy Corp., Alameda, CA 94501.

Administration of 0.4 mg/kg of MPTP into one internal carotid artery produces hemiparkinsonism in monkeys. In such a model, the side ipsilateral to MPTP administration becomes severely depleted of DA, while the side contralateral serves as a "normal" control. As we previously reported (Bankiewicz KS, et al. Life Sci. 1986), unilateral administration of 0.8 mg/kg results in overlesioned HPD monkeys (HPD+) which display bilateral signs of the PD. In this study, we further characterise HPD+ monkeys using behavioral and in vivo biochemical methods. 17 Rhesus monkeys (age 5-16; weight 3.92-14.86 kg; 6 female, 11 male) were studied during 9-18 months after MPTP lesion. The monkeys received an intracarotid infusion of 2-4 mg of MPTP supplemented by 0.4 mg/kg i.v. to induce HPD+, which still enabled them to sustain themselves. Apomorphine-induced turning, arm use, general activity measurements, clinical recordings using rating scale, in vivo microdialysis and CSF levels of HVA were examined. After the MPTP treatment, all of the monkeys showed progressively bilateral signs (freezing, bradykinesia, decreased defense reaction, posture and gait problems), and hemiparkinsonian signs with predominant use of the arm ipsilateral to the side of MPTP administration (rigidity, action tremor and decreased use of contralateral arm, ipsilateral spontaneous circling and apomorphine-induced contralateral turning). The CSF HVA levels were increased in the first hours after the MPTP administration with subsequent decrease of 40-60% 3 days later. Extracellular levels of dopamine and HVA as measured by microdialysis in caudate and putamen nucleus on the ipsilateral side were not detectable. Local amphetamine administration induced DA and HVA peaks only on the contralateral side, suggesting total lack of any remaining innervation on the ipsilateral side. This side, modelling the end stage of human PD, could be used for grafting DA-producing cells for studying DA replacement therapy, while the partially-lesioned contralateral side, modelling an advanced stage, could be used for studying trophic factor-releasing cells and inducing a trophic reaction.

546.16

CCK FACILITATES METHAMPHETAMINE -INDUCED DOPAMINE OVERFLOW IN RAT STRIATUM AND FETAL NIGRAL GRAFTS. Y. Wang* and S.L. Perng. Dept. of Pharmacology, National Defense Medical Center, Taiwan.

The purpose of this study was to investigate the electrochemical interactions of cholecystokinin (CCK) and methamphetamine (MA)-induced dopamine (DA) overflows in rat striatum. High-speed chronoamperometric recording techniques using Nafion-coated carbon fiber electrode were used to evaluate extracellular DA concentration in the striatum. CCK-8S, CCK-8US, MA and DA were locally applied directly to the striatum of urethane-anesthetized Sprague-Dawley rats. We found that CCK potentiated MA-induced DA release in the anterior striatum. This reaction is likely mediated through CCK-A receptors because only CCK-8S, but not CCK-8US, enhanced MA reactions. Replacement of Ca^{++} with Mg^{++} antagonized this reaction, suggesting that the modulation of MA by CCK is Ca^{++} dependent. Both MA-induced DA release and CCK modulatory effects were disappeared in the striatum after unilaterally lesioning the medial forebrain boundle with 6-OHDA lesions. The unilaterally lesioned rats were later transplanted with fetal ventral mesencephalon. We previously found that the zone of normalized dopamine clearance was considerably larger than that of normalized release in the anterior striatum after fetal nigral transplantation, which may be contributed to the partial reinnervation from the transplant. In the present study, we found that the modulation of DA release by CCK was regenerated in the zone of normalized release after fetal nigral transplantation. However, CCK did not increase MA-induced DA release in the partially innervated area. In conclusion, these findings suggest that the not only DA release process but also CCK modulatory mechanism were regenerated after fetal nigral transplantation.

546.17

ISOLATION OF MESENCEPHALIC PROGENITOR CELLS FROM RAT; REGULATION OF THE DEVELOPMENT OF THE DA PHENOTYPE. P.M. Carvey*, D.H. Lin, and L.R. Ptak. Dept. Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center. Chicago, IL 60612.

Reynolds and Weiss (Science 255:1707;1992) reported the isolation and partial characterization of epidermal growth factor (EGF) responsive striatal progenitor cells. Using their procedure, we have isolated mesencephalic progenitor cells. E-14.5 rat mesencephalon was isolated and plated onto 100 x 20 mm petri plates (1300 cells per cm^2) that had not been pre-treated with extracellular matrix products. The cells were grown in media containing 20 ng/ml EGF. Less than 0.1% of the cells survived and went on to form clusters of cells if left undisturbed for 14 days. These mitotically active cells have been successfully passaged for 8 months. When cultured on ploy-1-lysine coated plates containing a complete media (10% FCS), the cells differentiated into glia and neuron-like cells. None of these cells stained positive for tyrosine hydroxylase (TH). Similarly, TH positive cells were not found after exposure to various trophic factors or following co-culture with E14.5 striatum. In contrast, co-culturing progenitor cells with fresh mesencephalon had a profound effect increasing the number of TH staining neurons ($F_{2,69} = 122.97$; $p < 0.01$) relative to mesencephalic mono-cultures. Mitochondrial dehydrogenase activity was unchanged in the co-cultures suggesting that mitotic activity was not responsible for the increase in TH neurons observed. The cultures were also negative for the NE marker DBH. These data suggest that a soluble or a cell contact mediated activity present in mesencephalon was responsible for converting the progenitor cells into a DA neuron phenotype. Like striatal cells, mitotic progenitor cells capable of developing into DA neurons exist within the mesencephalon.

546.18

FETAL NIGRAL GRAFT-INDUCED RESTORATION OF GABAERGIC NEUROTRANSMISSION IN THE BASAL GANGLIA IN A RAT MODEL OF PARKINSON'S DISEASE. B.A. Flumerfelt*, W. Rushlow, C. Naus and N. Rajakumar, Department of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The basal ganglia exert their function mainly via disinhibitory GABAergic circuits. However, very little is known about the changes that occur in GABA neurotransmission in Parkinson's disease, and virtually nothing is known about such changes following dopamine supplementation by grafted fetal dopaminergic neurons. In the present study, GAD mRNA levels were compared in neurons of the entopeduncular nucleus (EPN) and the substantia nigra pars reticulata (SNR) in normal, unilaterally 6-hydroxydopamine-lesioned and grafted rats by means of in situ hybridization using $^{[35]}$S labeled cRNA probes. Autoradiographs revealed a moderate elevation of GAD mRNA levels in the ipsilateral SNR, particularly in the lateral half of the nucleus, while the ipsilateral EPN showed a slight increase. Nine months after transplantation of fetal nigral neurons, the GAD mRNA levels remained significantly elevated in the ipsilateral SNR, but the ipsilateral EPN appeared similar to those of normal rats. These data indicate that nigral grafts placed in the striatum differentially influence GAD mRNA levels in the EPN and SNR, and suggest a necessity for additional dopaminergic grafts in the nigra for complete restoration of basal ganglia function. *[Supported by the Parkinson's Foundation of Canada].*

546.19

XENOTRANSPLANTATION OF PORCINE VENTRAL MESENCEPHALIC NEUROBLASTS RESTORES FUNCTION IN PRIMATES WITH CHRONIC MPTP-INDUCED PARKINSONISM. L.H. Burns,*[1] P. Pakzaban,[1] T.W. Deacon,[1] J. Dinsmore,[2] O. Isacson.[1] Neuroregeneration Laboratory[1], McLean Hospital, Belmont, MA 02178, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115; Diacrin Inc.[2], Charlestown, MA 02129.

To investigate the potential of xenogeneic neuroblasts as an alternative to human cells for therapeutic neural transplantation, we evaluated the long-term survival and function of porcine fetal ventral mesencephalon (VM) cells transplanted into the striatum of parkinsonian monkeys. Four *Macaca fascicularis* were rendered parkinsonian by chronic intermittent low-dose intravenous injections of MPTP over a period of up to 15 months. Average daily locomotor activity, measured by photo-cell beams, decreased to 5 - 25% of normal values and remained unchanged for the 3 months preceding transplantation. Parkinsonian signs included hypokinesia, bradykinesia, rigidity and tremor. During the same period, dopamine fiber density in the striatum, as assessed by the ligand ^{11}C-CFT and PET, decreased to 20 - 30% of pre-MPTP values. Monkeys were transplanted unilaterally in the caudate and putamen with cell suspensions derived from the ventral mesencephalon of E28 porcine fetuses. To prevent xenograft rejection, monkeys were either immunosuppressed with cyclosporine A (CsA) or were implanted with cells pre-treated with anti-porcine MHC class I antibody fragments (Fab). One monkey received otherwise identical cells without any immunosuppression.

At 3 - 4 months after xenotransplantation, the CsA-treated monkey and the 2 Fab-treated monkeys showed increased locomotor activity and increased striatal ^{11}C-CFT signal on PET. The non-immunosuppressed monkey did not show an increase in locomotor activity or dopaminergic PET signal (compared to pre-transplant values). These data indicate that porcine mesencephalic neuroblasts can restore motor function and replenish dopaminergic fibers in parkinsonian primates.

547.1

Fast axonal transport is not influenced by exposure to pulsed electromagnetic fields before or after injury. Betty F. Sisken*, Janet L. Walker and Jane M. Jacob. Center for Biomed. Engineer. and Dept. of Anat. and Neurobiol., U. Kentucky, Lexington, KY 40506 and Dept. of Anat. Sci., U. Oklahoma HSC, Oklahoma City, OK 73190.

Fast axonal transport (FAXT) is responsible for the bidirectional movement of proteins within the nerve terminal and axon. Anterograde transport by FAXT of neurotransmitter-associated proteins and membranous organelles to nerve terminals occurs at a rate of ~410 mm/d. Pulsed electromagnetic fields (PEMF) have been shown to enhance axonal outgrowth in sciatic nerve when applied before or after injury, although the mechanism of this effect is not known. In this study, we examined the effects of acute PEMF treatments on fast axonal transport rates in the sciatic nerve of young Sprague-Dawley rats under 2 conditions: in uninjured (non-operated) nerves and 1 d after a crush of the nerve behind the knee. Rats were placed between Helmholtz coils for 15 min/d for 2 d preceding isotope injection or for 60 min on the day of isotope injection. While maintaining core body temperature at 37°C, [^{3}H]-proline was stereotactically microinjected into the spinal cord; rats were killed 2.5-5.0 hr later. After tissue solubilization and liquid scintillation spectroscopy, transport profiles indicate that acute treatments with PEMF had no effect on fast transport rates in uninjured or injured sciatic nerves. These studies are in agreement with others indicating that fast axonal transport rates are not altered in response to crush injury. They also show that PEMF effects are not related to fast transport mechanisms. Studies on slow axonal transport using a similar paradigm are underway. Supported by NIH NS 29621-02 (BFS).

547.2

FAST AXONAL TRANSPORT IN CORTICOSPINAL (CS) AXONS OF THE RAT. Daniel L. O'Donoghue*, Elizabeth G. Lease and Jane M. Jacob. Dept. of Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK 73190.

The axonal transport of proteins from the soma to the axon terminal is one of the most important functions of a neuron. In the peripheral nervous system (PNS), the fast transport rate of neurotransmitters and membranous organelles occurs at ~410 mm/d (Ochs, J. Physiol. 227:627, 1972). In this study, we directly measure the fast axonal transport rate in the longest axon tract in the central nervous sytem (CNS), the CS tract. Male Sprague-Dawley rats (4-10 wks old) were anesthetized with Chloropent and their body temperature maintained at 37°C. Intracortical microinjection of [^{3}H]-proline was made into sensory-motor areas containing CS neurons. Rats were killed 3-5 hrs later and the CS tract removed, cut into consecutive 1 mm segments, the non-protein radioactivity removed, and the tissue solubilized. After liquid scintillation spectroscopy, transport profiles indicated that the fast transported wave of radiolabeled proteins moved at ~267 mm/d (n=9). Fast transport rates in adolescent animals (4 wks) were no different than those in 10 wk rats. These data are in agreement with previous studies indicating that transport rates in the CNS are slower than those in the PNS.

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547.3

THE DIFFUSIBLE FAST AXONALLY TRANSPORTED DRG PROTEINS: DO THEY REPRESENT COMMON OR UNIQUE SECRETORY PRODUCTS? B. Tedeschi, S. Mulugeta and R.P. Ciavarra. Departments of Anatomy & Neurobiology and Microbiology & Immunology, East. Virg. Med. Sch., Norfolk, VA 23501.

Fast axonal transport (FT) is believed to represent the translocation of granules/vesicles in the neuronal secretory pathway. In the frog DRG/sciatic nerve preparation, we have previously differentiated the diffusible FT protein subset from the membranous protein subset. In the present study we addressed two questions: (1) Which diffusible DRG FT proteins are unique relative to the secretory products of non-neuronal nerve cells? and (2) Which diffusible DRG FT proteins are unique relative to other neuronal FT proteins? Neuronal or non-neuronal nerve proteins were pulse-labelled with ^{35}S-methionine and time allowed for FT (in the case of neuronal proteins). Radiolabelled diffusible proteins were collected in a bath surrounding nerve and bath/nerve proteins were subsequently analyzed by two-dimensional gel electrophoresis (2D-PAGE) and fluorography. Results showed that: (1) only one (10%) of the diffusible DRG FT proteins exhibited a possible identity with a diffusible non-neuronal sciatic nerve protein and (2) all of the diffusible DRG FT proteins were FT by retinal ganglion cell neurons. These results suggest that diverse neuronal populations may package a similar set of soluble FT proteins and that these diffusible products are quite different from the secretory products of non-neuronal nerve cells.

547.4

SYNAPTOPHYSIN AND SV2 ARE INCORPORATED INTO SYNAPTIC VESICLES VIA DIFFERENT ROUTES IN NGF-TREATED PC12 CELLS. J.-H. Tao-Cheng*. Lab. of Neurobiology, NINDS, NIH, Bethesda, MD 20892.

Transport and sorting of synaptic vesicle (SV)-associated proteins and divergence of SV and dense core granule (DCG) biogenesis have been explained by conflicting models. The present report characterizes the distribution of two such proteins, synaptophysin and SV2, by pre-embedding EM immunocytochemistry (ICC) in NGF-treated PC12 cells.

Two subcellular organelles had striking differences in ICC labeling: (1)DCG, the endocrine granules in PC12 cells, were intensely labeled for SV2 but only weakly for synaptophysin. Throughout the cell body and neurites, 60-80% of all DCG's were labeled for SV2, often with multiple grains of silver enhanced gold particles per DCG; while only 5-10% of DCG's were labeled for synaptophysin, and mostly with a single grain. (2) Pleomorphic, clear vesicles (~50-200 nm) in the shaft of the neurites were intensely labeled for synaptophysin, but minimally for SV2. These vesicles probably are in transit between the cell body and the terminals.

In contrast, in varicosities along the neurites and at neuritic terminals, two other organelles, (1) clusters of SV's and (2) irregular-shaped vesicles, most likely endosomes, were intensely labeled for both synaptophysin and SV2.

These results, combined with previous reports on SV and DCG biogenesis in PC12 cells, suggest that (a) the bulk of synaptophysin is transported through the neurites by way of pleomorphic vesicles, while the bulk of SV2 is transported by DCG's, (b) once they reach their functional destination, the neuritic terminals and the varicosities, both proteins are sorted into synaptic vesicles, probably through local vesicle membrane recycling and via endosomes.

547.5

DISTRIBUTION AND REGULATION OF LOW MOLECULAR WEIGHT GTP BINDING PROTEINS *rab*3A & *rab*3B IN THE RAT BRAIN. F. Nothias*, O. Stettler[1], J-D Vincent and Ph. Vernier. Inst. A. Fessard, CNRS, 91198 Gif-sur-Yvette & [1]INSERM U334, CEA-SHFJ, 91400 Orsay; France.

*Rab*3A and B belong to the ras-related superfamily of small GTP-binding proteins and are mainly present in nervous and endocrine tissues. These proteins appear to be involved in the control of a late step of exocytosis during which vesicules become docked to the plasma membrane. Physiological studies showed that these proteins may differentially regulate tonic and phasic exocytosic. This data prompted us to examine the brain distribution of mRNA encoding *rab*3A and B. *In situ* hybridization revealed that both mRNAs are heterogeneously distributed in the brain but overlap in most areas, although *rab*3B-mRNA is generally less abundant, except in the pituitary and olfactory bulb. This distribution was quantitatively ascertained by competitive PCR in the same tissues. In addition, double *in situ* hybridization showed that *rab*3A and B mRNAs are essentially colocalized within individual neurons.

During lactation, both *rab*3A and B mRNAs are upregulated in the magnocellular hypothalamic system in which increased stimulations are associated with the pulsatile release of oxytocin from the neurohypophysis. During lactation, an increase of rab3B mRNA was also observed in the anterior pituitary. Western blots confirmed these results and revealed that *rab*3A and B proteins exhibit different apparent molecular weights due to a post-translational modification.

547.6

RIBOSOMES BEARING SHORT GAP-43 NASCENT PEPTIDES BIND TO NUCLEAR AND MICROSOMAL SUBCELLULAR FRACTIONS. R. Moore-Batchelder, H. Pestana, and J. Denny*. Division of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249.

We have transcribed a linearized pGEM3 vector containing a full-length GAP-43 cDNA insert and have translated the purified, uncapped mRNA in vitro using reticulocyte lysate and 35S-methionine. A nuclear fraction isolated from cultured PC12 cells and a microsomal fraction isolated from either PC12 cells or from canine pancreas were added to translation mixtures both co-translationally and post-translationally at 30°C. The canine microsomes were specifically rough microsomes. In the co-translational experiment fractions were present during a 1 hour translation that was subsequently blocked either with cycloheximide or puromycin/high salt. In the post-translational experiment fractions were incubated with mixtures for 1 hour after the translations had been blocked with cycloheximide. In all cases there was an enrichment of GAP-43 nascent peptides of molecular weight 22 kDa and below in high-speed Airfuge pellet fractions relative to all larger nascent peptides and full-length GAP-43 and also relative to pellets obtained from translation mixtures that did not receive any subcellular fraction. These results suggest that short GAP-43 nascent peptides are competent for interaction with membranes while longer peptides are not.

547.7

ARCHITECTURAL EDITING OF GABA RECEPTOR α1 AND β3 SUBUNIT DETERMINE LOCALIZATION IN PC12 AND COS-7 CELLS. N.L. Peterson*, R. Salas and K.J. Angelides. Depts. Cell Biol. and Biochem., Baylor College of Med., Houston, TX 77030.

Several subunits and their isoforms have been identified encoding the γ-amino-butyric acid receptor (GABAR). Assembly of specific subunits leads to different pharmacological properties and cell surface localization. Despite the potential to assemble both homo- or heteromeric receptors in nonneuronal cells, there is emerging evidence that assembly and expression of GABAR subunits in neurons may be restricted. We have examined the sorting, cell surface expression, and localization of the α1 and β3 GABAR subunits in PC12 and cos-7 cells for clues of how receptor assembly of specific GABAR subunits affects localization and membrane mobility in neurons. PC12 cells transfected with cDNA encoding the α1 subunit retain the α1 subunit in an intracellular compartment whereas PC12 cells transfected with cDNA encoding the β3 subunit sort it to the plasma membrane and form clusters on both cell bodies and processes. After transfection of both α1 and β3 in PC12 cells, both proteins are found on the cell surface co-localized in clusters . In contrast to oocytes and epithelial cells, neuron-like cells express at their plasma membranes a more restricted repertoire of GABAR complexes. Moreover, we show that the localization of GABAR in discrete domains can be conferred by the β3 subunit. Cos-7 cells, used to study the intracellular localization at higher resolution, sort GABAR subunits similar to PC12 cells. The location of α1 in Cos-7 cells is consistent with an ER pattern suggesting that α1 may contain an ER retention signal. The β3 subunit, able to redirect α1 from the intracellular compartment to the cell surface, was not able to rescue a truncated α1 containing only the first two transmembrane domains. These results suggest that neurons likely have a mechanism by which specific complexes are assembled, requiring all the transmembrane domains, and architecturally edited before expression on the cell surface. Supported by 2F32NS09198-03 and NS28072.

547.8

SORTING AND CELL SURFACE EXPRESSION OF NMDA RECEPTOR SUBUNITS

Biserka Mulac-Jericevic, Lefkothea Karaviti* and Kimon J. Angelides. Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Neurons are highly polarized cells with a complex morphology, were proteins are targeted to discrete regions of the cell surface. At synaptic spines N-methyl-D-aspartate receptors (NMDAR) appear to be clustered at high density mediating the events of glutamate neurotransmission. Molecular cloning has identified several cDNAs encoding NMDAR subunits NR1, NR2A, NR2B, NR2C and NR2D. The NR1 subunit is essential in a heterologus configuration for electrophysiological and pharmacological characteristics of NMDAR. There is emerging evidence that the subunit composition of ligand-gated channels can determine sorting and targeting of the receptors. In order to study how neurons process NMDAR we have transfected COS-7 and neuron-like PC12 cells with cDNA encoding the NR1 subunit. We have used subunit specific antibodies, indirect immunofluorescence, and light and confocal microscopy to determine the cellular distribution of the NMDAR subunit in transfected cells. In COS-7 and PC12 cells NR1 is retained intracellularly when expressed alone. The majority of NR1 protein is sequestered in the Golgi compartment and a small fraction is found in lysosomal vesicles. When transfected cells were treated with Brefeldin A to dissociate the Golgi apparatus, NR1 was redistributed into the ER. The presence of NR1 beyond the ER in transfected COS-7 and neuron like PC12 cells strongly suggests that the protein is folded appropriately and has been processed into a transport-competent configuration. The NR1 subunit was not detected on the surface of these transfected cells by immunofluorescence or immunobloting of biotinylated surface proteins. These results raise the possibility, that the presence of more than one subunit is necessary for cell surface delivery of functional NMDAR and expression of the complex is regulated by availability of the other NMDAR subunits. Supported by grant NS28072 of HFSP.

547.9

EFFICIENT EXPRESSION OF FOREIGN PROTEINS IN A HUMAN NEURONAL TISSUE CULTURE SYSTEM. D.G. Cook*, R.S. Turner, V.M-Y Lee, & R.W. Doms. Dept Pathology, Univ. of Pennsylvania, Philadelphia, PA 19104

There is a great deal of interest in studying the cell biology of neurons. However, because neuronal cell lines have been difficult to obtain and because large cultures of purified primary neurons are difficult to prepare, many standard cell biological studies have not been feasible. Furthermore, it is often difficult to express foreign genes in neurons. In an effort to overcome these disadvantages, we have characterized vaccinia virus (VV) directed expression of foreign proteins in the human cell line NTera 2 (NT2). Using retinoic acid, NT2 cells differentiate to form large, highly purified (>99%) cultures of mature neurons (NT2-N). We found that NT2-N cells are readily infected by recombinant VV, yet they maintain their polarized morphology and die at a rate matching non-infected controls. To determine if foreign proteins can be expressed and properly processed in NT2-N cells they were infected with recombinant VV expressing the HIV-1 glycoprotein, gp160, under the control of a VV early/late promoter. Gp160 was easily detected 1 to 4 days post-infection by western blot, pulse-labeling, and immunofluorescence microscopy. Gp160 was transported to the Golgi apparatus, efficiently cleaved into gp120 and gp41 subunits, and expressed on the cell surface. Despite efficient expression the infection was non-productive (virus particles were not produced).

The ability of NT2-N neurons to efficiently express foreign proteins, with few of the cytophathic responses normally associated with VV infection, provides a novel opportunity to address a wide array of cell biological questions in a system of pure human neurons.

547.10

RNA SIGNALS AT THE CORTICAL SURFACE OF THE ISOLATED MAUTHNER CELL AXON. E. Koenig* and R. Martin. Dept. of Physiology, Univ. at Buffalo, Buffalo, NY 14214 and Electron Microscopy Section, Univ. of Ulm, Ulm, Germany.

Although RNA extracts derived from microscopic samples of isolated Mauthner (M) cell (C) axoplasm contain heavy and light cytoribosomal RNAs, comprising ~25% of the total RNA, ribosomes are not detected in randomly selected EM sections of this axon (Koenig (1979) *Brain Res.* 174:95). Either ribosomes may be in such low density that they are ordinarily overlooked, or they may be restricted in distribution to a particular region normally considered unlikely, such as the cortical domain near the plasma membrane. In order to investigate the latter possibility, we have examined the surface of isolated MC axoplasm, using (1) fluorescence microscopy to evaluate staining with YOYO-1, a fluorescent, cyanine nucleic acid-binding dye, and (2) electron spectroscopic imaging (ESI) to evaluate signals originating from polyphosphates characteristic of ribosomes. YOYO-1 staining of the MC axon shows a complex pattern of irregular strand-like axially oriented arrays on the surface containing strongly fluorescent ribonuclease-sensitive puncta. In preliminary ESI analysis, crazing thin sections of the cortical surface of isolated MC axons show areas exhibiting intense phosphorus signals characteristic of ribosomes, embedded in a homogenous "ribbon-like" structure. These areas, however, are encountered infrequently, which may reflect sampling problems at the ultrastructural level. Subaxolemmal ribosomes suggests a potential link between the actin-based cortical matrix and translational machinery. Supported in part by BNS 9010251 from NSF.

547.11

ENTEROPATHOGENIC *ESCHERCHIA COLI* DEPOLARIZES INFECTED HELA CELLS. H. Yan, D.A. Mathers*, M.A. Stein and B.B. Finlay. Departments of Physiology and Biotechnology, University of British Columbia, Vancouver, BC Canada V6T 1Z3.

Enteropathogenic *Escherchia coli* (EPEC) causes severe diarrhea in children. EPEC attach intimately to gut intestinal epithelial cells and mediate cytoskeletal rearrangements characterized by the formation of pedestal-like structures and localized degeneration of brush border microvilli. We have applied patch-clamp methodology to characterize the electrical events occurring during the early interactions of EPEC with epithelial cells.

HeLa epithelial cells were incubated with EPEC strain E2348/69 for 30 minutes at 37°C and washed extensively. Cells with attached EPEC colonies were used for whole-cell recordings, made at 21-24°C with patch electrodes containing (in mM): 135 KCl 5 NaCl 1 MgCl, 10 HEPES and 3 EGTA. Cells were bathed in a saline containing (in mM): 135 NaCl 4 KCl 1.8 CaCl, 1 MgCl, 10 HEPES and 5 glucose. The mean resting membrane potential (V_{REST}) of HeLa cells infected with EPEC was -24 mV. Cells incubated with supernatent from EPEC cultures, normal culture medium, or an EPEC mutant (*cfm*14-2-1) that does not mediate cytoskeletal rearrangements, showed mean V_{REST} values of about -48 mV. The results suggest that membrane depolarization is an important component of the pathogenicity of EPEC.

548.1

NADPH-DIAPHORASE HISTOCHEMISTRY OF RAT CHOROID PLEXUS. A. Chodobski*, J. Szmydynger-Chodobska, P.R. Monfils, A.Y.-J. Lin, M.P. Rahman, and C.E. Johanson. Program in Neurosurgery, Department of Clinical Neurosciences and Central Research Laboratory, Brown University/R.I. Hospital, Providence, RI 02903.

NADPH-diaphorase (NADPH-d) has been frequently colocalized with neuronal isoform of nitric oxide synthase (NOS), a synthetic enzyme for nitric oxide (NO), and is, therefore, considered as a histochemical marker for the latter enzyme. In the present study, we demonstrate the colocalization of NADPH-d-positive staining with NOS in rat choroid plexus (see accompanying abstract by J. Szmydynger-Chodobska et al.). Rats were perfused transcardially with 4% paraformaldehyde and brains were postfixed for 4 h at 4 °C. Parts of the choroidal tissue were dissected out and incubated at 37 °C for 3 h in PBS (pH 7.6) containing 0.1 mg/ml of nitro blue tetrazolium and 1 mg/kg of NADPH. Triton X-100 was added (0.6%) to improve tissue penetration. NADPH-d-positive nerve fibers were found to accompany anterior choroidal artery and its branches, as well as smaller arteries within the choroidal stroma. Choroidal venules and veins were devoid of NADPH-d staining; however, larger veins draining blood from the inferior part of the choroid plexus, proximal to the basal vein, had NADPH-d-positive innervation. Endothelial cells were stained as well. In addition, a deposition of the reaction product (formazan) was found in the cytosol of choroidal epithelial cells, with nuclei being free of staining. In conclusion, NADPH-d was shown to be colocalized with NOS in the choroid plexus. The presence of NADPH-d in different choroidal cell types suggests the multiple NO-mediated regulatory mechanisms in choroidal tissue. Supported by NIH Grant NS 27601.

548.2

IMMUNOHISTOCHEMICAL LOCALIZATION OF NITRIC OXIDE SYNTHASE IN RAT CHOROID PLEXUS. J. Szmydynger-Chodobska*, A. Chodobski, A.Y.-J. Lin, M.P. Rahman, B. Mayer, P.R. Monfils, and C.E. Johanson. Program Neurosurg., Dept. Clin. Neurosci. and Central Res. Lab., Brown Univ./R.I. Hospital, Providence, RI 02903, USA, and Inst. Pharmacol. Toxicol., Univ. Graz, A-8010 Graz, Austria.

We have previously shown that nitric oxide (NO) mediates some hemodynamic actions of angiotensin II (AII) in the choroid plexus. In the present study, we demonstrate by immunohistochemistry the presence of NO synthase (NOS), a synthetic enzyme for NO, in rat choroidal tissue. Anti-NOS antibody was raised in rabbits against purified porcine brain NOS. It recognizes neuronal NOS in several mammalian species, including the rat, and does not cross-react with inducible NOS. Rats were perfused transcardially with 4% paraformaldehyde and brains were postfixed for 4 h at 4 °C. Parts of the choroidal tissue were dissected out and incubated for 68 h at 4 °C with primary antibodies (1:2500). The procedures which followed were performed at room temperature. Biotinylated donkey anti-rabbit IgG was used to detect primary antibody, followed by incubation with streptavidin-biotin-peroxidase complex. To obtain color reaction, diaminobenzidine was used. NOS-positive nerve fibers were found to be associated with the anterior choroidal artery and its branches, as well as with smaller arteries located within the choroidal stroma. Choroidal veins and venules were devoid of NOS-containing innervation. Endothelial cells were not stained, indicating that the antibody does not cross-react with endothelial form of NOS. Choroidal epithelial cells expressed intense, distinctive staining of their cytosol. These results provide anatomical evidence supporting the physiological observations of AII interactions with NO synthesis in the choroid plexus. Supported by NIH Grant NS 27601 and by research funds from the R.I. Hospital.

548.3

OSMOTIC OPENING OF BLOOD-BRAIN BARRIER TO ENDOGENOUS ALBUMIN. A.W. Vorbrodt, D.H. Dobrogowska and A.S. Lossinsky*. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Early changes leading to the opening of the blood-brain barrier (BBB) to endogenous albumin were studied using a new quantitative immunocytochemical procedure. Hyperosmolar L(+)arabinose solution (1.8M) was infused into carotid arteries of rats which were killed 1, 5 and 30 min afterwards. Brain samples were immersion-fixed and embedded at low temperature in Lowicryl K4M. Ultrathin sections were exposed to anti-rat albumin antiserum followed by protein A-gold. The density of immunosignals (gold particles per μm^2) was recorded over four compartments: vascular lumen, endothelial cells (ECs), subendothelial space including basal lamina (BL), and the adjacent neuropil. The labelling density of the vessel lumen was considered to represent 100% of the circulating albumin. Morphometric and quantitative analysis indicated that in control animals, only 0.4-0.6% of the circulating albumin appears in the subendothelial space, whereas as soon as 1 min after infusion, this value increases to 3% followed by a further increase to 25% and 56% after 5 and 30 min respectively. The main routes of albumin escape are through the damaged ECs cytoplasm (including vesicles and penetrating indentations) rather than through modified interendothelial junctions. A slow increase of immunosignals in the adjacent neuropil suggests that the BL represents a serious obstacle for escaping albumin which seems to get trapped in this structure. (Supported by the National Institute on Aging, grant RO1-AG 10279-03).

548.4

EFFECTS OF HYPERTONIC SALINE INFUSION ON CEREBRAL PERFUSION IN NORMONATREMIC AND HYPONATREMIC RATS. S. Adler, D. Williams and J.G. Verbalis*. University of Pittsburgh School of Medicine and Carnegie Mellon University, Pittsburgh, PA 15213.

Rapid increases in plasma sodium (P_{Na}) and osmolality can disrupt the blood brain barrier (BBB). Recent studies from our laboratories have shown that this occurs at lower thresholds in hyponatremic (HN) than in normonatremic (NN) rats. The present experiments studied the effect of similar increases in P_{Na} on cerebral perfusion in HN and NN rats. NN (n=6), acute HN (n=8), and chronic HN (n=10) rats received iv infusions of either 700 mM or 500 mM NaCl. Both cortical and white matter perfusion were measured by arterial spin tagging in a 4.7 Tesla NMR magnet before and at 30, 60, and 90 min during the infusion. P_{Na} increased 22, 20, and 23 mmol/L, respectively, and arterial pressures remained constant throughout. Arterial pCO_2 rose slightly in all groups (from 61 to 63 in NN, 57 to 64 in acute HN, and 47 to 48 mm Hg in chronic HN). Cerebral perfusion increased significantly in both regions in each group, but the increases occurred more rapidly and were greater in the HN rats at all times. For example, by 60 min perfusion had increased in cortex and white matter by 36% and 27% in NN, by 62% and 56% in acute HN, and by 60% and 51% in chronic HN. Because local increases in brain perfusion can affect the BBB, the increased perfusion produced by hypertonic NaCl infusions may contribute to the BBB disruption caused by the induced hyperosmolality, and both of these factors may therefore participate in the demyelination that frequently accompanies overly rapid correction of hyponatremia.

548.5

GLYCATION INCREASES THE PERMEABILITY OF PROTEINS ACROSS THE BLOOD NERVE AND BLOOD BRAIN BARRIERS. Joseph F. Poduslo*, Geoffry L. Curran. Molecular Neurobiology Lab. Mayo Foundation, Rochester, MN 55905.

We have demonstrated increased permeability across the blood nerve barrier (BNB) of albumin after glycation with D-glucose (*PNAS* 89 (1992) 2218). The generality of this observation was evaluated by measuring the permeability coefficient-surface area product (PS) after correction for the residual plasma volume (V_p) across the BNB, as well as the blood brain barrier (BBB), for nerve growth factor (NGF) and human IgG after *in vitro* glycation with D-glucose, using an i.v. bolus injection technique in the cannulated brachial vein and artery of normal adult rats. Glycated proteins (gNGF and gIgG) had significantly decreased circulating plasma half-lives compared to the non-glycated proteins. The PS across the BNB obtained for gNGF was significantly increased compared to NGF with a 2.0 fold increase observed after 8 weeks of glycation and a 5.1 fold increase at 21 weeks of glycation. The V_p measurement for NGF and gNGF across the BNB was not significantly different at 8 weeks of glycation but was 1.3 fold greater at 21 weeks of glycation. The PS across the BBB for gNGF was 2 fold greater than NGF with a glycation time of 8 weeks and 3.2-3.6 fold greater with a glycation time of 21 weeks for six different brain regions. No changes were observed in the V_p for any of the brain regions for gNGF compared to NGF. The PS across the BNB for gIgG compared to IgG was significantly greater with a 4.1 fold relative increase. The PS across the BBB for gIgG ranged from a 2.8 fold increase for the thalamus to a 5.1 fold increase for the caudate putamen when compared to IgG. No significant differences were observed for the V_p values. These data demonstrate that glycation can enhance the permeability across the BNB and BBB of proteins with widely varying M_T and function. Since the glycation of NGF does not appear to affect its neurotrophic activity, systemic deliver of gNGF might be useful for treating a variety of neurodegenerative diseases. Similarly, the glycation of immunoglobulins might be a convenient procedure for delivery of a variety of antigens into the nervous system.NS14304

548.6

SELECTIVE OPENING OF THE BLOOD-TUMOR BARRIER: ROLE FOR RMP-7 IN CHEMOTHERAPY. T. Inamura, T. Nomura, P.J. Elliott*, R. T. Bartus & K.L. Black. Brain Res. Inst., UCLA Medical Center, Los Angeles, CA 90024 & Alkermes Inc., 64 Sidney St., Cambridge, MA 02139.

Current treatment of brain tumors is limited by the presence of cerebral capillary endothelial cells within the tumor tissue. Such cells have tight junctions which comprise the blood-tumor barrier. Enhanced drug delivery to tumors has been achieved previously by opening these junctions with hyperosmotic agents. However, such regimens have also allowed cytotoxic drugs access to healthy tissue. Recently, we have developed a bradykinin analog, RMP-7, which has been shown to selectively open tight junctions between cerebral endothelial cells.

In the current study, female Wistar rats (170-210g) with striatal RG2 tumors were given a labeled chemotherapeutic agent carboplatin, along with a 15min infusion (3ml/h) of RMP-7 (1ug/kg/min). Using quantitative autoradiography we found that RMP-7 produced a significant ($p<0.01$) two fold increase in carboplatin in the tumor, compared to vehicle-treated rats. No differences between RMP-7 and vehicle-treated groups were found in any other brain region examined. Similar data was obtained with other anti-tumor agents. Furthermore, treatment of other tumor-bearing rats with RMP-7 and chemotherapeutic drugs prolonged the survival time of such animals.

Thus, RMP-7 has the potential to be an adjunct to current neuro-oncology therapy due to (1) its selectivity for tumor cells over normal healthy tissue and (2) the probability that it may reduce the cytotoxic side-effect profile of anti-tumor drugs, by allowing lower therapeutic doses to maintain current (or greater) efficacy.

548.7

THE BRADYKININ AGONIST RMP-7 ENHANCES THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER: EVIDENCE FROM ELECTRON MICROSCOPY EXPERIMENTS. E. Sanovich*[1], P.M. Friden[2], R.T. Bartus[2] and M. Brightman[1]. Lab. of Neurobiology, NINDS, NIH, Bethesda, MD 20892[1] and Alkermes, Inc., 64 Sidney Street, Cambridge, MA 02139[2].

RMP-7, a novel bradykinin agonist, was tested for its ability to open the blood-brain barrier (BBB). Balb C mice were infused for 2 minutes via the jugular vein with La^{3+} (MW 139), in the form of $LaCl_3$ (5 mM), with or without RMP-7 (5 µg/kg). After 10 minutes, the mice were fixed with aldehydes and the brains processed for electron microscopy, so as to trace the pathway opened by RMP-7. Midbrain and cerebral cortex were examined for the presence of La^{3+} in tight junctions (TJ), basal lamina (BL) and perivascular spaces (PVS).

In control animals, La^{3+} did not penetrate the PVS and only occasionally labeled the BL. In contrast, the BL was labeled in more vessels and the PVS around many vessels were penetrated in the RMP-7 group. In some RMP-7 specimens, La^{3+} penetrated deep into the neuropil surrounding the vessel. These observations indicate that RMP-7 permeabilizes the BBB to La^{3+} by an intercellular route. Dextran (MW 3,000) appears to follow the same route. Is transcytosis involved as well?

Labeling of endocytic vesicles by La^{3+} was no greater in the RMP-7-treated mice; transcytosis, therefore, is not responsible for the movement of La^{3+} across the endothelium. In contrast, the number of TJ labeled and the depth of penetration through series of junctions were greater in RMP-7 mice than controls. Thus, RMP-7 may open the barrier by way of TJ. Whether the TJ is the exclusive pathway is being further examined.

548.8

ASSESSMENT OF BLOOD-BRAIN BARRIER CHOLINE TRANSPORT USING THE *IN SITU* BRAIN PERFUSION TECHNIQUE. DAVID D. ALLEN*. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Choline is an important constituent of membrane phospholipids and is a precursor to the neurotransmitter acetylcholine. Previous *in vivo* studies have found only a single low affinity transport system (K_m 200-500 µM) at the blood-brain barrier (BBB), whereas *in vitro* studies using isolated capillaries or cultured endothelial cells have found only a high affinity system (K_m 2-20 µM) or a mixture of the two. To address this discrepancy, the concentration dependence of unidirectional choline transport into brain was determined using the *in situ* rat brain perfusion technique (Takasato *et al*, 1984). Brains were perfused for 60 sec with physiological saline containing 0.0125 µM ^{3}H-choline and 0-20 mM unlabeled choline. At the end of perfusion, brains received a 15 sec wash with tracer-free fluid to remove extravascular isotope. Under these conditions, the concentration dependence of choline uptake was best described by a model with a single saturable system (V_{max} = 3.2 nmol/min/g, K_m = 39 µM) and a small nonsaturable uptake component (K_D = 9.9 x 10^{-5} ml/sec/g). Saturable choline uptake was hemicholinium sensitive and sodium dependent. In summary, the results suggest that saturable choline uptake into brain is mediated by a single, rather high affinity system with a K_m consistent with previous *in vitro* studies (K_m < 50 µM). As the K_m is on the same order as plasma choline concentration (5-20 µM), brain choline uptake may be subject to transport saturation and modulation under different physiologic conditions.

548.9

CLEARANCE OF ACIDIC AMINO ACIDS FROM CEREBROSPINAL FLUID, USING VENTRICULO-CISTERNAL PERFUSION IN THE ANAESTHETIZED RAT, A DEVELOPMENTAL STUDY.
H. Al-Sarraf, J.E. Preston*, M.B. Segal. Sherrington School of Physiology, UMDS, London SE1 7EH, UK.

The acidic amino acids, aspartate and glutamate, are excitatory neurotransmitters in CNS. The clearance of this group of amino acids from cerebrospinal fluid of adult and neonatal (7-day old) rats was investigated. Ventriculo-cisternal perfusions with ^{14}C-amino acids and ^{3}H-dextran were carried out for up to 90 min. Uptake of the amino acid by the whole brain was measured and the loss to blood was calculated (Davson *et al*, 1982).

	Lost (A)	Brain(B)	Blood (A-B)
Neonate	22.8±4.8	10.1±3.0	12.7±2.1
Adult	64.7±7.4	7.1±3.32	57.6±4.5

The percentage loss of ^{14}C-glutamate from the perfusion fluid during 90 min (A), Loss to brain (B), and to blood (A-B). Values are mean± S.E., (n=2-4).

Aspartate followed a similar pattern to glutamate, indicating a greater acidic amino acid efflux from CSF to blood in adult than in neonatal rats. However, there was no significant change in the loss to brain tissue with age ($p>0.05$).

548.10

DIFFERENTIAL EXPRESSION OF α-ACTIN mRNA AND IMMUNOREACTIVE PROTEIN IN BRAIN MICROVASCULAR PERICYTES AND SMOOTH MUSCLE CELLS. R. J. Boado* and W. M. Pardridge. Department of Medicine and Brain Research Institute, UCLA Schhol of Medicine, Los Angeles, CA 90024.

Hypertension has been linked to opening of the blood-brain barrier and may be related to the expression of the smooth muscle (sm) α-actin gene in contractile cells at the brain microvasculature. However, the cellular origin (i.e., endothelial cells, pericytes, sm cells) of the α-actin mRNA in the brain microvasculature is not clearly identified. Therefore, we investigated the abundance of actin mRNA by Northern blot analysis. The relative abundance of the α-actin 1.7 Kb transcript was: cultured pericytes>endothelial primaries (which contain both endothelial cells and pericytes) = freshly isolated microvessels. The α-actin mRNA was absent in a cloned bovine brain endothelial cell line. All samples showed the characteristic 2.1 Kb transcript corresponding to cytoplasmic β and γ isoform mRNA. The cellular distribution of the sm α-actin immunoreactive (IR) protein was studied by immunocytochemistry in cytospun/ isolated brain microvessels with a monoclonal antibody directed to the sm α-isoactin. This antibody reacted strongly with pre-capillary arterioles of microvessels, whereas no immunostaining was observed in either endothelial cells or in pericytes. Conclusion, the α-actin mRNA is expressed in cultured brain microvascular pericytes and in endothelial primaries, but the immunoreactive α-actin protein is not expressed in brain microvascular pericytes in vivo. These data suggest that either (a) the α-actin gene is induced in cultured brain pericytes, or (b) the α-actin mRNA in pericytes in vivo is subject to translational repression resulting in no detectable α-actin protein under normal physiologic conditions.

548.11

G_s AND G_i LEVELS ARE ALTERED BY AGING IN RAT BRAIN MICROVESSELS. P. Moore and P. Grammas*. Dept. of Pathology, Univ. of Oklahoma HSC, Oklahoma City, OK 73104

Guanine nucleotide-binding regulatory proteins (G proteins) play a central role in receptor-mediated signal transduction. These membrane proteins couple activated receptors to messenger enzymes, including adenylyl cyclase. In aging, altered response to agonists that elevate cAMP may reflect abnormal receptor-effector coupling. The objective of this study was to examine G protein expression in the cerebral microvasculature of aged rats. Microvessels were isolated from the cerebral cortices of young adult (1-3 month) and aged (>18 month) rats and membranes subjected to ADP-ribosylation. Cholera toxin and pertussis toxin were used to identify G_s and G_i respectively, and the two isoforms quantified by autoradiography of SDS-PAGE gels. The results indicated that the level of $G_{i\alpha}$ increased significantly ($P<0.01$) in microvessels from aged rats (65%) while the level of $G_{s\alpha}$ was lower (40%) in aged animals. These results demonstrate differential changes in G protein expression in aged animals and suggest that alterations in signal coupling may underlie some age-related alterations of the blood-brain barrier. (Supported by NS grant 30457, OCAST and the Glenn Foundation).

548.12

POSSIBLE DETERMINANTS OF VESSEL PHENOTYPES IN MUSCLE GRAFTED TO BRAIN. S. Naito, L. Chang and M. Brightman*. LN., NIH., Bethesda, MD. 20892.

The ability to determine a vessel's phenotype is decreased in adult tissue; some vessels in mature skeletal muscle grafted to mature choroid plexus are of the choroidal, fenestrated (FV) type rather than exclusively of the continuous (CV) muscle type. It is reported here that when the graft is from an E14 fetal rat, about 80% of the vessels are CV, like those of muscle and brain. Only a very few CV can be immunostained for endothelial barrier antigen and are, accordingly, brain derived. By E16, about 70% are FV, like those of host choroid plexus. Thus, between E14 and E16, a purported "conversion" factor that converts invading FV to CV, is diminished. To see whether the FV are extensions of choroidal blood vessels or converted graft vessels, fetal tissue was labeled with bromo - deoxyuridine. VEGF (vascular endothelial growth factor) may also be a vessel type determinant. It is known that VEGF is associated with FV and and that its mRNA is in the choroid plexus. By in situ hybridization, we find that mRNA to VEGF receptors is in choroid plexus but not in the graft. It is likely, therefore, that the tissue conversion factor, rather than VEGF, determines a vessel's phenotype during regeneration of blood vessels supplying muscle grafts.

548.13

ACIDIFICATION DECREASES $[CA]_i^{2+}$ IN RAT BRAIN CORTICAL ENDOTHELIAL CELLS D.Freyer, M.Weih, S.Weikert, J.G.Schulz, A.Villringer U.Dirnagl*, Dept. of Neurology, Humboldt University Berlin, FRG.

The brain extracellular and intracellular pH changes under various physiological (f.e. neuronal activation) and pathophysiological (f.e. ischemia) conditions. We investigated whether the extra- or intracellular pH affects $[Ca]_i^{2+}$ in rat brain cortical endothelial cells (DIV 0-14), which might link pH changes to $[Ca]_i^{2+}$ dependent mechanisms. For imaging of intracellular pH and $[Ca]_i^{2+}$ we used a confocal laser scanning microscope (Biorad MRC 600) and the fluorescent dyes BCECF-AM and FLUO-3-AM. Acidification of extracellular fluid (pH 7,4 to 6,8) led to a similar decrease of $[Ca]_i^{2+}$ but smaller decrease of pH_i than intracellular acidosis induced by the NH_4^+ prepulse technique . The results are summarized in the table:

means ± S.D. *= p < 0,05 cp. to control	FLUO-3 ΔF	BCECF ΔF
Extracellular acidosis	-25,3% ±10,3% *	-17,4 % ± 13,1% *
	n=37 cells, 2 preparations	n=18 cells, 2 preparations
Intracellular acidosis	-29,5% ± 11,5% *	-26 % ± 15,7% *
	n=13 cells, 1 preparation	n=20 cells, 3 preparations

We conclude that both extracellular and intracellular acidosis are capable of influencing intracellular free calcium in cortical endothelial cells and may thereby affect subsequent release of vasoactive substances like prostaglandines or nitric oxide. Supported by the DFG (Di 454-4/2).

548.14

EFFECT OF ENDOTHELIN ON ATPase ACTIVITY IN CEREBRAL CAPILLARY ENDOTHELIUM. N. Kawai, T. Yamamoto, H. Yamamoto, R.M. McCarron and M. Spatz*. Stroke Branch, NINDS, NIH, Bethesda, MD 20892.

Capillary ATPase activity has been thought to control the homeostasis of water-electrolytes in the brain. Endothelin-1 (ET-1) has recently been implicated in changes of blood-brain barrier (BBB) permeability. This reports describes receptor (ET_A)-mediated stimulation of ouabain-sensitive (OS) and ouabain-insensitive (OI) $^{86}Rb^+$ uptake (as a measure of Na^+-K^+-ATPase activity) by ET-1 and ET-3 in cultured rat brain capillary endothelial cells (BCEC). The uptake of $^{86}Rb^+$ (0.2 μCi/well) was determined in confluent BCEC (grown in 96-microwell plates) incubated in M199 with HEPES for 5 min at room temperature. A concentration-dependent increase of $^{86}Rb^+$ uptake induced by ET-1 or ET-3 [EC_{50} = 0.73 ± 0.17 (6) and 12.89 ± 3.69 (6), respectively] was inhibited by the ET_A receptor antagonist (BQ123) but not by the ET_B receptor antagonist (IRL 1038). Ouabain (ATPase inhibitor) and bumetanide (Na^+-K^+-Cl^- cotransport inhibitor) decreased ET-1-stimulated $^{86}Rb^+$ uptake into BCEC by 35% and 65%, respectively. Complete inhibition was seen with both of these agents. Similar results were observed with PMA, a PKC agonist. Ameloride [5-(N-ethyl-N-isopropyl)], inhibitor of the Na^+-H^+ antiporter, decreased both the OS and OI ATPase induced by ET-1, suggesting a linkage of the Na^+/H^+ exchanger with Na^+-K^+ ATPase and Na^+-K^+-Cl^- cotransport systems. The inhibition of ET-1-stimulated OS and OI $^{86}Rb^+$ uptake with staurosporin (PKC antagonist) indicates that ET-1-induced ATPase activity is mediated by PKC. These results suggest that the effect of ET-1 on capillary OS and OI Na^+-K^+-Cl^- systems may play a role in water-electrolyte disturbances in brain injury.

548.15

BLOOD-BRAIN BARRIER PERMEATION AND *IN VIVO* BRAIN RECEPTOR OCCUPANCY OF THE CALCIUM CHANNEL BLOCKER, SNX-111 (SYNTHETIC ω-CONOPEPTIDE MVIIA). K. Gohil, T.J. Abbruscato, Jr. T. Singh, D. Silva, G. Miljanich, R. Newcomb, L. Nadasdi, and T.P. Davis*, NEUREX Corporation, Menlo Park, CA 94025 and Dept. of Pharmacology, Univ. of Arizona, College of Medicine Tucson, AZ 85724.

The highly charged 25 amino acid peptide, SNX-111, is a potent blocker of N-type calcium channels and is neuroprotective in brain ischemia models when administered i.v. (e.g., ~1mg/kg). The blood-brain barrier (BBB) permeability of SNX-111 and its closely related Met^{12}->$norLeu^{12}$ analog, SNX-194, was assessed with three models: 1) Permeation through brain capillary endothelium using cultured brain microvessel endothelial cells (BMEC; Banks, et al., *Peptides* 13 (1992). 2) Amounts in rat brain *in vivo* using reversed phase HPLC after i.v. injection of ^{125}I-SNX-111, cardiac perfusion, and solid phase extraction. 3) Brain receptor site occupancy using receptor autoradiography in brain sections from naive rats or rats receiving transient four-vessel occlusion (4VO) following i.v. administration of SNX-111. Permeability of SNX-194 through the BMEC was 0.0007cm/min. *In vivo* brain amounts of SNX-111 were stable between 10 to 20 minutes after injection (0.0032 +/-0.0009% (cortex) and 0.0062 +/-0.0029% (midbrain) of injected peptide/g tissue). Brain amounts decay to <0.001%/g tissue 1-2hr after i.v. injection. Assuming a homogeneous distribution these data translate to a maximal brain concentration of 30-50nM after i.v. injection of 10mg/kg. The *in vitro* and *in vivo* data are consistent with the expected BBB permeation for a peptide the size of SNX-111. In brain sections, the most pronounced inhibition of binding of ^{125}I-SNX-111 by the injected SNX-111 was in hypothalamus, septum, medial striatum, and ventral hippocampus in 4VO-treated brains. The occupancy of receptor sites after 4VO may be related to the neuroprotective efficacy of SNX-111. In conclusion, despite low BBB permeability, SNX-111 is neuroprotective at moderate i.v. doses due to its high affinity for N-type calcium channels.

548.16

IN VITRO AND *IN VIVO* BLOOD-BRAIN BARRIER PERMEABILITY OF MU SELECTIVE, OPIOID ANTAGONIST CTAP AND AGONISTS DPDPE AND BIPHALIN: EVIDENCE FOR IMPROVED PERMEABILITY BY HALOGENATION. T.J. Abbruscato, S.J. Weber*, V.J. Hruby, and T.P. Davis, Departments of Pharmacology and Chemistry, University of Arizona, Tucson, AZ 85724, OREAD Laboratories, Inc., Lawrence, KS 66047

The enhancing effect of halogenation on blood-brain barrier (BBB) permeability was investigated for three receptor selective, opioid peptide ligands: CTAP (D-F-C-Y-D-W-R-T-Pen-T), DPDPE (Y-D-Pen-G-F-D-Pen), and biphalin ((Y-D-A-G-F-$NH)_2$). A significantly ($P<0.01$) greater percent of the halogenated peptide compounds reached the perfused mouse brain after 30, 60, 120min by both I.P. and I.V. administration when compared to non-halogenated parent. *In vitro* bovine brain microvessel endothelial cells (BMEC) were used to model the BBB. Permeability coefficients were calculated from passage across the BMEC confluent monolayers. A positive correlation was apparent between *in vitro* BMEC and *in vivo* distribution for halogenated CTAP and DPDPE. Nearly a two fold increase in the permeability coefficient was observed for the halogenated analogues of CTAP, DPDPE, and biphalin. (see data below).

Peptide Analogue	Permeability Coeff.	I.P. % Inj	I.V.% Inj
CTAP	4.55 ± 2.6	0.023	-----
[p-Cl Phe] CTAP	7.60 ± 2.0	0.036	-----
DPDPE	49.24 ± 2.18	------	0.064
[p-Cl Phe4] DPDPE	82.76 ± 3.46	------	0.178
Biphalin	55.00 ± 4.98	------	0.089
[p-Cl Phe4.4'] Biphalin	92.00 ± 5.88	------	------

These data show that halogenation leads to dramatic improvement in BBB penetration of three structurally distinct opioid ligands. Supported by NIDA #DA-06284

548.17

THE UPTAKE OF DOPAMINE BY THE CAUDATE NUCLEUS OF PERFUSED GUINEA PIG BRAIN. C.L. Martel*, J.B. Mackic, L.K. Klaidman, M.H. Weiss, J.G.McComb, J.D. Adams and B.V. Zlokovic. Depts. Neurol. Surg. and Pharmacol. and Divn. Neurosurg. CHLA, USC Sch. Med., Los Angeles, CA 90033.

Our studies in the guinea pig, Cavia porcellus, have demonstrated that, contrary to the conclusions drawn from studies in rats, [^{3}H]-dopamine ([^{3}H]-DA) does enter the brain by a specific, saturable uptake mechanism which is independent of the enzyme monoamine oxidase-B (MAO-B) and is inhibited by several D_1 and D_2 DA-receptor antagonists. In order to determine the molecular form of DA uptake, a high pressure liquid chromatography analysis was performed according to the method of Kalivas [J. Pharm. Exp. Ther. (1985) 235: 544-550] on the brain tissue of control guinea pigs and guinea pigs perfused for ten minutes with 10 μM DA. The levels of DA and its two main metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were quantitated in several different brain regions. In the caudate nucleus of the DA-perfused animals, there was an increase in the DA concentration of more than 75% from control levels, but there was no change in the levels of HVA or DOPAC. In non-striatal brain tissue, there was no significant change in the concentrations of DA or either of its main metabolites. This suggests that DA does indeed cross the blood-brain barrier intact, with minimal metabolic breakdown, but in a regionally selective manner. (Supported by TRDRP grant 2RT0071).

548.18

THYROID HORMONES EXPRESS HIGH AFFINITY FOR BOTH THE THYROID HORMONE AND LARGE NEUTRAL AMINO ACID TRANSPORTERS OF THE BLOOD-BRAIN BARRIER. M. Hokari and Q.R. Smith*. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda MD 20892.

Thyroid hormones are taken up into brain by a saturable transporter at the blood-brain barrier (BBB). This transporter has been suggested to be distinct from that for large neutral amino acids (System L), even though both sets of compounds show marked structural similarities. To examine this further, the kinetics of thyroid hormone and amino acid uptake into brain were examined simultaneously using the in situ rat brain perfusion technique (Takasato et al., 1984). Brains were perfused for 10-30 s using physiologic saline containing L-[^{125}I]T_3, L-[^{14}C]leucine and 0-1 mM cold compound. Permeability-area products were calculated from the quantity of tracer accumulated within the nervous system by the end of the perfusion. The results demonstrated that T_3 is taken up into brain with high affinity (V_{max} = 0.16 ± 0.02 nmol/min/g, K_m = 0.26 ± 0.03 μM) by a saturable mechanism that is sensitive to triiodothyroacetic acid (10 μM) but not to BCH (1 mM) - the defining substrate of the L System. T_3 was also a high affinity inhibitor of the BBB large neutral amino acid transporter (K_i = 1.0 ± 0.1 μM). At 30 μM, T_3 inhibited [^{14}C]leucine uptake by >95%. The results indicate that [^{125}I]T_3 binds with high affinity to both the thyroid hormone and large neutral amino acid transport systems and may be of use in identifying and labeling the carrier proteins.

548.19

VIRAL INDUCED DEMYELINATION: A ROLE FOR THE BLOOD-BRAIN BARRIER?

Egleton R.D, Dawson J, Butt A.M. Amor S+ & Segal M.B*
Physiology and +Immunology, UMDS, London SE1 7EH, U.K.

In multiple sclerosis (MS), dysfunction of the blood-brain barrier (BBB) has been observed, and may be major factor in this disease. Using the Semliki Forest virus (SFV) model of MS in the Balb-C mouse, we have measured the permeability of the BBB to ^{14}C mannitol during the course of the infection stage, in controls and following treatment with cimetidine, a histamine H_2 antagonist.

SFV animals were perfused at 3, 5 and 10 days post inoculation (PID) with a Ringer/albumin solution containing ^{14}C mannitol and the unidirectional transfer coefficient (Kin) for mannitol calculated

	SFV PID 5	SFV + Cimetidine
Brain	+ 74.20 %	+ 53.50 %
Spinal cord	+ 40.84 %	+ 1.35 % *

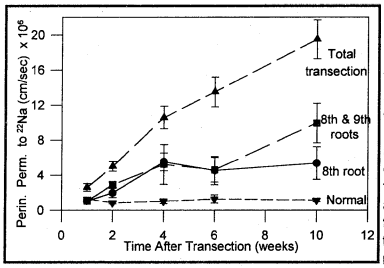
Table 1 The % increase of mannitol permeability across the BBB for PID5 and cimetidine treated animals compared to control (*$p < 0.001$, Students t-test compared to PID5).

The histamine H_2 antagonist cimetidine significantly reduced the permeability changes at PID 5. The opening of the BBB as indicated by the increase in the Kin for mannitol corresponds to the viremia and accumulation of virus in the brain as seen by others.

548.20

ENDONEURIAL REGULATION OF PERINEURIAL PERMEABILITY: EVIDENCE FROM PARTIAL NERVE TRANSECTION STUDIES. [1]J. R. Steiner, [1]A. Weerasuriya and [2]M. E. Michel*. [1]Division of Basic Sciences, Mercer Univ. School of Medicine, Macon, GA 31207, and [2]NINDS, NIH, Bethesda, MD 20892.

The monofascicular mid-thigh portion of the frog sciatic nerve has major contributions from the 7th, 8th and 9th spinal roots. Comparisons of compound action potentials reveal that each of these roots contributes about 30% of the myelinated fibers in the sciatic nerve. The effect of transecting either the 8th, or 8th and 9th, or all three roots on perineurial permeability was examined using an *in vitro* technique (*Brain Research*, 173, 503-511, 1979); the index of permeability was ^{22}Na. The results are graphed as mean ± S.E. (n = 5 or 6). The initial increase in permeability is probably associated with the clearing of myelin debris from the endoneurium, and the later increase to the absence of fibers in the underlying endoneurium. It is difficult to reconcile these data with the notion that the permeability of the blood-nerve interface is altered in an all or none fashion with an increase in its permeability being considered as a breakdown of its barrier properties. The data are more consistent with the prospect that perineurial permeability is regulated in a graded manner by endoneurial components, which release factors that affect the rate of turnover of perineurial tight junctions (NIH RO1 NS30197).



PRESYNAPTIC MECHANISMS IV

549.1

ARE LONG LATENCY RECURRENT EPSPS RECORDED IN THE IMMATURE HIPPOCAMPUS MONOSYNAPTIC EVENTS? K. Smith and J.W. Swann*. The Cain Foundation Laboratories, Department of Pediatrics, Division of Neuroscience, Baylor College of Medicine, Houston, TX, 77030.

The immature hippocampus has a great propensity for generating electrographic seizures. One possible explanation could be that there is an age dependent difference in the recurrent excitatory network. Our laboratory, has been investigating the local network through paired intracellular recording from immature CA_3 neurons. Minislices of CA_3 from immature (9 - 14 days old) rats were bathed in 1.7 mM penicillin to block inhibition. In 152 neuronal pairs (304 cells) 8% revealed monosynaptic interactions. The onset of the epsps ranged from 0.7 - 3.3 msec. Many of our epsps had latencies longer than 2 msec. However, when compared with polysynaptic epsps; they did not fluctuate in latency, their durations were short, they followed one for one at high presynaptic spike frequencies, and the probability of transmission was high. We therefore attempted to determine why some monosynaptic epsps had long latencies. We hypothesized that the long latencies were due to slow conduction velocities in CA_3 recurrent axons. To determine this we investigated the conduction velocity of the Schaffer collateral fibers by extracellular recordings of the presynaptic fiber volley. The experiments were conducted at 33° C in the presents of CNQX and APV. In 6 experiments conduction velocities of 0.14 - 0.33 m/sec were recorded. Based on a mean value of 0.20 m/sec, an action potential would be expected to travel 500 μm in 2.5 msec. Therefore, our results suggest the longer latency epsps are likely to be monosynaptic events and the delays are due to slow conduction velocities in the developing recurrent axons of the hippocampus. (Supported by NIH Grant NS18309).

549.2

Presynaptic influences on the decay of excitatory synaptic currents. **S. Mennerick* and C.F. Zorumski. Dept. of Psychiatry and Program in Neuroscience, Washington Univ. Med. Sch., St. Louis, MO 63110.**

Since synaptic glutamate (Glu) transients are very brief, the decay of excitatory synaptic currents (EPSCs) is thought to represent the average elementary lifetime of a receptor channel bound only once by transmitter molecules. Our results suggest that in hippocampal microcultures under conditions of enhanced transmitter release, decays of evoked non-NMDA EPSCs can reflect prolonged Glu actions. In neurons treated with cyclothiazide (CYZ) to enhance non-NMDA receptor sensitivity, inhibition of Glu uptake with DL-Threo-3-hydroxyaspartate (THA, 30 μM) differentially prolonged currents of large quantal content. The optimal EPSC decay time constant was increased in THA by 12.4 vs 2.7 msec in bath solutions containing 3 mM vs. 0.75 mM Ca^{++}. THA had no effect upon CYZ-treated miniature EPSC decays in cells where evoked responses were prolonged by THA. These results suggest that uptake plays a greater role in clearing Glu after release of many quanta, presumably because of higher or prolonged Glu concentrations. CYZ-treated EPSCs became faster with decreased release even in the absence of THA: (decay = 51.9 msec and 26.2 msec in 3 mm and 0.75 mM Ca^{++}). In cells untreated with CYZ, a subset of slowly decaying EPSCs were preferentially prolonged by THA. Depressing release speeded EPSCs and reduced the THA effect upon slow EPSCs. Comparison of presynaptic versus postsynaptic inhibition of EPSCs indicated that the speeding of EPSCs with reduced release was not due to differences in the changing current amplitude with high and low release. Therefore, diffusion, uptake, and receptor desensitization are insufficiently rapid to limit the decays of evoked EPSCs to those of miniature EPSCs. The results are consistent with studies suggesting that multiple quanta can interact with overlapping or closely spaced postsynaptic receptor domains.

549.3

DIFFERENTIAL REGULATION OF PRESYNAPTIC PLASTICITY BY αCaMKII IN THE CA1 REGION OF HIPPOCAMPAL SLICES. B.G. Frenguelli*, A. Smith, A.J. Silva & P.F. Chapman† Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and †University of Minnesota, Minneapolis, MN 55455.

Paired-pulse facilitation (PPF) and post-tetanic potentiation (PTP) are presynaptic forms of short-term plasticity. We have studied PPF and PTP in mice heterozygous for the α isoform of CaMKII (αCaMKII). These mice, in contrast to homozygous α CaMKII mutants, show no deficiency in long-term potentiation (LTP), the induction of which is postsynaptic in CA1 neurons. Extracellular and whole-cell recordings of PPF and PTP were made from the CA1 region of hippocampal slices, under standard *in vitro* conditions, taken from wild-type mice (WT) and heterozygous mice (HET). Extracellular field recordings revealed a striking deficit in the extent of PPF across 50-250 ms interpulse intervals (IPI) in HET (eg at 100 ms IPI, WT, 158 ± 7%, n=13 slices; HET, 134 ± 2%, n=22, $p<0.001$) even under conditions of GABA receptor blockade. Whole-cell (-60 mV) recordings in the absence of GABA inhibition confirmed these observations (eg at 100 ms IPI, WT, 185 ± 11% n=5 cells; HET, 130 ± 10% n=7, $p<0.05$). PTP was elicited, in the presence of AP5, by theta burst stimulation (TBS) consisting of two 4 shock, 100 Hz bursts separated by 200ms. To sample PTP, 15 shocks were given at 1 Hz commencing 1 s after the last burst. Surprisingly, TBS elicited PTP which was significantly *greater* in HET than in WT in both field (WT, 121 ± 2% n=9; HET, 140 ± 5% n=10, $p<0.01$) and whole-cell (WT, 102 ± 2% n=4; HET, 122 ± 3% n=5, $p<0.05$) recordings. These observations reflect a specific impairment of synaptic function since other aspects appear normal. For example, the effects of reductions in extracellular Ca^{2+} were identical in WT and HET slices, as was the ratio of fEPSP slope to fiber volley amplitude. Thus, α CaMKII can modulate synaptic transmission in a manner dependent upon the immediate history of the terminal.

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549.4

What accounts for the variance of miniature synaptic current amplitude? M. Frerking, S. Borges, and M. Wilson*. Section of Neurobiology, Physiology, and Behavior, University of California, Davis CA 95616.

Isolated amacrine cells from embryonic chick retina form autapses in culture. In the presence of TTX, TEA, and internal Cs^+, these autapses are capable of Ca^{2+} dependent evoked and spontaneous GABA release. Spontaneous, discrete $GABA_A$ currents (minis) represent, on average, the opening of less than 20 channels. As in other cell types, the mini amplitude distribution is positively skewed; cable filtering is unlikely to account for this skew, since these neurons are electrically compact. To determine how much of the observed variance in the mini distribution is due to the summed properties of individual release sites, we have examined regions of the cell in isolation.

Minis were restricted to a small region of an isolated neuron by focal superfusion of a Ca^{2+} containing saline. Multiple Gaussian peaks are evident in distributions of focally restricted minis in all cells examined, with little variance unaccounted for by uncorrelated noise, and we estimate on the basis of this low variance that the probability of channel opening is between 50 and 100%, implying a small number of receptors per release site. When compared to whole cell mini distributions, focally restricted minis show a reduced variance, mainly due to the absence of large minis; however, both distributions have a similar mode. We calculate that the frequency of mini release is too low to support the possibility that the large events seen in the whole cell distribution are random coincidences. We suggest on this basis that some of the variance seen in the whole cell distribution is not due to the sum of independent events at different release sites. Supported by EY04112 (MW) and an NSF predoctoral fellowship (MF).

549.5

THE EFFECT OF ELECTROTONIC STRUCTURE ON QUANTAL AMPLITUDE AT CENTRAL SYNAPSES. A.A.V. Hill*, G.W. Davis, C. Bigelow, R.K. Murphey. Neuroscience and behavior program, Univ. of Massachusetts at Amherst, MA 01003.

We examined a distributed central synapse in the cricket cercal sensory system. Dual, simultaneous intracellular recordings were made from the lateral and medial dendrites of the cricket interneuron, MGI, while stimulating an identified sensory neuron (SN), 3c. This SN makes synaptic contacts at two electrotonically separated sites, the lateral and medial dendrites of MGI. Thus, an electrode in the lateral dendrite can detect EPSPs that originate from the nearby site and EPSPs that originated from a distant site --the medial dendrite. Therefore, the amplitude histogram contains the true quantal amplitude, *q*, of nearby synapses and the smaller apparent quantal amplitude of the distant synapses due to the electrotonic decay of potential. Isolation of EPSPs originating solely from each group of release sites was achieved by comparing EPSP amplitudes recorded simultaneously. If the amplitude of an EPSP was much greater in one dendrite than in the other dendrite then the EPSP was assumed to originate from that dendrite. The *q* of EPSPs that arose near the electrode in either dendrite were of the similar amplitude. Since the input resistances of these two dendrites are comparable, this suggests that the underlying quantal synaptic conductances are very similar. This result shows that the *q* is uniform for the terminals of a single SN.

We performed compartmental simulations of the distributed synaptic input to MGI. The simulations showed that our method of isolating EPSPs that originated solely on one dendrite was reliable. The simulations also showed that the evoked amplitude histogram, seen from a single electrode, in one dendrite is greatly affected by the presence of synapses located on the other dendrite. We were unable to fit the resulting simulated amplitude histograms with a simple binomial model of release. We suggest that a statistical model, the sum of two binomials, that takes into account the electrotonic structure of the postsynaptic neuron is required.

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549.6

TARGET REGULATION OF THE PROBABILITY OF PRESYNAPTIC RELEASE IN THE CRICKET CNS. G.W. Davis* and C. Bigelow and R.K. Murphey. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Our results demonstrate that a single SN is capable of making synapses with different probabilities of release at different target interneurons, MGI and 10-3. A single identified SN was stimulated and at least 500 EPSPs were recorded simultaneously from MGI and 10-3. The simple binomial was fit to each amplitude distribution using the Expectation-Maximization algorithm with four free parameters; the binomial parameters *n* and *p*, the quantal size, *q*, and the variance of the background noise. Goodness of fit was assessed using both chi squared and Kolmogorov-Smirnov analysis. The contacts to MGI had a lower probability of release, p, compared to the terminals from the same SN that contacted 10-3. The difference in *p* at the terminals of a single SN contacting MGI and 10-3 appears to determine the dynamic properties of these synapses. SN terminals contacting MGI showed paired-pulse facilitation while terminals contacting 10-3 showed paired-pulse depression. The majority of the change in EPSP amplitude during paired-pulse facilitation and depression can be accounted for by changes in *p*. Furthermore, when transmitter release was reduced in low calcium, high magnesium saline the probability of release at these synapses was reduced and synaptic depression was abolished. The difference in the probability of release and dynamic properties for synapses contacting MGI and 10-3 was consistent for 5 identified SNs that contacted either interneuron alone (N=13). These results indicate that the probability of presynaptic transmitter release is regulated locally, at the synapse by an interaction with the postsynaptic cell. We further hypothesize that such a local regulation of *p* is responsible for determining the characteristic short term dynamic release properties of these central synapses during development. Supported by NSF grant #BNS 90-96180.

549.7

Comparison of Quantal Analysis Methods at Crayfish, *Drosophila* and Rat Hippocampal Synapses: Measurements of Charge, Amplitude and Direct Counts of Events. B.A. Stewart*, R.L. Cooper, J.M. Wojtowicz, S. Wang and H.L. Atwood. Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A8.

In crayfish neuromuscular synapses low transmitter output permits direct counting of quanta released by stimulation. This is often the preferred method of estimating the quantal content "*m*". We have compared this method with two alternative methods: (1) measurements of the peak amplitude and (2) measurements of current area (or charge). These two methods have been used in the past in other preparations but not compared directly. To verify their accuracy we compared all three methods on data sets from the crayfish neuromuscular junction and found consistently that the areas but not the peak amplitudes give accurate results. In the crayfish and larval *Drosophila* preparations extracellular focal macropatch recordings over visible neuromuscular varicosities were used, whereas in the rat, whole cell clamp recordings were used. The deficiency of the amplitude method is most acute when quantal release is not synchronous as in crayfish synapses. In the rat dentate gyrus synapses the area method gave substantially higher estimates of "*m*" since it included the late, asynchronous release. Another advantage of the area method is a reduced noise level and partial compensation for spatial decrement along dendrites. Finally, the area and peak amplitude methods were compared at the *Drosophila* synapses and excellent agreement between the two was found. These synapses have high output and appear to release transmitter synchronously. We conclude that in general the area method offers an adequate substitute for the direct estimate of quantal content. Funded by NSERC, MRC and NCE of Canada.

549.8

PRESYNAPTIC GABAERGIC CONTROL OF EXCITATORY AXON ACTIVITY REVEALED BY BLOCK OF GAD. H. Golan* and Y. Grossman. Department of Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel.

Block of glutamate decarboxylase (GAD) as well as GABA receptor (GABA R) have epileptogenic effect. In order to study the effects of reducing GABA levels in the inhibitory terminal, inhibitory and excitatory postsynaptic potentials (IPSPs, EPSPs) were recorded intracellularly from fibers in the opener muscle of crayfish walking leg. The corresponding synaptic currents, (IPSCs, EPSCs) were recorded by loose patch clamp technique. 90 min exposure to mercaptopropionic acid or aminooxyacetic acid, GAD blockers in this system, reduced IPSP amplitude by 52% (n=6) and conductance by 63.3%. The maximal inhibition of EPSP amplitude decreased by 40% (n=10). Quantal analysis of the IPSCs revealed a decline in the quantal content due to 27% (n=5) reduction in the probability of release at each active zone. Quantal current was not affected. Presynaptic inhibition of EPSCs was reduced by 62%. Reduction in presynaptic inhibitory tonus induced by perfusion with GAD or GABA R blockers (90 min) generated hyperactivity of the excitatory terminal manifested in one of the following responses: doubled EPSC amplitude, increase of potentiation, or spontaneous activity. Our data show that blockage of GABA synthesis reduces pre- and postsynaptic GABA release and inhibition. Lack of tonic GABA input on the excitatory terminal increases its excitability and release. Supported by grant from the Israel Planning and Grants Committee,.

549.9

WHOLE CELL RECORDINGS OF EPSPS IN RAT TRIGEMINAL MOTONEURONES IN-VITRO. J.C.Curtis & K.Appenteng & M-Y Min (SPON: Brain Research Association) Dept. of Physiology, Univ. of Leeds, Leeds, UK.

We have used the whole cell patch recording method to record synaptic activity in rat trigeminal motoneurones in tissue slices (500μm thickness) from animals aged 8 days. Electrodes were filled with a potassium gluconate solution and data only accepted from motoneurones when the seal resistance was at least 2 GΩ, access resistance < 50MΩ and membrane potential at least -50 mV. There was a high incidence of spontaneous activity in the motoneurones. The mean interval between single spontaneous EPSPs in normal ACSF was 100 ms, but this increased to 350 ms in slices bathed in TTX (0.6 μM). EPSP activity obtained in the presence of TTX was completely abolished by bath application of CNQX (10 μM). Amplitude distributions of single spontaneously occurring EPSPs recorded in either normal ACSF or in the presence of TTX were skewed and peaky. The mean quantal amplitudes in the different cells ranged from 210 to 405 μV. Averaged EPSPs elicited by single presynaptic neurones in single motoneurones ranged from 33 - 76 μV. Examination of individual sweeps comprising the averages revealed that the small averaged EPSPs were due to a high incidence of failures of transmission. The above data suggests that transmission at excitatory synapses at trigeminal motoneurones may be quantal in nature, that the quantal amplitude as determined at the soma may be relatively uniform for glutaminergic inputs onto the motoneurones, and that alterations in the probability of transmitter release are likely to provide a powerful means of modulating transmission at these synapses.

549.10

SPONTANEOUS SYNAPTIC CURRENTS IN VISUALIZED RAT HIPPOCAMPAL NEURONS. Z. Xiang* and T. H. Brown. Depts. of Psychol. and Cell.& Mol. Physiol. Yale Univ., New Haven, CT. 06520.

This laboratory has long been interested in the structure and physiology of the mossy-fiber (mf) system of the hippocampus (Brown et al, 1979; Brown and Johnston, 1983; Johnston and Brown, 1983; Barrioneurovo et al, 1986; Griffith et al, 1986; Claiborne et al., 1993; Yu and Brown, 1994). Our recent analysis of the quantal mechanism of mf LTP expression supports the hypothesis of a presynaptic process that increases the mean number of released quanta (Xiang et al, 1994).

Using sharp electrodes, this lab had previously examined spontaneous miniature synaptic potentials and currents in CA3 pyramidal neurons, but the signal-to-noise-ratio was poor. Here we have begun to re-examine spontaneous synaptic activity in CA3 neurons using whole-cell recordings. The cells were directly visualized using infrared (IR) differential interference contrast (DIC) video microscopy, switching from our previous (fixed-stage) inverted configuration (Keenan et al, 1988) to an upright one (40X water-immersion objective).

The recordings were restricted to cells judged to be healthy based on their contrast under video microscopy. We restricted the analysis to events judged to be electrotonically near to recording site based on 10-90 rise time (<1.5 msec). With the soma clamped at about -80, most of the spontaneous events were in the range of 10 - 50 pA, although occasionally we saw very large spontaneous events (>200 pA), even in the presence of 1 μM tetrodotoxin, which should block evoked release. We are currently trying to characterize the quanta specifically associated with the mf synapses. Supported by NSF, NIH & ONR

549.11

COMPUTATION OF LONG-DISTANCE PROPAGATION OF POISSON PROCESS-ELICITED IMPULSES. K. Moradmand and M.D. Goldfinger*. Dept. of Physiology & Biophysics, Wright State University, Dayton, OH 45401-0927.

The Hodgkin-Huxley/Cable theory formalism was used to compute propagating impulses in a 100.μm-diameter axon (λ=3245.μm) as previously described (1). Computation parameters were: Δx=405.63 μm; ($\Delta x/\lambda$)=0.125; T=10°C. Total axon length was 8.1 cm (25λ) or 101.4 cm (312.5λ). The temporal integration step (=1.0 μsec) provided a single-spike point-to-point conduction velocity averaging 6.61 m/sec (with σ=0.053 m/sec) except near the sealed ends. The Poisson stimulation train of 0.2 msec-duration, constant-amplitude pulses had a mean rate of 10.6 msec (σ=9.6 msec; coeff.var.=0.91), single-exponential Interevent Distribution (IID) falling limb, and an Expectation Density (ED) consisting of a maintained noisy (+/- 1σ) plateau. Poisson stimulation yielded variability in elicited action potential amplitudes. For short interspike intervals (5.2-8. msec), the second-spike peak was attenuated by as much as 37.mV; for 10-15 msec intervals, second-impulse amplitude was slightly (~1.mV) increased. Impulse amplitude did not change for larger intervals. Using paired stimuli, short interspike intervals (5-11 msec) increased in duration and longer interspike intervals (12-16 msec) decreased in duration with propagation distance.

With Poisson stimulation, in the 25λ cable, the IID consisted of a deadtime, short-interval peak, and monotonic falling limb. The ED consisted of a deadtime, short-interval peak, and noisy-maintained plateau. However, both IID and ED varied with propagation distance: longer propagation distance shifted IID and ED modes to a larger time range, as expected by the slower and variable conduction velocity of the second of a short-interval impulse pair. Over a larger conduction distance (306λ, ~1m), the IID included a major symmetrical peak, and the ED developed harmonics. These computations confirmed (2) that wide-band impulse codes can change with propagation distance. Ref's.: (1) M.D. Goldfinger, Biophys. J., 50:27, 1986; (2) S.A. George, Biol. Cybern. 26:209, 1977. Supported by NSF BCS-9315856(MDG).

549.12

VISUALIZATION OF NERVE TERMINAL STAINING WITH FM1-43 TRIGGERED BY BLACK WIDOW SPIDER VENOM (BWSV).
A.W. Henkel and W. Betz.* Dept. of Physiology
University of Colorado Medical School, Denver, CO 80262

Frog motor nerve terminals can be stained with the fluorescent styryl dye FM1-43 in an activity dependent fashion. The dye, evidently, is taken up into vesicles while they undergo cycles of exo- and endocytosis in response to nerve stimulation. BWSV is well known to trigger massive exocytosis at synapses. This action does not depend on free extracellular calcium ions. However, calcium is required for subsequent endocytosis.

Here we show that frog nerve terminals take up FM1-43 when they are exposed to BWSV in the presence of calcium. Frog nerve muscle preparations were pre-incubated in normal frog Ringer containing BWSV (0.3 gland/ml) until the muscles started to twitch (about 30 minutes). FM1-43 (2 μM) was added for five additional minutes. The muscles were then washed for 1 hour. The FM1-43 staining pattern was indistinguishable from control preparations stimulated via the nerve without BWSV. When the same experiment was performed in the absence of extracellular calcium only a minute quantity of dye was taken up into the nerve terminals and the synapses looked swollen and puffed. These observations support previous electron micrograph data which suggest an important role for calcium in endocytosis of BWSV poisoned nerve terminals.
Supported by a HFSPO Long Term Fellowship award to AWH and NIH grant NS 10207 to WB.

PRESYNAPTIC MECHANISMS V

550.1

PAIRED PERFORATED PATCH RECORDINGS IDENTIFY THE TRANSMITTER RELEASED FROM SACCULAR HAIR CELLS.
B. Yazejian*, Z.J. Zhou and A. D. Grinnell, Jerry Lewis Neuromuscular Research Center and Jules Stein Eye Institute, UCLA, 90024-1770.

The transmitter released by saccular hair cells has been suggested to be glutamate but this has never been proven directly. We have used glutamate receptor-rich catfish retinal horizontal cells as detectors of transmitter released from frog saccular hair cells. Simultaneous perforated patch recordings were made of voltage-activated calcium currents from isolated hair cells and of the responses in closely apposed horizontal cells. The magnitudes of the hair cell presynaptic calcium currents were correlated with the amplitudes of the horizontal cell responses. The current responses in the horizontal cells appeared to have a linear current-voltage relationship in the absence of extracellular Mg^{2+} and reversed near the cationic reversal potential. Our results are consistent with the hypothesis that the hair cell transmitter is glutamate or some closely related excitatory amino acid. This approach will allow the identification and characterization of both the hair cell transmitter and the mechanism of its release.
Supported by NS 30673

550.2

MODULATION OF PRE-AND POST-SYNAPTIC CURRENTS MEASURED WITH THE PERFORATED PATCH METHOD AT A VERTEBRATE SYNAPSE.
A. D. Grinnell* and B. Yazejian, Jerry Lewis Neuromuscular Research Center, UCLA, 90024-1770.

We have been using double perforated patch recording techniques to study the mechanisms of transmitter release at synapses formed in culture between varicosities on Xenopus motor neuron neurites and muscle cells. This system has proven to have many advantageous features in common with the classic squid giant synapse preparation. Most notable of these is the ability to measure directly the presynaptic calcium currents responsible for transmitter release. In addition, we are able to measure the quantal content of evoked release, the dependence of release on the presynaptic calcium current and on the extracellular calcium concentration. Furthermore, we have shown the level of modulation of release and presynaptic calcium current by both adenosine and norepinephrine and the sensitivity of these to applications of omega conotoxin and dihydropyridines. Finally, we have studied the calcium current dependence of depression and facilitation by giving paired presynaptic voltage clamp steps to evoke equal magnitude calcium currents.
Sponsored by NS 30673

550.3

ANALYSIS OF CHANGES IN PRESYNAPTIC CURRENTS DURING SLOW DEVELOPING POTENTIATION IN *APLYSIA*.
M.V. Storozhuk and S.M. Fredman*. A.A. Bogomoletz Institute of Physiology, Kiev, Ukraine and Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

Brief high frequency stimulation of the A-B neuron synapse in the cerebral ganglion of *Aplysia* evokes a long-lasting increase in synaptic transmission, slow developing potentiation (SDP). Work to date indicates that SDP is due to changes in the presynaptic A neurons. Although there are transient alterations in the action potential following tetanic stimulation (4, 500 msec 20 Hz trains), these changes are poorly correlated with the time course of increases in EPSP amplitudes in B neurons. There is no significant broadening of the falling phase of the A neuron action potential. The input resistance of the B neurons does not change during SDP. Inducing SDP while voltage clamping A neurons reveals a decrease in total outward current. SDP is not due to the inactivation of the early K^+ current, I_A. SDP persists even when I_A is inactivated by clamping A neurons to a holding potential of -35 mV. Usually only 5-15% of the total current changed after high frequency stimulation. As the relative changes are small, it is likely that a specific current is altered. Subtracting the total current during SDP from that during preceding control stimulations reveals an inward difference current that peaked at ~12 msec after the start of a step from -35 to +25-30 mV and then decayed. The change in current was time-dependent and slowly diminished after tetanization. This current may be due to either the inactivation of a fast outward current with a voltage-dependence different from that of I_A or an increase in a high voltage activated Ca^{2+} current. Since SDP can be partially blocked by L-type Ca^{2+} channel antagonists, this latter possibility is presently being investigated.
This work was supported by NINDS grant NS28199 and NIGMS (MBRS) grant GM08037 to SMF.

550.4

FACILITATION STUDIES ON CRAYFISH NEUROMUSCULAR SYNAPSES: EFFECT OF SEROTONIN AND CAFFEINE.
K. Judd, L. Crawford, M. Lui and S.J.Velez*. Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The facilitation properties of the largest excitor neuron innervating the superficial flexor muscles of the crayfish Procambarus clarkii were examined by measuring the junction potential sizes (jp's) elicited in muscle fibers while stimulating the neuron at 1 and 10 Hz. In Ringers solution, the jp's obtained at 10 Hz were larger than those obtained at 1 Hz, and these were stable during 15 minutes of constant stimulation. In a 10^{-5} M solution of serotonin (5-HT) in Ringers, jp's obtained at both 1 and 10 Hz were larger than controls, and both were again stable during constant stimulation. In a 25 mM solution of caffeine in Ringers, there was no change from controls in the initial jp's recorded at 1 and 10 Hz stimulation, but during the 15 minutes of continuous stimulation the jp's decreased by more than 50% of their initial values. Serotonin's presynaptic depolarization, by increasing calcium entry upon stimulation, could enhance the initial jp's recorded at 1 and 10 Hz, yet this is not affecting the stability of the facilitation mechanisms. Caffeine's known effect of releasing calcium from internal stores suggest that interfering with the intracellular free calcium concentration is not affecting the initial release of transmitter but over time will have an effect on the stability of the facilitation mechanisms, which are believed to involve residual calcium levels.

550.5

CLOSE PRESYNAPTIC ACTIVE ZONES MAY ENHANCE FACILITATION

J.L. Winslow*[1,2], R.L. Cooper[2], C.K. Govind[3], J. Pearce[3], L. Marin[2], H.L. Atwood[1,2]. [1]Biomedical Eng., [2]Physiology Dept. [3]Life Sciences, Scarborough College, U. of Toronto, Toronto, Ont. M5S 1A8.

Voltage activated Ca^{2+}, Ca_V, channels, normally activate neurotransmitter release. Freeze fracture micrographs of crayfish presynaptic active zones, AZs, show Ca_V channels and K_{Ca} channels as prominent intramembrane particles. Reconstructed serial EM sections of varicosities from crayfish opener muscle axons show 0-5 AZs per synapse, with different separations. To evaluate separation effects on faciliation due to spatial summation of Ca^{2+} diffusing from two adjacent zones, we used a computational preparation. We video-digitized a freeze-fractured image of an AZ, then traced the channels. The ratio of Ca_V to K_{Ca} channels was set at 2:1. Using a reaction diffusion model for Ca^{2+} and Ca^{2+} binding proteins (non diffusable buffer) with Ca_V channels arranged as traced, we calculated the 3D concentration of Ca^{2+} for one AZ, then for two separated AZs. With Ca^{2+} diffusion coefficient as in H_2O and no binding sites, all Ca^{2+} diffuses away 0.2 ms after channel closing. With 0.5-1.6 mM total Ca^{2+} binding proteins, residual concentration of Ca^{2+} increases 5-7 times the resting value 0.1 μM with successive stimulation. When two AZs centers are separated by 200 *nm*, the Ca^{2+} concentration midway between the AZs is sufficient to potentially cause vesicle release. *Thus, in single synapses adjacent AZs may promote transmitter release and facilitation.* (See Cooper, R.L. et al., this meeting.) Funded by NSERC, MRC, & NCE of Canada.

550.6

STRUCTURAL CORRELATES OF QUANTAL PARAMETERS AT CRUSTACEAN NEUROMUSCULAR JUNCTIONS.

R.L. Cooper*, J.L. Winslow, C.K. Govind[1], J. Pearce[1] L. Marin & H.L. Atwood. Dept. of Physiology, Med. Sci. & Life Sci. at Scarborough College[1], Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A8.

We investigated the role of calcium in determining the probability of transmission and whether "complex" synapses have a higher probability of transmission. Synaptic currents are recorded from single varicosities with a macropatch electrode to determine quantal parameters of synaptic release. Reconstruction of recorded crayfish excitatory terminal varicosities shows complex (more than one active zone) and simple (only one active zone) synapses. Complex synapses have also been observed in a variety of crustacean species at inhibitory, excitatory and presynaptic inhibitory neuromuscular junctions (NMJ). . In the reconstructions of serial sectioned macropatched terminals active zones (AZs) of complex synapses are often close together. In freeze fracture replicas, two AZs containing intramembranous particles (Ca_v and K_{ca} channels) also occur close together. Cooperative interaction of between closely spaced AZs may occur at complex synapses. Thus, $[Ca^{2+}]_i$ would be greater at sites of vesicle release after an impulse at complex synapses. We propose that the probability of release is higher at sites where AZs are in close apposition and that the number of pairs of close AZs may represent the anatomical correlate of the quantal parameter "*n*" (number of release sites) at low frequencies of stimulation. At higher frequencies of stimulation, more distant AZs and single AZs may acquire higher probability of release, thus giving the nerve terminal a mechanism for grading synaptic output with frequency (see also Winslow, J.L. *et al.*, this meeting). Funded by MRC, NCE & NSERC of Canada.

550.7

EVIDENCE THAT EXCITABILITY CHANGES IN PRESYNAPTIC FIBERS MAY AFFECT PAIRED-PULSE FACILITATION IN HIPPOCAMPAL SLICES

J.F. Storm* & R. Lipowsky *Inst. of Neurophysiol., University of Oslo, Norway.*

Facilitation is a widespread synaptic phenomenon, usually attributed to presynaptic mechanisms. Excitatory synapses in hippocampal slices also show facilitation, even during minimal stimulation of afferent fibers, but it has not been extensively tested whether this is an entirely synaptic process, or whether there may also be variable excitation or conduction in the presynaptic axons. If so, this may have implications also for the study of other synaptic processes. Encouraged by suggestions from D. Kullmann and B. McNaughton, we have performed experiments to test these possibilities.

Whole cell recordings were obtained from CA3 and CA1 pyramidal cells in slices from young rats, while paired stimuli of equal, low intensity (Stim.1 = Stim.2; interval 40-60 ms) were given from a small glass pipette. In CA1 cells, stimulation of presynaptic fibres in *str. radiatum*, elicited excitatory postsynaptic currents (EPSCs), which usually showed clear facilitation; i.e. EPSC2 was larger and/or showed fewer failures than EPSC1. To test whether this difference could be partly due to variable axon excitability or conduction, fibers in *str. radiatum* were stimulated in a similar way while recording from CA3 somata, giving short-latency, presumably antidromic, action potentials. In response to paired pulses, these spikes often showed fewer failures to Stim.2 than to Stim.1. This effect persisted even after blocking excitatory and inhibitory synaptic transmission by reducing the [Ca]/[Mg] ratio (0.1 mM Ca/6.5 mM Mg) or by adding CNQX and bicuculline. Similar effects were also seen with antidromic stimulation of CA1 cells or by "direct" stimulation close to CA3 or CA1 somata. These results suggest that the initiation and/or conduction of action potentials in hippocampal axons may change during repetitive stimulation, and that these effects may add to and influence paired-pulse facilitation and other synaptic phenomena. [Supported by NFR.]

550.8

RELEASE PROBABILITY IS CHANGED BY ALTERING EXTRACELLULAR CA^{2+}, BUT PAIRED PULSE FACILITATION IS NOT, AT PYRAMID-INTERNEURONE SYNAPSES IN NEOCORTICAL SLICES.

Alex Thomson*, David C. West and Jim Deuchars, Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

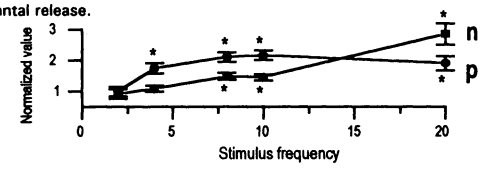
Single axon connections between pyramidal cells and interneurones were recorded using pairs of biocytin-filled sharp electrodes in slices of neocortex. Typically, EPSPs elicited in an interneurone by spikes in a single pyramid exhibited profound paired pulse facilitation and a high probability of failures of transmission to single presynaptic spikes in standard medium (2.5mM Ca^{2+}). The effect of raising extracellular Ca^{2+} on the EPSPs elicited in one class of interneurones, ie. bursting, sparsely-medium spiny interneurones, was studied. When extracellular Ca^{2+} was raised to 5mM, the proportion of failures of transmission to the first spike decreased and averaged amplitudes were almost doubled. However, the 2nd EPSP also increased in amplitude and paired pulse facilitation was not reduced. These data contrast strongly with those obtained from pairs of synaptically connected pyramidal neurones. EPSPs elicited in postsynaptic pyramids typically exhibited paired pulse depression that was augmented when extracellular Ca^{2+} was raised. In addition, unlike pyramid-pyramid EPSPs, the time course of EPSPs recorded in this class of interneurones was increased.

550.9

INCREASE IN NEUROSECRETORY ACTIVE SITES DURING FACILITATION.

M.K. Worden, D.A. August, and J.T. Hackett*. Dept. Mol. Physiol. and Biol. Physics. UVa. Health Sci. Ctr., Charlottesville, VA 22908

The classic Del Castillo and Katz model for quantal release has as its basis the statistical parameters " n", the number of active quantal release sites and "p", the probability for release to occur. Unfortunately, these values are subject to spatial and temporal variations which may render them useless. We have used a method (Provan & Miyamoto, 1993) which avoids these problems to measure facilitation of neurosecretion at a lobster neuromuscular junction. Extracellular recording during steady state conditions were made from synaptic sites using macropatch electrodes with tip diameters of 20 μm. The excitatory nerve was stimulated at a constant frequency for each trial in a range between 1 and 20 Hz. Unbiased estimates of quantal release parameters were computed. Both "n" and "p" increased for stimulus frequencies at and above 4 Hz ($p<0.001$, t-test). Spatial variance in "p" was found to increase up to 4 Hz, and then to reach a plateau. In sum, unbiased estimates of "n" and "p" were obtained and both increased in proportion to stimulus frequency, while the spatial variance in "p" was nearly constant. Facilitation results from an increase in both the active release sites and the probability of quantal release.



550.10

SHORT-TERM PLASTICITY OF EXCITATORY SYNAPSES BETWEEN HIPPOCAMPAL NEURONS IN DISSOCIATED CELL CULTURE.

D. D. Cummings[1*] and M. A. Dichter[1-3] [1]The David Mahoney Institute of Neurological Sciences, [2]Dept. of Neurology, University of Pennsylvania School of Medicine and [3]Graduate Hospital, Philadelphia, PA 19104.

Excitatory synaptic transmission mediated by either NMDA, non-NMDA, or both receptors in our very low density embryonic hippocampal neuron cultures exhibits paired-pulse depression at interstimulus intervals between 0.1 and 4 seconds in paired whole cell patch clamp recordings. However, paired pulse depression(PPD) is much less predictable at excitatory synapses than at inhibitory synapses (Wilcox, Buchhalter, and Dichter, 1994). Consistent with classical experiments, lowering extracellular Ca from 2.0 to 0.5 mM decreases the probability of PPD and raises the probability of paired pulse facilitation(PPF) at 1 sec. interstimulus intervals. The unexpected occurrence of PPD and post tetanic depression (PTD) in $[Ca]_O$=2.0 mM in this elemental synaptic preparation and in other CNS preparations (Thomson and West, 1993) suggests that this may be an important biological property of excitatory synapses. Some experiments demonstrate a relative increase in the frequency in mini-EPSCs accompanying PPD or PTD. A correlation between decreased evoked response amplitude and increased mini-EPSC frequency suggests that short-term plasticity of evoked response amplitude may be dissociated from changes in spontaneous release of neurotransmitter. Alternatively, post-synaptic mechanisms such as desensitization of AMPA receptors may mask facilitation of the evoked response. Supported by NS24260(M.A.D.).

550.11

ANESTHETICS PRODUCE DIFFERENTIAL EFFECTS ON EPSP FACILITATION. S.M. Amagasu*, A.A. Mikulec, F.A. Monroe and M. B. MacIver* Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Anesthetic effects on excitatory postsynaptic potential (EPSP) facilitation were studied as a measure of actions on presynaptic release. Effects produced by clinically relevant concentrations of halothane, isoflurane, and thiopental were compared on Schaffer-collateral evoked dendritic EPSPs recorded in CA 1 of hippocampal brain slices. Paired stimulus pulses were used to produce EPSP facilitation. In agreement with previous studies, synaptically evoked discharge of CA 1 neurons was blocked by halothane (1.2 vol %, 1 rat MAC), isoflurane (1.4 vol %), and thiopental (100 μM). The block produced by the anesthetics was associated with a 22 ± 1.3%, 26 ± 2.4%, and 23.4 ± 8.5% depression of single-pulse EPSP amplitudes by halothane, isoflurane, and thiopental respectively. Halothane increased EPSP facilitation from control values of 130% to 142 ± 6.7%, while isoflurane and thiopental reduced EPSP facilitation to 123 ± 3.1% and to 121 ± 11.3% respectively. Effects on EPSP facilitation demonstrate anesthetic actions on glutamate nerve terminals, since facilitation is known to involve only presynaptic mechanisms. The similar effects on EPSP amplitude but differential effects on facilitation provide further support for a Multisite Agent Specific (MAS) mechanism of anesthetic action.

Supported by NIH GM49811.

550.12

TRANSIENT INWARD CURRENT IN CULTURED RAT EMBRYO MUSCLE MIMICKING QUANTAL SECRETION PROCESS.

J. Vautrin,* A.E. Schaffner and J.L. Barker.
Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Current- and voltage-clamp, whole-cell patch recordings in myotubes and 20μm-diameter myoballs from 18-22 day old rat embryos exhibited dihidropyridine, Mg^{2+}- (10mM) and TTX- (1μM) sensitive transient inward currents (TICs). TICs were either spontaneous, or evoked, appearing during depolarizing commands, and after both depolarizing and hyperpolarizing pulses. The delay to TIC occurring during the pulse was an hyperbolic function of the stimulating pulse amplitude with a minimum of about 200μs. The delay until TIC occurring after a short (0.5-2ms) pulse could be as long as 500ms and its variability increased with its average value. At depolarized holding potentials the peak amplitudes of TICs occurring milliseconds after the end of hyperpolarizing pulses were a direct function both of the duration and the amplitude of the pulse, the gradation suggesting adequate control of the potential by the clamp system. At more hyperpolarized holding potentials the peak amplitude of TICs occurring during (longer) depolarizing pulses remained independent on the membrane potential as if they were unclamped. However, the delay and time course remained dependent of the potential at which these TICs occurred suggesting some control of the potential by the clamp amplifier and that the TIC peak amplitude is not controlled by membrane potential. With short constant liminal pulses, TICs showed all-or-none behavior, as well as discrete fluctuations in amplitude mimicking quantal and subquantal fluctuations in synaptic signals. Intriguingly, both facilitation and depression occurred in paired pulse protocols. How TICs are related to synaptic transmission is a challenge for future study.

550.13

ECTOPIC ACTION POTENTIALS IN HIPPOCAMPAL AND DENTATE NEURONS UNDER CONTROL CONDITIONS. D.D. Mott*, S.F. Stasheff, D.V. Lewis and W.A. Wilson, Depts. of Ped. (Neurology), Neurobiol., Med. and Pharmacol., Duke Univ. and V.A. Med. Ctrs, Durham, N.C. 27710.

We have previously reported that induction of electrographic seizures (EGS) using kindling-like stimulation increased the occurrence of ectopic action potentials (baseline spikes) in CA3 pyramidal cells. These ectopic action potentials appeared to be generated in the axon terminal by a depolarizing GABA response. However, it was also noted that in 37% of the CA3 neurons sampled, ectopic action potentials occurred prior to EGS induction. We now report the presence of ectopic action potentials under control (non-hyperexcitable) conditions in other cell types in both the hippocampus and dentate gyrus.

Intracellular responses were recorded from neurons in the rat hippocampal slice preparation using sharp microelectrodes. Ectopic action potentials were identified as action potentials which arose sharply out of the baseline with no preceding depolarization and continued to occur despite somatic hyperpolarization. Ectopic action potentials were present in 31 out of 160 sampled granule cells (19%). Similarly, ectopic action potentials were observed in area CA1 in both pyramidal cells and fast spiking interneurons in the pyramidal cell layer. In these cells ectopic action potentials occurred both spontaneously and following orthodromic and antidromic stimulation. Evoked ectopic action potentials occurred primarily during the $GABA_A$ IPSP and manipulations designed to reduced GABAergic inhibition blocked ectopic action potentials, demonstrating their dependence on GABAergic potentials.

These results suggest that the occurrence of ectopic action potentials may be more widespread than previously thought and that these action potentials may contribute to cellular responses under normal (non-hyperexcitable) conditions.

Supported by NS27488, NS17771 and the Veterans Administration

550.14

ZINC ENHANCES ACTION POTENTIAL INDEPENDENT GABA RELEASE FROM INHIBITORY TERMINALS IN THE DENTATE GYRUS. E. Buhl*[1] and I. Mody[2]. [1]MRC Anatomical Neuropharmacology Unit, Oxford University, U.K., and [2]Depts. of Anesthesiology/Pain Mgmt. & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

Depending on the developmental stage, zinc is known to exert various effects on GABAergic inhibition. The effects of zinc range from diminishing GABA responsiveness in the absence of the γ-subunit, to enhancing inhibition secondary to its action on the excitability of inhibitory interneurons. We have examined the possibility whether zinc modulates the release of GABA from inhibitory nerve terminals when the excitability of interneurons *per se* is unlikely to be affected.

Recordings were obtained from dentate gyrus granule cells in conventional (400 μm thick) coronal adult rat brain sections using whole-cell patch clamp techniques. Miniature inhibitory postsynaptic currents (mIPSC) were recorded in the presence of 1 μM TTX, 10 μM CNQX and 40 μM D-AP5, with 130 mM CsCl, 10 mM Hepes, and 2 mM $MgCl_2$ containing electrodes. During a given recording, access resistance was frequently monitored, and experiments were only selected for analysis if access resistance did not change throughout the recording period. Furthermore, event detection accuracy was ensured by analyzing the frequency and amplitude of events at two different holding potentials. Zinc (100-200 μM) was applied through bath perfusion, and produced a significant enhancement of mIPSC frequency. In 20% of the recordings, zinc induced dramatic bursts of mIPSCs, causing a 10-20-fold increase in event frequency during burst periods. As expected from the presence of the $GABA_A$ receptor γ-subunit on adult granule cells, zinc had no effect on the decay kinetics, 10-90% rise times, and amplitudes of mIPSCs.

Our findings demonstrate a presynaptic effect of zinc on GABA release most likely occurring at the level of inhibitory terminals of GABAergic neurons. Such modulation of tonic GABA release by zinc, may play an important role in the regulation of excitability in the dentate gyrus.

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LONG-TERM POTENTIATION: PHYSIOLOGY VI

551.1

MECHANISMS OF SELECTIVE LONG-TERM POTENTIATION OF EPSCs IN INTERNEURONS OF STRATUM ORIENS IN RAT HIPPOCAMPAL SLICES. M. Ouardouz* and J.-C. Lacaille, Center for Research in Neurological Sciences and Department of Physiology, University of Montréal, Montréal, Qc, Canada H3C 3J7.

Mechanisms of long-term potentiation (LTP) have been extensively studied in hippocampal pyramidal cells, but the involvement of local inhibitory cells remains largely unknown. In the present study, the effects of tetanization were examined on excitatory postsynaptic currents (EPSCs) in different hippocampal interneurons. Rat hippocampal slices (300 μm thickness) were submerged in a chamber mounted on an upright microscope and CA1 interneurons were identified visually with Nomarski optics in str. oriens (OR-cells) or lacunosum-moleculare (LM-cells). EPSCs evoked by electrical stimulation of afferent fibers (-80 mV, 0.033 Hz) were recorded in whole-cell mode (recording solution in mM: 140 $CsCH_3SO_3$, 5 NaCl, 1 $MgCl_2$, 10 HEPES, 1 EGTA, 2 ATP, 0.4 GTP). Following tetanic stimulation (100 Hz, 1s) paired with postsynaptic depolarization (-20 mV), EPSCs in OR-cells were significantly increased in amplitude for 10 min (137.7 ± 7.0% of control [mean ± sem], n=11) to 30 min (129.9 ± 1.3% of control, n=5). Similar stimulation did not potentiate EPSCs in LM-cells (91.6 ± 8.2% of control, n=7). LTP of EPSCs in OA-cells was blocked by intracellular application of 25 mM BAPTA (88.2 ± 7.4% of control, n=5), and by bath application of i) 25 μM AP-5 (88.3 ± 4.2% of control, n=8), ii) 500 μM MCPG (98.6 ± 8.4% of control, n=5), and iii) 100 μM L-NAME (83.4 ± 6.4% of control, n=5). These results suggest that excitatory synapses of interneurons in str. oriens display selective long-term potentiation via activation of NMDA and metabotropic glutamate receptors, postsynaptic elevation of Ca^{2+} and NO synthesis. Thus, various inhibitory cells may participate differently in hippocampal long-term potentiation.

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551.2

FEED-BACK SYNAPTIC PLASTICITY IN HIPPOCAMPAL ST. ORIENS-ALVEUS INHIBITORY NEURONS G. Maccaferri* and C. J. McBain, Laboratory of Cellular and Molecular Neurophysiology, NICHD National Institutes of Health, Bethesda, MD 20892.

We studied the effect of low (10 min, 1 Hz) and high frequency (4 X 1 s, 100 Hz) stimulation of the st. radiatum on the synaptic responses of st. oriens-alveus (O-A) interneurons. Hippocampal slices were prepared from 15-22 days old rats and whole-cell current-clamp recordings were performed on a total of 17 interneurons in the presence of 1 μM bicuculline. In addition, the extracellular field potential in the pyramidal cell layer was continuously monitored. Activation of st. radiatum afferents elicited excitatory post-synaptic potentials (EPSP) or action potentials commencing ~ 2.5 ms after the peak of the field population spike (fPS). Following the recording of stable synaptic responses for 10 minutes at 0.1 Hz, the low-frequency protocol was applied and both EPSP and fPS amplitudes were strongly depressed (~ 70%) for the entire duration of the recording (up to 30 min). Subsequent treatment with the high-frequency protocol could restore or even potentiate the original baseline response. Identical results were obtained when the recording electrode contained 10 mM EGTA in the absence of Ca^{2+} or when the phosphatase inhibitor microcystin LR (10 μM) was added. In contrast, bath application of the NMDA receptor antagonist D-AP5 (50 μM) could prevent both the EPSP and the fPS depression. These data indicate that st. O-A interneurons can undergo long-term depression (LTD) and long-term potentiation (LTP) without requiring an increase of intracellular Ca^{2+} concentration or phosphatase activity. These results, together with the delayed onset of the interneuron EPSP when compared to the fPS peak, demonstrate that the LTD and LTP observed are indirect phenomenons driven by the recurrent collaterals of CA1 pyramidal cells.

551.3

LONG TERM POTENTIATION OF RECURRENT INHIBITION ONTO HIPPOCAMPAL CA 1 PYRAMIDAL CELLS IN VITRO. H.C.R. Grunze*, D.G. Rainnie, E.F. Hearn, R.W. McCarley, R.W. Greene. Harvard Medical School & VAMC, Department of Psychiatry, Brockton MA 02401, USA

Modulation of recurrent inhibition is critical not only for the normal function of highly excitable regions of the brain, especially the limbic system, but may also may be important in prevention of excitotoxic damage. Post-mortem data indicate reduced numbers of GABAergic interneurons while MRI data show marked volume reductions in cortical and especially limbic gray matter in schizophrenics, suggesting a possible role of excitotoxic damage in this disorder, and the need for careful study of the dynamics of recurrent inhibition. In the present study, standard extracellular (n= 26) and intracellular (n= 8) recordings from in vitro brain slices of rat hippocampi showed that recurrent inhibition onto CA1 neurons is enhanced by NMDA- mediated long term potentiation (LTP), and that this LTP persists for periods greater than 20 minutes. The LTP was evoked by a tetanus applied to the Stratum oriens (100 Hz for the extracellular and 400 Hz for the intracellular recordings) and resulted in a mean enhancement of the recurrent inhibitory drive of 19 ± 9.8 % in 23/26 recordings (extracellular recordings) and 32.5 ± 24.7 % in 8/10 neurons (intracellular recordings). Furthermore, this LTP was completly blocked by the NMDA antagonists 2- amino-phosphonovaleric acid (APV), MK- 801, phencyclidine (PCP) and N-Acetyl-L-aspartyl-L-glutamic acid (NAAG). Presumably, the tetanic stimulus activated excitatory recurrent CA1 axon collaterals synapsing on inhibitory interneurons that, in turn, elicited the inhibitory postsynaptic potentials (IPSPs) in the recorded CA1 neurons. We thus infer that the LTP (indexed by the increased amplitude of the evoked IPSP) occurs at the excitatory CA1 to inhibitory interneuron synapse. Blocking the LTP of recurrent inhibition may explain the increased behavioral excitability in rats seen on exposure to PCP, whose effects in man mimic positive schizophrenic symptoms.

551.4

LONG-TERM POTENTIATION OF IPSPs IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. Z. Xie*, S. Yip, and B.R. Sastry, Neuroscience Research Laboratory, Department of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3

Previous reports from this laboratory showed that tetanic stimulation of the stratum radiatum induced LTP of not only the excitatory postsynaptic potentials (EPSPs) but also of the inhibitory postsynaptic potentials (IPSPs) in CA1 neurons. In the present study, experiments were conducted to further examine LTP of the IPSPs in guinea pig hippocampal CA1 neurons. In control neurons, tetanic stimulation (100 Hz, 1s) of the stratum radiatum caused LTP of the EPSP and the fast IPSP but not of the slow IPSP (n=8). In CA1 neurons injected with BAPTA (a Ca^{2+} chelator) (n=6) or K-252b (a PKC inhibitor) (n=8), the tetanic stimulation induced a larger LTP of the fast IPSP and LTP of the slow IPSP, but did not cause LTP of the EPSP. While tetanic stimulation of the stratum radiatum induced LTPs of the EPSP and the fast IPSP in control neurons, the IPSPs evoked by the stimulation of the alveus were not changed (n=4). LTP of the fast IPSP was reduced, and LTP of the slow IPSP was blocked in BAPTA- (n=6) or K-252b- (n=6) injected CA1 neurons when the tetanus was applied in the presence of APV. In the presence of APV (50 μM) and CNQX (20 μM), stimulation of the stratum radiatum presumably evokes monosynaptic IPSPs. When the tetanic stimulation was given in the presence of these drugs, LTP of the fast-, but not of the slow- IPSP, was observed in control as well as BAPTA- or K-252b-injected neurons (n=6 each).

These findings suggest that LTP of the IPSPs in CA1 neurons is modulated not only by postsynaptic $[Ca^{2+}]i$ and PKC activity but also by presynaptic conditions (changes in interneuron excitability or LTP of excitatory synapses on the interneurons?). There appear to be differences between LTPs of the fast and the slow IPSPs. This study was supported by a grant from the NIH (NS 30959) to B. R. S. and Canadian M. R. C. Studentships to Z. X. and S. Y.

551.5

HOMOSYNAPTIC AND HETEROSYNAPTIC CHANGES IN DRIVING OF DENTATE GYRUS INTERNEURONS FOLLOWING BRIEF TETANIC STIMULATION Richard A. Tomasulo* Departments of Neuroscience and Neurology, University of Virginia, Charlottesville, VA 22908.

The inhibitory circuit within the hippocampal dentate gyrus (DG) can change its net inhibitory efficacy following brief tetanic stimulation of either the perforant path (PP) or the dentate commissural pathway (CP) (J. Neurophysiol. 69:165). To further explore the possible contribution of inhibitory circuit plasticity to information storage, we measured, in urethane-anaesthetized rats, changes in interneuron driving that followed conditioning regimens similar to those of the previous study. The primary measure of synaptic drive was the firing latency of DG interneurons isolated with an extracellular tungsten recording electrode. Stimulating electrodes were placed in the ipsilateral angular bundle and the contralateral dentate hilus. Identification criteria included high spontaneous firing rate, short duration action potential, firing to PP stimulation at low latency and threshold, multiple firing, and CP firing latency below 6 msec. Data were collected in 20-minute periods during which we activated the CP and PP inputs alternately. Conditioning regimens were control, PP tetany at spike threshold, PP tetany well above spike threshold, CP tetany, and paired tetany. Paired conditioning was given only after both inputs had been individually conditioned. (Conditioning stimulation: 400Hz, 20 msec.) Both CP and paired tetany consistently reduced CP firing latency ($p = .03$ and .01). PP latency decreased to paired and to high-intensity PP tetany, but the mean changes were not significant. Individual cases suggested heterosynaptic interactions. The results indicate limited plasticity of interneuron driving in vivo under conditions that alter the E-S relationship and change commissural inhibitory efficacy. We suggest that plasticity of the inhibitory circuit contributes to information storage in the hippocampus. Supported by K08NS01438 from NINDS to RT.

551.6

GABAERGIC LONGTERM POTENTIATION IN RAT BRAINSTEM SLICES Steven R. Glaum* & Penelope A. Brooks Dept. Physiology, Royal Free Hospital School of Medicine, London, U.K. & * Dept. Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL. 60637

Whole-cell patch recordings were made in the nucleus tractus solitarii (NTS) in transverse brainstem slices from rats. Monosynaptic $GABA_A$-receptor mediated inhibitory postsynaptic currents (IPSCs) or potentials (IPSPs) were evoked (0.1-0.2 Hz) by electrical stimulation within and medial to the tractus solitarius in the presence of ionotropic glutamate receptor (GluR) antagonists (10μM DNQX, 50μM D-AP5). Low frequency (5-50 Hz) tetanic stimulation resulted in posttetanic- (PTP) (<5min, 222.4 ±18.3% control, n=39) and longterm- (LTP) potentiation (≥15min, 175.3±8.6% at 15 min posttetanus, n=26) of the IPSP/Cs. In the majority of cells, a 20Hz tetanus resulted in LTP persisting 20-40 min, but was ocassionally observed to persist up to the duration of the recording (~2hr). LTP was not due to alterations in the reversal potential of IPSP/Cs and the IPSP/C could be completely blocked by the $GABA_A$ antagonist bicuculline. Furthermore, current responses to micropressure application of the $GABA_A$-receptor agonist muscimol were unaltered in cells displaying LTP. Unlike excitatory LTP in the hippocampus, GABAergic LTP was evokable in the presence of the metabotropic GluR antagonist α-methyl-4-carboxyphenylglycine (MCPG, 200μM). Although we have previously shown that presynaptic $GABA_B$ receptors inhibit GABAergic transmission in the NTS (Brooks et al., J. Physiol. 457:115-129, 1992), paradoxically, induction of LTP was blocked in the presence of the $GABA_B$-receptor antagonists 2-OH-saclofen (400μM) or CGP35348 (3-amino-propyl-(diethoxymethyl)) phosphinic acid (100μM). PTP was unaffected by $GABA_B$-receptor antagonists. These data suggest that neuronal or terminal excitability of GABAergic interneurons in the NTS is enhanced following brief periods of increased frequency of activation *in vitro*. This phenomenon may be involved in the temporal modulation of autonomic reflex sensitivity observed during certain behavioral states, such as the defense reaction.

551.7

THE RELATIONSHIP BETWEEN SHORT-TERM AND LONG-TERM POTENTIATION OF INHIBITORY SYNAPTIC TRANSMISSION IN RAT VISUAL CORTEX. Y. Komatsu*. Dep. of Physiology, Kyoto Prefectural Univ. of Med., Kamigyoku, Kyoto 602, Japan.

High-frequency activation of inhibitory synapses can induce short-term (STP) or long-term potentiation (LTP) of inhibitory synaptic transmission in rat visual cortical cells. To study the relationship between the STP and LTP, inhibitory postsynaptic potentials evoked by layer IV stimulation were recorded from layer V cells of developing rat visual cortex slices where excitatory synaptic transmission was blocked by glutamate receptor antagonists. Strong high-frequency conditioning stimulation of layer IV commonly induced LTP while weak one induced LTP or STP that decayed to control levels over 20-40 minutes. A weak conditioning stimulation, which first elicited STP, elicited LTP when it was applied again after the potentiated responses had returned to the control level (20-30 minutes). After LTP was saturated by repetitive application of strong conditioning stimulation, STP was still induced. These results suggest that different mechanisms are responsible for STP and LTP although they may be partly common.

551.8

KCL induced LTP in CA3-CA1 cultures: a possible role for NO

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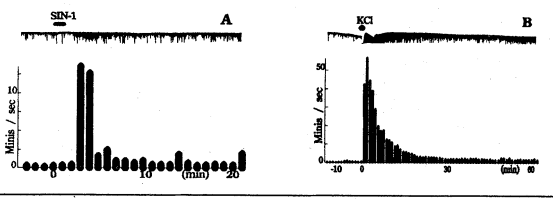
LTP of miniature EPSCs (mini) frequency can be elicited in hippocampal CA3-CA1 cultures by local application of Glutamate or Sucrose (0 Mg++). We studied the role of NO in this LTP by recording minis in the whole cell configuration (TTx 0.5 μM present). Local application of NO donors (SIN-1, 0.05-10 mM, 60 sec) increased mini frequency in the absence of any stimulated pre or post synaptic activity (n=9/15 ; APV 25 μM)(A). The onset of the effect was always delayed (~30-60 sec lag from SIN-1 washout) and displayed a burst like pattern throught out the experiments. These features and the requirement of high dosage of SIN-1 might result from the absence of concurrent pre-synaptic activity in most of the boutons. We used 90 mM KCl pulses (0.2 sec applications, 3 times with 5 sec intervals) to synchronize pre- and post-synaptic activation. This elicited a strong and reliable LTP (2 to 10 fold increase in mini frequency for more than 60 min, n=5/6) (B). Block of postsynaptic Ca2+ rise (5-10 mM BAPTA n=8/8) prevented mini frequency potentiation, possibly by inhibiting the production of the retrograde messenger(s). We have now begun to test if NO and other putative retrograde messengers can restore LTP in BAPTA blocked cells where presynaptic activity is present and dissociated from the postsynaptic Ca++ rise.



551.9

HEME OXYGENASE-2 MUTANT MICE AS MODELS FOR STUDYING EFFECTS OF THE DEPLETION OF ENDOGENOUS CARBON MONOXIDE, A PUTATIVE NEURONAL MESSENGER . K. D. Poss, C. Chen*, and S. Tonegawa. Center for Cancer Research and Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Recent research has implicated carbon monoxide (CO) as being a retrograde messenger in the mammalian hippocampus. The major physiological supply of CO depends on the activity of microsomal heme oxygenases, of which there are two isoforms. These enzymes degrade heme molecules to release biliverdin, free iron, and CO. We are currently studying heme oxygenase-2 (HO-2), which is highly expressed in many neuronal populations of the brain. Using homologous recombination in embryonic stem cells, we have generated mice with null mutations in HO-2, in order to remove this source of CO postulated to have roles in neuronal activity. Homozygous HO-2 mutants are viable and are indistinguishable from wild-type litter mates upon sight. Our initial experiments will attempt to determine whether or not endogenous CO normally acts as a type of neurotransmitter. Neuroanatomy and synaptic transmission are therefore being studied in the mutant mice. We are also measuring LTP in HO-2 mutants. The possible role of HO-2 and CO in spatial learning will be investigated, by testing HO-2 mutants in tasks such as the Morris water maze. In addition, second messenger systems reported to be modulated by CO are being examined in the mutants, to determine possible mechanisms of CO action.

551.10

A NOVEL CHOLINERGIC INDUCTION OF LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS. J.M. Auerbach* and M. Segal. Dept. of Neurobiology, The Weizmann Institute, Rehovot 76100, Israel.

Long-term potentiation (LTP) is a lasting increase in the efficacy of synaptic transmission and is assumed to underlie plastic changes associated with learning and memory. As acetylcholine (ACh) is known to be involved in cognitive processes of learning and memory, we studied the long lasting effects of ACh on synaptic transmission in submerged hippocampal slices using intra- and extracellular recording techniques. Here we describe a novel action of the muscarinic agonist carbachol (CCh). Submicromolar concentrations of CCh produced a gradually developing, long-lasting increase in the CA1 excitatory postsynaptic potential (e.p.s.p.) and population spike when continuously applied for 20 minutes, while higher concentrations caused an immediate but reversible depression. This potentiation, named muscarinic LTP (LTP_m) was N-methyl-D-aspartate (NMDA) receptor independent, was independent of influx of extracellular calcium, but was dependent on release of calcium from intracellular stores and on the activation of kinases. LTP_m can be induced in the complete absence of synaptic stimulation, as well as in the absence of intact CA1/CA3 connections. Saturation experiments showed that the mechanisms of LTP_m and tetanus-induced LTP converge. These observations provide a direct link between ACh and mechanisms of synaptic plasticity.

551.11

TAURINE INDUCES A LONG LASTING INCREASE OF SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. M. Galarreta[1], J. Bustamante[2], R. Martín del Río[1] and J.M. Solís[1]*. [1]Depto. Investigación, Hosp. Ramón y Cajal, and [2]Depto. Fisiol., Fac. Medicina, U.C.M., Madrid, Spain.

Although taurine is one of the most abundant amino acids in the brain of mammals, its physiological role in the nervous system is not well defined. The best known electrophysiological effect of taurine is to act as a weak agonist on $GABA_A$ receptors. Our purpose has been to study the actions of taurine on evoked synaptic potentials. Experiments were carried out in the CA1 region of rat hippocampal slices continuously perfused in a submerged chamber. The basic observation was that bath application of taurine (10 mM) for 30 min induced, after a transitory decrement, an enhancement of field excitatory postsynaptic potentials (fEPSPs) that lasted after taurine washout (146±9% after 1h washout, n=15). The following features related with this taurine action can be pointed out: 1) fEPSPs increase is dependent on taurine concentration (5-10 mM) and taurine perfusion time (10-30 min), 2) fEPSP potentiation develops in the presence of $GABA_A$ or NMDA antagonists, 3) N-methyltaurine, a taurine analogue, does not induce fEPSP potentiation, 4) during taurine perfusion the afferent volley (AV) also shows a long lasting increase in amplitude, 6) fEPSP enhancement is not fully explained as a function of the AV increase, as it is demonstrated by the AV/fEPSP relations before and after taurine, 7) the taurine-induced potentiation of the AV is also observed in 0 Ca^{2+} 12 mM Mg^{2+}, 8) intracellular recordings of evoked EPSPs and monosynaptic evoked IPSPs show long term increases of both EPSPs and IPSPs amplitudes after taurine perfusion, while input resistance does not change significantly.These results could be explained, at least partially, through a taurine effect on glutamatergic and GABAergic presynaptic fibers, and show a new action of taurine consisting in a long lasting enhancement of synaptic transmission. (Supported by F.I.S.S. 93/0565).

551.12

CONDITIONS FOR LTP INDUCTION STUDIED WITH PERFORATED PATCH-CLAMP METHODS IN VISUAL CORTICAL SLICES OF YOUNG RATS. T. Tsumoto* and Y. Yoshimura. Dept. Neurophysiol., Biomedical Research Center, Osaka University Medical School, Suita, 565 Japan.

For induction of long-term potentiation (LTP) of synaptic efficacy, the membrane potential of postsynaptic neurons during synaptic inputs is suggested to be a critical factor. To test this suggestion, we prepared thin slices (140-150 µm thick) of visual cortex of rats aged 9 to 18 days for whole-cell voltage-clamp recordings from layer 2/3 neurons. Nystatin, which makes the membrane of a cell-attached patch electrically permeable (Horn and Marty, J. Gen. Physiol. 92, 145, 1988), was added to the pipette solution at 200 µg/ml. Excitatory postsynaptic currents (EPSCs) evoked by test stimulation applied to an adjacent layer 2/3 neuron at 0.1 Hz were recorded for 5-10 min after an establishment of perforated patch-clamp recording. Then, test stimulation was paired with postsynaptic depolarization at 1 Hz for 1 min. In 11 of the 15 cells in which the membrane potential was depolarized to -20 mV, LTP of EPSCs was induced following pairing. LTP of EPSCs was observed in 5 of the 14 cells depolarized to -40 mV. No LTP was observed when the postsynaptic cells were clamped at -60, -70 or -90 mV. Thus, the induction of LTP may depend on postsynaptic depolarization more than about -40 mV. Significant LTD was not induced in any of the cells tested with pairing stimulation of neighboring 2/3 cells. A question of whether this is also the case in synapses from layer 4 neurons to layer 2/3 neurons is being addressed with similar pairing procedures.

551.13

IS ADULT CORTICAL PLASTICITY MEDIATED BY INHIBITORY GATING OF LONG-TERM POTENTIATION IN HORIZONTAL PATHWAYS? J.P. Donoghue*, C.D. Aizenman, G. Hess Dept. Neurosci.., Brown Univ., Providence, RI 02912; Dept. Animal Physiology, Jagiellonian Univ. Krakow, Poland.

Adult sensory and motor cortical representations are capable of rapid reorganization, but neither the mechanism nor the site for this plasticity is clear. Reorganization of motor cortex (MI) has been obtained by local blockade of inhibition within MI, which implicates inhibitory neurons in plasticity and suggests that intrinsic cortical circuits contain necessary components. We investigated whether persistent changes in the functional interactions of MI neurons can arise by long-term potentiation (LTP) of synaptic transmission in horizontal pathways within layers II/III and whether the occurrence of horizontal LTP is gated by cortical inhibitory circuitry. Field potentials (FPs) evoked in horizontal pathways were examined in adult rat MI slices. Focal, transient application of the GABAA receptor antagonist, bicuculline methiodide (BMI) immediately before theta-burst stimulation (TBS) resulted in LTP. Simultaneous stimulation of horizontal pathways on either side of the recording site yielded larger LTP (34% increase in FP amplitude), with greater reliability (88% of cases), than stimulation of horizontal pathways from one side alone (24% increase; 57% of cases). Nearby recording sites that did not receive BMI expressed no LTP. Intracellular recordings confirmed the long lasting enhancement of EPSPs following TBS (4 of 9 neurons). Next, the hypothesis that activity in vertical pathways can replace the BMI requirement for LTP induction was tested. Combined TBS of the horizontal pathway and the vertical pathway leading from deep layer III to layers II/III produced LTP in the horizontal pathway FP (21%, 94% of cases). The vertical pathway FP usually potentiated as well (15%, 83% of cases). LTP was blocked reversibly by 50 µM APV.

These data demonstrate the potential for LTP in horizontal pathways of MI and suggest that inhibition may gate synaptic modification. This gate may be opened by conjoint activity with vertical connections which presumably decreases local inhibition. The results demonstrate the importance of temporal interactions among cortical pathways for cortical plasticity and also support the conclusion that intrinsic connections form a substrate for cortical reorganization. Support: NS22517.

551.14

SYNAPTIC TRANSMISSION BETWEEN DESCENDING FIBERS AND LUMBAR MOTONEURONS OF THE FROG: FACILITATION AND LONG-TERM DEPRESSION OF SINGLE-FIBER EPSPS. A.E.Dityatev*[1,2], V.M.Kozhanov[2] and H.P.Clamann[1], Dept. of Physiology, Univ. of Bern[1], CH-3012, Switzerland; Inst. of Evolutionary Physiology & Biochemistry[2], 195271 St. Petersburg, Russia

Twenty-five data sets of motoneuronal EPSPs evoked by intracellular stimulation of descending fibers of the ventral lateral column were analyzed. Reticulospinal fibers (n=13) were identified by stimulation of the reticular formation; the remaining fibers (n=12) were presumably propriospinal. In both subsets of data there was considerable variability of the mean amplitude (from 44 to 4100 µV) and shape of averaged EPSPs (half-width from 4.9 to 23 ms). Amplitude of the propriospinal EPSPs was higher; in 4 cases mean amplitudes were bigger than 1 mV. There was strong positive correlation between rise-time and half-width and negative correlation between the rise-time and amplitude (for EPSPs with amplitude < 1 mV), implying variability in location of synaptic boutons.

When a test stimulus was evoked in a single fiber 40 ms after a conditioned stimulus, it produced weak facilitation of reticulospinal EPSPs (ratio between mean amplitudes of test and conditioned EPSPs was 1.21±0.22). For the propriospinal fibers (mean ratio 1.05±0.21), facilitation was observed in only a half of studied connections. In the remaining synapses, which all had high amplitude EPSPs, there was weak depression of the test EPSP. Stimulation of a descending fiber at 5 Hz produced strong depression of the EPSP in 1-2 min. Recovery time after 5-10 min of stimulation was from 15 to 30 min (n=4) and in one case recovery was not complete 45 min after termination of 5 Hz stimulation. Ratio between amplitudes of test and conditioned EPSPs was not changed but half-width was increased within 5 min after the stimulation. We suggest that presynaptic mechanisms mediate the depression, and the effect is stronger at more proximal synapses. Supported by SNF.

551.15

LONG-TERM POTENTIATION AND KINDLING-INDUCED POTENTIATION IN THE FREELY MOVING GUINEA PIG. G.C. Teskey* and P.A. Valentine. Behav. Neurosci. Res. Grp., Dept. of Psychology, Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

This study was undertaken to examine both long-term potentiation (LTP) and kindling-induced potentiation (KIP) in the same structures in freely moving guinea pigs. Guinea pigs were chosen because they exhibit a number of differences in electrical kindling as compared to rats. Male and female, mixed strain guinea pigs were chronically implanted with bipolar stimulating and recording electrodes. Animals had stimulating electrodes implanted in either the olfactory bulb (OB) or lateral olfactory tract (LOT) and recording electrodes in the piriform cortex. Following a two week recovery period, a series of baseline input/output (I/O) response curves were generated. These were based on the application of rectangular 200 µsec pulses at 11 intensities thru the stimulating electrodes. The potentials evoked in the piriform cortex were analyzed for maximum peak and minimum valley amplitudes. LTP experimental animals received high frequency stimulation (HFS) trains once a day for a total of four days. Kindling experimental animals received a total of 10 stimulations, one per day, consisting of 1 msec biphasic square wave pulses at 60 Hz, for 2 seconds. In these animals the resulting afterdischarges (AD's) were recorded and the convulsive behaviour scored. Post-manipulation I/O's were recorded for several weeks. We report potentiation of the piriform evoked responses to HFS and kindling of the LOT but not to HFS and kindling of the OB. Supported by NSERC and AHFMR.

551.16

A DEVELOPMENTAL EXAMINATION OF LTP IN FREELY-MOVING RATS. J.D. Bronzino*, R.J. Austin-LaFrance and P.J. Morgane. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

This study examined the establishment, maintenance, and decay of LTP across the perforant path/dentate granule cell synapse in freely-moving rats at 15, 30, and 90 days of age. Measures of population spike amplitude (PSA) and population EPSP slope were used to assess the magnitude and duration of LTP obtained from each age group. At 15 days of age, significant potentiation of both EPSP slope and PSA measures was obtained 15 min. after tetanization. This enhancement was maintained without significant change over the first 24 hrs. post-tetanization, after which these measures rose steadily during the 5 day recording period. To determine what percentage of this rise resulted from tetanization and what percentage resulted from normal development, input/output curves were recorded daily from non-tetanized pups as they matured from PND 16-29. Subtraction of developmental increases indicated that tetanization effects decayed to baseline between days 4 and 5 post-tetanization. At both 30 and 90 days of age, significant enhancement of both EPSP slope and PSA measures was obtained 15 min. after tetanization. These levels were significantly below those obtained from 15 day olds and were maintained without significant change for only 3-5 hrs., decaying to baseline within 24 hrs. Results indicate that LTP of both the EPSP slope and PSA can be reliably established in 15-day old rats, and that both the magnitude and duration of this LTP is significantly greater than that obtained from juveniles or adults. The immature functional status of GABAergic interneuronal synaptic contacts with the granule cell population is hypothesized as a possible mechanism underlying both the greater magnitude and longer duration of LTP obtained from 15 day old animals. **Supported by NSF Grant # BCS-9208128.**

551.17

MOSSY FIBER LONG-TERM POTENTIATION IN CONSCIOUS, FREELY MOVING ANIMALS: TIME COURSE AND INDUCTION PARAMETERS. **E.J. Barea-Rodriguez*, B.E. Derrick and J. L. Martinez, Jr. Department of Psychology, University of California, Berkeley, CA 94720.**

Previously we reported that in the anesthetized animal, mossy fiber long-term potentiation (MF-LTP) is frequency dependent (J. Neurosci.,in press), because a minimum of 30 pulses at 100 Hz are needed to induce MF-LTP. In the present study, we investigated the stimulation parameters necessary for the induction of MF-LTP in conscious, freely moving animals with indwelling electrodes. We found that unlike for the anesthetized preparation, 30 pulses at 100Hz did not induce MF-LTP but that seizures were induced. By contrast, when the animals were placed in a novel environment, the administration of two 100 Hz trains induced MF-LTP, without the occurrence of seizures. This potentiation decayed within 24 hrs. We propose that the physiological mechanisms associated with hippocampal theta activity which are present in novel environment, prevent the occurrence of seizures and facilitate the induction of MF-LTP.
Supported by F32-MH10641 to EJBR, and DA04195 and the Rennie fund to JLM.

551.18

SYNAPTOGENESIS INDUCED BY HIPPOCAMPAL MOSSY FIBER LONG-TERM POTENTIATION. M.L. Escobar*, E.J. Barea-Rodriguez, J.A. Reyes, B.E. Derrick, and J.L. Martinez, Jr. Department of Psychology, University of California, Berkeley, CA 94720.

Evidence that neural activity may cause the formation of synapses in the adult brain has come from studies of limbic seizures and kindling in the rat hippocampus (TINS 13: 312-318, 1990; J. of Neurosci. 9: 2795-2803, 1991). It has been shown that long term potentiation (LTP) of mossy fiber responses in area CA3 of the rat hippocampus constitutes an opioid receptor-dependent form of hippocampal synaptic plasticity (Brain Res. Bull., 27: 219-223, 1991). In the present study, Timm sulfide silver technique was used to examine whether LTP at the mossy fiber-CA3 synapse affects structural reorganization and synaptic formation in the adult rat hippocampus *in vivo*. Our results show that seven days after LTP induction, a prominent band of Timm's staining is present bilaterally in the infrapyramidal band but is more prominent in the contralateral CA3 (*stratum oriens*). In addition, we found that naloxone (an opioid receptor antagonist) reverses this effect. Stimulation induced increases in Timm's staining, not associated with LTP, was also evident. The increase in Timm's staining which we interpret to be synaptic reorganization, becomes apparent as soon as 1 hour after the LTP induction and is quite pronounced after 7 days. These results indicate that synapses are formed in response to neural activity associated with LTP thus, experience-dependent information storage could involve local, activity-dependent synaptogenesis.
Supported by DA04195 to JLM, Rennie Fund of the University of California, F32-MH10641 to EJBR and CONACYT of Mexico to MLE.

551.19

GENE EXPRESSION ASSOCIATED WITH HIPPOCAMPAL MOSSY FIBER LONG-TERM POTENTIATION *D.T. Rivera*, B.E. Derrick, W. Meilandt, M.R. Rosenzweig and J.L. Martinez, Jr.* Departments of Psychology and Cell and Molecular Biology, University of California, Berkeley, CA 94720.

Long-term potentiation (LTP) is a long lasting form of synaptic plasticity that currently is considered a viable model of memory storage in the mammalian brain. Such sustained plastic changes are likely maintained by alterations in gene expression. To study gene expression associated with LTP, we induced mossy fiber LTP unilaterally in anesthetized rats in the presence of the NMDA receptor antagonist (+/-)-CPP, while the contralateral hippocampus was not potentiated and was used as a control. Three hrs after LTP induction, mRNAs were isolated from both hippocampi. A cDNA library was generated from the potentiated hippocampal tissue. This library then was subtracted from cDNAs amplified *in vitro* from the contralateral, non-potentiated, control hippocampus. This subtraction yielded 44 subtracted clones. Partial screening of these clones for LTP-association using Northern blot analysis revealed one clone preferentially hybridized to the same gene expressed in a hippocampus of a separate rat in which mossy fiber LTP was induced. We termed this gene LAG-19 for LTP-associated gene 19. Restriction endonuclease analysis of this gene identified its size as 5600 bp. Thus far, partial DNA sequencing of LAG-19 reveals no homology to published sequences in the Genbank nucleotide database. Future analysis will focus on continued screening and on the identification of other subtracted clones. Supported by DA 04195 (JLM), NSF-DIR 9101951 (DTR), NIDA DA04795 (MRR).

552.1

IDENTIFICATION OF A NOVEL G-PROTEIN COUPLED RECEPTOR IN THE VENTRAL TEGMENTUM BY PCR. M.E. Charlton* and R.S. Duman. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine , New Haven, CT 06508.

Previously we described the PCR based identification of cDNA fragments encoding novel G-protein coupled receptors in the rat brain (Soc. Neuro. 19 Abstract #634.8). PCR of cDNA derived from the ventral tegmentum with degenerate primers to the third and sixth transmembrane domains (Libert *et. al.*, Science 244:569, 1989) resulted in the amplification of several previously identified (e.g. beta$_2$-adrenergic, dopamine D_2, neuromedin K) as well as several novel PCR clones. One of these clones, VT 15-20, was chosen for further study. Sequence analysis revealed that this cDNA fragment is most homologous at the nucleotide and amino acid levels to the IL-8 receptor (approximately 40%). Northern blot analysis of rat spleen RNA detected a single major RNA species of approximately 2.0 kb in length. The relative abundance of this clone was determined by ribonuclease protection assay: levels of VT 15-20 mRNA were highest in the spleen, lung, heart, and liver with lower levels in the thalamus, pons, frontal cortex, and hippocampus. In addition, VT 15-20 mRNA was also detected in neuroblastoma (locus coeruleus-like) and hemopoietic (HL-60) cell lines. The cellular distribution pattern of this clone is being determined by *in situ* hybridization. We are currently in the process of isolating a full length clone encompassing VT-15-20 from a rat spleen cDNA library. Further study will include characterizing the ligand specificity, pharmacology, and regulatory properties of the receptor.

552.2

IDENTIFICATION OF A PUTATIVE CHEMOKINE RECEPTOR FROM RAT LOCUS COERULEUS BY PCR. S. R. Marsh*, M. E. Charlton, and R. S. Duman. Laboratory of Molecular Psychiatry, Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Studies in our laboratory have examined the molecular and cellular determinants which underlie the function of the locus coeruleus (LC), the major noradrenergic nucleus in brain. One approach is to identify and characterize novel G protein-coupled receptors from this brain region using PCR. cDNA derived from the LC, and other discrete regions of rat brain, was PCR amplified using a set of degenerate primers derived from the conserved third and seventh transmembrane-spanning domains of the IL-8, δ-opioid, angiotensin II, somatostatin, substance P and substance K receptors. The resulting PCR products (ranging from 300 to 1000 bp) were then subcloned and identified by sequencing. Some of these clones corresponded to previously published sequences, such as the SSTR-2 receptor and a D4-like receptor. Another clone (LCR3-12), amplified from LC cDNA, shared properties with other G protein-coupled receptors and was most homologous (52% identical over a 100 amino acid stretch) to the human C-C chemokine receptor type 1 (Neote *et al.*, Cell 72, 415, 1993). RNAse protection analysis showed high levels of LCR3-12 mRNA in rat spleen and lung, with lower levels in kidney, liver, pons, thalamus, midbrain, olfactory bulb, and cerebellum. Northern blot analysis revealed two primary transcripts of approximately 1.7 and 4.8 kb. Further studies are in progress to isolate a full length cDNA clone for LCR3-12, and then to investigate its ligand-binding and functional properties.

552.3

MOLECULAR CLONING OF A NOVEL CANDIDATE G PROTEIN-COUPLED RECEPTOR FROM RAT BRAIN. Z. H. Song*, W. S. Young, M. J. Brownstein and T. I. Bonner. Lab. of Cell Biology, NIMH, NIH, Bethesda, MD 20892

To clone novel G protein-coupled receptors, degenerate primers designed from the regions conserved between SKR6 (a cannabinoid receptor) and edg-1 (a published orphan receptor) yet distinguishing them from other receptors, were used to amplify the DNA prepared from a rat cerebral cortex cDNA library. rCN3, one of the clones generated by PCR, had amino acid sequence features typical of G protein-coupled receptors. Using a radiolabeled PCR fragment of rCN3 as a probe, a full-length cDNA was isolated from the rat cerebral cortex library. This 1.8 kb full-length cDNA of rCN3 contained a 1098 base pair open reading frame, encoding 363 amino acid residues. Sequence analysis demonstrated that rCN3 possesses a number of structural characteristics of rhodopsin-like G protein-coupled membrane receptors, including seven putative hydrophobic transmembrane domains and six connecting loops. Comparing the sequence of rCN3 with other G protein-coupled receptors revealed that rCN3 is similar to GPCR1 and GPCR21 (61% amino acid identity in transmembrane region one through carboxy-terminal), two other published orphan G protein-coupled receptors. Therefore, rCN3, together with GPCR1 and GPCR21, may belong to a novel G protein-coupled receptor subfamily with identical or closely related endogenous ligand. Northern and in situ hybridization experiments demonstrated that rCN3 mRNA is present in the rat brain, with particularly high levels of expression in striatum and retrosplenial cortex. The precise physiological role of rCN3 awaits elucidation of its ligand and signal transduction pathways.

552.4

CLONING, CHROMOSOMAL LOCALIZATION AND EXPRESSION MAPPING STUDIES OF HUMAN GENES ENCODING G PROTEIN-COUPLED RECEPTORS. M.Heiber, T.Nguyen, J.Docherty, H.Heng, R.Cheng, A.Marchese, G.Shah, X.Shi, L-C. Tsui, S.George and B.O'Dowd* Addiction Res. Found. and Dept. of Pharm., Univ. of Toronto, and The Research Institute, Hospital for Sick Children, Toronto, ON, Canada M5S 1A8

Following the cloning of the opioid receptors (OR) μ, κ and δ, we searched for related receptors. We used oligonucleotides based on the OR to amplify human genomic DNA using PCR, and we have isolated fragments encoding 11 unique GPCR genes. We have obtained full length clones containing 9 of these genes and each of the encoded receptor structures most closely resemble peptide binding GPCR. Two genes designated #11 and #12 shared 70% identity with each other and each shared 40% identity in the transmembrane (TM) regions of the opioid receptors. Gene #11 was mapped to C20 (q13.3) and gene #12 to C10 (q11.2-q21.1). mRNA transcripts from these genes are expressed in the frontal cortex, and in-situ hybridization histochemistry revealed expression of gene #12 in the human pituitary and mouse brain demonstrated expression in the PVN of hypothalamus. Gene #33, mapped to C15 (q21.6), encoded a receptor with identity to the OR in the TM regions. Northern analysis revealed #33 transcripts were located in the hippocampus, and in situ analysis in the rat brain revealed expression in the dentate gyrus and the CA 1-3 regions of hippocampus. Gene #64, mapped to C6 (q21-22.1), was expressed in the human caudate. Gene #14 mapped to C19 (q13.2-13.3), gene #18 to C17 (q21.1 to 21.3), but neither gene was expressed in brain regions. Gene #53 was mapped to C1 (p36). We predict that the discovery of these receptors represent and advance in the understanding of the functions of peptide transmitter systems in brain.

552.5

CHARACTERIZATION OF THE RAT CEREBELLAR ENDOTHELIN$_B$ (ET$_B$) RECEPTOR USING THE NOVEL AGONIST [^{125}I]BQ3020 M.F. Jarvis*, A.A. Assal, and G.E. Martin. Rhône-Poulenc Rorer Central Research, Collegeville , PA 19426

The complex binding of radiolabeled endothelin isopeptides (ET-1, ET-2, ET-3) in rat brain has indicated the existence of multiple affinity subtypes of the ET$_B$ receptor. Vasodilation has been associated with an interaction with a "super high affinity" ET receptor subtype whereas vasoconstriction is mediated via a different "high affinity" receptor subtype. Recently, a linear peptide fragment of ET-1, N-acetyl-[Ala^{11,15}]ET-1(6-21) (BQ3020) has been identified as a potent and ET$_B$-selective agonist. The present studies were conducted in order to characterize the binding of [^{125}I]BQ3020 to the ET$_B$ receptor in rat cerebellum. [^{125}I]BQ3020 (0.1 nM) bound with high specificity (90% of total) and selectivity for the ET$_B$ receptor. ET-1, ET-2, and ET-3 inhibited 0.1 nM [^{125}I]BQ3020 binding with equivalent affinity (K$_i$ values = 55 - 110 pM). The sarafotoxins S6$_a$, S6$_b$, and S6$_c$ also potently inhibited [^{125}I]BQ3020 binding (K$_i$ values = 55 - 2000 pM). The ET$_A$-selective antagonist, BQ123 (10 μM) did not significantly inhibit [^{125}I]BQ3020 binding. Ligand saturation studies indicated that [^{125}I]BQ3020 bound a single class of recognition sites with very high affinity (Kd = 31 pM) and limited capacity (Bmax = 570 fmol/mg protein). High affinity 0.1 nM [^{125}I]BQ3020 was reduced by 40-50% in the presence of 1 mM guanine nucleotides. ET-1 was found to produce a biphasic inhibition curve in competing for 0.5 nM [^{125}I]BQ3020. This ET-1 inhibition curve was made monophasic and shifted to the right in the presence of 1 mM GTP-γ-S The guanine nucleotide sensitivity of [^{125}I]BQ3020 binding suggests that the different functional consequences of ET$_B$ receptor activation may be mediated by different affinity states of the receptor.

552.6

CHARACTERIZATION OF CORTICOTROPIN RELEASING FACTOR RECEPTORS FROM HUMAN SMALL CELL LUNG CARCINOMA (SCLC) CELLS. K.D. Dieterich, D.E. Grigoriadis and E.B. DeSouza*, Neurocrine Biosciences, Inc. San Diego, CA 92121.

Numerous peptides, growth factors and receptors have been identified in small cell lung carcinoma (SCLC) cells which account for 25 % of bronchogenic neoplasms. We examined the radioligand binding, second messenger and mRNA characteristics of corticotropin releasing factor (CRF) receptors in a variety of human SCLC cell lines and compared their characterisitcs to CRF receptors in the mouse pituitary tumor cell line AtT-20.The human SCLC cell lines NCI H-69, H-82, H-146, H-209, H-345, H-446, and H-510A all demonstrated specific [^{125}I]Tyr^0-ovine CRF ([^{125}I]oCRF) binding which was linear with increasing protein concentrations, saturable, reversible and of high affinity. NCI H-82 showed the highest level of specific [^{125}I]oCRF-binding (about 60 % of total binding). Scatchard analysis revealed a single class of binding sites in NCI-H82 and AtT-20 cells with K$_D$ values of 263 ± 48 and 285 ± 75 pM and B$_{max}$ values of 74 ± 7 and 70 ± 13 fmol/mg protein, respectively. [^{125}I]oCRF-binding sites on NCI-H82 cells demonstrated pharmacological rank order of potencies for a variety of CRF-related peptides comparable to those seen in AtT-20 cells. Ovine, bovine and rat/human CRF all inhibited the binding with similar K$_i$ values (1 nM) while the peptide antagonist α-helical oCRF had a K$_i$ value of 27-49 nM. VIP, GHRH, secretin and the de-amidated peptide bovine CRF(1-41)OH did not inhibit the binding. [^{125}I]oCRF-binding was inhibited by guanine nucleotides suggesting a coupling of receptors to guanine nucleotide binding proteins. CRF stimulated cAMP production in NCI-H82 and AtT-20 cells with EC$_{50}$ values of about 2 nM. Northern blot analysis of total RNA revealed the presence of a 2.6kb mRNA band in NCI-H82 cells corresponding to the CRF-R1 variant of the recently cloned human CRF receptor. In summary, the data demonstrate the presence of CRF receptors in SCLC cells with kinetic, pharmacological, second messenger and mRNA characteristics comparable to those in pituitary and brain and suggests the utility of this cell line for CRF-related studies.

552.7

PHARMACOLOGICAL CHARACTERIZATION OF RAT AND HUMAN CORTICOTROPIN- RELEASING FACTOR (CRF) RECEPTORS EXPRESSED IN STABLE CELL LINES D.E. Grigoriadis*, C.W. Liaw, T. Oltersdorf and E.B. De Souza, Neurocrine Biosciences Inc., San Diego, CA 92121.

Over the past decade, substantial evidence has accumulated from both laboratory and clinical studies implicating corticotropin-releasing factor (CRF) as a physiological mediator of stress responses or stress-induced disorders. Recently, using an expression cloning approach a CRF receptor cDNA was isolated from a human ACTH-secreting pituitary adenoma. Full length human and rat CRF receptor c-DNAs were subcloned in the mammalian expression plasmid pCDM-7 amp containing the human cytomegalovirus (CMV) promoter or a modified version of pCDM-7 in which the CMV promoter was replaced with the RSV promoter. These constructs were co-transfected with pSV-2neo into two different cell backgrounds, COS-7 and LtK-, which do not normally express CRF receptors, using the calcium phosphate precipitation method resulting in the generation of stable cell lines. CRF receptors expressed in these cell lines demonstrated reversible, saturable, high-affinity binding to CRF with the pharmacological and functional characteristics comparable to those found in a variety of animal or human tissues. $[^{125}I]$oCRF bound to a single population of binding sites in LtK- cells transfected with human and rat CRF receptors with apparent affinities (K_D) of 130 and 168 pM and receptor densities of 97 and 588 fmol/mg protein respectively. The pharmacological rank order of potencies of CRF and related peptides was identical to the established profile for the CRF receptor in the rat frontal cortex. In addition, CRF receptors transfected into these cell lines were coupled to a guanine nucleotide binding protein and when incubated with CRF, could stimulate the production of cAMP from these cells with an EC_{50} of approximately 1 nM. Furthermore, CRF-stimulated cAMP production could be inhibited by specific and selective peptide antagonists to the CRF receptor. The stable expression of CRF receptors in clonal cell lines represents an unique opportunity for the discovery of non-peptide agonists or antagonists to the human receptor for the possible treatment of CRF-mediated disorders.

552.8

COLORIMETRIC ASSAY FOR RAPID SCREENING OF CRF RECEPTOR LIGANDS C. W. Liaw, D. E. Grigoriadis, E. B. De Souza, T. Oltersdorf* Neurocrine Biosciences, Inc. San Diego, CA 92121.

Corticotropin releasing factor (CRF) is the primary regulator of ACTH secretion and other pro-opiomelanocortin-derived peptides from the anterior pituitary gland. The receptor for corticotropin releasing factor is known to be coupled to G_S and transduces its signal through stimulation of cAMP production. Here we describe the characterization of several stable rat and human CRF receptor-expressing LVIP2.0Zc cell lines which also contain an exogenous cyclic AMP responsive β-galactosidase reporter gene construct. CRF receptor activity was assayed by measuring the induction of β-galactosidase in response to CRF. Rat/human and bovine CRF stimulated β-galactosidase activity in a dose dependent manner with EC_{50} values of ~0.1 nM; the biologically weak deamidated analog of bovine CRF was ~ 500-fold less potent. The pharmacological specificity of the interaction was further demonstrated by the dose-dependent inhibition of CRF-stimulated β-galactosidase activity by CRF receptor antagonist, [D-Phe^{12},Nle^{21,38}, Ala^{32}] r/hCRF(12-41). The magnitude of the maximal response to CRF varied among individual cell lines. This variation was independent of the level of CRF receptor expression, but reflected differences in the intrinsic activity of adenylate cyclase. In contrast to most cyclic AMP assay systems, the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine decreased the CRF induced β-galactosidase activity when used in the context of the assay regimen described here. Since the assay can be easily performed in a high throughput 96-well plate format, these cell lines provide an efficient way for the identification of CRF receptor agonists and antagonists.

552.9

PRESENCE OF CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS ON HUMAN PERIPHERAL BLOOD CELLS AND MONOCYTE LINES. P.J. Reynolds, D.E. Grigoriadis, T.W. Lovenberg*, T.T. Wong, D.P. Behan, E.B. DeSouza, and P.J. Conlon, Neurocrine Biosciences, Inc. San Diego, CA 92121.

Corticotropin releasing factor (CRF), a 41-amino acid peptide, plays a significant role in integrating the stress-related and inflammatory responses to immunological agents such as viruses, bacteria, or tumor cells through its coordinated actions in the nervous, endocrine and immune systems. In addition to its effects on the hypothalmus-pituitary-adrenal (HPA) axis, CRF has recently been reported to exacerbate inflammatory responses. *In vitro* CRF has been described to enhance pro-inflammatory cytokine production. *In vivo,* anti-CRF antibody treatment was shown to lessen the recruitment of cells to an inflammatory site. In an effort to better understand the mechanism(s) of action whereby CRF may be involved in inflammation, CRF-receptor binding studies were carried out on immune cell populations. Specific, saturable high affinity binding of CRF to human peripheral blood cells was observed. CRF-receptors were also detected on the human monocyte-like cell lines THP-1 and U937. No appreciable CRF binding was seen on HuVec cells and the human promyelocytic cell line HL-60. The presence of the CRF receptor was confirmed on peripheral blood mononuclear cells and monocyte lines using RT-PCR. Given that peripheral mononuclear cells express CRF receptors, further studies are underway to determine which immune cell populations express CRF receptors, and whether these receptors are modulated during an immune response; and if so, is receptor modulation due to pro-inflammatory cytokine production? These studies are likely to further our understanding of the role of CRF in inflammation.

552.10

CORTICOTROPIN RELEASING FACTOR-BINDING PROTEIN IN HUMAN BRAIN: IDENTIFICATION AND CHARACTERIZATION IN ALZHEIMER'S DISEASE. D.P. Behan, J.C. Troncoso, N. Ling*, and E.B. De Souza, Neurocrine Biosciences, Inc. San Diego, CA 92121 (D.P.B. and E.B.D.S.) and Neuropathology Lab, Johns Hopkins University School of Medicine, Baltimore, MD 21205 (J.C.T.).

Corticotropin releasing factor (CRF), a 41-amino acid peptide, has been implicated in cognitive function and in neurologic illness such as Alzheimer's disease (AD). Overall, large deficits in CRF and a reciprocal up-regulation of CRF receptors in brain areas affected in AD have been observed. A membrane-associated form of a binding protein with high affinity for CRF (CRF-binding protein; CRF-BP) and the ability to inactivate CRF has recently been identified in brain areas (cerebral cortex, amygdala and hippocampus) that are affected in AD and are involved in learning and memory processes. The present study was designed to characterize and quantify CRF and CRF-BP in postmortem brain samples of Alzheimer's patients and age-matched controls. CRF-BP levels were measured in NP-40 solubilized membranes of occipital, parietal, frontal and temporal cerebral cortex using a specific two-site ligand immunoradiometric assay. CRF-BP levels in cerebral cortex ranged from 300 - 700 ng/g wet weight and approximately 70% - 85% of the total CRF-BP activity was membrane associated. Pharmacological characterization of the CRF-BP activity solubilized from cerebral cortex revealed a rank order of binding potency [human CRF ~ α-helical ovine CRF(9-41) > human CRF (6-33) >> ovine CRF] identical to that seen for recombinant human CRF-BP. As previously reported, CRF levels were decreased (>50% reductions) in all areas of the cerebral cortex examined in AD as compared to age-matched controls; no significant alterations in the pharmacological characteristics or levels of CRF-BP were noted in the same tissue samples between groups. In summary, these data provide evidence for the presence of CRF-BP in normal and AD brain tissue and suggest that deficits in CRF may be due to decreased CRF synthesis and/or increased degradation rather than neuronal loss or that CRF-BP is mainly confined to a separate group of non-CRF neurons which are spared in dementia.

552.11

EVIDENCE OF FUNCTIONAL GROWTH HORMONE-RELEASING FACTOR RECEPTORS IN RAT HYPOTHALAMUS. L. Boulanger* and P. Gaudreau. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center and University of Montreal, Montreal, Canada, H2L 4M1.

Recent reports suggest the existence of GRF receptors mediating endocrine and behavioral actions of this peptide, in the hypothalamus. Our goals were to characterize GRF binding sites in hypothalamus homogenates and to determine if this binding leads, as in the anterior pituitary, to the stimulation of adenylate cyclase (AC) activity. In our assay conditions (Tris buffer pH 7.4 containing $MgCl_2$, EDTA, sucrose, diprotine and BSA, ≈ 200 μg prot./tube, 1 h, 4°C), [^{125}I-Tyr^{10}]hGRF(1-44)NH_2 was stable and specific binding represented 40-50% of the total binding. This binding was temperature and time-dependent, reversible and saturable. The affinity was hGRF(1-29)NH_2 > Nα-Ac-[His^1,D-Arg^2] >> [Ala^1] ≈ [Ala^6] ≈ [Ala^{10}]. VIP and PACAP were inactive at 0.1 μM. In our AC assay conditions (Lefrançois and Gaudreau, J. Chromatogr., 1993), hGRF(1-29)NH_2 elicited a concentration-dependent production of cAMP. Although the efficacy of GRF was much lower in the hypothalamus than in the pituitary, its potency was similar in both tissues (EC_{50} ≈ 40 nM). The antagonist, Nα-Ac-[His^1,D-Arg^2]GRF(1-29)NH_2 did not stimulate AC activity, but substantially inhibited the response to hGRF(1-29)NH_2. Although these results support the existence of functional hypothalamic GRF receptors, they still have to be localized to establish their involvement in the regulation of GRF secretion and control of appetite.

552.12

CELLULAR UPTAKE OF INTRACEREBROVENTRICULARLY INJECTED ANTISENSE OLIGODEOXYNUCLEOTIDES INTERNALLY LABELED WITH BIOTIN OR DIGOXIGENIN. F. Yee*, H. Ericson, D.J Reis and C. Wahlestedt. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell University Medical College, New York, NY 10021.

Recently, we have demonstrated that intracerebroventricular (*icv*) administration of antisense oligodeoxynucleotides (ODNs) specific for the rat neuropeptide Y (NPY) - Y1 receptor and N-methyl-D-aspartate (NMDA)-R1 receptor subunit resulted in decreases of the respective binding levels (Wahlestedt, *et al., Science.* 259:528, 1993; Wahlestedt *et al., Nature.* 363:260, 1993). In addition, treatment with the NPY-Y1 antisense ODNs was shown to produce anxiety-like effects, and treatment with the NMDA-R1 antisense ODNs reduced focal ischemic infarctions in rats.

In contrast, the intracellular fate of *icv* injected ODNs has not been well characterized. In the present study, we used biotin or digoxigenin to label the rat NPY-Y1 and NMDA-R1 receptor antisense ODNs at an internal position, and examined their cellular uptake at several timepoints (*i.e.* 15 min, 1, 2, 4 and 6 hrs) after *icv* administration. Because the biotin antibody detected endogenous biotin in addition to the biotinylated antisense ODNs, we used digoxigenin to label the antisense ODNs. We found the most intensely stained cells near the injection site, along with labeling of the vasculature and surrounding cells, which were mainly in ventral cortical regions.

The stability of the *icv* injected antisense ODNs were examined by performing recovery experiments, and a portion of the intact digoxigeninated antisense ODNs were detected in the cytoplasmic and nuclear fractions of rat brain extracts.

552.13

EXPRESSION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE RECEPTOR SUBTYPES IN SUPERIOR CERVICAL GANGLION NEURONS. K. M. Braas* and V. May. Dept. of Anatomy & Neurobiology, Univ. of Vermont Coll. of Medicine, Burlington, VT 05405.

Pituitary adenylate cyclase activating polypeptides (PACAP) belong to the vasoactive intestinal polypeptide (VIP)/secretin/glucagon family of bioactive peptides. The PACAP type I receptor is specific for PACAP, while the type II receptor displays similar affinity for PACAP and VIP. Splice variants of the type I PACAP receptor differentially stimulate adenylate cyclase and phospholipase C. The PACAP peptides exerted potent regulation of superior cervical ganglion (SCG) neuropeptide Y (NPY) and catecholamine (CA) expression. PACAP38 and PACAP27 stimulated sustained secretion of NPY and CA from cultured rat SCG neurons; neurosecretory responses to PACAP were approximately 100-fold more potent than to VIP. To identify the population of responsive SCG neurons, binding was determined using biotinylated PACAP38. Over 60% of the SCG neurons were labeled and may be responsive to PACAP regulation. To distinguish the expression of type I PACAP receptor isoforms, the presence or absence of either one or two 84 bp cassettes was examined using reverse transcription and polymerase chain reaction (RT-PCR) with mRNA from fetal, neonatal, adult and cultured SCG cells. The predominant molecular form of PACAP receptor mRNA identified by RT-PCR contained one 84 bp cassette; further hybridization studies are necessary to identify the specific insert. No PCR products were observed using primers specific for the VIP (type II) receptor. These studies demonstrate that SCG neurons express specific type I PACAP receptor mRNA, bind PACAP and respond to PACAP stimulation. Supported by HD-27468.

552.14

DISTINCT RECOGNITION RESIDUES FOR BENZODIAZEPINE AND PEPTIDIC CCK RECEPTOR ANTAGONISTS DETERMINED BY SITE DIRECTED MUTAGENESIS. E. Malatynska, R.J. Knapp, D.Stropova, P. Peterson, E. Varga, H.I. Yamamura and W.R. Roeske*. University of Arizona Dpt. of Pharmacology, Tucson, AZ 85724.

CCK-B receptor antagonists are important as anxiolytics, neuroleptics antinociceptive and antisatiety drugs. This abstract describes the possible recognition sites for CCK receptor antagonists with different chemical structure. The rat CCK-B receptor cDNA was obtained by PCR using primers derived from the sequence first described by Wank et al. (PNAS 89:8691:, 1992). The cDNA was cloned in the PCRII vector and then transferred to pSV-Sport1 expression vector. COS-7 cells transfected with this recombinant vector expressed high levels (Bmax = 210 fmol/mg protein) of the CCK-B receptors. The expressed receptor was fully functional since SNF 8702 (CCK-B selective ligand) increased free cytoplasmic calcium levels with an A50 value of 66 pM. Here we used the recombinant rat CCK-B/pSV-Sport1 vector to produce single aminoacid mutation of Val^{349} residue located on VIth TMD to Leu using the unique site elimination method. Selectivity of the benzodiazepine derivatives L365,260 for CCK-B and L364,718 for CCK-A receptors results from the presence of Val^{349} or Leu at this position, respectively (Beinborn et al., 1993). It was tested here whether this residue is also important for peptidic CCK-B receptor antagonist. For Val^{349} CCK-B receptor the IC-50 values of L364,718 , L365,260 and PD134308 (peptoid) were as follows 617 nM, 25.3 nM and 18 nM, respectively. These values agree with CCK-B receptor antagonists profile. For mutant Leu^{349} CCK receptor the IC-50 values of L364,718 (96.4 nM) and L365,260 (424.2 nM) were inverted while the IC-50 value for PD134308 (26 nM) was unchanged. Thus, the antagonist profile for the benzodiazepine derivatives switch to that of CCK-A receptor while the affinity for PD134308 remains as that of CCK-B receptor. The conclusion of this study is that benzodiazepine and peptidic CCK antagonists have distinct recognition residues.

552.15

XENOPUS OOCYTES INJECTED WITH RAT BRAIN POLY (A^+) RNA EXPRESS ADRENOMEDULLIN-SENSITIVE RECEPTORS. Y. Uezono*, H. Kobayashi#, T. Nagatomo@, H. Yamashita@, A. Wada# and F. Izumi. Depts. of Pharmacol. and @1st Physiol., Sch. of Med., Univ. of Occup. and Environ. Health, Kitakyushu 807; #Dept. of Pharmacol., Miyazaki Medical College, Kiyotake 889-16, Japan.

Adrenomedullin (AM), a 52 amino acid peptide, has been recently discovered from human pheochromocytoma which has a hypotensive effect via an elevation of cAMP. AM is localized in several tissues other than pheochromocytoma such as brain, lung, kidney and structurally homologous to the calcitonin gene-related peptide (CGRP) and amylin, the peptides known to exert their effects via increasing cAMP. Little is known, however, about the receptors for AM. We have reported that the protein encoded by the cystic fibrosis transmembrane conductance regulator gene (CFTR), the Cl^- channel activated by A-kinase, can be used as a sensitive reporter for cAMP changes in *Xenopus* oocytes (Receptors and Channels 1:233 (1993)). In the present study to see whether brain expresses the AM receptors, we injected cRNA for the CFTR along with poly (A^+) RNA from rat brain into oocytes. Three days after injection, rat AM resulted in a concentration-dependent increase in Cl^- current via CFTR channels with the maximal response at 10^{-7}M. In the same oocyte, both CGRP and amylin 10^{-7}M also caused CFTR currents. The potencies for the CFTR currents of the three peptides were CGRP > AM >> amylin. The present results suggest this is a novel method for functional expression cloning of the receptors; although it is not known whether all three peptides have their own receptors or share several types of common receptors.

552.16

CLONING THE GUINEA PIG CALCITONIN RECEPTOR. A. Sarkar* and I.M. Dickerson. Dept. Phys. and Biophys., Univ. Miami Sch. Med., Miami, FL.

Calcitonin is a 32 amino acid peptide hormone which interacts with both central and peripheral calcitonin receptors. The calcitonin receptor is a member of the class of 7-transmembrane G protein-coupled receptors. The deduced amino acid sequences of rat, human and porcine calcitonin receptors were aligned. From this alignment, degenerate oligonucleotide primers were designed for the polymerase chain reaction (PCR). The primers were denoted CTR-1, CTR-2, and CTR-3. CTR-1 is the upstream PCR primer located in the proposed N-terminal extracellular domain. CTR-1 is a 27mer and is 2048-fold degenerate. CTR-2 is the downstream PCR primer located just prior to the first transmembrane domain, and is a 131,072-fold degenerate 27mer. CTR-3 is the primer for reverse transcription, located between the proposed transmembrane loops II and III and is a 30mer with 2048-fold degeneracy. Reverse transcription was performed on guinea pig kidney and cerebral poly A^+ mRNA using CTR-3 as a primer. An aliquot of the reverse transcription reaction was used as template for the PCR reaction in which CTR-1 and CTR-2 served as primers. The resulting PCR products were cloned into the plasmid pAMP-1 (GIBCO-BRL). DNA was isolated from 24 colonies, and digested with restriction enzymes that flank the cloning region. The subcloned PCR products were the expected size of 222bp. Twenty-four individual subclones representing kidney and cerebral PCR amplified products were selected for sequencing. DNA sequencing revealed that all clones were identical, with >90% identity to previously cloned calcitonin receptors. This indicates that the guinea pig, like the human and pig, may contain a single calcitonin receptor isotype, unlike the rat, which contains two calcitonin receptors isotypes. Our subcloned PCR product is denoted pgp.CTR. Northern Blots probed with pgp.CTR insert identified a 4.0 Kb RNA, in agreement with the size reported for the porcine and human calcitonin receptor RNA. The sequence of the calcitonin receptor will be compared to the known calcitonin receptors, and the pharmacological properties of the guinea pig calcitonin receptor will be determined.

552.17

TRANSIENT CEREBRAL ISCHEMIA INDUCES OVEREXPRESSION OF OXYTOCIN RECEPTORS IN THE GERBIL HIPPOCAMPUS. M. Dubois-Dauphin*, C. Bouras, and P. Vallet. Dept. of Physiology, University Medical Center, 1211 Geneva, Dept. of Psychiatry, Div. of Morphology, 1225 Geneva, Switzerland.

Using an iodinated oxytocin (OT) antagonist and light microscopy autoradiography, oxytocin receptors were detected in the brain of adult male gerbils following transient cerebral ischemia (6 min.). Either immediately or 10, 30, 100 hrs later, the animals (3/series) were reanesthetized and their brain quickly removed and frozen in isopentan. Section were incubated with 0.05 nM of [^{125}I]-labelled-d(CH_2)$_5$[Tyr(Me)^2, Thr^4, Tyr-NH_2^{9}] OVT with or without an excess of OT in order to determine the non specific binding.
In control animals, specific binding occurred in various regions of the brain (bed nucleus of the stria terminalis, amygdaloid nuclei, anterior periventricular hypothalamus, subiculum, dorsal motor nucleus of the vagus nerve). In the hippocampus, binding sites were localised in *stratum oriens* and *stratum radiatum*. The labelling covers uniformly these layers suggesting that oxytocin receptors could endow glial cells rather than interneurons. Hippocampal pyramidal cell layers were devoid of binding sites. After 10-100 hours of survival following ischemia, a marked increase of the density of oxytocin binding sites was detected in the *stratum oriens* dorsal to the CA1 pyramidal layer in the anterior hippocampus. A delayed neuronal death with a drastic loss of neurons was observed in the CA1 layer after 48 hours of survival following ischemia. Concomitantly, an increase of GFAP immunoreactivity was observed in this region and in the *stratum oriens* as well. Thus, the increase in the density of oxytocin receptors in the *stratum oriens* following transient cerebral ischemia appears to be associated with glial cells and could be related to neuronal death occurring in the pyramidal CA1 layer.

552.18

PHARMACOLOGICAL CHARACTERIZATION OF BRAIN OXYTOCIN RECEPTORS (OTRs) IN RATS. L.M. Flanagan-Cato* and S. J. Fluharty, Depts. of Psychology, Animal Biology, Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Phil., PA 19104

Brain OTRs have been implicated in a variety of behaviors, e.g. sexual and parental. Previous characterizations of peripheral OTRs have suggested diverse binding properties of OTRs in reproductive tissues. Little is known, however, about the functional properties of rat brain OTRs. In the present studies, the pharmacological profile of rat brain OTRs was assessed. Brains from 10-day old rats were homogenized and the membranes prepared for incubation with the radioligand ^{125}I-ornithine vasotocin (^{125}I-OVTA). Saturation isotherm studies with Scatchard analysis determined the density and affinity of brain ^{125}I-OVTA binding sites (B_{max}=24 fmol/mg protein; K_D=50 pM). The specificity of binding was confirmed by competition with unlabelled OT (K_D=2.01$\pm$0.08 nm, n=5). The rank order potency of brain OTR agonist binding was OT > AVP > thr^{4}gly^7-OT(n=3). OT binding was best fit with a two-site model, with approximately 50% high affinity sites (K_D=0.19 pM), and 50% low affinity sites (K_D=10.80 nM). Preliminary studies using nonhydrolyzable analog of GTP, GTPμS, resulted in monophasic OT binding with only the low affinity component, which indicates that brain OTRs, like their peripheral counterparts, are coupled with G-proteins. * Supported by NS23986 and MH43787.

552.19

HUMAN Y-79 RETINOBLASTOMA CELLS EXPRESS PACAP RECEPTORS. M.C. Olianas*[1], M.G. Ennas[2], S. Petruzzelli[3] and P. Onali[1], Dept. Neurosciences[1], Dept. Cytomorphol.[2], Inst. Internal Medicine[3], University of Cagliari, Cagliari, Italy.

Recently, the presence of receptors for pituitary adenylate cyclase activating peptide (PACAP) has been demonstrated in the retinas of different mammalian species (Onali & Olianas, Brain Res. 641, 132, 1994). In the present study we report that in human Y-79 retinoblastoma cells PACAP-38 and PACAP-27 are potent stimulators of adenylyl cyclase activity with EC50 values of about 20 and 150 pM, respectively. VIP is much less potent (EC50 = 40 nM) than PACAPs, whereas secretin (1 μM) is inactive. The adenylyl cyclase stimulation by PACAP-38 is potently counteracted by the specific PACAP receptor antagonists PACAP 6-38 and PACAP 6-27. Cytochemical studies using biotinylated PACAP-38 demonstrate the diffuse presence of PACAP binding sites in Y-79 cells. These results support the idea that PACAP may act as a neurotransmitter in the retina and indicate that Y-79 retinoblastoma cells may represent an useful model for studying the possible effects of the peptide on retinal cell function and differentiation.

552.20

VIP AND PACAP BIND WITH HIGH AFFINITY TO HUMAN GLIOBLASTOMA CELLS. T.W. Moody', S. Jakowlew, P.Chung, M. Fridkin and I. Gozes. Biomarkers and Prevention Research Branch, National Cancer Institute, Rockville, MD 20850; Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel; and Department of Chemical Pathology, Sackler School of Medicine, Tel Aviv, Israel.

Human glioblastoma cells contain peptide (Bombesin/GRP) (BB/GRP) and growth factor (epidermal growth factor) (EGF) receptors. The actions of BB and EGF are reversed by synthetic antagonists such as (Psi[13,14], Leu[14])BB and monoclonal antibody (mAb) 108 respectively. Here the effects of vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP) were investigated. ^{125}I-VIP bound with high affinity to U-138 cells and specific ^{125}I-VIP binding was inhibited with high affinity by VIP, PACAP and VIPhybrid (IC_{50} values of 5, 10 and 500 nM respectively). In contrast, specific ^{125}I-PACAP-27 binding was inhibited with high affinity by PACAP and PACAP(6-38) (IC_{50} = 10 and 30 nM) but not VIP (IC_{50} = 1000 nM). These data suggest that ^{125}I-PACAP may bind to PACAP type-I (PACAP) and ^{125}I-VIP may bind to PACAP type-II (VIP) receptors. Northern analysis of poly A+ mRNA from U-138 cells indicated major 5.5, 2.4 and 1.3 bands using a VIP receptor cDNA probe. VIP and PACAP elevated cAMP and the increase in cAMP caused by VIP was reversed by VIPhybrid whereas the increase in cAMP caused by PACAP was reversed by PACAP(6-38). The effects of VIP and PACAP on the growth of U-138 cells will be discussed. These data suggest that VIPhybrid and PACAP(6-38) function as VIP and PACAP receptor antagonists respectively on glioblastoma cells.

PEPTIDES: PHYSIOLOGICAL EFFECTS II

553.1

HYPOTHALAMIC AND EXTRAHYPOTHALAMIC CORTICOTROPIN-RELEASING HORMONE mRNA EXPRESSION IN COLD STRESSED RATS. S. Makino[1], K. Fukuhara[2], M. A. Smith*[3], and P. W. Gold[1]. Clinical Neuroendocrinology Branch[1], Biological Psychiatry Branch[3], NIMH; Clinical Neuroscience Branch[2], NINDS; National Institutes of Health, Bethesda, MD 20892.

Exposure to cold is a potent stimulus of the hypothalamo-pituitary-thyroid axis. Yet it is still controversial whether cold stress activates the hypothalamo-pituitary-adrenal (HPA) axis. On the other hand, the regulation of extrahypothlamic corticotropin-releasing hormone (CRH) in the stressful situations needs to be elucidated. To address these issues, we exposed rats to cold (-3°C) continuously for 0.5, 1, 3, 6, 24 hours and 5 days, and examined CRH mRNA expression in the paraventricular nucleus (PVN), central nucleus of the amygdala (CEA) and bed nucleus of the stria terminalis (BNST) by using in situ hybridization histochemistry. Plasma levels of corticosterone increased transiently at 0.5 h, then returned to normal by 1 h. CRH mRNA in the PVN and POMC mRNA in the anterior pituitary did not change up to 5 days. This indicates that cold stress may transiently induce CRH or ACTH secretion, but may not be enough to stimulate CRH or ACTH synthesis. By contrast, CRH mRNA in the CEA and dorsolateral subdivision of the BNST decreased at 6 and 24 hours exposure to cold (maximally decreased by 25% and 45% at 24 h compared to control in the CEA and BNST, respectively). These data suggest that (1) cold is a mild stressor, if any, of the HPA axis, (2) but that cold stress can modulate extrahypothalamic CRH.

553.2

CRF-INDUCED EXCITATION OF NEURONAL FIRING RATES IN LOCUS COERULEUS NEURONS IS ATTENUATED BY SYSTEMIC ADMINISTRATION OF APRAZOLAM. J.C. Landry, Z.N. Stowe, M.J. Owens, P.M. Plotsky* and C.B. Nemeroff. Laboratory of Neuropsychopharmacology, Dept. of Psychiatry and Beh. Sciences, Emory Univ. Sch of Medicine, Atlanta, GA 30322.

Corticotropin-releasing factor (CRF) neurons are believed to integrate the endocrine, autonomic, immunologic, and behavioral responses of mammals to stress. Investigations have shown that both direct and intracerebroventricular (ICV) administration of CRF excites LC neurons. Benzodiazepines (BZD) have been shown to decrease LC neuronal firing rates after direct, ICV, or systemic administration. However, only systemic administration of BZD attenuates sensory-evoked activity of LC neurons. In the present study, the effects of ICV CRF on LC neuronal firing rates and responsiveness to GABA were assessed. Furthermore, the effects of systemic administration of alprazolam on CRF-induced excitation and GABA-induced inhibition were studied. Individual extracellular neuronal recordings were obtained from spontaneously active LC neurons (n=17) in chloral hydrate anesthetized (400 mg/kg, i.p.) male rats (250-350 gm). ICV CRF (5 μg) produced an increase in firing rate that peaked at 20-30 minutes post injection; recordings were continued for up to 60 minutes. Systemic administration of alprazolam (0.25 mg/kg; [n=3]; 0.5 mg/kg, [n=5], i.p.) attenuated CRF-induced excitation. At 0.5 mg/kg of alprazolam, the CRF-induced excitation was reduced by 31.8% (n=5). Throughout the experiment, GABA (0.25 M, pH 3.7) was iontophoretically (5-30 nA) applied in a 30 second pulse every 90 seconds. All neurons studied were inhibited by direct application of GABA. Interestingly, no significant change in GABA-induced inhibition was observed either after ICV CRF or systemic alprazolam administration.

These findings are consistent with the action of systemic BZD to attenuate increases in LC neuronal firing rates associated with stimulation. The ability of alprazolam to attenuate CRF-induced excitations of LC neurons supports the hypothesis suggesting that CRF may mediate LC responses to stress and that alprazolam may exert a portion of its therapeutic effect via actions on CRF. Supported by Daland Fellowship from American Philosophical Society to ZNS and NIMH MH 42088 to CBN.

553.3

CORTICOTROPIN-RELEASING FACTOR (CRF) INCREASES HIGH-VOLTAGE ACTIVATED (HVA) CALCIUM CURRENTS IN ACUTELY DISSOCIATED NEURONS OF THE CENTRAL AMYGDALA (CeA). **B.Yu* and P.Shinnick-Gallagher. Dept. of Pharmacology & Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77555**

Since CRF and the CeA mediate certain stress responses in the brain, we tested CRF on calcium currents in CeA neurons. When held at -80 mV, HVA currents peaked at +10 mV and averaged 298pA ± 17 (n=106) in 3 mM $[Ca^{2+}]_o$. Nifedipine (5 μM) inhibited 53% ± 4 of peak current (held at -40 mV). However, not every CeA neuron possessed L-type HVA currents. ω-CTX GVIA (1-2 μM) inhibited 24% ± 5 of the peak I_{Ca}, indicating N-type currents were present, while ω-CTX MVIIC (200 nM) inhibited 30 ± 4% of the peak I_{Ca}, suggesting the presence of Q-type currents. No P-type current was recorded since ω-Agatoxin (100-200 nM) did not reduce I_{Ca}. These data suggest that the HVA calcium currents of CeA neurons are comprised of N-, L-, and Q- but not P-type of calcium currents. CRF (1-400 nM) enhanced HVA calcium currents in approximately 50% CeA neurons recorded. However, in the remaining neurons, CRF had no effect. CRF enhanced both peak and steady state HVA calcium currents. CRF (50 nM) increased the peak HVA 23 ± 4% (n=9). No shift was observed in the peak of the current-voltage relationship. The CRF-induced increase in HVA calcium currents is concentration-dependent and the estimated EC_{50} is 9.7 nM. The CRF receptor antagonist (α-helical CRF_{9-41}, 3 μM), which did not alter calcium currents itself, blocked the CRF-induced increase of HVA calcium currents. These data suggest that CRF enhances calcium currents through a receptor mechanism and could thereby facilitate neuronal communication through CeA neurons during the stress response. (supported by NS 29265).

553.4

INTRACISTERNAL (IC) CRF ANTAGONIST INCREASES GASTRIC VAGAL EFFERENT DISCHARGE (GVED) IN URETHANE-ANESTHETIZED RATS. H.P.Kosoyan, J.Y.Wei, M.Gunion* & Y.Taché. CURE/Gastroenteric Biol. Ctr., VA Wadsworth Med. Ctr. & Dept. of Med. & Brain Res. Inst., UCLA, Los Angeles, CA 90073.

We previously showed that IC CRF (21 and 63 pmol) decreases GVED by 17.4 and 24.0% respectively in rats. Urethane induced an increase of hypothalamic CRF transcripts and secretion and low basal activity of GVED. **Aim:** To assess the influence of the new CRF antagonist, [DPhe[12],Nle[21,38],C^{α}-MeLeu[37]]$rCRF_{12-41}$ given IC on GVED in urethane-anesthetized rats. **Method:** Male SD rats (280-320 g) were injected with urethane (1.5 g/kg, im) and implanted with a catheter into the cisterna magna for IC(15μl) injection. A strand of the ventral gastric branch of the vagus was cut distally to record multi-unit efferent discharge. Nerve signals were sent to a window discriminator and counted on computer. Maximum 1 min peak changes from basal GVED were calculated and expressed as Mean ± SEM peak changes from baseline. IC injections of distilled water (15 μl, pH=7.0, 37°C) and CRF antagonist (25 μg/rat) over 15-30 sec were performed at 10-35 min intervals and thereafter CRF was injected IC. **Results:** IC vehicle (n=11) had no significant effect on basal GVED (101.6 ± 1.4%, $p>0.05$) while IC injection of CRF antagonist increases GVED to 143.6 ± 3.8% (n=8). The excitatory response peaked within 15 ± 3.2 min (n=8), and lasted more than 2 h. CRF (21 pmol/rat) injected IC at 5 min, 95 ± 5 min, 125 ± 5 min and 176 ± 35 min after the CRF antagonist had no significant effect on GVED, while the injection of CRF (63 pmol/rat) at 185 ± 15 min after the CRF antagonist produced a significant inhibition of GVED to 82.2 ± 5.4 % (n=4). **Conclusions:** These results suggest that IC [DPhe[12],Nle[21,38],C^{α}-MeLeu[37]]$rCRF_{12-41}$ antagonizes both endogenous and exogenous CRF and urethane-induced tonic inhibition of GVED through brain CRF pathways. (Supported by NIH grants NS28433 & DK30110).

553.5

SOMATOSTATIN EFFECT ON GUINEA-PIG DORSAL THALAMIC NEURONS IN VITRO. R. Rai* and R. Llinás, Dept. of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Spontaneous and voltage-evoked electroresponsiveness were examined using sharp microelectrode intracellular recordings in dorsal thalamus neurons in guinea-pig slices. A few minutes following bath application of 2μM somatostatin superfusion, a characteristic increase in low-threshold calcium spike activity was noticed in specific thalamic nuclei. The results could be observed, at the spontaneous-electrical-activity level, as a marked tendency towards intrinsic oscillation, often occurring from a membrane potential similar to the initial non-oscillating level, where low-threshold activity was only observed after membrane hyperpolarization in the order of 5 to 10 mV. In other cases the increase of oscillatory activity would occur on depolarizations or hyperpolarizations of a few millivolts from the initial resting-potential level. The results suggest that such changes in the resting level of the cell, which are either too small, or in the wrong direction, to affect directly the level of voltage-dependent inactivation of the type-T calcium channels responsible for such low-threshold activity were not the decisive factor effecting the excitability changes.

Similar results were obtained by testing neuronal excitability directly, via transmembrane current pulses delivered from different holding potentials. In somatostatin the voltage dependence for low-threshold calcium-spiking initiation was found to decrease by close to 30% with reference to the normal voltage level at which low-threshold spikes are generated. The results suggest that the level at which low-threshold calcium spikes is triggered in thalamic neurons may be regulated by means other than transmembrane voltage. Somatostatin did not affect all thalamic neurons, moreover cells that responded were localized in a group probably related to somatostatin innervations.

The results indicate that neuropeptides may exercise regulation of intrinsic membrane properties onto well-defined sets of thalamic nuclei without affecting others, and so, may modulate global brain activity by acting on the thalamocortical system in a manner different from that generated by conventional synaptic input. NIH /NS13742.

553.6

SOMATOSTATIN (SS) EFFECTS ON CYTOSOLIC CALCIUM CONCENTRATION ($[Ca^{2+}]_c$) IN PC12 CELLS.

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Recent findings suggest that, among neuropeptides, SS plays an important role in the modulation of nerve cell functional activity. The control of $[Ca^{2+}]_c$ has long been recognized as a fundamental mechanism of cell activation. In the present study, the effects of SS on $[Ca^{2+}]_c$ have been studied by conventional fluorimetry in PC12 cells loaded with Fura-2 in the presence of 2mM Ca^{2+}. In all the experiments, the application of either Ca^{2+} ionophores or high K^+ concentration has been used to increase the $[Ca^{2+}]_c$. Our results can be summarized as follows: i) the application of the tetradecapeptide form of SS, SS-14 (10^{-9}-10^{-6}M), induces a dose-dependent decrease of $[Ca^{2+}]_c$; ii) similar effects are induced by the application of SS analogs (SMS 201-995; D-Trp,D-Cys-SS; D-Trp-SS; MK 678; CGP 23996); iii) pretreatment with a SS antagonist (Cyclo-SS) prevents the effects of SS-14 application. These observations suggest that SS may modulate nerve cell functional activity by a specific inhibition of Ca^{2+} channels. In addition, experiments with selective blockers of different voltage-dependent Ca^{2+} channels (D 600, ω conotoxin, GVIA, nitrendipine, nifedipine) suggest that this inhibition is mediated by Ca^{2+} channels of the L-type.

553.7

EFFECTS OF CYSTEAMINE ON REGIONAL CHOLINE ACETYLTRANSFERASE ACTIVITY IN RATS. K. Kuki, H. Kaneda, Y. Komurasaki, K. Sakai* and K. Maeda. Dept. of Psychiatry, Kobe Univ. Sch. of Med., Kobe 650, Japan.

It is well-known that somatostatin (SS), one of brain neuropeptides, is decreased in cerebrospinal fluid or postmortem brain with Alzheimer's disease. Dysfunction of cholinergic system in Alzheimer's disease is also reported. The relation between SS and cholinergic system is not obvious yet in Alzheimer's disease. In this study, we investigated the effects of cysteamine, which depleted brain SS contents, on regional choline acetyltransferase (CAT) activity in rats.

Male Wistar rats (300-350 g) were used. Cysteamine was dissolved in saline and adjusted to pH 7.4. 90, 150, 300 mg/kg cysteamine and saline for controls were injected intraperitoneously (n=5). Four hours later, rats were decapitated and the brains were dissected into the olfactory bulb (Ob), anterior cortex (Ac), temporal cortex (Tc), posterior cortex (Pc), hippocampus (Hc) and striatum (St) with Glowinski's method. SS and neuropeptide Y (NPY) contents of each region were measured by radioimmunoassy and CAT activity by Fonnum's method. Statistical analysis was performed by student t-test.

As a result, SS contents in the hippocampus were only decreased significantly (Mean ± SE of % of controls were 33.8 ± 16.2, 39.7 ± 12.9, 18.4 ± 2.4 % at 90, 150, 300 mg/kg, $p < 0.05$). However, NPY contents and CAT activity did not have changes in any regions.

Our results suggest that cysteamine may not have effects on CAT activity directly in the brain regions and the decrease of hippocampal SS contents may not, too.

553.8

SELECTIVE EFFECTS OF NEUROTENSIN IN THE PREFRONTAL CORTEX FOLLOWING DOPAMINERGIC STIMULATION IN THE NUCLEUS ACCUMBENS. D. Mcgrath*J. Arnold A. Drumheller and F.B Jolicoeur
Departments of Psychiatry and Pharmacology, Faculty of Medicine, Univ. of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4

Neurotensin has been shown to markedly reduce the behavioral hyperactivity which appears 2 hr after intra-accumbens administration of the potent dopamine receptor agonist, 2-amino-6,7-dihydroxy-1,2,3,4 tetrahydro-naphthalene (ADTN) (Jolicoeur et al, Neuropeptides 6:143,1985). In order to better characterize this finding, the effects of bilateral intra-accumbens administration of ADTN (12.5μg) on concentrations of norepinephrine (NE) dopamine (DA), its metabolites DOPAC and HVA, serotonin (5-HT) and its metabolite 5-HIAA were examined in terminal regions of dopaminergic fibers, including prefrontal cortex, amygdala, septum, globus pallidus, striatum and the nucleus accumbens itself. Following ADTN administration, neurochemical changes were assessed at 2 hr, when animals displayed a marked hyperactivity. Results indicate that intra-accumbens admin-istration of ADTN results, 2 hr later in significant decreases in concentrations of NE, DA, DOPAC and HVA as well as of 5-HT and 5-HIAA in all regions. In a follow-up experiment, neurotensin (3.5μg) was injected intraventricularly 20 min prior to the neurochemical determinations performed at 2 hr after intra-accumbens administration of ADTN. Except for the prefrontal cortex, neurotensin accentuated the observed neurochemical changes in all regions. In the prefrontal cortex, neurotensin also further reduced concentrations of NE, 5-HT and 5-HIAA; however, the peptide systematically reversed the decreases in DA, DOPAC and HVA produced by ADTN in this region. These findings suggest that: a) dopaminergic stimulation in the accumbens results in widespread and enduring neurochemical changes; and b) neurotensin appears to have a selective neurochemical action in the prefrontal cortex in these circumstances.

553.9

DOPAMINE-INDUCED INHIBITION IS ATTENUATED BY IONTOPHORECTICALLY APPLIED NEUROTENSIN IN THE NUCLEUS ACCUMBENS OF RATS - *IN VIVO* STUDY. Z.N. Stowe*, J.C. Landry, Z.L. Tang, and C.B. Nemeroff. Laboratory of Neuropsychopharmacology, Dept. of Psychiatry and Beh. Sciences, Emory Univ. Sch. of Medicine, Atlanta, GA 30322.

Neurotensin (NT), an endogenous tridecapeptide, possesses a pharmacobehavioral profile that is similar to antipsychotic drugs. Previous electrophysiological data suggest that both direct and intracerebroventricular (ICV) application of NT produces predominantly excitatory responses in dopamine (DA) neurons in the ventral tegmental area (VTA). Direct administration of NT consistently antagonized DA-induced inhibitions in the VTA. In contrast, McCarthy et al (Gen. Pharmacol. 10 331-333;1979) found that iontophoretic application of NT either had no effect or inhibited the firing rate of DA-sensitive neurons in the nucleus accumbens (NAS). The effects of iontophorectically applied NT on DA-induced inhibitions in individual neurons in the NAS were studied. Extracellular neuronal recordings were obtained from spontaneously active neurons in the NAS in chloral hydrate anesthetized (400 mg/kg, i.p.) male rats (250-350 gm). DA-sensitivity was confirmed via iontophoretic application (15-35 nA) of DA (0.1M, pH 4.0). A total of 12 neurons were included in the data analysis [percent change = -26.3±12.4]. Direct iontophoresis (25-40 nA) of NT (0.5mM, pH 6.0) did not produce any significant alteration in firing rates (n=9) [percent change = -0.46±18.7]. However, direct application of NT antagonized DA-induced inhibition in all neurons tested (n=6).

These findings provide electrophysiologic evidence that NT antagonizes the effects of DA in the NAS. These findings support the hypothesis that NT receptor agonists may prove to be useful adjuncts in the treatment of psychotic symptoms, purportedly related to excessive mesolimbic DA tone. SR 48692 was a gift from D. Gulley, Sanofi Recherche, France. Supported by the Scottish Rite Schizophrenia Research Program, a Daland Fellowship from the American Philosophical Society to ZNS and NIMH MH-39415 to CBN.

553.10

NEUROTENSIN EXCITATION OF SEROTONERGIC NEURONS IN THE RAT DORSAL RAPHE NUCLEUS IN VITRO.

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Immunohistochemical and autoradiogaphic studies have shown that neurotensin (NT) and its receptors are heavily represented in the dorsal raphe nucleus (DRN), especially in its ventral part. The purpose of the present study was to test, in brain slices containing the DRN, the influence of NT on the activity of serotonergic (5-HT) neurons. The cells were identified by the ability of the α_1-agonist phenylephrine (PE) to induce firing, and serotonin to reduce this effect. In extracellular recordings, after washout of PE, NT (10 nM - 10 μM) induced a dose-dependent increase of the firing rate of 5-HT neurons (EC_{50} ~ 150 nM; maximum effect ~ 1 μM). This response was not homogeneous since excitations were mainly observed in the ventral part of the DRN. The response to NT desensitized rapidly for some 5-HT neurons whereas it was very prolonged for others. Calyculin A, a phosphatase inhibitor, increased the desensitization of the NT response, suggesting a phosphorylation step in this phenomenon. In the presence of PE, the NT response was occluded, possibly due to shared transduction mechanisms. In intracellular recordings using KCl (2 M) electrodes, NT induced a depolarisation with little or no apparent change in membrane resistance.

In conclusion, NT has an excitatory effect on 5-HT neurons in the DRN which desensitizes rapidly in some cells; this desensitization may involve receptor phosphorylation since it is enhanced by the phosphatase inhibitor calyculin A.

553.11

NEUROTENSIN REVERSES LOCOMOTOR INHIBITION PRODUCED BY MICROINJECTIONS OF GBR-12909 INTO THE MEDIAL PREFRONTAL CORTEX.

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It has been postulated that increased dopamine (DA) activity in the medial prefrontal cortex (mPFC) exerts an inhibitory influence over DA release in the nucleus accumbens leading to a reduction in locomotor activity. In addition, it has been demonstrated that neurotensin (NT) modulates DA-stimulated cell activity in the mPFC (Beauregard, M. *et al., Neuroscience*, 47:613-619, 1992). Thus, experiments were designed to test two hypotheses: 1) that an increase of DA in the mPFC causes locomotor inhibition and 2) that NT modulates DA-influenced locomotor activity in the mPFC. Bilateral cannula guides were implanted into the mPFC in pentobarbital/chloral hydrate anesthetized LS/IBG mice. Open field behavior was monitored in 5 minute blocks for a total of 30 minutes 24 hours post surgery in an automated open field apparatus (Omnitech). The distance traveled within the first 15 minutes after injection of drug was the measure used for analysis. The subjects, naive to the apparatus, were injected bilaterally with either GBR-12909, a selective DA uptake blocker, neurotensin, or a combination of the drugs. Drugs were delivered in a volume of 100 nl per side over a 30 second period and were prepared in normal saline. GBR-12909, in doses ranging from 30 fmols to 1 nmol per side, produced a U-shaped dose response curve. A maximum inhibition of 47% of saline-injected control values was observed at a dose of 3 pmols. The highest dose tested of GBR-12909 (1 nmol) caused the animals to become significantly activated to ca. 129% of control values. Higher doses of GBR-12909 could not be tested owing to the insolubility of the drug at higher concentrations. Doses of NT from 0.01 fmols to 1 nmol were tested and found to have no dose-related effect on locomotor activity. NT, when co-injected with 3 pmols GBR-12909, dose-dependently reversed the inhibitory effect of GBR-12909. A dose of 3 pmols NT completely abolished the locomotor inhibition produced by GBR-12909. In conclusion, these results are consistent with the hypothesis that the DAergic system of the mPFC regulates locomotor activity. Furthermore, these results suggest that NT modulation of DA function in the mPFC may be important for the regulation of locomotor activity. (This work was supported, in part, by USPHS grants AA00079 and AA07330.)

553.12

NEUROTENSIN (NT), N-ACETYL-L-ASPARTYLGLUTAMATE (NAAG) AND BETA-ENDORPHIN (BE) MODULATE BASAL AND NMDA-STIMULATED [^{3}H]DA RELEASE FROM SLICES OF GUINEA PIG NUCLEUS ACCUMBENS (NAC), PREFRONTAL CORTEX (PFC) AND STRIATUM (STR).

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Dopaminergic activity is thought to be disturbed in schizophrenia; positive symptoms may be associated with hyperactivity in nucleus accumbens, while negative symptoms may be due to hypoactivity in frontal cortex. The striatum, although not involved in the symptomatology of schizophrenia, is the substrate for unwanted side effects of typical antipsychotic medication. We sought to identify potential endogenous regulators of DA release that might produce differential effects in these brain areas. Using a superfusion system, we tested the peptides NT (0.1nM-1μM), NAAG (5-500μM) and BE (0.1nM-1μM) for their effects on basal and 100μM NMDA-stimulated [^{3}H]DA release in slices prepared from guinea pig brain. NT enhanced basal release in NAC, PFC and STR at all concentrations tested. NT (maximally at 10-100nM) also potentiated 100μM NMDA-stimulated [^{3}H]DA release in a biphasic manner from all regions. NAAG enhanced basal [^{3}H]DA release in NAC, PFC and STR but to a lesser extent than NMDA. BE enhanced basal [^{3}H]DA release in STR and NAC (PFC not yet tested) but to a lesser extent than NMDA. NAAG (5-500μM) or BE (0.1nM-1μM) did not enhance 100μM NMDA-stimulated [^{3}H]DA release in these regions. These results suggest that NT, NAAG and BE may all be endogenous regulators of DAergic neurotransmission and therefore may be potential therapeutic targets for antipsychotic drug development. Supported by NARSAD and NIDA.

553.13

CHOLECYSTOKININ- AND NEUROTENSIN-EVOKED CATIONIC CURRENTS IN SUBSTANTIA NIGRA DOPAMINERGIC NEURONS ARE MEDIATED BY IP_3-INDUCED CALCIUM RELEASE.

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To understand the ionic and molecular mechanisms by which sulfated cholecystokinin octapeptide (CCK-8) and neurotensin (NT) modulate the electrical activity of substantia nigra (SN) dopaminergic (DA) neurons, whole-cell patch-clamp recordings were used to study electrophysiological effects of CCK-8 and NT on acutely isolated SN DA neurons. Both CCK-8 and NT excite SN DA neurons via increasing a nonselective cationic conductance. CCK-8- and NT-evoked inward currents were inhibited by the intracellular perfusion of GDP-beta-S (1 mM). In DA neurons internally perfused with GTP-gamma-S (0.5 mM), the inward currents produced by CCK-8 and NT became irreversible. Pretreating DA neurons with 500 ng/ml pertussis toxin(PTX) did not significantly affect the ability of both peptides to induce cationic currents. Intracellular application of heparin(2 mg/ml), an inositol (1,4,5) trisphosphate (IP_3) receptor antagonist, and buffering intracellular calcium with the Ca^{++}-chelator BAPTA (10 mM) suppressed cationic currents evoked by CCK-8 and NT. Dialyzing DA neurons with protein kinase C (PKC) inhibitors, staurosporine and PKC(19-31), failed to prevent CCK-8 and NT from generating cationic currents. It is concluded that PTX-insensitive G-proteins mediate neuropeptide-induced enhancement of cationic conductance of SN DA neurons. The coupling mechanism via G-proteins is likely to involve the generation of IP_3, and subsequent IP_3-evoked Ca^{++} release from the intracellular store results in activating the nonselective cationic conductance.

553.14

THE NEUROCHEMICAL AND BEHAVIORAL PHARMACOLOGY OF SYSTEMICALLY ACTIVE REDUCED BOND NEUROTENSIN(8-13) MIMETICS.

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The C-terminal hexapeptide fragment of neurotensin (NT(8-13)) has been shown to bind to the neurotensin receptor and retain full agonist activity. Structure-activity studies on a variety of NT(8-13) mimetics resulted in the discovery of compounds which behaved as NT receptor agonists *in vitro*, and had a variety of behavioral effects consistent with NT agonist activity when administered systemically. The reduced bond isostere PD 149760 was shown to have high affinity for the NT receptor found in new born mouse brain (Ki = 1.13nM) and was effective at inhibiting calcium mobilization (EC_{50} = 362nM). Its putative prodrug PD 149163 effectively inhibited spontaneous locomotor activity in the mouse (ED_{50} = 0.2 mg/kg IP) and acetic acid induced stretching (ED_{50} = 0.05 mg/kg IP). A related analog, PD 147113 was also shown to have potent systemic activity in the acetic acid induced stretching paradigm (ED_{50} = 0.08 mg/kg IP). These effects were not reversed by naloxone or by the NT antagonist SR 48692. PD 147113 also increased the firing rate of A-10 DA cells *in vitro* at a concentration of 10 nM and elevated DOPAC overflow in the nucleous accumbens as measured by intracerebral microdialysis *in vivo* (2 mg/kg SC). PD 149163 and PD 147113 also inhibited feeding in the rat (ED_{50} = 0.2 mg/kg IP). These findings demonstrate systemically active NT agonists may have utility as antipsychotic, analgesic or anorectic agents.

553.15

EFFECTS OF A SYSTEMICALLY ADMINISTERED NEUROTENSIN PEPTIDE AGONIST ON DA AND DOPAC TURNOVER IN THE RAT NUCLEUS ACCUMBENS AND STRIATUM.

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Recently, novel analogues of neurotensin have been synthesized which, unlike native neurotensin, are capable of producing changes in locomotor activity and brain neurochemical turnover when administered i.p. and s.c. (Neurosci. Abs. 123.14, 1992). These modified peptides appear to have greater stability in circulation and/or efficiency in crossing the blood brain barrier. This study evaluated the effect of one of these stable peptide agonists, a modified C-terminal hexapeptide of neurotensin, (Me)-Arg-Lys-Pro-Trp-tLeu-Leu (MNT 8-13) on dopamine (DA) and DOPAC overflow in the nucleus accumbens and striatum of urethane-anesthetized rats as measured by intracerebral microdialysis (ICMD). In untreated animals, MNT 8-13 (2.5mg/kg s.c.) produced variable changes in DA and DOPAC in both regions. However, in those animals pre-treated with pargyline (50mg/kg i.p.), DA levels increased 69% in the nucleus accumbens and 30% in the striatum, while DOPAC values remained relatively unchanged following MNT 8-13 injection. We conclude that under certain conditions, stable neurotensin analogues are able to preferentially modulate DA turnover in the mesolimbic system of rats following peripheral administration.

553.16

SUBSTANCE P (SP) AND THE μ SELECTIVE OPIOID AGONIST, DAMGO, ARE PHYSIOLOGICAL ANTAGONISTS WITHIN THE VENTRAL PALLIDUM (VP).

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It has been demonstrated with various paradigms that SP and opioids are likely physiological antagonists (e.g., SP antagonists block behavioral symptoms of morphine withdrawal; enkephalins attenuate pain by blocking release of SP). Axons from the nucleus accumbens (NA) neurons that contain SP and enkephalin-like immunoreactivity synapse on the VP neurons. Both SP and μ opioid receptors are found within the VP. Our previous work demonstrated that SP increases and opioids decrease firing rate of the VP neurons. To ascertain possible interactions between opioids and SP within the VP, the present study employed single neuron extracellular recordings and microiontophoresis of DAMGO (10mM) and SP (1mM) in chloral hydrate anesthetized rats. Sixty-one VP neurons were tested with both compounds. Forty-eight percent of these were sensitive to DAMGO, and 49% to SP. As observed previously, microiontophoretically applied DAMGO decreased, and SP increased, neuronal activity. Thirty-three percent of the neurons tested were sensitive to both SP and DAMGO, and 30% to one peptide only. Ejection currents ranging between 5-35nA were used to generate current-response curves for both compounds. E_{max} for DAMGO was 41% below the baseline firing with an E_{Cu50} at 15nA. SP E_{max} was 47% above the baseline and the E_{Cu50} 20nA. A near-threshold current of SP, co-iontophoresed with multiple current levels of DAMGO, reduced DAMGO-induced responses to the pretreatment firing rates. Similar treatments with DAMGO attenuated neuronal responses to SP. This study 1) verified opposite roles of SP and μ opioid agonists in regulation of neuronal activity within the VP, 2) suggests colocalization of opioid and SP receptors (implying convergence of enkephalinergic and SP-ergic projections from NA on to single VP neuron), and 3) implicates physiological antagonism between SP and opioids within the VP. Work supported by DA0525 to TCN.

554.1

MODULATION OF INTRACELLULAR CALCIUM BY BRADYKININ IN N1E-115 CELLS. Jay S. Coggan* and Stuart H. Thompson. Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

Murine N1E-115 neuroblastoma cells release calcium from IP_3 - sensitive internal stores in response to bradykinin (BK), but not the selective B1 receptor agonist des-arg^9 BK. This suggests that the B2 receptor type is expressed. Low concentrations (<500 pM) of BK elicit responses that are slow in onset (20-30 sec), long in duration (2-4 min) and exhibit low frequency calcium oscillations (20-50 sec periods). When N-methyl-D-glucamine is substituted for extracellular Na in order to block Na/Ca exchange, the calcium oscillations in response to BK (500 pM) have shorter periods but are less profound. High concentrations of BK (> 1 nM) result in rapidly rising calcium concentrations which smoothly decay to baseline within one minute in the continued presence of agonist. Short duration application of BK (30 sec.) elicits similar calcium transients, but responses to subsequent applications exhibit marked desensitization. In the absence of extracellular calcium, the responses to bradykinin display the same characteristics. Trace averages from several cells indicate that there is no difference between responses in the presence or absence of extracellular calcium. Calcium influx, therefore, is not associated with the release of calcium from intracellular stores in response to BK. Supported by NIH NS14519.

554.2

BRADYKININ-INDUCED OSCILLATIONS OF CYTOSOLIC CA^{2+} ACTIVITY AND OF MEMBRANE POTENTIAL IN RAT GLIOMA CELLS. G. Reetz and G. Reiser*. Institute of Physiological Chemistry, University of Tübingen, Hoppe-Seyler Str. 4, 72076 Tübingen, Germany.

In fura-2 loaded rat glioma cells (C6-4-2) continuously superfused with bradykinin (bk), rhythmic membrane hyperpolarisations and $[Ca^{2+}]_i$ oscillations (1/min) were observed.

The initial $[Ca^{2+}]_i$ response to bk resulted from $InsP_3$-induced Ca^{2+} release, whereas the subsequent oscillations were dependent on both $InsP_3$-sensitive Ca^{2+} stores and Ca^{2+} influx. Simultaneous intracellular recording of the membrane potential showed that the oscillation of $[Ca^{2+}]_i$ and of the membrane potential were synchronous. The oscillations were affected by the K^+ equilibrium potential and by blocking of $K^+(Ca^{2+})$ channels. This indicates a potentiation of Ca^{2+} influx by membrane hyperpolarisation due to activation of $K^+(Ca^{2+})$ channels. Mn^{2+} quench experiments gave additional evidence for influx of Ca^{2+}.

We conclude that Ca^{2+} is periodically released from Ca^{2+} stores and subsequently opens $K^+(Ca^{2+})$ channels. The stores are refilled by the Ca^{2+}-ATPase and by influx of Ca^{2+} across the cell membrane, augmented by membrane hyperpolarisation. The oscillations were also influenced by hypotonic and by hypertonic medium. Thus cell volume probably serves as a negative feedback regulator during the falling phase of $[Ca^{2+}]_i$. These experiments are discussed with respect to physiological functions of glial cells in volume regulation during ischemia or stroke.

554.3

EVIDENCE FOR A NEUROGENIC COMPONENT IN BRADYKININ B1 RECEPTOR-MEDIATED PLASMA EXTRAVASATION DURING TURPENTINE INFLAMMATION IN THE RAT. M.J.K. Walker and M.N. Perkins*. Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, U.K.

Although bradykinin (BK) B1 receptors are not involved in the acute activation of nociceptive neurones, several studies have demonstrated that following persistent inflammation B1 receptors are induced and contribute to inflammatory pain. The B1 receptor agonist, des-Arg^9 BK, is inactive in naive animals, but it has been found to increase nociceptive responses following an inflammatory insult in rats. In addition to the sensitisation and activation of nociceptive afferents, kinins also mediate other symptoms of inflammation including oedema formation.The experiments presented here have investigated whether B1 receptors induced during inflammation also mediate plasma extravasation (PE). PE was examined in rats that had been injected with 50% turpentine into a single hind paw 72 hours prior to testing, and was measured as the extravasation of ^{125}I-BSA following intraplantar injections of des-Arg^9 BK. Des-Arg^9 BK (100-300 pmol/paw) induced a significant increase in PE in both the ipsilateral and contralateral hind paws of turpentine-treated, but not naive rats. An increase in PE was observed 60 min., but not at 5 or 30 min. following des-Arg^9 BK administration. This effect was reversed by the selective B1 antagonist, des-Arg^9-Leu^8 BK. A neurogenic component of des-Arg^9 BK-mediated PE was revealed by pre-treating rats with capsaicin in order to deplete C-fibres. Capsaicin pre-treatment reversed the B1-mediated PE, but had no effect on the baseline extravasation in controls. Inhibition of des-Arg^9 BK-mediated PE by co-administration of a NK1 receptor antagonist, CP 99994, suggests that B1 receptor-mediated PE involves the activation of C-fibres and the release of substance P. These results are consistent with a systemic induction of B1 receptors following local persistent inflammation. They also suggest that B1 receptor activation initiates a chain of events which ends with a neurogenically-mediated PE.

554.4

CHARACTERIZATION OF A NOVEL HYPERPOLARIZING RESPONSE TO SUBSTANCE P IN FERRET SENSORY NEURONS. M.S. Jafri and D. Weinreich*. Dept. Pharmacol. & Exp. Ther., Univ. Maryland Sch. Medicine, Baltimore, MD 21201.

Substance P (SP) depolarizes some primary afferent neurons situated in trigeminal or dorsal root ganglia. This study represents the first characterization of a hyperpolarizing response to SP recorded in primary afferent neurons, those located in the nodose ganglion.

In intact and in acutely dissociated neurons, 100 nM SP hyperpolarized the membrane potential by ~10% (-7.0 ± 0.7 mV) and decreased the membrane input resistance by ~30% (-6.1 ± 1.3 MΩ). The hyperpolarization or outward current produced by SP was recorded in 105 of 132 cells (80%) and was dose-dependent (EC_{50} = 68 nM). The SP effect was reversibly blocked by 10 nM CP96,345, an NK-1 antagonist and was mimicked by 100 nM ASMSP, an NK-1 agonist. The response was not affected by 100 nM SR48968, an NK-2 antagonist. The SP effect was reversibly abolished by lowering extracellular Ca^{2+} (≤10 µM) and reversibly blocked by the K^+-channel blockers apamin, 4-aminopyridine, Ba^{2+}, Cs^+, and TEA. E_{rev} for the SP current was -85 ± 2 mV and varied with $[K^+]_o$ in a Nernstian fashion (slope = 55 mV/10-fold $\Delta[K^+]_o$). Our results show that SP activates an NK-1 receptor coupled to a Ca^{2+}-dependent outward K^+ current. We have observed SP-like immunoreactivity associated with ferret nodose neurons and therefore propose that SP receptors on these neurons may function as inhibitory autoreceptors.

554.5

EFFECTS OF THE TACHYKININ NK_2 RECEPTOR ANTAGONIST SR48968 ON RAT SPINAL MOTONEURONES. G.Baranauskas*, A.Nistri. Biophys. Lab., Int. Sch. Adv. Studies (SISSA), Via Beirut 4, 34013 Trieste, Italy.

Several studies suggest the existence of the tachykinin NK_2 receptor subtype in the rat spinal cord. To clarify this issue and to study the possible involvement of NK_2 receptors in synaptic transmission, the specific NK_2 receptor antagonist SR48968 was used during intracellular recording from functionally-identified lumbar motoneurones (rest potential 74±2 mV, mean±s.e.m.) of the neonatal rat (P6-12) spinal cord continuously superfused *in vitro* at room temperature. All drugs were bath-applied. In all cells SR48968 (0.5 µM) had no effect on resting membrane potential or resistance but it did reduce the depolarisation (50±10%; n=5), the increase in input resistance (10±5%; n=5) and the baseline synaptic activity induced by the specific NK_2 receptor agonist [βAla^8]NKA_{4-10} (200 nM). SR48968 did not affect analogous changes produced by substance P-methylester (200 nM) and [MePhe^7]NKB (200 nM), specific agonists for NK_1 and NK_3 receptors, respectively. For synaptic transmission studies two different types of dorsal root stimulation was used to recruit either Aß fibers alone or C fibers as well. Additionally, 1 Hz trains (25 s) were employed to elicit slowly incrementing depolarizations with slow recovery (windups). In the presence of SR48968 no change in response amplitude for all three types of stimulation was detected, although a modest reduction (16%) in the duration of the depolarization tail following windup was noted. Our study confirms the existence of SR48968 sensitive NK_2 receptors in rat spinal cord and suggests that they play a relatively minor role in synaptic transmission from dorsal root afferents to motoneurones. Supported by CNR and INFM.

554.6

IS THE GALANIN-INDUCED INCREASE IN POTASSIUM CONDUCTANCE AND DECREASE IN BARIUM CURRENTS OCCURRING IN MUDPUPPY PARASYMPATHETIC NEURONS MEDIATED BY TWO DIFFERENT RECEPTORS? L. A. Merriam*, J. M. Mulvaney and R. L. Parsons. Dept. of Anatomy and Neurobiology, Univ. of Vermont, Burlington, VT 05405.

Galanin increases an inwardly rectifying potassium conductance (G_K) and decreases voltage-dependent barium currents (I_{Ba}) in mudpuppy parasympathetic neurons. In the present study, we have determined the concentration dependence of galanin and sensitivity to pertussis toxin and the chimeric ligand galantide to determine whether galanin's actions are mediated by the activation of a single receptor or different receptors. All experiments were done on dissociated neurons from the cardiac ganglion of the mudpuppy *Necturus maculosus* using the perforated patch mode of the whole cell voltage clamp technique. We found that the EC_{50} for the activation of G_K (~44 nM) is 100-fold greater than the EC_{50} for the inhibition of I_{Ba} (~0.4 nM). Pretreatment with PTX (~10 µg/ml for 24-36 hrs) inhibited both actions of galanin. Galantide (10^{-9}-10^{-6}M) antagonized the increase in potassium conductance by 10^{-6}M galanin without any measurable agonist action. In contrast, galantide (10^{-8}-10^{-6}M) produced a concentration-dependent decrease in I_{Ba}. I_{Ba} could be further decreased by 10^{-7}M galanin in the presence of galantide. These results suggest that although activation of a G protein is involved in both actions of galanin, two different galanin receptors may mediate the increase in potassium conductance and decrease in I_{Ba} in mudpuppy neurons. Supported by NS23978.

554.7

BARORECEPTOR REFLEX MODULATION BY THE GALANIN FRAGMENT (1-15). Z. Díaz, J.A. Narváez*, P.B. Hedlund, J.A. Aguirre, N. Yanaihara, S. González-Barón and K. Fuxe. Dept. of Physiology, Faculty of Medicine, University of Málaga, Spain; Lab. of Bio-Organic Chemistry, School of Pharmaceutical Sci. University of Shizuoka, Japan and Dept. of Neuroscience, Karolinska Institutet, 104 01 Stockholm, Sweden.

Recently we have reported that the galanin (1-15) [GAL (1-15)] administered centrally elicites dose-related vasopressor responses and counteracts the vaso-depressor responses induced by galanin (1-29) [GAL (1-29)]. For a better understanding of the role of the galanin peptide family on central cardiovascular control we have studied the modulation of the baroreflex responses (BRR) by both galanin molecules. Groups of anaesthetized rats received injections in the lateral ventricle (i.v.t.) of 0.1 nmol of GAL (1-15), 0.3 nmol of GAL (1-29), 3 nmol of GAL (1-29) or aCSF alone (control group) by means of an automatic injection pump (30 µL/3 min). BRR was elicited before and after i.v.t. injections by intravenous bolus injections of few differents dosis of L-phenylephrine (LPE) (33 to 6,25 µg/kg b w) administered in a random manner. Mean arterial pressure (MAP) and heart rate (HR) changes were recorded from a catheter in the femoral artery. BRR sensitivity was evaluated from the slope of the regression lines related to the maximal reflex bradycardia due to the transient hypertension induced by LPE. GAL (1-29) did not induce any statistical changes in the slopes compared with the controls at any dose tested. However, i.v.t. injection of 0.1 nmol of GAL (1-15) produces a decrease of BRR sensitivity ($p<0.02$) when compared with the control of the GAL (1-29) groups ($p<0.05$). The blocking effect of BRR by GAL (1-15) might explain in part its vasopressor action and may indicate a major role for this galanin fragment in central cardiovascular regulation could be suggested possibly acting on specific receptor subtypes for N-terminal fragments of galanin.

This work was supported by Spanish CICYT (Sal 91-0458 and PB91-0769) and by the Swedish MRC (04X-715).

554.8

AN *IN VITRO* INVESTIGATION OF THE EFFECTS OF ORG 2766 ON MEDIAL VESTIBULAR NUCLEUS NEURONS IN GUINEA PIG. D.P.D. Gilchrist, C.L. Darlington* and P.F. Smith. Dept. of Psychology and Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

Vestibular compensation is a process of behavioural recovery that follows removal of the peripheral vestibular receptors in one inner ear. The spontaneous recovery is correlated to a return of resting activity in medial vestibular nucleus (MVN) neurons on the ipsilateral side to the lesion. In behavioural studies systemic administration of adrenocorticotrophic hormone fragment 4-10 (ACTH-(4-10)) can accelerate the compensation process. ACTH-(4-10) may produce its effects by acting directly on MVN neurons, as these neurons respond to ACTH-(4-10) *in vitro* at concentrations ranging from 10^{-10} to 10^{-14} M. Our own recent behavioural studies have shown that the synthetic ACTH-(4-9) analogue, Org 2766, can accelerate compensation at smaller doses than ACTH-(4-10). The present study investigated the effect of Org 2766 on MVN neurons *in vitro*. Using standard electrophysiological techniques MVN neurons in coronal brainstem slices from guinea pig were recorded before and after superfusion with Org 2766 at concentrations of 10^{-8}, 10^{-10} and 10^{-12} M. The mean resting rate of all neurons recorded was 11.65 Hz (n=49). No neurons responded to Org 2766 at a concentration of 10^{-8} M (6/6). At a concentration of 10^{-10} M only 25 % (5/20) of neurons responded to Org 2766 superfusion. At a concentration of 10^{-12} M 33% (4/12) of neurons responded. For those neurons that did respond to Org 2766, the predominant response was a decrease in firing. Although the direction of the response to Org 2766 is the same as for ACTH-(4-10), the size of the response and the number of cells that have responded is comparatively small. In some cases the resting activity did not return to the control level indicating the concentration may have been toxic. Since Org 2766 produces its effects at smaller doses than ACTH-(4-10) in behavioural studies, it will be necessary to continue the *in vitro* investigations using lower concentrations of Org 2766. Alternatively the finding may indicate that although Org 2766 and ACTH-(4-10) produce a similar effect in a behaving animal, the site of action may differ.

554.9

100-FOLD MORE POTENT VIP AGONIST AND ANTAGONIST A. Davidson*, G. Lilling, R. Glazer, A. Ticher, I.E. Ashkenazi, S. Rubinraut, M. Fridkin, D.E. Brenneman and I. Gozes Sackler Med. Sch., Tel Aviv Univ.; Weizmann Inst. Rehovot, Israel; SDMP, LDN, NICHD, NIH, Bethesda, MD 20892.

Vasoactive intestinal peptide (VIP), a regulator of neuronal survival, is found in brain areas associated with the control of memory and biological rhythms. To distinguish VIP receptors that mediate distinct VIP functions, potent analogues were designed. Using the replacement of Met_{17} with Nle and the addition of stearyl moiety, an analogue was devised that exhibited a 100-fold greater potency than VIP in the promotion of neuronal survival of spinal cord neurons. This agonist increased neuronal survival via a cAMP-independent mechanism. Identical modification of a VIP hybrid antagonist ($neurotensin_{1-6}VIP_{7-28}$) also resulted in a 100-fold greater potency in blocking VIP-mediated increases in neuronal survival. Blockade of circadian activity rhythms was limited to VIP antagonists that could inhibit VIP-mediated increases in cAMP. In addition to these lipophilic peptides providing a means for receptor discrimination, these compounds offer a new strategy in the treatment of neurodegenerative diseases or disturbances in biological rhythms.

554.10

A SYNTHETIC PEPTIDE DERIVED FROM LAMININ MODULATES THE ELECTRICAL PROPERTIES OF NEURONS

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The ECM-molecule laminin plays a role in brain development as well as in neurodegeneration and regeneration in the mature CNS.

We investigated the effect of a synthetic decapeptide derived from a neurite-outgrowth-promoting domain of the B2-chain of laminin (P20) on the electrical properties of neocortical pyramidal neurons. 30 pyramidal neurons were recorded intracellularly in *in vitro* slice preparations from adult rat neocortex and stimulated synaptically or by depolarising current pulses. 19 neurons were treated with P20 and 11 neurons with a control-peptide of the same molecular size.

In the presence of P20, 85 % of the neurons tested showed a significant increase in input resistance (R_N). In 60% of them R_N increased by more than 20%. The strong rise in direct excitability was associated with an increase in inward rectification. The peak conductance of the IPSP was slightly reduced in 60% of the neurons. In 10 out of 19 neurons spreading depressions occurred terminating the recording period. No significant changes were observed during the application of the control-peptide.

Our results indicate that the synthetic peptide derived from a neurite-outgrowth-promoting domain of the B2-chain of laminin alters the electrical properties of neocortical neurons, making them more excitable. Laminin or related smaller peptides may have neuromodulatory functions in the adult CNS.

554.11

EFFECTS OF BRAIN-DERIVED PEPTIDES ON SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS H. Xiong*, J.M. Wojtowicz and A. Baskys. Depts. of Physiology and Psychiatry, Univ. of Toronto and Clarke Inst. of Psychiatry, Toronto, Ontario M5S 1A8, Canada

We have demonstrated previously that a neuroprotective drug Cerebrolysin™ (CB) (EBEWE Pharmaceuticals, Austria) exerts a profound depressant action on synaptic transmission in rat hippocampal slices via $GABA_B$ receptors (Soc. Neurosci. Abstr. 19:1525, 1993). However, this $GABA_B$ effect accounts for only ~40% of the CB-induced depression, suggesting that other mechanisms may also be involved. The aim of this study is to test our hypothesis that CB may also act on opioid receptors.

Evoked field potentials (FPs) were recorded in the CA1 of rat hippocampal slices. Bath application of CB (10µl/ml) reduced the initial slope (mV/ms) of the FPs to 26.4 ± 3.6% of control ($n=19$, $p<0.01$, ± s.e.m.), followed by a long-lasting enhancement (0.5 - 2 hrs.) to 117.2 ± 5.9% of control ($n=19$, $p<0.01$). Application of broad spectrum opioid receptor antagonist naloxone attenuated this CB-induced depression in a dose dependent manner, suggesting involvement of opioid receptors in the mediation of CB-induced depression. To find the possible agonist component in the CB mixture, the effects of DAMGO (Mu selective) and Dynorphin A (1-8) (DYN, kappa selective, also high affinity for delta subtype) peptides on synaptic transmission in the CA1 were tested and compared with the effects of CB. Preliminary results have shown that DYN (1 - 3 µM) reduced FP initial slope to 60.4 ± 10.2% of control ($n=13$, $p<0.01$), followed by an enhancement (113.1 ± 7.9% of control, $p=0.06$). In contrast, DAMGO (2 - 4 µM) caused a small reduction of FP slope (83.6 ± 4.6% of control, $n=9$, $p>0.05$). The results shown that both DYN and CB had similar effects with respect to time course and enhancement, suggesting that CB may contain kappa and/or delta receptor agonist components. These components may play a role in preventing brain damage and cognitive impairment.

Supported by EBEWE Initiative, Austria and MRC of Canada.

554.12

CENTRAL NON-OPIOID MOTOR EFFECTS OF DYNORPHINS: ANTAGONISM BY NMDA RECEPTOR ANTAGONISTS. V. K. Shukla S. Lemaire* and M. Dumont . Dept. of Pharmacology, University of Ottawa, Ottawa, K1H 8M5, CANADA.

Central administration of Dyn A(1-13) and related peptides in mice produced dose dependent non-opioid motor effects characterized by wild running, jumping, circling and barrel rolling. Animals showed one or more of these effects within 10 min after i.c.v. administration of these peptides. The doses which produced motor effects in 50 % mice (ED_{50}) with Dyn A(1-13), Dyn A(1-17), Dyn A(1-8), Dyn Ia, Dyn A(2-13), [Ala^1]Dyn A(1-13) and Dyn A(6-10) were 14.40, 14.32, 40.08, 3.08, 28.82, 26.00 and >100 nmol/mouse, respectively. Dyn A(1-13) (20 nmol/mouse)-induced motor effects were completely blocked by the co-administration of NMDA antagonist, dextrorphan (0.2-0.6 nmol/mouse). Co-administration of other NMDA antagonists (nmol/mouse) like dextromethorphan (0.2-1), MK-801 (0.1-0.3), CPP (0.075-0.3) and HA-966 (0.5-8) also partly blocked Dyn A(1-13)-induced motor effects. All the NMDA antagonist tested were less effective at doses exceeding the peak protection levels because of their intrinsic *per se* toxicity. The results indicate that the NMDA receptor is involved in the non-opioid motor effects of Dyn and related peptides. Supported by the Medical Research Council of Canada.

554.13

MELANOTROPIC AND OPIOID PEPTIDE EFFECTS ON NORADRENERGIC NEURONS IN THE CAUDAL DORSOMEDIAL MEDULLA. J.A. Carr* and K.J. Gregg. Dept. Biol. Sci., Texas Tech Univ., Lubbock, TX 79409-3131

We used an *in-vitro* approach to investigate the influence of opiate and melanotropic peptides on the activity of noradrenergic neurons in the A2 area of the caudal brainstem of the rat. [Nle^4, D-Phe^7]α-MSH (NDP-MSH) had no effect on the synthetic activity of noradrenergic neurons in the A2 as gauged by *in-vitro* L-DOPA accumulation in caudal dorsomedial medulla (CDMM) explants. NDP-MSH reduced the norepinephrine (NE) content of explants treated with NSD-1015 but had no effect on basal or K^+-stimulated release of endogenous NE from CDMM slices *in vitro*. β-endorphin (βE) reduced K^+-induced release of NE from CDMM slices and naloxone attenuated this effect. The μ-opioid agonist [D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin (DAGO) had no effect on basal NE release but significantly reduced K^+-stimulated NE release from CDMM slices. These results suggest that activation of μ-opioid receptors inhibits depolarization-induced NE release from noradrenergic nerve terminals in the CDMM. Melanotropin receptors do not modulate NE release, but may influence NE metabolism in the CDMM. Supported by a grant from the Howard Hughes Medical Institute through the Undergraduate Biological Sciences Education Program and a grant from the TTU Research Enhancement Fund.

554.14

PRESYNAPTIC DELIVERY OF CGRP BY TRANSFECTED PC12 CELL CO-CULTURES. E. S. Schweitzer*, C.-J. Jeng, and Susan J. H. Tao-Cheng**. Dept. of Anatomy & Cell Biology, UCLA Medical School, Los Angeles, CA 90024 and **Laboratory of Neurobiology, NINDS, NIH, Bethesda, MD 20892.

In order to examine the role of CGRP in synapse formation and maintenance, we have engineered PC12 cells to synthesize and secrete CGRP from regulated (presynaptic) secretory vesicles. We have previously shown that the transgenic CGRP co-localizes with norepinephrine-containing vesicles on density gradient fractionation, and is secreted upon depolarization of the PC12 cells in the presence of calcium. We have now shown by immunoelectron microscopy that the CGRP is contained exclusively in dense-core vesicles in both undifferentiated and NGF-treated PC12 cells. CGRP is absent from the synaptophysin- and SV2-rich small clear vesicles. The CGRP in the dense-core vesicles is therefore presumably the source of the CGRP secreted from the cells upon stimulation.

We have now co-cultured wild-type and CGRP-expressing PC12 cells with C2 myotubes, to examine the effect of the CGRP on the induction of ACh receptors and the formation of ACh receptor clusters. Preliminary evidence indicates that the location of the ACh receptor clusters on the surface of the myotubes is correlated with the presence of PC12 cell presynaptic terminals. We are examining these co-cultures for evidence of a specific effect of CGRP on the rate or extent of ACh receptor clustering.

554.15

Pancreastatin Stimulates Cobalt Uptake by Hippocampal Neurons. C. S. Toomim*& W. R. Millington. Division of Molecular Biology and Biochemistry, Univ. of Missouri-Kansas City, Kansas City, MO 64108.

Pancreastatin, a 49 amino acid peptide derived from chromogranin A, is widely distributed in neural and endocrine tissues. Pancreastatin modulates the secretion of insulin and other endocrine hormones; however, no evidence of its function in brain exists to date. We tested whether pancreastatin activates hippocampal neurons by using a histochemical assay which measures cobalt uptake through calcium permeable channels in tissue slices (Pruss et al. Neuron 7:509, 1991). Our initial experiments confirmed that kainic acid, a glutamate receptor agonist, produced a dose-dependent accumulation (1-100μM) of cobalt in hippocampal neurons of the dentate gyrus and Ammon's horn (CA). Co-incubation with the glutamate receptor antagonist, kynurenate (5mM) blocked the response completely.

Pancreastatin also produced a concentration related activation of cobalt uptake, albeit at considerably lower concentrations (0.5,1,10 or 100nM) than kainate. The regional distributions of pancreastatin and kainate responsive neurons were similar; Pancreastatin (500pM) stimulated cobalt uptake in areas CA1 and CA2, whereas higher concentrations (1-100nM) also promoted cobalt uptake into CA3 and pyramidal and hilar cells of the dentate gyrus. At low concentrations, cobalt uptake was visible only in small portions of the dendrites, whereas, at higher concentrations, the cell bodies were also labelled. Interestingly, pancreastatin stimulated cobalt uptake was also blocked by kynurenate (100μM) suggesting that a glutamate receptor may be involved in the response. These data demonstrate that pancreastatin activates hippocampal neurons, suggesting that it may function as a neurotransmitter. Supported by the Scientific Education Partnership of the Marion Merrell Dow Foundation.

554.16

PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP STIMULATES cAMP-DEPENDENT TRANSCRIPTION AND PROMOTES SURVIVAL OF CEREBELLAR GRANULE NEURONS "IN VITRO". F. Rene, F. Barthel, J.M. Felix, J.L. Roberts, A.C. Gore*, and J.P. Loeffler. Lab. of Physiology, URA 1446, 21 rue Rene Decartes, 670 Strasbourg Cedex France. *Mt. Sinai Medical Center, New York, NY 10029-6574.

We have studied the effects of PACAP on the survival and on the transcriptional regulation of rat cerebellar granule cells in culture. Incorporation studies using (^{3}H)leucine and (^{3}H)uridine demonstrated that PACAP acted as a survival factor for cerebellar granule neurons which were prepared from 8 or 11-day-old rats respectively. This neurotrophic effect appears to result from PACAP receptor isoforms that activate the adenylate cyclase rather then phospholipase C. Indeed, the survival effect is mimicked by Forskolin (5x10^{-6}M) but not by TPA (10^{-7}M). To investigate this mechanism, we chose to analyze long term effects at the transcriptional level. We used a chloramphenicol acetyl transferase (CAT) reporter gene driven by the minimal cAMP responsive element (CRE: TGACGTCA). We have shown that after transfection into cerebellar neurons, this construct is efficiently stimulated by PACAP27 (10^{-8}M) or PACAP38 (10^{-8}M). This induction is mediated by the cAMP regulatory pathway. Indeed, cAMP measurements have shown that the activation of PCAP receptors increases cAMP level in a dose-dependent manner. Further co-transfection studies with mutated PKA regulatory subunits have shown that induction of CRE/tK-CAT by PACAP was suppressed when the cAMP-dependent protein kinase (PKA) was inactivated with a dominant inhibitory mutant of this kinase. Finally, by using a yeast transcriptional activator GAL4-CREB fusion protein, we demonstrated that the transcriptional effect of PACAP is mediated by the transacting factor CREB. In summary, we demonstrated that PACAP is a survival factor for cultured cerebellar granule neurons and these trophic effects may depend on transcriptional regulation primarily mediated by cAMP.

554.17

ENDOTHELIN-1 ACTIVATES CHEMOSENSORY TYPE I CELLS AND POTENTIATES THE HYPOXIC RESPONSE IN RABBIT CAROTID BODY. J. Chen, L. He, B. Dinger and S.J. Fidone*. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Endothelin (ET) and ET mRNA have been localized to diverse structures in the central and peripheral nervous systems, and ET has been demonstrated to evoke to release of catecholamines (CA) from adrenal chromaffin cells and corpus striata. In many tissues, the effects of ET are mediated by G-protein coupled receptors which initiate the hydrolysis of phosphatidylinositol. In the present study, we have examined the effects of ET-1 on inositol-phosphate (IPn) formation, CA release and carotid sinus nerve activity elicited from *in vitro* superfused rabbit carotid bodies where vascular effects are eliminated. Submicromolar and micromolar concentrations (0.01, 0.1, 0.5 and 1.0 μM) of ET-1 elevated the accumulation of IPn 1.3-, 2.1-, 4.7- and 5.3-fold ($p<0.01$), respectively. Similar concentrations (0.1 and 1.0 μM) of ET-1 potentiated the hypoxia-evoked release of CA by 1.36- and 2.16-fold ($p<0.05$), respectively. Finally, CSN activity evoked by hypoxic superfusion solutions (equilibrated with 20% O_2) was elevated by 31.2% ± 5.8% ($\bar{X}$ ± SEM, $p<0.001$) in the presence of 1 μM ET-1. In preliminary experiments, ET-3 appeared to be less potent with respect to IPn formation, CA release and CSN discharge. The data suggest that ET actions are mediated by elevated intracellular Ca^{2+} concentrations and protein kinase C in chemosensory type I cells. Endothelins may act as physiological antagonists to atrial natriuretic peptide and nitric oxide, which are endogenous to the carotid body and exert powerful inhibitory influences on the carotid chemoresponse . Supported by USPHS grants NS12636 and NS07938.

554.18

BINDING OF THE NEMATODE FMRFamide-LIKE NEUROPEPTIDE, SDPNFLRFamide, TO *PANAGRELLUS REDIVIVUS* MEMBRANES. J.P.Davis, J.W.Bowman, T.G.Geary, A.R.Friedman, M.J.Larsen, D.E.Lowery* and D.P.Thompson. Upjohn Laboratories, Kalamazoo, MI 49001.

SDPNFLRFamide (PF1), originally isolated from the free-living nematode *P.redivivus*, induces profound flaccid paralysis in neuromuscular segments of the parasitic nematode, *Ascaris suum*, at 1-10 ηM. We conducted binding studies to characterize the receptor(s) for this peptide using [^{3}H]PF1 (134 Ci/mmol) and membrane preparations from *P.redivivus* (whole worm) and *A.suum* (muscle, hypodermis, ovaries). Among the *A.suum* tissue preparations, a low level of specific binding was detectable only in the hypodermal membranes. Scatchard analysis of [^{3}H]PF1 binding to *P.redivivus* membranes indicated both a high and low affinity binding site for this peptide. The high affinity site is characterized by a K_d of 2.7 ηM and B_{max} of 8.5 fmol/mg protein. K_d and B_{max} values of 23.3 ηM and 58.3 fmol/mg protein, respectively, were obtained for the low affinity site. Specific binding in these studies accounted for 40% of the total bound, and total binding was 1% of the label added. Competition binding studies revealed that, in the *P.redivivus* membrane preparation, the affinity of PF1 is equal to that of SADPNFLRFamide (PF2), another neuropeptide isolated from *P.redivivus* that has inhibitory effects on *A.suum* muscle.

554.19

NEUROMUSCULAR EFFECTS OF NEMATODE FMRFAMIDE-LIKE PEPTIDES IN *ASCARIS SUUM*.
J.W. Bowman, D.P. Thompson, A.R. Friedman, C.A. Winterrowd, A.K. Ichhpurani, K.L. Blair* and T.G. Geary. The Upjohn Co., Kalamazoo, MI 49001.

FMRFamide (Phe-Met-Arg-Phe-NH_2) related peptides (FaRPs) are widely distributed in invertebrates, but have not yet been found in vertebrates. Several unique FaRPs have been identified in nematodes, including the free-living organisms *Caenorhabditis elegans* and *Panagrellus redivivus* and the parasitic species *Ascaris suum*. We investigated the physiology of one of the peptides isolated from the free-living worms, PF1 (SDPNFLRFamide), and also confirmed reports that two *A. suum* FMRFamide-like peptides, AF1 and AF2, have a biphasic effect (relaxation followed by excitation) on muscle strips prepared from this parasite. We show that an intact nerve cord (dorsal or ventral) is required for this response. In contrast, PF1 is strictly inhibitory in *A. suum* muscle strips (with or without nerve tissue) and does not require Cl^-. Inhibitors of nitric oxide synthase (NOS) blocked the effects of PF1 on this preparation. We detected NOS activity in whole *P. redivivus* and in *A. suum* hypodermis tissue and identified a specific binding site for [^{3}H]PF1 in these tissues. We propose that PF1 acts on the hypodermis to release NO and thus inhibit muscle activity in *A. suum*.

554.20

STRUCTURE-ACTIVITY RELATIONSHIPS OF NEMATODE FMRFamide-LIKE NEUROPEPTIDES IN *ASCARIS SUUM*.
D.P.Thompson*, A.R.Friedman, J.W.Bowman, T.G.Geary, M.F.Kellman, M.L.Ware, E.Bullock and A.K.Ichhpurani. Upjohn Laboratories, Kalamazoo, MI 49001.

We examined the structure-activity relationships of two FMRFamide-like neuropeptides found in nematodes, AF1 (KNEFIRFamide) and PF1 (SDPNFLRFamide). We substituted alanine for each amino acid to generate a series of analogs that could pinpoint residues important for biological activity. We also tested a series of PF1 analogs in which each amino acid was replaced with its *d*-isomer. Truncated AF1 and PF1 analogs were similarly analyzed. Activity was measured in an *Ascaris suum* muscle strip preparation.

For both AF1 and PF1, elimination of the C-terminal amide abolished activity. In the AF1 series, N-terminal deletions or extensions had unfavorable effects on potency. Ala substitution at any position markedly reduced potency. KNAFIRFamide and KNEFARFamide showed only inhibitory effects, lacking the pronounced excitatory phase characteristic of AF1. For the PF1 analogs, ala substitution in the 4 N-terminal positions had little effect on activity. A similar pattern was found for the *d*-isomers, except that SD(*d*P)NFLRFamide and SDP(*d*N)FLRFamide were inactive. Substitution in either series in the C-terminal half was generally deleterious with the exception of SDPNFARFamide, which was at least as active as PF1, and SDPN(*d*F)LRFamide, which showed excitatory activity, completely opposite of the effects of PF1.

CATECHOLAMINE RECEPTORS VI

555.1

EFFECTS OF AGING AND ALCOHOL CONSUMPTION ON DOPAMINE D1 RECEPTORS IN MULTIPLE BRAIN REGIONS OF FISCHER 344 RATS.
J. M. Woods* and M.J. Druse-Manteuffel. Molecular & Cellular Biochem. Loyola U. Med. School, Maywood, IL 60153.

We examined the separate and combined effects of chronic alcohol consumption and aging on dopamine D1 receptors in the rostral and caudal striatum, nucleus accumbens, frontal cortex, ventral pallidum and globus pallidus of Fischer 344 rats. [^{3}H]-SCH 23390 was used to radiolabel dopamine D1 receptors on twenty micron brain sections. Brains were obtained from 5, 14 and 24 month Fischer 344 rats that were pair-fed a control or ethanol-containing liquid diet on a chronic basis prior to sacrifice. The relative density of [^{3}H]-SCH 23390 binding was determined using the NIH Image program.

The results of these experiments demonstrated an age-related decrease in dopamine D1 receptors in both the rostral and caudal striatum and in the frontal cortex. This age-related decline was typically found in both control and ethanol-fed rats. In comparison to control rats, binding was significantly decreased in the ventral pallidum of ethanol-fed rats. No significant differences were found in the K_D for [^{3}H]-SCH 23390 binding.

This research was supported by the USPHS - AA08451.

555.2

EFFECTS OF AGING AND ALCOHOL CONSUMPTION ON DOPAMINE D2 RECEPTORS IN BRAIN REGIONS OF FISCHER 344 RATS.
M.J. Druse-Manteuffel* and N. Tajuddin. Molecular & Cellular Biochem. Loyola U. Med. Sch., Maywood, IL 60153.

The present study examined dopamine D2 receptors in multiple brain areas of 5, 14 and 24 month Fischer 344 rats that were pair-fed a control or ethanol-containing liquid diet prior to sacrifice. Additional rats consumed the control liquid diet ad libitum. The brain areas examined include the rostral and caudal striatum, nucleus accumbens, substantia nigra, ventral pallidum and globus pallidus. [^{3}H]-Spiperone was used to radiolabel dopamine D2 receptors on twenty micron brain sections. Binding was determined using the NIH Image program.

These experiments demonstrated an age-related decrease in dopamine D2 receptors in the rostral striatum of both control and ethanol-fed rats. There was also an age-related decrease in D2 receptors in the caudal striatum. Although [^{3}H]-spiperone binding to D2 receptors was comparable in most brain areas of control and age-matched ethanol-fed rats, binding in the ventral pallidum of the ethanol group was generally lower. No significant age or alcohol-related differences were found in the K_D. There were also no significant difference in D2 receptors in age-matched control rats that consumed the liquid diets on a pair-fed or ad libitum basis.

This research was supported by the USPHS - AA08451.

555.3

EFFECTS OF PRENATAL IV COCAINE AND GENDER ON D_2 AND D_3 RECEPTORS IN THE STRIATUM AND NUCLEUS ACCUMBENS.
D.R. Wallace*, C.F. Mactutus and R.M. Booze, Dept. of Pharmacology, College of Medicine and College of Pharmacy, University of Kentucky, Lexington, KY 40536.

The effect of maternal cocaine treatment on the density of D_2 and D_3 receptors was examined in the striatum and nucleus accumbens of male and female offspring. Intravenous cocaine injections were given on gestational days 8-14 (3 mg/kg X 1 daily) and on gestational days 15-20 (3 mg/kg X 2 daily; Mactutus *et al., NTT*:1994). Pups (Sprague-Dawley) were sacrificed on postnatal day 35 and striatum/nucleus accumbens tissue removed and frozen (tissue homogenates) or whole brains were blocked and frozen (receptor autoradiography). Binding analysis for D_2 receptor density was performed with [^{125}I]-epidipride (0.5 nM) and D_3 receptor density was determined by [^{3}H]-(+)-7-OH-DPAT (5 nM). All analyses were carried out at room temperature (21±1°C) for 90 minutes and terminated either by rapid filtration (homogenates) or by two successive 2 minute washes in ice-cold buffer (autoradiography). Densities of both D_2 and D_3 receptors, as determined by quantitative densitometry, were based on appropriate standards which were co-exposed with tissue sections. Overall, female pups demonstrated a 52% higher density of striatal D_3 receptors, relative to male pups. A significant drug effect ($F_{1,5}$=80; $p \leq 0.003$) was apparent when analyzing D_3 receptors in the striatum. Dopamine D_3 receptor density was unchanged in the nucleus accumbens. These results suggest that prenatal cocaine administration alters D_3 receptor density across gender and region, and moreover, that a gender difference exists with females exhibiting a higher density of striatal D_3 receptors in comparison to males. Prenatal IV cocaine-induced alterations of presynaptic striatal D_3 receptors could result in significant gender-related differences in D_3 receptor-modulated dopamine release/synthesis. (Supported by DA06638 and DA09160)

555.4

EFFECTS OF QUINOLINIC ACID LESIONS ON THE DISTRIBUTION OF D3 RECEPTORS IN RAT BRAIN
Catherine Chen*, W.D. Essman, S. McElligott, M.P. Kung, H. Kung and P. McGonigle Depts. of Pharmacology and Radiology, University of Pennsylvania, Phila., PA 19104.

Quinolinic acid lesions of the cell bodies in the nucleus accumbens (NAc) were used to examine the cellular localization of D3 receptors in the rat brain. A unilateral injection of quinolinic acid (150 nmol/0.5 µl) was administered into the NAc (ML+1.5; AP+1.5 from bregma ; DV-6.8 from the dura) at a rate of 0.1 µl/min. The contralateral NAc received a vehicle injection. Eight days later, the animals were sacrificed and coronal sections were processed for histology and quantitative autoradiography. D3 and D2 receptors were labelled with [^{125}I]-7-Trans-OH-PIPAT and [^{125}I]-NCQ-298, respectively. A marked loss of neuronal perikarya was observed in the shell and core of the NAc surrounding the site of injection. The density of D3 receptors was reduced in both the shell (dorsal - 60±8%; ventral - 43±4%) and the core (dorsal - 54±10%; ventral - 42±6%) of the NAc. Quinolinic acid has been shown to eliminate GABAergic efferent neurons at this dose, suggesting that D3 receptors are on the cell bodies or dendrites of these cells. The density of D2 receptors in the NAc was not altered by the lesion, indicating that D2 and D3 receptors are not co-localized on these cells. The density of D3 receptors was also reduced in the ipsilateral substantia nigra pars compacta (45±11%) and the ventral tegmental area. This result suggests that D3 receptors are also located on the terminals of striatonigral fibers originating in the NAc. The D1 receptor has a similar cell body and terminal localization, suggesting that D1 and D3 receptors may be co-expressed by some cells in the NAc. (Supported by USPHS GM 34781 and NS-24538)

555.5

REPEATED COCAINE ADMINISTRATION INCREASES DOPAMINE RECEPTOR-REGULATED ADENYLYL CYCLASE ACTIVITY IN RAT CAUDATE PUTAMEN. E.M. Unterwald*, J.J. Fillmore, and M.J. Kreek. The Rockefeller University, New York, NY 10021.

Cocaine binds to the dopamine transporter and prevents the reuptake of dopamine into presynaptic dopaminergic terminals. Repeated daily cocaine administration to rats for 14 days has been shown to upregulate dopamine D_1 receptors (Unterwald et al., 1994). Adenylyl cyclase activity was measured to investigate the functional consequences of cocaine-induced dopamine receptor changes. Male Fischer rats were injected three times daily at one-hour intervals with saline or cocaine HCl (15 mg/kg, ip) for 1, 7, or 14 days. The ability of dopamine and SKF-82958, a selective D_1 receptor agonist, to stimulate adenylyl cyclase in the rostral portion of the caudate putamen was examined using a cAMP radioligand binding assay in crude membrane preparations. Dopamine, 10^{-8} to 10^{-4} M, produced a dose-dependent increase in cAMP formation with a maximum stimulation of 207 $\pm$ 2.7% over basal levels in saline-injected rats. Administration of cocaine did not significantly effect dopamine-stimulated cyclase activity. SKF-82958, 10^{-8} to 10^{-4} M, produced a dose-dependent increase in cAMP formation with a maximum stimulation of 192 $\pm$ 5.9% over basal levels. Administration of cocaine for 1 or 7 days did not significantly alter SKF-82958-stimulated cyclase activity. However, 14 days of cocaine administration significantly increased the stimulation of cyclase activity by SKF-82958. These findings suggest that chronic, repeated cocaine administration results in an enhancement of D_1 receptor-mediated effector function in the rostral caudate putamen. [Supported by grants from NIDA (EMU and MJK) and the Aaron Diamond Foundation (MJK)].

555.6

DOPAMINE-INDUCED DEPOLARIZATION OF MEDIAL PREFRONTAL CORTICAL NEURONS. W.-X. Shi* and B.S. Bunney. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Dopamine (DA) innervation in the prefrontal cortex (PFC) is particularly sensitive to stress and has a powerful influence on subcortical DA systems. Malfunction of this system has been suggested to play a critical role in the pathogenesis of schizophrenia. To understand how DA may act in the PFC, we made whole cell recordings from rat medial PFC neurons in an interface brain slice preparation. Confirming the result of a previous intracellular study (Penit-Soria et al., *Brain Research* **254**:263-274, 1987), we found that DA dose-dependently (1-200 μM) depolarized most mPFC neurons located in layers V and VI (30/34, ranging from 2.5 to 15 mV). This effect of DA persisted in the medium containing TTX (1 μg/ml, n=3) or zero Ca^{2+} and 4 mM Mg^{2+} (n=2), suggesting a direct effect of DA on the recorded cell. Using electrodes containing Lucifer Yellow (0.1%), nine DA-responding neurons were intracellularly labeled, six of them were identified as pyramidal neurons and three as non-pyramidal neurons, suggesting that DA affects both projection and interneurons in the PFC. Surprisingly, however, the depolarizing effect of DA was not mimicked by either the D_1 agonist SKF38393 (1-10 μM, n=4) or the D_2 agonist quinpirole (1-10 μM, n=5) or the mixture of the two (n=2). The mixed D_1/D_2 agonist apomorphine (10 μM) also produced no effect on PFC neurons (n=3). Furthermore, neither the D_1 antagonist SCH23393 (10-30 μM, n=7) nor the D_2 antagonist eticlopride (10 μM, n=2) blocked the effect of DA. These preliminary results suggest that the depolarizing effect of DA seen in PFC neurons is not mediated by the known DA receptors that should be sensitive to the DA active agents used above. Whether DA produces this effect by acting on a new type of DA receptor or non-specifically on other transmitter receptors remains to be determined. Supported by PHS award MH28849, the NPF, the NARSAD, and the State of Connecticut.

555.7

EXPRESSION OF D_1 RECEPTOR-MEDIATED INHIBITION OF MIDBRAIN DA NEURONS REQUIRES CO-ACTIVATION OF POSTSYNAPTIC D_2 RECEPTORS. P.L. Smith, W.-X. Shi, B.S. Bunney*. Depts of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We previously reported that low doses of the D_1 antagonist SCH23390 partially but significantly reversed *d*-amphetamine-induced inhibition of substantia nigra dopamine (DA) neurons in paralyzed rats (low cervean isolé preparation) and suggested that D_1 receptors are involved in the feedback control of DA neurons. However, we also showed that the D_1 agonist SKF38393 alone had no consistent effect on DA cells. Since *d*-amphetamine by releasing DA activates both D_1 and D_2 receptors and SKF38393 acts on only D_1 receptors, we hypothesized that the expression of the D_1-mediated effect on DA neurons may require co-activation of D_2 receptors. To test this hypothesis, we examined the effect of SKF38393 in animals pretreated with the D_2 agonist quinpirole. Quinpirole potently inhibited DA neurons. To avoid a complete inhibition, low doses of quinpirole (<40 μg/kg, i.v.) were first used to produce a partial inhibition. In most of these cells (8/9), however, SKF38393 (20 mg/kg, i.v.) produced no effect on the remaining activity of the cell. Considering the fact that DA autoreceptors are much more sensitive to a DA agonist than postsynaptic DA receptors, we further postulated that a D_1-mediated effect depends on activation of postsynaptic D_2 receptors and that the doses of quinpirole used were not high enough to activate those receptors. To examine this possibility, we injected quinpirole slowly to desensitize DA autoreceptors. By doing this, we were able to injected 40 μg/kg or more of quinpirole while keeping the cell active. In most of these cells (8/9), SKF38393 clearly produced a further inhibition of firing, which could be completely reversed by SCH23390 (20-80 μg/kg, i.v.). These results suggest that activation of D_1 receptors has an inhibitory effect on DA neurons and the expression of this effect requires co-activation of D_2 receptors most likely located on non-DA neurons. Supported by MH28849, the NPF, the NARSAD, and the State of Connecticut.

555.8

THE D2 RECEPTOR AGONIST QUINPIROLE EXCITES NEURONS OF THE GLOBUS PALLIDUS IN FREELY-MOVING RATS. K. C. Hooper*, D. A Banks, and G. V. Rebec. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

We have previously shown that selective stimulation of D2 receptors by systemic administration of the D2 agonist quinpirole (LY-171555) inhibits the activity of neurons in the striatum of awake, behaving rats (Hooper et al., Soc. Neurosci. Abstr. 1992 18:995).

To assess how this change in striatal activity affects neuronal activity in the globus pallidus, an output nucleus of the basal ganglia that receives an inhibitory projection from the striatum, we monitored single-unit activity in the globus pallidus of freely-moving male rats in response to the systemic administration of quinpirole at a dose known to inhibit striatal activity (1.0 mg/kg). Our results indicate that quinpirole excites neurons in the globus pallidus of awake rats. This effect probably occurs through a disinhibitory mechanism since GABAergic striatal cells, which project to the globus pallidus, are inhibited by quinpirole. Consistent with this view, preliminary data indicate that the quinpirole-induced excitation of pallidal neurons is reversed by eticlopride, a D2 receptor antagonist.

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555.9

DOPAMINE RELEASE FROM HUMAN CEREBRAL CORTEX SLICES IS MODULATED BY D_2-TYPE AUTORECEPTORS. E. Fedele, °P. Severi, °A. Ruelle and M. Raiteri*. Institute of Pharmacology and Pharmacognosy, University of Genova, Viale Cembrano 4, 16148 Genova and °Division of Neurosurgery, Galliera Hospital, Via A. Volta 8, 16128 Genova, Italy.

In this study we have investigated the existence and the pharmacological profile of dopamine autoreceptors in superfused, electrically-stimulated slices of human neocortex prelabelled with [^{3}H]DA. Human cerebral cortex tissue was obtained from patients undergoing neurosurgery to remove subcortical tumours. Samples of frontal (1), parietal (2) and temporal (4) cortex from 5 males and 2 females (aged 32-57 years) were used. [^{3}H]DA release was elicited using a continuous electrical stimulation protocol (3 Hz, 2 ms, 24 mA). Full concentration-response curves for agonist(s), either in the presence or in the absence of (-)sulpiride, along with the appropriate controls were done on cerebral tissue obtained from a single patient. Since no significant differences both in the evoked overflows and in the drugs' effects have been observed among the various cortical region, the data from the different experiments were pooled. Quinpirole (0.01 - 10 μM) was able to inhibit [^{3}H]DA overflow in a concentration-dependent manner, the EC_{50} being 25 nM and the maximal effect, reached at 10 μM, approx. 80%. On the contrary, SKF 38393 was devoid of any significant effect up to 10 μM. The concentration-response curve of quinpirole was shifted to the right in the presence of (-)sulpiride (0.1 and 1 μM). The calculated apparent pA_2 value was 8.26 (8.51 at 1 μM and 8.02 at 0.1 μM). (-)Sulpiride (1 μM) did not affect on its own [^{3}H]DA release. In conclusion, this study represent the first demonstration that in human brain the release of dopamine from mesocortical nerve terminals is controlled by receptors sensitive to D_2 receptor ligands thus indicating the presence of presynaptic autoreceptors of the D_2 type which, however, do not seem, under our experimental conditions, to be tonically activated by the released neurotransmitter. Supported by grants from the Italian M.U.R.S.T.

555.10

SYNERGISTIC ACTIVATION OF DOPAMINE SOMATODENDRITIC AUTORECEPTORS BY PRAMIPEXOLE, A D3-PREFERRING AGONIST, AND U-91356A, A D2-PREFERRING AGONIST. M.F.Piercey*, M.W.Smith, S.R.Haadsma-Svensson, and W.E.Hoffmann. CNS Diseases Research, The Upjohn Company, Kalamazoo, Michigan, 49001 USA.

Membranes from CHO cell lines expressing D_1, D_2, D_3, and D_4 receptors were used to demonstrate preferential affinities of dopamine agonists U-91356A and pramipexole to D_2 and D_3 receptors, respectively, whereas apomorphine bound with good affinity to all subtypes. Using dye-filled glass microelectrodes, DA neurons in SNPC were identified by classical electrophysiological/neuroanatomical criteria (Bunney *et al.*, JPET 155:560, 1973). Apomorphine (ED50 = 13 μg/kg i.v.), pramipexole (ED50 = 66 μg/kg i.v.), and U-91356A (ED50 = 32 μg/kg i.v.) all depressed DA neuronal cell firing in the SNPC. U-99194A, the D_3-selective antagonist (J. Neurotransm. 94:11-19, 1993), selectively bound to D_3 receptors, and more effectively blocked apomorphine than it blocked either U-91356A or pramipexole. Pramipexole and U-91356A were combined in solution such that their final concentration in the mixture was inversely proportional to their relative individual potencies. The resulting potency (ED50 = 15 mg/kg) was equal to that for apomorhine and greater than for either compound alone. Moreover, U-99194A more effectively antagonized the pramipexole/U-91356A combination than it did either of the compounds given alone. It is concluded that there is a D_2-D_3 synergism at the somatodendritic autoreceptor level.

555.11

SELECTIVE DOPAMINE D3 RECEPTOR ACTIVATION BY 7-OH-DPAT INDUCES HYPOMOTILITY AND DECREASES STRIATAL DOPAMINE RELEASE BUT NOT METABOLISM IN AWAKE RATS. R.R.Gainetdinov, T.D.Sotnikova, T.V.Grekhova and K.S.Rayevsky*. Institute of Pharmacology RAMS, Baltiyskaya 8, Moscow125315, Russia

We have previously found a rather good correlation (r=0.75,p<0.02) between the abilities of acute typical and atypical neuroleptics to affect preferentially dopamine (DA) metabolism or release in the rat dorsal striatum in vivo and their relative potencies at D2 and D3 receptor. The present microdialysis study in awake Wistar rats with selective DA D3 agonist 7-OH-DPAT (KiD3 - 0.78 nM; KiD2 - 61 nM for CHOcells; Levesque et al.,1992, PNAS 89:8155) gives a further support for the hypothesis that DA D3 but not D2 autoreceptors are responsible for the presynaptic regulation of DA release in rat striatum. Using quantitative microdialysis "point of no net flux" method we were able to estimate the interstitial free concentration (IFC) of 7-OH-DPAT in the dorsal striatum. 7-OH-DPAT as well as DA, DOPAC and HVA were detected by HPLC/ED. In 20 min after i.p. administration of 7-OH-DPAT in dose 6mg/kg its IFC reached maximum: 1918+299 nM (n=4). The marked decrease of DA release (to 20-30% of basal) and metabolism (to about 50%) was also observed. Due to approximately linear dependence of the dose -dialysate concentration observed it may be inferred that 7-OH-DPAT dose of 0.05 mg/kg gives IFC which would not exceed 16 nM. Importantly,that 7-OH-DPAT in this dose produced significant decrease of DA release (to about 60%), but failed to affect metabolites level in the rat striatum, whereas in dose 0.25 mg/kg (calculated IFC 80 nM) decreased, both DA release and metabolism approximately to 30 and 70%, respectively. The threshold dose for the significant decrease of DA release was 0.025 mg/kg (calculated IFC 8 nM), while even in a dose 0.01 mg/kg (calculated IFC 3.2 nM) the drug induced hypomotility. In conclusion,7-OH-DPAT at the dose range well below the affinity to DA D2 receptor induces hypomotility and decreases DA release but not its metabolism in the dorsal striatum of awake rats.

555.12

MODULATION OF MESOLIMBIC, MESOCORTICAL AND NIGROSTRIATAL DOPAMINE RELEASE AND SYNTHESIS BY DOPAMINE D_3 AUTORECEPTORS: INFLUENCE OF THE SELECTIVE D_3 ANTAGONIST, S 14297. A. Gobert*, J.-M. Rivet, V. Audinot, J.-L. Peglion and M.J. Millan. Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, France.

Dopaminergic autoreceptors control the activity of ascending dopaminergic pathways. Here, we employed the novel, selective D_3 antagonist, S 14297, to evaluate the potential contribution of D_3 receptors to this action. As a measure of synthesis, the ratio of the levels of the dopamine metabolite, DOPAC, were compared to those of dopamine (DA) in the cortex, nucleus accumbens, olfactory bulb and striatum of (male) rats. For determination of DA release, its levels were measured in dialysates of the nucleus accumbens and striatum of freely-moving rats. Doses are in mg/kg, s.c.. The selective D_3 agonists, 7-OH-DPAT (0.0025 - 0.04) and quinpirole (0.01 - 0.63) potently increased DA:DOPAC ratios whereas the midly preferential D_2 agonist, piribedil was weakly active (0.16 - 10.0). Further, across these and other agonists, for each tissue, correlation coefficients for potency in decreasing synthesis were higher with affinity at cloned D_3 receptors (r = 0.81 - 0.91) than cloned D_2 receptors (r = 0.65 - 0.79) and were very high with potency in evoking hypothermia (0.96 - 0.98). S 14297, which slightly enhanced turnover of DA alone, dose-dependently (0.31 - 10.0) prevented the influence of 7-OH-DPAT (0.16) upon DA turnover. In contrast, its stereoisomer, S 17777, which displays 20-fold lower affinity at D_3 sites, was inactive. 7-OH-DPAT (0.16) greatly (- 80 %) reduced dialysate levels of DA in the accumbens and striatum. This action was markedly inhibited by S 14297 (1.25) which, alone, failed to modify levels of DA. S 17777 (20.0) was inactive. In conclusion, D_3 autoreceptors inhibit synthesis and release of DA in ascending dopaminergic projections and S 14297 acts as an antagonist at these sites. These data support a role of D_3 receptors in the modulation of mood and in the pathogenesis of psychiatric and motor disorders.

555.13

INTERACTIONS BETWEEN D_3-PREFERRING AGONISTS AND ANTAGONISTS IN BIOCHEMICAL AND BEHAVIORAL MODELS IN RATS. K.A.Svensson*[1], N.Waters[2], M.P.Stone[1], J.E.Myers[1] and A.Carlsson[2]. [1]CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001, and [2]University of Göteborg, Göteborg, Sweden.

Behavioral experiments in rats show that the D_3-preferring agonists (+)-7-OH-DPAT and pramipexole were highly efficacious in reducing exploratory activity as compared to D_2 agonists. In the same animals, the accumulation of DOPA or the release of dopamine in various brain regions was not affected by the ED_{50} dose causing reduced locomotor activity. In contrast, for the more D_2-preferring agonists, such as apomorphine, (+)-3PPP, and quinpirole, there was a closer correlation between doses that reduce locomotor activity and doses that reduce DOPA accumulation and dopamine release. A similar trend was also observed for the D_2-selective compound U-91356. The data suggest that the D_3-selective agonists, in contrast to the more D_2-selective agonists, are able to reduce locomotor activity at doses that do not affect dopamine release and/or synthesis. Furthermore, these data are in line with the hypothesis that the D_3 receptor is postsynaptic with an inhibitory influence on rat locomotor activity. Our present work focuses on the interaction between D_3-preferring agonists and antagonists on behavioral and neurochemical parameters in order to shed further light on the possible functional role of the D_3 receptor.

555.14

S 14297, A SELECTIVE, POTENT AND STEREORESOLVED LIGAND ANTAGONIST AT HUMAN D_3 RECEPTORS ABOLISHES 7-OH-DPAT-INDUCED HYPOTHERMIA IN THE RAT. V. Audinot, J.-L. Peglion, J.-M. Rivet*, J. Vian, J.-F. Prost, M. Spedding and M.J. Millan, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, France.

Evaluation of the functional significance of dopamine D_3 receptors has been hindered by the lack of a selective antagonist and by the absence of a model of their activation in vivo. This study addressed these questions employing the novel naphtofurane, (+)- S 14297. At cloned, human D_3 or D_2 receptors transfected into CHO cells, using [^{125}I]-iodosulpiride as a radioligand, S 14297 showed high selectivity and potency at D_3 vs D_2 sites: Kis (nM) = 13 and 297 as compared to its (-)-isomer, S 17777 (406 and 3544), AJ 76 (70 and 154), haloperidol (2 and 0.5) and the agonists, 7-OH-DPAT (2 and 103), quinpirole (6 and 265) and piribedil (243 and 126). Scatchard analysis suggested S 14297 to bind competitively; Kd/Bmax (+ 30 nM, S 14297) = 3.6 nM/2.2 pmoles/mg protein vs (without S 14297) 1.0/2.1. In (male) rats, 7-OH-DPAT (0.01 - 0.63 mg/kg, s.c.) elicited hypothermia, an action potently mimicked by quinpirole (0.04 - 2.5) whereas piribedil (0.63 - 10.0) was weakly active. For a total of 8 agonists, potency correlated powerfully with affinity at D_3 receptors (0.84) but not D_2 receptors (0.49). S 14297 (0.04 - 1.25), but not S 17777 (> 10.0), mimicked haloperidol (0.01 - 0.16) and AJ 76 (0.16 - 2.5) in abolishing hypothermia induced by 7-OH-DPAT (0.16). Across 8 antagonists, the correlation was very high for D_3 affinity (0.97) but less pronounced for D_2 affinity (0.82). S 14297 (up to 20.0 mg/kg, s.c.) elicited neither catalepsy nor prolactin secretion indicating a lack of D_2 antagonist properties *in vivo*. Further, antagonist potency in eliciting prolactin secretion correlated better with affinity at D_2 (0.98) than D_3 (0.81) sites. The affinity of S 14297 at diverse monoaminergic receptors was > 80-fold lower than at D_3 receptors. In conclusion, D_3 receptors mediate hypothermia in the rat and S 14297 is as a selective, potent and stereoresolved antagonist of D_3 receptors *in vivo*.

555.15

EFFECTS OF THE D_3-PREFERRING DOPAMINE AGONIST PRAMIPEXOLE ON REGIONAL BRAIN ENERGY METABOLISM AND THEIR RELATIONSHIP TO DISTRIBUTION OF ^{3}H-PRAMIPEXOLE BINDING SITES. M.Camacho-Ochoa*, D.L.Evans, E.L.Walker, S.Mattichak, and M.F.Piercey. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

In studies with cloned subtypes of the D2 receptor subfamily, Pramipexole (PPX) has higher affinity for the D_3 as compared to the D_2 and D_4 subtypes, and none for D_1 receptors (Neurosci Abs 19:80, 1993). Using Sokoloff's quantitative 2-deoxyglucose (2DG) autoradiography method (J Neurochem 28:897, 1977), PPX, 0.3 mg/kg, i.v., increased energy metabolism in several basal ganglia regions (substantia nigra, subthalamic nucleus), but increased it even more in sensory and motor cortex. No significant effects were observed in mesolimbic DA areas (n. accumbens, olfactory tubercle, islets of Calleja, VTA), or in primary areas of nigrostriatal innervation (caudate, globus pallidus). Receptor binding autoradiography was used to evaluate distribution of ^{3}H-PPX (5 nM, 62 Ci/mmole) binding sites within rat brain. Talipexole, 10 µM, was used to evaluate nonspecific binding. ^{3}H-PPX binding sites were found in the islets of Calleja, an area dominated by D_3 receptors (PNAS 89:8155, 1992), nucleus accumbens, and olfactory tubercle, as well as in the caudate nucleus, a DA postsynaptic area high in D_2 receptors, but in which D_3 pharmacological effects also occur (Neurosci Abs 19:80, 1993). ^{3}H-PPX binding sites were low in VTA and substantia nigra, areas rich in DA cell bodies, and in sensory and motor cortex. Thus, increases in energy metabolism occurred in areas low in ^{3}H-PPX binding sites, but not in areas with the highest ^{3}H-binding site concentrations. The data emphasize the ability of 2DG autoradiography to measure downstream functional effects of pharmacological agents.

555.16

EFFECTS OF THE DOPAMINE D3 SELECTIVE AGONIST R-(+)-7-OH-DPAT IN THE INTRACRANIAL SELF-STIMULATION (ICSS) PARADIGM T. Kling-Petersen*[1], E. Ljung[1], N. Waters[1] and K. Svensson[2] [1]Dept. of Pharmacology, Medicinaregatan 7, 413 90 Göteborg, Sweden and [2]CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001, USA.

The dopamine D3 selective agonist R-(+)-7-OH-DPAT produced a significant increase in reward thresholds over a wide dose range (0.094 - 96.0 nmol/kg, s.c.) similar to the effects seen with the classical D2 antagonist haloperidol (0.005 - 0.16 mg/kg, i.p.) when tested in rats trained to self-stimulate in a rate-intensity brain stimulation reward paradigm. At higher doses, 7-OH-DPAT produced a significant facilitation of reward thresholds.

7-OH-DPAT, at doses that per se produced a increase in reward threshold, significantly enhanced d-amphetamines facilitatory effect on brain stimulation reward when administered in combination with d-amphetamine (0.25 or 1.0 mg/kg, s.c.). This is in contrast to haloperidol that antagonize the facilitatory effects of d-amphetamine.

The data from the ICSS model corroborate earlier observations that 7-OH-DPAT is capable of producing hypolocomotion over a wide dose range (Svensson, K. et al, J Neural Transm, 95, 71, 1994) without the concomitant reduction of brain dopamine release and synthesis seen after D2 selective agonists. The data support the hypothesis that the functional D3 receptor is a postsynaptic receptor with a psychomotor inhibitory function in the rat.

555.17

CHARACTERIZATION OF A SELECTIVE DOPAMINE D3 RECEPTOR AGONIST LIGAND, [3H]PD 128907. H. C. Akunne[1*], P. Towers[2], G. J. Ellis[2], D. Dykstra[3], H. Wikstrom[3], L. Wise[4], T. Heffner[1] and T. Pugsley[1]. Neurosci [1]Pharm/ [4]Chem, Parke-Davis Pharm. Res., Div. of Warner-Lambert Co., Ann Arbor, MI, 48105 USA; [2]Cardiff Lab, Amersham Corp, Forest Farm, Cardiff, Wales CF4 &YT and [3]Dept Of Med Chem, Univ of Groningen, NL-9713 AW Groningen, Netherlands.

A selective dopamine (DA) D3 receptor agonist ligand, PD 128907 [(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H[1]benzopyrano4,3b]-1,4-oxazin-9-ol] exhibits about a 1000-fold selectivity for human D3 receptors (K_i, 1 nM) versus human D2 receptors (K_i, 1183 nM) using [^{3}H]spiperone as the ligand. Binding studies with [^{3}H]PD 128907, prepared as a selective probe for the D3 receptor, showed saturable, high affinity binding in human D3 receptors expressed in CHO-K1 cells with a K_d of 0.99 nM and a binding density (B_{max})of 826 fmol/mg protein (in the absence of Na^+). Under the same conditions, there was no specific binding in CHO-K1 cells expressing human D2 receptors. [^{3}H]PD 128907 binding in all these studies was monophasic with the Hill coefficients close to unity. The K_d and B_{max} in the rat olfactory tubercle membranes, presumably rich in D3 receptors, were 1.3 nM and 116 fmol/mg protein respectively; while in the rat striatal membranes, the K_d was 3.8 nM and the B_{max} was 298 fmol/mg protein. There was no specific binding in the rat striatal membranes in the presence of 120 mM NaCl (a condition that inhibits binding to the D2 receptor); in comparison the binding parameters were not significantly different in olfactory tubercle and CHO-K1-D3 cell membranes in the presence or absence of NaCl. This suggests that [^{3}H]PD 128907 labels D3 receptors specifically in the presence of NaCl without $MgCl_2$. The rank order of potency for inhibition of [^{3}H]PD 128907 binding with reference DA agents was consistent with the pharmacology for D3 receptors. This highly selective new ligand possesses high specific activity, high specific binding activity with low non-specific binding and therefore should be useful for characterizing further the DA D3 receptors.

555.18

POSSIBLE INVOLVEMENT OF DOPAMINE D3 AUTORECEPTORS IN THE BEHAVIORAL EFFECTS OF PD 128907. A.E. Corbin*, J.N. Wiley and T.G.Heffner. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

PD 128907 has been described as a dopamine (DA) D3 receptor agonist-like compound (D3 Ki=1.1 nM, D2 Ki=1183 nM, D1 Ki>10000 nM; DeMattos et al., Soc Neurosci Abstr, **19**, 77, 1993). Previous studies in rats have shown that PD 128907 inhibited DOPA accumulation (Ibid.) and decreased striatal DA release as measured by microdialysis (Cooke et al., Soc Neurosci Abstr, **19**, 77, 1993). The present studies show that PD 128907 reduces spontaneous locomotor activity (LMA) in mice (ED50=0.75 mg/kg ip) and rats (ED50= 0.03 mg/kg sc). However, at higher doses, LMA inhibition diminishes in rats, a profile similar to the DA D2 agonist apomorphine and the DA D3 agonist-like compound (+)-7-OH-DPAT. Doses of PD 128907 that reduce LMA in rats also reduce DOPA accumulation in the GBL/NSD model (ED50s=0.014 and 0.37 mg/kg sc in mesolimbic area and striatum) and reduce DA overflow in awake rats in the microdialysis model (28-52% decreases at 0.03-0.1 mg/kg sc). PD 128907 dose-dependently reverses the LMA stimulation produced by the indirect DA agonist amphetamine in mice (ED50=0.3 mg/kg ip) and rats (ED50=0.01 mg/kg sc). At higher doses, PD 128907 produces stereotyped behavior (sniffing at 1 mg/kg sc) and reverses the behavioral sedation produced by the DA depleting agent reserpine (ED50=6.3 mg/kg sc). The high D3 potency of PD 128907 suggests that at low doses, this drug may be acting at D3 receptors to inhibit spontaneous as well as amphetamine-stimulated LMA. The correlation of decreased DA synthesis and release with these low dose behavioral effects of PD 128907 suggests mediation by presynaptic D3 receptors. High dose studies reveal DA agonist-like effects which may be mediated by stimulatory actions at D2 receptors or D2 and D3 receptors.

555.19

Olanzapine compared to clozapine and haloperidol as dopamine and serotonin receptor antagonists in rats. Susan K. Hemrick-Luecke*, Frank P. Bymaster and Ray W. Fuller. Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN 46285 U.S.A.

Receptor occupancy in rats was estimated by the ability of test drugs (given ip) to counteract effects of EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline), which irreversibly inactivates dopamine D1 and D2 receptors as well as serotonin (5HT)2A receptors. EEDQ (10 mg/kg, sc) increased striatal concentrations of 3,4-dihydroxyphenyl-acetic acid (DOPAC) at 24 hrs. Haloperidol at 0.3 mg/kg increased striatal DOPAC concentrations at 1 hr and antagonized the prolonged EEDQ increase in DOPAC as well as the EEDQ-induced decrease in radioligand binding to D2 receptors. Haloperidol at doses up to 3 mg/kg did not block the effects of EEDQ on radioligand binding to striatal D1 or cortical 5HT2A receptors. Clozapine increased striatal DOPAC at 10 mg/kg, but a higher dose (60 mg/kg) only partially antagonized the EEDQ-induced increase in DOPAC. Clozapine was more potent in antagonizing the EEDQ-induced decrease in cortical 5HT2A receptors as compared to striatal D1 or D2 receptors. Olanzapine increased striatal DOPAC at 1 mg/kg, whereas a higher dose (10 mg/kg) was needed to block the EEDQ-induced increase in DOPAC. Olanzapine blocked EEDQ-induced decreases in radioligand binding to striatal D2 and cortical 5HT2A receptors at lower doses than required to block effects on striatal D1 receptors. Neuroendocrine measurements are compatible with these findings. Haloperidol antagonized the dopamine D2 receptor-mediated increase in rat serum corticosterone by pergolide, with an ED50 of 0.18 mg/kg, whereas the ED50 values for olanzapine and clozapine were 3 mg/kg and >10 mg/kg. The quipazine-induced rise in serum corticosterone, a 5HT2A receptor-mediated effect, was blocked by olanzapine and clozapine with ED50 values of 0.57 mg/kg and 2.6 mg/kg but not by haloperidol (at 1 mg/kg). These data show that olanzapine, an atypical antipsychotic drug candidate, has lower occupancy of dopamine D1 and D2 receptors than of 5HT2A receptors *in vivo*. In this respect, olanzapine is like clozapine, but in all cases, olanzapine was more potent than clozapine.

555.20

LEVODOPA BUT NOT BROMOCRIPTINE INDUCES TRE- AND CRE-BINDING ACTIVITIES IN THE RAT STRIATUM.

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Long-term treatment of Parkinson's disease with levodopa is associated with a high incidence of adverse effects such as wearing-off phenomenon and dyskinesia. Bromocriptine, on the other hand, has been reported to produce these effects less frequently. In order to elucidate the effects of levodopa and bromocriptine on transcription factors, which may produce long-lasting changes in the responsibility of central dopaminergic system, we examined the effect of these compounds on 12-*O*-tetra-decanoylpholbol 13-acetate-responsive element (TRE)- and cyclic AMP-responsive element (CRE)-binding activities by using gel-mobility assay. Administration of levodopa (100 mg/kg; ip) with benserazide (50 mg/kg; ip) to rat increased both TRE- and CRE-binding activities in the dorso-lateral aspect of the caudate-putamen. Bromocriptine (2.5, 5mg/ kg) did not induce TRE- and CRE-binding activities. As has been reported on c-*fos* expression in the dopamine depleted striatum, levodopa, a mixed D-1/D-2 dopamine receptor agonist, but not bromocriptine, a D-2 agonist, may induce TRE- and CRE-binding activities.

555.21

REGIONAL BRAIN GLUCOSE METABOLISM FOLLOWING α2 ADRENERGIC BLOCKADE IN BIPOLAR MALES. Mark E. Schmidt, John A. Matochik*, Robert C. Risinger, Philip Mitchell, William Z. Potter. Section on Clinical Pharmacology, NIMH, Bethesda,MD, 20892.

Several medications used to treat mood disorders have indirect effects on the α2 adrenergic receptor. Moreover, altered regulation of this receptor has been postulated to play a pathophysiologic role in depression. Comparing FDG PET scans before and after an infusion of the α2 antagonist idazoxan (IDX) may provide a possible measure of central α2 function. Using this method, we hope to test hypotheses regarding α2 function in psychiatric disorders and therapeusis. Six males with Bipolar Mood Disorder underwent FDG scans before and after infusion of IDX, 200 mcg/kg. 5 templates comprised of 62 regions of interest (ROIs) were applied to 5 matching transaxial planes from each PET scan. Baseline global metabolism was significantly lower ($p<.01$) in the patient group (9.57± 1.51) compared to a control group of 13 normal males (11.71± 1.14). Absolute metabolic rate significantly increased in 13 ROIs including 6 prefrontal and 4 occipital ROIs ($p<.05$). There was a trend for an increase in global metabolic rate after IDX ($p<.10$). In comparison, healthy males showed no effect on global metabolic rate, also showed significant increases in occipital ROIs, but showed no change or a significant decrease in metabolism in frontal ROIs. While preliminary, our finding of lower baseline global metabolism replicates previous findings in bipolar depressed patients. The regional findings suggest common responses to α2 blockade in occipital regions but group differences notably in prefrontal cortex.

555.22

EFFECTS OF ADRENOCEPTOR AGONISTS ON ELECTRICAL SLOW WAVES AND PHASIC CONTRACTIONS OF CAT COLON CIRCULAR MUSCLE. K. Venkova and J. Krier*, Dept. Physiol., Michigan State Univ., East Lansing, MI 48823

The effects of isoproterenol, phenylephrine and norepinephrine on spontaneous electrical slow waves (ESW) and associated phasic contractions (PC) of colon circular muscle were studied using intracellular microelectrode recordings and isometric force measurements. Atropine (0.5 μM) and hexamethonium (1 μM) were present to prevent interaction with cholinergic neurons. ESW consist of an upstroke phase followed by a plateau and repolarization . Muscle cells near the submucosal border had resting membrane potential of 71 ± 2 mV. ESW had a mean rate of upstroke depolarization of 308 ± 33 mV/s, an amplitude of 32 ± 3 mV , duration of 6-20 s and a rate of repolarization of 60 ± 6 mV/s. The frequency of ESW and PC ranged between 4-6 cycles per min. Isoproterenol (0.1-1 μM) reduced the duration of ESW and the amplitude of PC by 51% and 83%, respectively. Phenylephrine (0.05-0.5 μM) increased the duration of ESW and the amplitude of PC by 40.5% and 34%, respectively. Norepinephrine (0.01-0.5 μM) resembled the effect of phenylephrine, increasing the duration of ESW by 20% and the amplitude of PC by 28%. All adrenoceptor agonists did not change the resting membrane potential, the amplitudes of ESW , the frequency of ESW and PC and the rate of upstroke depolarization and repolarization of the ESW. The effects of isoprenaline were blocked by propranolol (1 μM) and the effects of phenylephrine and norepinephrine were blocked by phentolamine (3 μM). Propranolol potentiated the excitatory effects of norepinephrine. Nitrendipine (1-10 μM) and verapamil (1-10 μM), antagonists of L-type Ca^{2+} channels, reduced the duration of ESW to 2.5-3 s and suppressed PC. Under these conditions, isoprenaline, phenylephrine or norepinephrine did not affect ESW and PC. The data indicate that ESW and PC of colon circular muscle are regulated by α- and β-adrenoceptors. The data suggest that adrenoceptor agonists cause changes in the duration of ESW and the amplitude of the associated PC by interacting with L-type Ca channels. (NIH-DK-29920)

556.1

INCREASED NITRIC OXIDE SYNTHASE ACTIVITY BY SIN-1 AND SODIUM NITROPRUSSIDE IN INTACT NEURONAL CELLS.
J. Hu* and E.E. El-Fakahany. Division of Neuroscience Research in Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455.

It has been shown that nitric oxide (NO) regulates NO synthase activity through negative feedback in cytosolic enzyme preparations in a variety of cell types. We tested the effects of the NO-generating compounds *S*-nitroso-*N*-acetyl-penicillamine (SNAP), 3-morpholinosydnonimine (SIN-1) and sodium nitroprusside (SNP) on NO synthase activity in intact neuroblastoma N1E-115 cells. SIN-1 and SNP at concentrations which elicit almost complete inhibition of NO synthase activity in cytosolic enzyme preparations of these cells only inhibited about 30% of the enzyme activity in intact cells, as measured by the conversion of [^{3}H]L-arginine into [^{3}H]L-citrulline during incubation for 45 min. Surprisingly, SIN-1 and SNP stimulated [^{3}H]L-citrulline formation when cells were incubated with the compounds for > 1.5 hours. Neither inhibitory nor stimulatory effects of SNAP on NO synthase were observed in the intact N1E-115 cellls. This is in contrast to the inhibitory effects of SNAP in cytosolic preparations of the enzyme. Measurement of the activity of lactate dehydrogenase indicated that there was no parallel increase in cell permeability in response to SIN-1 or SNP. The stimulation by SIN-1 and SNP of NO synthase activity was completely blocked by 3 mM EGTA, suggesting that an influx of Ca^{2+} is activated by the compounds in intact N1E-115 cells. Moreover, hemoglobin, superoxide dismutase and deferoxamine attenuated the effects of SIN-1, but not those of SNP, on [^{3}H]L-citrulline formation. Our results demonstrate that NO-generating compounds might somehow enhance Ca^{2+} influx, resulting in an up-regulation of NO synthase activity. These effects might be dependent on the chemical nature of the NO species released by the compounds.

556.2

TRANSMITTER MODULATION OF NMDA-ACTIVATED NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN RAT FRONTAL CORTEX.
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We have previously reported that activation of classical N-methyl-D-aspartate (NMDA) receptors can stimulate NOS activity in the frontal cortex. In this study we focused on other transmitters that may activate NOS or modulate the NMDA-activation of NOS. We found that of the transmitters examined, glutamate and acetylcholine (ACh) receptor agonists were the only ones to affect NOS activity. Glutamate stimulated NOS activity and this response is blocked by NMDA antagonists, but was unaffected by non-NMDA antagonists. These data are substantiated by the fact that AMPA, kainic acid (KA) and quisqualic acid (Quis) did not increase NOS activity, even in the presence of diazoxide which prevents desensitization of the glutamate receptors, suggesting that the glutamate response was due to activation of the NMDA receptor in this preparation.

Norepinephrine, dopamine and γ-aminobutyric acid (GABA) had no effect on either the basal or NMDA-activated NOS. However, while the basal NOS activity was unaffected by ACh agonists, 1 mM carbamylcholine chloride, a mixed ACh agonist, inhibited the NMDA response completely. This inhibition was not reversed by atropine, nor was it mimicked by muscarine. Nicotine (30 μM) inhibited the NMDA response, but this inhibition was not complete, perhaps due to receptor desensitization. The inhibitory effect of nicotine was insensitive to hexamethonium and d-tubocurare. Complete pharmacological characterization of this apparent novel nicotinic mechanism is currently under investigation. Supported by DA-02073.

556.3

SIMPLE GUANIDINE ANTAGONISTS OF NITRIC OXIDE SYNTHASE.
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The role of nitric oxide synthases (NOS) in the function of the nervous system under normal and ischemia/reperfusion conditions is a topic of great interest. The current known antagonists of NOS are limited in that they lack selectivity between the brain, endothelium and macrophage NOS isoforms. Thus, selective antagonists would be useful tools to elucidate the role of the three NOS isoforms. Using the ^{3}H-L-arginine to ^{3}H-L-citrulline conversion assay (PNAS 87, 682-685, (1990)), compounds were assayed for inhibition of rat cerebellar , mouse macrophage, and human endothelial NOS. In our initial search for novel antagonists of NOS, we speculated that simple guanidines would be a reasonable starting point. The screening results for a series of substituted guanidines will be presented. Briefly, we have discovered small simple guanidines which inhibit the NOSs in the micromolar range (similar to methylarginine and nitroarginine), but are not selective versus a particular isoform. We will present results which will demonstrate that all of the NOS isoforms are inhibited by small cyclic guanidines and that inhibition is very dependent upon the size of the molecule.

556.4

ISOLATION OF NITRIC OXIDE SYNTHASE (NOS) IN THE RAT UTERUS.
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The presence of NADPH-diaphorase-positive nerves in the rat uterus, and the role of nitric oxide (NO) during pregnancy, suggest that neurons containing NO may be important in regulating myometrial activity, uterine blood flow and other reproductive functions. NOS was isolated from the uterus of adult female rats by diethylaminoethyl (DEAE) column and further purified by 2',5'-ADP agarose. The chromatographic properties revealed two isoenzymes, NOS_1 and NOS_2. The molecular weights of both isoenzymes was approximately 155 Kd by SDS PAGE which was similar to NOS_1 and NOS_2 from rat brain. The enzymes required NADPH, Ca^{+2} and calmodulin as cofactors. However, in the absence of calmodulin and/or calcium NOS_1 was reduced by 96%, while NOS_2 was reduced by 70%. This maximal enzyme activity was similar for brain. These results demonstrate that the rat uterus contains NOS and the fundamental kinetic constants, Km and Vmax, are similar to brain NOS. The uterine NOS is NADPH-dependent and different enzyme isoforms are suggested by the calmodulin-dependence and trifluoroperazine-dependence of both uterine and brain NOS_1.

556.5

PHOTONEURAL REGULATION OF RAT PINEAL NITRIC OXIDE SYNTHASE
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Nitric oxide (NO) appears to play a role in the regulation of cGMP synthesis in the rat pineal gland. Pineal nitric oxide synthase (NOS) is dependent on calcium for its activity, and all its biochemical characteristics are similar to the constitutive form of the enzyme present in the cerebellum. The factors regulating the expression of the constitive form of NOS in the central nervous system remain largely unknown. We report here a photoneural regulation of NOS activity in the rat pineal gland. In the absence of the adrenergic stimulation following constant light exposure (LL) or denervation (bilateral superior cervical ganglionectomy), pineal NOS activity is markedly reduced. A maximal drop is measured after 8 days in LL (- 80% *versus* control). When rats are housed back in normal light:dark conditions (LD 12:12), pineal NOS activity returns to normal after 4 days. A partial decrease in pineal NOS activity is also observed when rats are placed for 8 days in LD 18:6 or shorter dark phases indicating that pineal NOS activity reflects the length of the dark phase. Because it is known that norepinephrine (NE) is released at night from the nerve endings in the pineal gland and this release is blocked by exposure to light, our data suggest that NOS is controlled by adrenergic mechanisms. Our observation may also explain the lack of cyclic GMP response to NE observed in animals housed in constant light.

556.6

INDUCIBLE NITRIC OXIDE SYNTHASE IN CA1 NEURONS.
I. Divac* and J. Regidor. Department of Medical Physiology, University of Copenhagen, Denmark, and Department of Morphology, University of Las Palmas de Gran Canaria, Spain.

Our results, obtained in collaboration with several colleagues, have shown that NADPH-diaphorase activity can be induced in the pyramidal CA1 neurons of the hippocampus by different agents. The effective agents are: electrical stimulation, mild stress, and direct injury of the hippocampus, either in vitro by slicing or in vivo by saline injections. After the induction, we found in hippocampal tissue an increased formation of cyclic GMP, indicating that the induced molecule has NOS enzymatic function. The induction of NADPH-diaphorase activity in CA1 neurons following intrahippocampal injections of saline can be abolished by pretreatment with anti-inflammatory drugs, dexamethasone or indomethacin, but not by a NOS inhibitor, L-NAME. Surprisingly, an antibody raised against the constitutive neuronal NOS did not stain the CA1 neurons which are demonstrably stained for NADPH-diaphorase. We conclude tentatively that a molecule, different from the constitutive NOS with both NADPH-diaphorase and NOS activities, can be induced in some neurons.

556.7

DISTRIBUTION OF NITRIC OXIDE SYNTHASE (NOS) IN RAT CENTRAL NERVOUS SYSTEM (CNS): A COMPARATIVE STUDY OF QUANTITATIVE AUTORADIOGRAPHY AND ENZYME ACTIVITY. S. Maayani*, G. R. Holstein, and P. Fernandez. Departments of Anesthesiology (S.M., P.F.) Neurology and Anatomy/Cell Biology (G.R.H.), Mount Sinai Medical Center, New York, NY 10029.

NOS is a ubiquitous enzyme in mammalian CNS. However, a quantitative assessment of its distribution is lacking. We have developed a novel method for quantitative autoradiography of [^{3}H]-nitroarginine binding. Data obtained with this method have been compared to the distribution of enzyme activity across rat CNS measured by the citrulline assay. The (100 μM) nitroarginine-sensitive NOS activity was assessed (24^{0}C, 10 min, 0.25-40 μM [^{3}H]-arginine) in three preparations: a crude homogenate and cytosolic and particulate fractions derived from it. Quantitative autoradiography of (100 μM) arginine-sensitive [^{3}H]-nitroarginine binding was done under the same NOS assay conditions with 50 nM [^{3}H]-nitroarginine, in 20 μm coronal cryostat sections of rat brain.

Km values appeared to be constant across the rat CNS (1-3 μM) while the Vmax (fmol/min mg tissue) varied substantially: the highest was observed in the cerebellum (100) and the lowest was found in the spinal cord (10). 20-40% of NOS activity was found in the particulate fraction, indicating an uneven distribution of the enzyme within cell compartments. Areas of highest binding included the olfactory tubercle, amygdala, fornix, substantia nigra, periaqueductal gray, and the cerebellar cortex. Intermediate binding levels were obtained in piriform cortex, hippocampus, septal nuclei, hypothalamus, globus pallidus, and the superior colliculus. Low binding was observed in frontal and parietal cortices, and the caudo-putamen. Arginine-sensitive [^{3}H]-nitroarginine binding varied between 65% of total binding (olfactory tubercle) to none (caudoputamen).

This combination of two quantitative methods for assessing NOS is proposed as a powerful tool to study the enzyme in mammalian CNS.

556.8

LOCALIZATION AND BIOCHEMISTRY OF NADPH-DIAPHORASE IN THE DEVELOPING CHICK RETINA: REGULATION BY CALCIUM IONS AND INHIBITION BY ARGININE ANALOGS R.Paes-de-Carvalho*, M.H. de Faria, J.L.M. do Nascimento and J.N. Hokoc.Dep. Neurobiologia, UFF and Inst.Biofisica C.Chagas Filho, UFRJ, Rio de Janeiro, Brazil.

NADPH-diaphorase (ND) activity, reported to be identical to Nitric Oxide Synthase (NOS), is found in specific groups of neurons in the CNS. Here we studied ND activity and localization in the developing chick retina. Enzyme activity was measured by spectrophotometry after incubating retinal homogenates with NADPH and Nitro blue Tetrazolium in Tris 50mM pH 8.1 at 37°C. ND histochemistry, performed incubating 4% paraformaldeyde-fixed cryostat tissue sections with the same compounds, revealed the presence of stained cells in retinas since 8 days of development (E8). An adult pattern was observed at E14, when photoreceptors and amacrine cells were ND positive. ND was detected biochemically in homogenates of E8, E11 and E14 retinas when we observed a 50% stimulation by Ca^{++} and 70% inhibition by L-N^G-Nitroarginine (NARG). The stimulation by Ca^{++} decreased at E17 and was not significant in post-hatched retinas. Inhibition curves obtained with increasing concentrations of NARG and L-N^5-(1-iminoethyl) ornithine (NIO) in the presence and absence of Ca^{++} suggested the presence of Ca^{++}-dependent and independent isoforms of NOS in the retina. The results showed that the ND biochemical assay is a simple and sensitive method for studying NOS activity and that different isoforms of NOS are expressed in populations of chick retinal cells during development. (supported by CNPq and PROPP-UFF).

556.9

NITRIC OXIDE SYNTHASE ACTIVITY IS INCREASED IN BRAIN IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY V.L. Raghavendra Rao , R. Audet and R.F. Butterworth* Neuroscience Res. Unit, Hôp. St-Luc (Univ. Montreal), 1058 St-Denis St., Montreal, Que.

Glutamatergic synaptic dysfunction has been proposed as a causal factor in hepatic encephalopathy. Increased glutamate release (Butterworth et al., *J. Neurochem. 56*, 1481, 1991) and decreased glutamate binding to NMDA receptors (Peterson *et al., J. Neurochem. 55*, 386, 1990) were previously reported in the brains of portacaval shunted rats. Such changes could lead to alterations in the second messenger systems coupled to glutamate receptors. As NMDA receptors have been shown to act via the nitric oxide/cGMP second messenger system, we studied the activities of nitric oxide synthase (NOS), in the brains of rats following portacaval anastomosis. Our results demonstrate that the activities of this enzyme were significantly increased in cerebral cortex (by 65%, $p<0.01$), cerebellum (by 54%, $p<0.01$), hippocampus (by 88%, $p<0.01$) and striatum (by 64%, $p<0.01$) of shunted rats compared to sham-operated controls. As the arginine transport is a prerequisite for nitric oxide production, we also studied [^{3}H]arginine transport into the cerebellar and cortical synaptosomes prepared from the brains of portacaval shunted and sham operated rats. [^{3}H]arginine uptake was significantly increased (by 45-55%, $p<0.01$) in both cortex and cerebellum. Alterations of the NOS system and the resultant increased production of nitric oxide and cGMP in brain could be of pathophysiologic significance in hepatic encephalopathy. (Supported by MRC Canada).

556.10

NITRIC OXIDE AND CYCLIC GMP ACTIVATE LOCUS COERULEUS NEURONS IN RAT BRAIN SLICES: ROLE OF CYCLIC GMP-DEPENDENT PROTEIN KINASE

J. H. Kogan*, J. Pineda and G. K. Aghajanian. Depts. of Cell. & Mol. Physiology, Psychiatry, & Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06508.

Nitric oxide (NO) has recently been shown to be a diffusible second messenger capable of stimulating soluble guanylate cyclase. Since the noradrenergic nucleus locus coeruleus (LC) is rich in the α_1 and β_1 subunits of soluble guanylate cyclase (Furuyama et al., Mol. Brain Res. 20, 1993) we investigated the role of the NO/cyclic GMP pathway in the LC. Extracellular electrophysiological recordings demonstrated that bath-applied 8-bromo-cyclic GMP (8Br-cGMP, 1mM) caused a gradual excitation of LC neurons; a more rapid response occurred when cyclic GMP (100μM) was introduced via patch pipettes. Similarly, during extracellular recordings, the NO donors sodium nitroprusside (SNP, 1mM) and S-nitroso-N-acetylpenicillamine (SNAP, 1mM) excited LC neurons. The SNP excitation could be occluded by a prior application of 8Br-cGMP indicating that SNP was acting through the NO/cyclic GMP pathway. Furthermore, H-8 (100μM), a protein kinase inhibitor with a relatively high affinity for cyclic GMP-dependent protein kinase (PKG), antagonized both the SNP and the 8Br-cGMP activation of LC neurons consistent with an action through PKG. These results suggest that there may be a physiological role for the NO/cyclic GMP pathway in the LC.

556.11

NITRIC OXIDE (NO) GAS ENHANCES TRITIATED NOREPINEPHRINE ([^{3}H]NE) RELEASE FROM RAT HIPPOCAMPAL SLICES. A.K. Stout* and J. J. Woodward. Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond VA 23298.

Previous studies in our lab have shown that NO gas enhances N-methyl-D-aspartate (NMDA)-stimulated release of preloaded [^{3}H]NE from rat brain slices in a dose-dependent, oxygen-sensitive, and cyclic GMP-independent manner. In this study we have attempted to determine the mechanism for the enhancement of neurotransmitter release seen with NO. NO-enhanced transmitter release was not due to buffer acidification or generation of NO degradation products, since reducing buffer pH below 7.3 inhibited NMDA-stimulated [^{3}H]NE release and nitrite or nitrate ions (3-100μM) had no significant effect on release. Carbon monoxide (10-300μM), another diatomic gas with properties similar to NO including heme binding and guanylate cyclase activation, had no significant effect on depolarization-induced [^{3}H]NE release. The NO effect was probably not due to mono-ADP-ribosylation of cellular proteins, since the ADP-ribosyltransferase inhibitors nicotinamide (10μM-10mM) and luminol (1μM-1mM) did not diminish the enhancement of transmitter release seen with NO. The NE reuptake inhibitor desmesthylimipramine (10nM-10μM) neither mimicked nor blocked the effect of NO, suggesting that NO was not acting via inhibition or reversal of the NE transporter. The metabolic inhibitors sodium azide (0.1-3mM), potassium cyanide (0.1-3mM), and 2,4-dinitrophenol (1μM-1mM) all dose-dependently enhanced NMDA-stimulated [^{3}H]NE release similar to NO. These results suggest that NO may enhance neurotransmitter release by inhibiting cellular respiration and altering calcium homeostasis. Supported by NIAAA AA08089 and NIDA Training Grant DA 07027.

556.12

CHARACTERIZATION OF NITRIC OXIDE-INDUCED [^{3}H]NOREPINEPHRINE RELEASE FROM HIPPOCAMPAL SLICES. G. Lonart* and K.M. Johnson. *Dept. of Pharmacol. and Toxicol. Univ. of Texas Med. Branch, Galveston, TX 77555*

We have previously reported that a long exposure to hydroxylamine, a nitric oxide (NO) generator, induced a tetrodotoxin-insensitive, Ca^{2+}-dependent release of [^{3}H]norepinephrine ([^{3}H]NE) from rat hippocampal slices (Lonart et al., *Eur. J. Pharmacol.*, 1992). These data suggested that NO induced the release of [^{3}H]NE directly from the noradrenergic terminals via exocytosis. However, we recently made observations which suggest the involvement of both NO-induced glutamate (Glu) release and non-classical [^{3}H]NE release via reverse NE transport. When we applied only a 10 min pulse of 100 μM hydroxylamine, which evoked a 10-fold increase in the [^{3}H]NE efflux, this response was reduced by about 80% by both 0.5 μM tetrodotoxin and 1 mM kynurenate, a Na^+-channel blocker and a non-selective ionotropic glutamate receptor antagonist, respectively. Ten μM CNQX and 100 μM GYKI 52446, competitive and non-competitive AMPA/kainate receptor antagonists, respectively, reduced the hydroxylamine response by about 50%. The NMDA receptor antagonists AP-5 and MK-801 reduced the hydroxylamine response only at concentrations which also inhibited [^{3}H]NE transport in hippocampal synaptosomes. However, CGS 19755, another NMDA receptor antagonist which had no effect on the [^{3}H]NE transport, did not influence the hydroxylamine-induced [^{3}H]NE efflux. Ten μM desipramine or 10 μM nomifensine, potent inhibitors of norepinephrine transport, reduced the hydroxylamine-evoked [^{3}H]NE efflux from hippocampal slices by about 80%. S-nitroso-L-cysteine, another NO generator (300 to 3,000 μM), stimulated the efflux of both [^{3}H]Glu and [^{3}H]NE from hippocampal synaptosomes. These data suggest that moderate NO stimuli can evoke [^{3}H]NE efflux both directly from the noradrenergic terminals and indirectly via releasing glutamate. Supported by DA-02073 (K.M.J.) and the P. and A. Forman Research Foundation (G.L.).

556.13

LIPOPOLYSACCHARIDE INHIBITS THE PRODUCTION OF NITRIC OXIDE IN RAT PHEOCHROMOCYTOMA CELLS. G. Cao#*, R.L. Prior#, J.G. Strain# and J.A. Joseph#. #USDA-ARS, HNRC at Tufts Univ. Boston, MA 02111 and *Dept. of Nutri. Sci., Univ. of Conn. Storrs, CT 06296

Nitric oxide (NO) is a recently recognized messenger molecule mediating diverse functions including vasodilation, neurotransmission, and antimicrobial and antitumor activities. NO is produced from L-arginine by NO synthase which has two isoforms, i.e. the constitutive NO synthase (cNOS) and inducible NO synthase (iNOS). The iNOS can be induced by bacterial lipopolysaccharide (LPS), phorbol esters, and some cytokines like TNF-α, IFN-γ and LI-1β. However, the mechanism involved in the negative regulation of the NO production is not clear. Here, we report an inhibition of 43% and 50% in nitrite production in U-10 cells, a variant of PC-12 rat pheochromocytoma cells, by adding 1 or 10 μg/ml LPS, respectively in the culture media for 3 days. Nitrite production was also inhibited by 43% in control cells not exposed to LPS but incubated with 0.5 mM N^G-methyl-L-arginine acetate, a compound that specifically and competitively inhibits NOS. The results suggested that the NO produced in these U-10 cells was from cNOS and could be negatively regulated by LPS.

556.14

CARBON MONOXIDE AS A NEUROTRANSMITTER: A SIMPLE, SENSITIVE, SPECIFIC RADIOMETRIC ASSAY FOR HEME OXYGENASE. E.E. Thompson* and S.H. Snyder The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Heme oxygenase (HO) catalyses the oxidation of heme to biliverdin and carbon monoxide (CO). Since CO can activate soluble guanylate cyclase, it is likely that HO plays a role in cGMP mediated signal transduction. To better study the role of HO in signal transduction, we have developed a new assay for HO which uses ^{55}Fe-labelled heme as substrate. As HO cleaves the heme ring to release Fe^{2+}, this assay directly measures released $^{55}Fe^{2+}$ bound to transferrin attached to Sepharose beads. As little as one pmol of $^{55}Fe^{2+}$ is readily detected, providing a sensitivity 50 to 100 times greater than conventional assays based on the absorbance of bilirubin formed from biliverdin by biliverdin reductase. The assay is specific, as no known brain enzyme other that HO releases Fe^{2+} from heme. The assay is simple to perform. Assays are conducted in the presence of the Sepharose-transferrin beads which are counted directly after incubation, centrifugation and washing. Thus, 100 to 200 assays can be performed in an hour. This assay permits detection of HO activity in very small brain samples and microwell samples of cell culture.

556.15

DOES CARBON MONOXIDE REGULATE CYCLIC GMP LEVELS IN THE RAT BRAIN IN VIVO? J.T. Laitinen* and K.S.M. Laitinen. Depts. of Physiol. and Pharmacol. & Toxicol., Univ. of Kuopio, POB 1627, FIN-70211 Kuopio, Finland.

Microdialysis method combined with a specific RIA was used to study *in vivo* regulation of brain cGMP levels by the putative gaseous messengers nitric oxide (NO) and carbon monoxide (CO). Anesthetized rats were implanted with the microdialysis probes in the frontal cortex (FC) or the cerebellum (CB). Basal cGMP production was similar in both regions but we observed marked differences in responses to the NO-donor sodium nitroprusside (SNP) or to CO. Most notably, SNP maximally elevated cGMP 3-fold in the FC but 90-fold in the CB. Perfusion with CO-saturated aCSF stimulated cortical cGMP levels transiently and reversibly but elicited an irreversible and delayed response in the CB. Perfusion with the NO-synthase inhibitor L-NAME (2 mM) suppressed cerebellar cGMP by 75 % but exhibited no potency in the FC. Quite opposite, local perfusion with the heme oxygenase (HO) inhibitor zinc protoporphyrin IX (ZnPP) (100 μM) suppressed basal cortical cGMP by 50 % but was without effect in the CB, suggesting that CO, generated by HO activity, might regulate cortical cGMP production *in vivo*. As ZnPP may have actions independent of HO inhibition, we have begun to address these questions by using more specific tools, including a microassay for HO activity in discrete brain regions, as well as antisense oligodeoxynucleotide targeting of HO gene *in vivo*. These tools are hoped to reveal whether endogenously produced CO participates in the maintenance of cortical cGMP levels in vivo.

556.16

MORPHINE ATTENUATES FORSKOLIN-INDUCED PREPROENKEPHALIN mRNA IN RAT STRIATUM: A QUANTITATIVE *IN SITU* HYBRIDIZATION STUDY. J.N. Simpson* and J. F. McGinty. Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Activation of the adenylate cyclase/cAMP second messenger system leads to an increase in preproenkephalin (PPE) and preprodynorphin (PPD) gene expression. Because opiate receptors are negatively coupled to adenylate cyclase, pro-opioid gene expression may be negatively influenced by opioid receptor agonists. We previously demonstrated an increase in striatal PPE and PPD mRNA levels as well as an increase in striatal phospho-CREB and FOS immunoreactivity following intracerebroventricular (ICV) administration of forskolin (Simpson and McGinty, Neuroreport, June, 1994). In a preliminary experiment, medial striatal PPE and PPD mRNA levels were measured in rats which received either unilateral ICV injection of H_2O-soluble forskolin (FSK, 10 mM/10 μl dH_2O) 1 h following a systemic injection of morphine sulfate (MOR, 25 mg/kg, n = 4) or unilateral ICV FSK only (n = 4). In the MOR/FSK group, a booster injection of MOR (10 mg/kg) was given 2 h after ICV FSK. The mRNA hybridization signal in the striatum ispilateral to ICV FSK was compared with hybridization signal in the contralateral striatum. FSK induction of PPE, but not PPD, mRNA was significantly attenuated by MOR. In a separate experiment, levels of striatal pro-opioid and and zif/268 mRNA were examined 4 h following ICV FSK (10 mM/10 μl saline, n = 5) or ICV saline (10 μl, n = 5). FSK significantly induced PPE, PPD, and zif/268 mRNA in the medial striatum. The finding that striatal zif/268 mRNA is induced by FSK supports previous reports that the promoter region of the zif/268 gene contains a CRE-like element. Results of a current study confirming morphine effects on forskolin-induced pro-opioid mRNA in the rat striatum as well as morphine effects on forskolin-induced zif/268 mRNA will be presented. Supported by DA 05470 (JNS) and DA 03982 (JFM).

556.17

REGULATION OF THE cAMP RESPONSE ELEMENT BINDING PROTEIN (CREB) IN THE LOCUS COERULEUS (LC) AND NUCLEUS ACCUMBENS (NAc) BY DRUGS OF ABUSE. K.L. Widnell*, D.S. Russell, D.W. Self, M.J.D. Miserendino, C. Konradi^, S.E. Hyman^, and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06508; ^Massachusetts General Hospital, Charlestown, MA 02129.

We have shown previously that chronic administration of morphine or cocaine alters certain G proteins and components of the cAMP intracellular messenger system. These changes occur in specific brain regions known from behavioral and pharmacological studies to mediate aspects of drug addiction (see *Neuron*, 11:995, 1993). We are currently investigating the molecular mechanisms involved in these adaptations by focusing on regulation of the transcription factor CREB in the LC and NAc. Morphine admininstration regulates CREB phosphorylation in the LC, as would be expected given morphine regulation of the cAMP pathway (*J. Neurochem.* 58:1168, 1992). CREB has been thought to be constitutively expressed in the brain, however we have found that chronic morphine increases levels of CREB immunoreactivity in the LC. Interestingly, in the NAc, chronic morphine decreases CREB immunoreactivity. Preliminary evidence suggests that chronic cocaine also influences CREB levels in this brain region. In order to test whether CREB is directly involved in the observed changes in signal transduction proteins, we are infusing antisense PS-oligonucleotides to CREB into the NAc. Chronic (5 day) antisense injections downregulate CREB immunoreactivity by approximately 50%; this decrease in CREB appears to be associated with regulation of protein kinase A and Giα, as well as Fos-like proteins as would be expected. These studies should further elucidate the role of CREB in morphine and cocaine regulation of intracellular target proteins implicated in drug addiction.

556.18

Molecular basis of dopamine-glutamate interactions in the rat striatum. C. Konradi[1]*, X.-M. Li[2], R. L. Cole[1] and S. E. Hyman[1], [1]*Laboratory for Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129 and* [2]*Department of Psychiatry, University of Saskatchewan, Saskatoon, S7N 0W0 Canada.*

Abnormalities in dopamine neurotransmission have been implicated in the pathogenesis of neuropsychiatric disorders including Parkinson's disease and schizophrenia. The goal of our studies is to elucidate the influence of glutamate on dopamine-mediated gene regulation in striatum. Knowledge of dopamine-glutamate interactions may provide novel targets of drug therapy.

We have previously demonstrated regulation of AP-1 binding proteins in rat striatum by dopamine agonists and antagonists. In addition, we and others have observed that D1 and D2 dopamine receptor-mediated *c-fos* expression is inhibited by pretreatment with the NMDA receptor antagonist MK-801. Conversely, in primary striatal cultures, low levels of glutamate that are insufficient to induce *c-fos* expression, significantly enhance dopamine mediated *c-fos* expression and AP-1 binding activity. This effect appears to be mediated by Ca^{2+}. Dopamine markedly induces cAMP in these cultures, while glutamate has no effect on cAMP levels. In addition, the Ca^{2+} ionophore A23187 was able to mimic the effects of glutamate. These data suggest that interactions between Ca^{2+} and the cAMP pathway may be critical in the regulation of striatal gene expression in response to dopaminergic drugs.

556.19

INDUCTION OF A NEWLY SYNTHESIZED CREB/ATF PROTEIN COMPLEX AFTER DOPAMINERGIC STIMULATION IN RAT PRIMARY STRIATAL CULTURES. R.L. Cole*, C. Konradi and S.E. Hyman. Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Boston MA 02115.

Chronic administration of the psychostimulant drugs cocaine and amphetamine results in slow onset, long term behavioral changes including sensitization, tolerance, and most significantly, a profound state of dependence. Our previous studies have focused on the initial events regulating the prodynorphin gene which is robustly and selectively induced by chronic (but not acute) administration of amphetamine in both dorsal and ventral rat striatum. To investigate the molecular mechanisms of gene regulation by dopamine in striatal neurons we have utilized rat primary striatal cultures. We have previously reported that in gel-shifts CREB binds to three functional CRE elements within the dynorphin promoter, which have been termed dynCRE1 (-1660/-1553), dynCRE2 (-1630/-1623) and dynCRE3 (-1546/-1539), and is phosphorylated in response to dopamine stimulation. We now report that, in addition, a lower molecular weight complex is induced. The appearance of this complex is blocked by pre-treatment with the protein synthesis inhibitors cycloheximide and anisomycin prior to dopamine stimulation, suggesting protein synthesis dependence. In supershift analysis, the new protein complex is recognized by a CREB antiserum, implying that the new complex may contain CREB protein heterodimerizing with a newly synthesized protein. This raises the intriguing possibility that switching of dimerization partners may be a critical mechanism regulating CREB activity, thus altering its effects on gene expression, either positively or negatively. However, this protein complex may also represent a homodimer of a newly-synthesized protein in the CREB/ATF family, which cross reacts with the CREB antiserum. This would suggest that novel transcription complexes are induced by striatal neurons after dopamine stimulation. Changes in the composition of transcription factor complexes and/or the appearance of new transcription factors are likely to be key events in the regulation of gene expression following chronic psychostimulant administration.

556.20

GLUCOCORTICOIDS ATTENUATE KAINATE-INDUCED AP1 DNA BINDING ACTIVITY IN RAT BRAIN. T.M. Unlap* and R.S. Jope . Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294.

Glucocorticoids, which are produced by the adrenal glands, play a vital role in enabling mammals adapt to stress. This process is facilitated by glucocorticoids binding to their receptors in the cytosol of target cells and translocating these receptors into the nucleus where they function as transcription factors and modulate gene expression. Persistent high levels of glucocorticoids, however, can have profound deleterious effects, the mechanisms of which are not clearly understood.

In order to elucidate the mechanism by which glucocorticoids affect neuronal signal transduction pathways, this laboratory has shown that depletion of circulating glucocorticoid levels in rats by adrenalectomy increases the induction of immediate early genes (IEG), genes whose expressions are altered minutes after a stimulus has been initiated and whose expressions are regulated by the AP1 transcription factor. AP1 consists of homodimers (Jun-Jun) or heterodimers (e.g. Jun-Fos). Furthermore, this laboratory has also shown that dexamethasone (1mg/kg) significantly reduced kainate-induced AP1 activation in rat brain without altering the levels of IEG proteins.

To determine if endogenous glucocorticoid levels affect kainate-induced AP1 activation, rats were treated with kainate (12 mg/kg) for 1.5hr prior to sacrifice either in the morning (6:30 A.M.) or in the afternoon (2:30 P.M.), when glucocorticoid levels are low or high, respectively, and AP1 activity was measured in the cortex and hippocampus using a gel mobility shift assay and a radiolabled oligonucleotide containing the AP1 binding site (TGACTCA). We found that kainate-induced AP1 activity was higher in the morning than in the evening (4 fold vs 1.8 fold over control) in the cortex but was the same at both times in the hippocampus. Since circulating glucocorticoid levels are higher in the afternoon than in the morning the reduced kainate-induced AP1 activation in the afternoon may be due to these high circulating glucocorticoid levels.

BEHAVIORAL PHARMACOLOGY II

557.1

BEHAVIORAL AND BIOCHEMICAL PROPERTIES OF GABA AND BENZODIAZEPINE RECEPTORS IN CBA AND C57 MICE. S. Liljequist* and G. Cebers. Dept of Clinical Neuroscience, Drug Dependence Res, Karolinska Institute, S-17176 Stockholm, Sweden.

C57Bl/6 male mice show "normal" habituation in a locomotor activity test situation, whereas CBA/Ca show no habituation. CBA mice also display increased latency in a passive avoidance situation and an increased sensitivity to the convulsive actions of the GABAergic antagonist picrotoxin. Receptor binding studies revealed no differences in benzodiazepine (BDZ) binding properties or in the ability of various BDZ receptor ligands to inhibit BDZ binding. Neither was there any differences in GABA- or pentobarbital-produced enhancement of BDZ binding. However, the binding of ^{35}TBPS (2 nM) was about 30% higher in forebrain microsacs of CBA mice as compared to C57 whereas no strain differences were noted in midazolam- or DMCM-produced modulation of ^{35}TBPS binding. Further experiments revealed no differences in GABA-produced influx of ^{36}chlorine. However, midazolam-induced enhancement of ^{36}chlorine influx was significanly higher in forebrain microsacs of C57 mice as compared to CBA mice. In contrast, the DMCM-produced a more marked reduction of GABA stimulated ^{36}chlorine in forebrain microsacs of CBA as compared to C57. Our current data indicate that genetic differences in the GABA/BDZ receptor system may explain at least some of the previously observed genetic differences in the behavioral actions of GABA receptor ligands in CBA and C57 animals.

557.2

FELBAMATE, A NOVEL ANTIEPILEPTIC AGENT, DOES NOT AFFECT COGNITION IN RODENTS BD Smith*, ME Grzelak & VL Coffin. *Schering-Plough Research Institute, Kenilworth NJ 07033.*

Felbamate (2-phenyl-1,3-propanediol dicarbamate; Felbatol, Taloxa) is a novel anticonvulsive agent recently approved by the FDA for treatment of epilepsy in the U.S. While the mechanism of action of felbamate has not been fully elucidated, recent evidence has accumulated to indicate that felbamate may act at the strychnine-insensitive glycine-binding site on the NMDA receptor complex. Since the NMDA receptor has been implicated in cognitive processes (i.e., LTP), the current study was designed to investigate the potential effects of felbamate on cognition.

Doses of felbamate up to 1000mg/kg sc did not produce deleterious effects in either mice or rats in passive-avoidance responding (PAR). In contrast, the noncompetitive NMDA antagonist MK-801 (dizocilpine) produced performance deficits at doses from 0.1 to 1.0mg/kg in both rats and mice. Both drugs prevented NMDA-induced convulsions (ED50=20.3 and 0.82, respectively). The therapeutic index (ratio of deficit-producing to anticonvulsant doses in ED50s) for dizocilpine was less than one-fold, while felbamate had a greater than 50-fold separation. Taken together, these results indicate that felbamate does not produce cognitive deficits at doses more than 50 times the dose needed to block seizure activity in rodents.

Animal	Therapeutic Ratio[a]	
Test-time after drug administration	dizocilpine	felbamate
rat (24h)	0.119	>50
mouse (24h)	0.078	>50
mouse (1h)	0.354	>50

[a]Dose producing PAR deficit/Dose to block NMDA -induced seizure

557.3

INHERITABLE INDIVIDUAL-SPECIFIC BEHAVIORAL FEATURES OF RATS AND EFFECTS OF PROPOFOL. R. Dirksen [1], B. Ellenbroek [1, 2], and A.R. Cools [2]. Inst. for Anesthesiology [1], and Dept. Psychoneuropharmacology [2]. University of Nijmegen, Nijmegen 6500HB, the Netherlands.

Inheritable differences in the physiological state of the brain that direct the display of behavior include those in the GABA transmission. This transmission is essential to effects of various general anesthetics, including propofol. The present study uses the 14th generation of pharmacogenetically selected APO-UNSUS and APO-SUS rats to evaluate whether the neurobiological profile of individual-specific features affects the response to propofol. We assessed the duration of abolishment of purposeful movements (APM) and the incidence of propofol-evoked behavior after three different doses i.v., each injected at a one week interval. One week later, the rats were anesthetized with urethane, and the effects of i.v. propofol on withdrawal reflexes, heart rate and blood pressures were measured. The induction of APM and magnitude of reflex inhibition representing the anesthetic type of effect were not different for the two types of rats. APO-UNSUS rats showed a greater heart rate variability and their systolic blood pressures were lower than APO-SUS rats. Propofol reduced heart rate variables stronger in APO-UNSUS rats, whereas blood pressures variables were reduced to a greater extent in APO-SUS rats. Cardiovascular responses serve to indicate the sensitivity of an individual to an anesthetic in clinical conditions, and the marked interindividual differences occurred at the same magnitude of anesthetic response. Drug-induced behavior differed among the two types of rats. APO-SUS rats showed a higher incidence of involuntary muscular contractions and vacuous chewing with tongue protrusion than the APO-UNSUS rats, whereas their incidence of grooming behavior was lower. The difference in these adverse reactions may relate to the mirror image in functionality of GABA transmission in the striato-nigro-collicular pathway for the two types of rats. Translated to the human condition, personality may affect the severity of side-effects of propofol.

557.4

STEREOSPECIFIC REQUIREMENTS FOR BINDING AND TRANSDUCTION OF BEHAVIORAL EFFECTS AT DIAZEPAM-INSENSITIVE GABA$_A$ RECEPTORS IN PIGEONS. J.B. Acri, G. Wong, J.M. Witkin* Drug Devel. Grp., Psychobiology Section, NIDA-IRP-ARC, NIH, Baltimore, MD 21224 and Laboratory of Neuroscience, NIDDK, NIH, Bethesda, MD 20814.

There is a positive correlation between binding at diazepam-insensitive (DI) benzodiazepine receptors and substitution for the discriminative stimulus effects of flumazenil (FLU) in pigeons. These experiments examined the binding affinities and discriminative stimulus effects of two benzodiazepine partial agonists (bretazenil and Ro 14-5974) and their R-enantiomers, Ro 18-2598 and Ro 14-7527, at diazepam-sensitive (DS) and DI receptors. The ability of compounds to substitute for the discriminative stimulus effects of FLU and midazolam (MDZ) were determined, to differentiate DS- from DI-mediated effects, with comparison to the full DS agonist, Ro 19-0528. Compounds were also tested for their ability to block MDZ effects. S-enantiomers bretazenil, Ro 14-5974, and Ro 19-0528 bound to both DI and DS receptors with nanomolar affinities, whereas R-enantiomers did not bind to either DI or DS receptors (Ki > 1000 nM). Consistent with DI binding affinity, bretazenil and Ro 14-5974 fully substituted for the discriminative stimulus effects of FLU, and Ro 19-0528 partially substituted. Bretazenil and Ro 14-5974 partially substituted for the discriminative stimulus effects of MDZ, and Ro 19-0528 fully substituted, consistent with actions as partial and full DS agonists. Both bretazenil and Ro 14-5974 partially blocked the discriminative stimulus effects of MDZ. The R-enantiomers did not engender FLU or MDZ responding, and did not block the discriminative stimulus effects of MDZ. Results indicated a stereospecific requirement for DI binding and transduction of behavioral effects, consistent with DI receptor-mediation of the discriminative stimulus effects of FLU. Further, this is the first report of DI effects for a full DS agonist, Ro 19-0528, and a non-parallel FLU substitution curve for this compound suggests the possibility of unique behavioral interactions that remain to be elucidated.

557.5

EVIDENCE FOR AN INVERSE AGONIST ACTION OF RITANSERIN ON ASSOCIATIVE LEARNING IN THE RABBIT. S. Welsh*, A. G. Romano, K. J. Simansky and J. A. Harvey. Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Drugs classified as partial agonists at 5-HT_2 receptors (e.g., LSD) have been demonstrated to enhance associative learning as measured by acquisition of the rabbit's nictitating membrane (NM) response. We have now examined the effects of the 5-HT_2 antagonist, ritanserin, on acquisition of the NM response and on its ability to antagonize the effects of LSD. Classical conditioning of the NM response was accomplished by pairing both light and tone CSs with a corneal airpuff US. Ritanserin (0.032 to 3.2 mg/kg, s.c.) given 1 hr prior to each conditioning session, significantly retarded acquisition of CRs in a dose-dependent manner. LSD (0.013 mg/kg, i.v.) given 20 minutes prior to each session significantly enhanced the acquisition of CRs. This enhanced acquisition of CRs produced by LSD was completely blocked by a dose of ritanserin (0.5 mg/kg) which when given alone had an intermediate effect in retarding CR acquisition. The ability of ritanserin to block the effects of LSD suggests that LSD produces an enhancement of learning through an action at the 5-HT_2 receptor. Control experiments indicated that ritanserin (0.5 mg/kg) given alone had no effect on nonassociative determinants of responding such as sensitization, pseudoconditioning or altered baseline rates. This dose of ritanserin did alter stimulus processing. There was a more than 10 dB elevation of the CS intensity threshold for eliciting CRs. Ritanserin had no effect on the US intensity threshold for eliciting URs but did significantly decrease the amplitude of the elicited UR. The results of these studies lead us to conclude that ritanserin blocks learning through an inverse agonist action at the 5-HT_2 receptor. Therefore, these data suggest that the activity of the serotonergic systems in brain are normally required for optimal learning. This research was supported by MH 16841.

557.6

FAILURE OF ACEA 1021 TO SERVE AS A DISCRIMINATIVE STIMULUS IN RATS. K.L. Shelton* and R.L. Balster Medical College of Virginia, Department of Pharmacology and Toxicology, Richmond, VA 23298-0613

NMDA antagonists have shown promise as treatments for a number of neurological disorders. However, one of the problems of NMDA antagonists, particularly those such as PCP which act by blocking the receptor associated ion channel, is their production of PCP-like psychological effects and abuse liability. Nonetheless, behavioral studies have shown potentially important differences among the different types of site-selective NMDA antagonists. To further examine the behavioral effects of glutamate antagonists, we attempted to train the novel quinoxalinedione glutamatergic antagonist, 5-nitro-6,7-dichloro-1,2,3,4-tetrahydroquinoxaline-2,3-dione (ACEA 1021), which has in vitro selectivity as an antagonist at the glycine modulatory site on the NMDA receptor, as a discriminative stimulus in rats. Fifteen adult, male Sprague-Dawley rats were used as subjects. The rats were initially trained to respond under a fixed ratio-1 schedule on both levers of a standard two-lever operant conditioning chamber for 45-mg food pellet reinforcers. After responding had stabilized under FR-1 conditions, discrimination training began. One lever was designated the saline lever and the other lever as the drug lever. Rats were injected once per day, 30 minutes prior to the training session with either 10 mg/kg ACEA 1021 or vehicle in a double alternation sequence (i.e. drug, drug, sal, sal). Over sessions, the FR requirement was gradually increased until a FR-16 schedule was in effect. After 60 training sessions, only one subject had reached the acquisition criteria of 9 out of 10 consecutive sessions during which the first FR had been emitted on the correct lever and 90% or more of the animal's total responses were on the correct lever. For 7 of the subjects, the training dose of ACEA 1021 was then increased to 15.6 mg/kg. After 60 additional training sessions, none of these animals had reached acquisition criteria. It has been shown that nearly all drugs with abuse liability can be trained as a discriminative stimulus in animals. The inability to train ACEA 1021 as a discriminative stimulus despite prolonged training at doses that decreased response rates compared to saline, provides evidence that it's interoceptive stimulus properties are weak at best and it may therefore have low potential for abuse.

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557.7

MODULATION BY INOSITOL OF CHOLINERGIC AND SEROTONERGIC-INDUCED SEIZURES IN LITHIUM TREATED RATS. M.B. Williams* and R.S. Jope, Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama at Birmingham, Birmingham, AL 35294

Lithium's inhibitory effect on inositol monophosphatase of the PI pathway was suggested to be involved in the mechanism of action of lithium, the treatment for bipolar affective disorder (Berridge et al, 1982). Inositol depletion occurs following toxic but not therapeutic doses of lithium. However, supplemental inositol attenuated seizures induced by acute lithium plus pilocarpine (PILO) (Tricklebank et al, 1991; Kofman et al, 1993). We used EEG measurements of seizures produced by PILO given to lithium-pretreated rats (acute or chronic) and determined the effect of myo- and epi-inositol on seizure latency. Myo-inositol (0-55 mmole, icv) dose-dependently increased latency to seizures induced by acute lithium (3 mmole/kg, ip) plus PILO (30 mg/kg, sc). Epi-inositol, which is not utilized for PI synthesis, blocked seizures in 50% of tested rats. In chronic lithium treated rats, inositol did not block seizures produced by PILO. Similarly to its effect on muscarinic agonists, lithium increased the response to R(-)-DOI, a selective 5HT2 (PI-linked) agonist (Williams and Jope, 1994). While DOI (8 mg/kg, i.p.) alone was not convulsive, lithium plus DOI evoked seizures. Myo-inositol (icv) blocked seizures in some rats treated with lithium and DOI.

557.8

IMIPRAMINE, CLOMIPRAMINE AND FLUOXETINE SHOW SIMILAR EFFICACY, BUT A DISTINCT MECHANISM OF ACTION IN THE LEARNED HEPLESSNESS PARADIGM Gambarana C.*, Ghiglieri O., De Montis M.G. and Tagliamonte A. Institute of Pharmacology, University of Siena, Siena, Italy.

The clinical efficacy of antidepressant drugs which act selectively upon the serotonergic system supports the hypothesis of the relevant role of serotonin in the pathogenesis of depression. Selective inhibitors of 5-HT uptake, as well as agonists of 5-HT_{1A} receptors, are active in some animal models of depression. Thus, in the present study we compared the effects of a long-term exposure to imipramine (IMI), clomipramine (CMI) and fluoxetine (FLU) on the learned helplessness (LH) model of depression. Experiments were carried out on male Sprague-Dawley rats. IMI (10 mg/kg i.p. twice a day), CMI (10 mg/kg i.p. twice a day) and FLU (10 mg/kg i.p. once daily) were administered for 15 days. Animals received 80 unavoidable electric shocks (1 mA) on the tail 2 h after the last treatment, and were tested for escape 24 h later. IMI, CMI and FLU showed the same efficacy in preventing the behavioral effects of repeated unavoidable stress. The acute inhibition of D_1 dopamine receptor activity (as obtained by injecting SCH 23390) completely prevented the effects of IMI, and only partially those of FLU. On the other hand, SCH 23390 antagonized the efficacy of CMI only in 50% of the animals. Moreover, the preventive effect of FLU and CMI was completely antagonized by the acute administration of pindolol (a 5-HT_{1A} antagonist), which failed to affect IMI antidepressant activity. Since pindolol also inhibits β-adrenoceptors, propranolol (a β-blocker wich does not compete with endogenous 5-HT) was acutely tested on IMI, CMI and FLU treated rats, and showed no effect. It was concluded that: a) β-adrenoceptors are not involved in the LH prevention by the three compounds tested; b) IMI prevents LH development through the selective activation of D_1 dopamine receptors; c) FLU and CMI act mainly at the level of 5-HT_{1A} receptor system.

557.9

POSSIBLE ROLE FOR THE 5-HT1B/D RECEPTOR IN THE MEDIATION OF ANTIDEPRESSANT-LIKE EFFECTS IN THE MOUSE TAIL SUSPENSION TEST. M. F. O'Neill, A. G. Fernández and J.M. Palacios*. Laboratorios Almirall, Cardener 68-74, Barcelona 08024, Spain.

The ability of many antidepressant treatments to increase synaptic concentrations of serotonin is believed to be the mechanism by which they exert their clinically beneficial effects. It is uncertain, however, via which receptor subtype(s) the increased concentrations of serotonin subsequently act. Several studies have suggested that agonists at 5-HT1B receptors such as RU 24969 demonstrate antidepressant-like actions in rodent tests. We set out to determine if RU 24969 could reduce immobility in the mouse tail suspension test and to compare the effects of two recently described compounds; WAY 100135 and GR 127935, selective antagonists at 5-HT1A and 5-HT1B receptors respectively, on the effects of this and other antidepressant treatments. All drugs were administered s.c. RU 24969 significantly decreased immobility at a dose of 3mg/kg. Neither antagonist alone had any effect in this test up to doses of 10mg/kg. WAY100135 (10mg/kg) had no effect on the decrease in immobility induced by paroxetine (1mg/kg) or RU 24969 (3mg/kg) while GR 127935 (10mg/kg) not only blocked the effects of the 5-HT1B agonist but also blocked the effects of paroxetine (1mg/kg) and imipramine (10mg/kg) in the TST. In conclusion, these results indicate that the 5-HT1B/D receptor may be involved in the mediation of the behavioural action of antidepressant agents.

557.10

EFFECTS OF 5-HT3 ANTAGONISTS AND 5-HT1A AGONISTS ON FIXED-CONSECUTIVE-NUMBER PERFORMANCE IN RATS. P.C. Mele, J.H. McDonough, P.J. Winsauer and C.G. Franz. Behavioral Sciences Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5603.

Similarities and differences in the behavioral effects of 5-HT3 antagonists and 5-HT1A agonists have been reported. The present study compared drugs from these classes in rats responding under a fixed-consecutive-number (FCN) schedule of reinforcement. The FCN schedule required 20 consecutive responses on one lever before a response on a second lever resulted in delivery of milk reinforcers during daily 30-min sessions. The selective 5-HT3 antagonists ondansetron (0.001-3 mg/kg, i.p., 15 min presession) and zacopride (0.01-10 mg/kg, i.p., 15 min presession) failed to alter either rates or accuracies of responding significantly. The mixed D2/5-HT3 antagonist metoclopramide (1-3 mg/kg, i.p., 30 min presession) and the selective 5-HT1A agonist 8-OH-DPAT (0.032-0.32 mg/kg, s.c., 15 min presession) dose-dependently reduced rates and accuracies of responding. The partial 5-HT1A agonist buspirone (0.3-10 mg/kg, i.p., 15 min presession) decreased response rates substantially but reduced accuracies only minimally. Within-session patterns of responding differed across drugs: 8-OH-DPAT disrupted responding early in the session and this was followed by recovery; metoclopramide reduced or eliminated responding after a period of unaffected or less affected responding early in the session; buspirone eliminated responding throughout the session. Selective 5-HT3 antagonists and 5-HT1A agonists differentially altered FCN performance in rats. Within each class, selective versus nonselective drugs could be distinguished.

557.11

EFFECTS OF CORTICOSTERONE AND DEXAMETHASONE ON SCHEDULE-INDUCED POLYDIPSIA IN ADRENALECTOMIZED RATS. F Cirulli[12]*, H. van Oers[1], E. R. De Kloet[3] and S. Levine[1]. [1]Dept. of Psychiatry, Stanford Univ. Sch. of Med., Stanford, CA 94305; [2] Lab. Fisiopatologia O. S., Istituto Superiore di Sanità, I00161 Rome, Italy; [3]Div. of Medical Pharmacology, Cent Bio-Pharm Sci., Univ. of Leiden, 2300 RA Leiden, The Netherlands.

Removal of circulating glucocorticoids by adrenalectomy prevents the normal acquisition of schedule-induced polydipsia (SIP), while corticosterone (CORT) administration reinstates this behavior in adrenalectomized (ADX) rats. These studies investigated which glucocorticoid (GC) receptor is responsible for mediating CORT effects on SIP. In Experiment I the effects of dexamethasone (DEX) and CORT on the acquisition of SIP were studied. DEX and CORT pellets (respectively 15 mg and 200 mg) were implanted subcutaneously in ADX rats. CORT but not DEX replacement was able to reinstate SIP in ADX rats. Because DEX binds almost exclusively to glucocorticoid receptors (GRs), while CORT binds to both GRs and mineralocorticoid receptors (MRs), results from Experiment I indicate that GRs alone are not sufficient for SIP acquisition. In Experiment II CORT pellets of different concentrations (1, 10, 50, 200 mg) were implanted in ADX rats in order to distinguish whether MRs alone, or a combination of GRs and MRs is required for SIP reinstatement. Results from Experiment II showed that the 1 and 10 mg CORT pellets were not able to reinstate SIP in adrenalectomized rats, while animals implanted with 50 or 200 mg pellets did show the behavior. These results indicate that occupancy of both MRs and GRs is required for SIP acquisition.

557.12

3α-ANDROSTANEDIOL INHIBITION OF SEXUAL RECEPTIVITY AND NOCICEPTION: EVIDENCE FOR A GABAERGIC MECHANISM. C.A. Frye*, K.R. Van Keuren & M.S. Erskine. Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215

Many steroids can act via intracellular receptors and at the GABA receptor complex (GBR). Progesterone (P) or its metabolites may initiate sexual receptivity and 5α-reduced androgens, particularly 3α-Androstanediol (3α-Diol), may terminate estrus in part via actions at the GBR. To investigate this hypothesis ovx rats (N=50) received 3α-Diol (0.6, 3.0, 6.0 and 7.5 mg/kg) or vehicle (10% (v/v) ethanol:propylene glycol) at 1000h (0h), and 2 SC injections of estradiol (E_2: 1 μg/0.2 ml in 10% ethanol) at 1300 (3h) and 1900 (9h) daily for 2 days. P (0.5, 1.0, 2.0 and 4.0 mg/kg) or sesame oil vehicle was given at 1430h after 2 days of 3α-Diol and E_2. Behavioral testing was carried out at 1830h, 48-49 hours after the first injection of E_2 and 4 hours after injection of P. 3α-Diol (3.0 and 6.0 mg/kg) significantly attenuated P's (2.0 and 4.0 mg/kg) facilitation of sexual behavior (Lordosis Quotient, Lordosis Rating, Proceptivity). P and 3α-Diol both produced analgesia as assessed via the radiant heat tailflick method. 3α-Diol at 3.0 mg/kg inhibited P-induced receptivity, uniformly produced maximum analgesia, and dramatically increased GABA-stimulated chloride flux into cortical synaptoneurosomes. As 3α-Diol binds poorly to its intracellular receptor, these data suggest that 3α-Diol may act via the GBR to inhibit sexual receptivity and promote analgesia.

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557.13

THE CCK_A RECEPTOR ANTAGONIST DEVAZEPIDE DECREASES RETENTION OF IMMOBILITY BY A GLUCOCORTICOID RELATED MECHANISM IN THE FORCED-SWIM TEST IN RATS. M. Ruiz-Gayo, F. Hernando and J.A. Fuentes*. Dept. of Pharmacology, Sch. of Pharmacy. Univ. Complutense. Ciudad Universitaria, 28040 Madrid, Spain

Post-stress occupancy of glucocorticoid receptors has been shown to influence information storage. This is a fact that seems to facilitate the manifestation of "depressive" signs in rats that suffer a second stressful session. Cholecystokinin (CCK) has been proposed as a second rank order modulator of the hypothalamus-pituitary-adrenal axis. With the aim to study whether endogenous CCK plays a role in behavioral patterns regulated by glucocorticoids, the effects of both CCK_A (MK-329, devazepide) and CCK_B (L-365,260) receptor antagonist on the retention of immobility in the forced-swim test were studied in rats.

Male Wistar rats, weighing 120-150 g, were forced to swim during 15 min into a methacrylate cylinder (18 cm diameter, 25 cm high) containing a water depth of 18 cm at 25º C. 24 hr later, animals were reintroduced during 5 min period. Drugs were dissolved in polyethylenglycol 200-ethanol (95/5) and given subcutaneously just before first swimming trial.

Devazepide (0.3 mg/kg), but not L-365,260 (1 mg/kg), was found to reduce the duration of immobility as compared to control in the 5-min retest session. As previously reported, the same effect was observed when the drug tested was the glucocorticoid G_{II} receptor antagonist RU-486 (mifepristone). Interestingly, the effect of devazepide was not only reversed by the CCK receptor agonist CCK_B (40 μg/kg), but also by the selective glucocorticoid G_{II} receptor agonist dexamethasone (0.03 mg/kg).

These results suggest that CCK, by acting upon CCK_A receptors, may be involved in the behavioral response to stress through a glucocorticoid related mechanism.

557.14

THE EFFECTS OF THE NEUROSTEROID, PREGNENOLONE SULFATE, ON SPONTANEOUS LOCOMOTOR ACTIVITY, SPATIAL LEARNING AND MEMORY. D .M. Kinsella, M. Kavaliers, K.-P. Ossenkopp and R. Shivers*. Neuroscience Program, University of Western Ontario, London, Canada.

The sulfated neurosteroid Pregnenolone Sulfate (PS) has been shown to have a variety of behavioral effects. Recently evidence has been presented that PS affects cognitive processes. The present study examined the effects of PS on spatial learning and memory in adult male rats. PS (0.5 and 1.0 mg/kg) and vehicle treated rats were tested in a Morris water maze task, where they had to learn the position of a hidden platform. PS treated rats were found to learn the task significantly faster than the saline vehicle controls, although the vehicle group had significantly better retention. The effects of PS on locomotor activity and swimming behavior were also examined. In a multivariate analysis of locomotor behavior, PS was found to cause a dose-dependent increase in activity. There were, however, no significant effects on swimming behavior. These results suggest that PS may affect spatial learning and locomotor activity. Supported by an NSERC to KPO and MK.

557.15

THE NITRIC OXIDE SYNTHASE INHIBITOR, N(G)-MONOMETHYL-L-ARGININE IN THE MPOA INCREASES SEMINAL EMISSIONS IN RESTRAINED SUPINE RATS AND REDUCES THE INCIDENCE OF COPULATION IN A COPULATION TEST. J. Moses* & E. M. Hull. Department of Psychology, State University of New York at Buffalo, Buffalo, NY 14260.

The nitric oxide synthase inhibitor N(G)-monomethyl-L-arginine (NMMA; 25, 50, 100, 200 and 400 μg) was microinjected into the medial preoptic area (MPOA) of male rats. In a test of ex copula genital reflexes, restrained supine rats exhibited a dose-dependent increase in seminal emissions which was significant at the 400 μg dose. This effect was not mimicked by the inactive isomer N(G)-monomethyl-D-arginine. In a subsequent copulation test, animals in the NMMA (400 μg) condition were significantly less likely to successfully initiate copulation than were animals in the vehicle condition. The results suggest that nitric oxide in the MPOA contributes to the regulation of male sexual behavior.

557.16

THE EFFECTS OF THE NOS INHIBITORS, 7-NITROINDAZOLE AND L-NITRO-ARGININE-METHYL ESTER, ON THE SEDATIVE/HYPNOTIC AND ANTINOCICEPTIVE EFFECTS OF PROPOFOL AND ETOMIDATE. S. Harris, S. La Marca and R.W. Dunn*. Ohmeda Inc., Pharmaceutical Products Division, 100 Mountain Ave., Murray Hill. NJ 07974.

It has previously been shown in rats that L-Nitro-Arginine-Methyl Ester (L-NAME) reduced the requirement for the inhalational anesthetic halothane (Johns et al., *Anesthesiology*, 77:779, 1992). Therefore, we examined the sedative/hypnotic and antinociceptive effects of intravenous (iv) anesthetics alone and in the presence of NOS inhibitors [L-NAME and 7-Nitroindazole (7-NI), iv]. ED_{50} values for anesthetic potency of propofol (P) and etomidate (E), as measured by the loss of righting (LOR) in rats, were determined to be 3.7 and 0.3 mg/kg, iv, respectively. Intravenous administration of L-NAME was devoid of anesthetic effects (10-1000 mg/kg, iv) while 7-NI produced a dose-dependent LOR with an ED_{50} of 20.4 mg/kg. However, pretreatment (30 min) with either L-NAME (100 mg/kg, iv) or a sub-anesthetic dose of 7-NI (10 mg/kg, iv) had no significant effect on the anesthetic potency of P (3.1 and 4.3 mg/kg, respectively) or E (0.34 and 0.56 mg/kg, respectively). Therefore, NOS inhibition did not reduce the requirement of the iv anesthetics tested. In addition, at the ED_{100} dose for LOR, both P (5 mg/kg) and E (1.23 mg/kg) lacked antinociceptive activity in the tail flick assay. At higher doses, P and E significantly increased tail flick latencies with antinociceptive A_{50} values equal to 11.1 and 5.4 mg/kg, iv, respectively. Pretreatment with either L-NAME (30 min) or 7-NI (15 min) at doses devoid of antinociceptive effects in rats (100 and 10 mg/kg, respectively), had no effect on E tail flick latency. However, L-NAME but not 7-NI, significantly reduced the A_{50} for P by 37% to 7.0 mg/kg, iv. These data show that NOS inhibition by either L-NAME or 7-NI has no effect on the anesthetic potency of P or E, but that the antinociception produced by anesthetic doses of propofol is enhanced by pretreatment with L-NAME.

558.1

ASSESSMENT OF DRUG-INDUCED REBOUND USING A THREE-LEVER DRUG-DISCRIMINATION TASK. W. F. Caul*, R. J. Barrett, E. M. Huffman, and J. R. Stadler. Department of Psychology and V. A. Medical Center, Vanderbilt University, Nashville, TN 37240.

Our previous research, using a two-lever drug-drug discrimination task, has shown that time-dependent, bidirectional changes in choice responding occur following administration of drug(e.g., Barrett et al., Psychopharm., 1992). Because one limitation of the two-lever procedure is that it is not possible to identify cue states that differ from than those associated with the training drugs, in the present study animals were trained on a three-lever task to discriminate among two drugs and vehicle.

Rats were trained over a 10-month period using a VI schedule of food reinforcement to discriminate among 0.5 mg/kg amphetamine , distilled water, and 0.03 mg/kg haloperidol. The dose-response functions for responding on each lever indicate that animals were responding on the basis of a continuum of dopaminergic activity. Subsequent to a large (10 mg/kg) dose of amphetamine, predominant responding shifted from the amphetamine lever at 4 hr, to the water lever at 8 hr, and then to the haloperidol lever at 20 and 24 hrs.

558.2

LOW DOPAMINERGIC AND HIGH CHOLINERGIC FUNCTION IN CAUDATE NUCLEUS DURING ETHANOL WITHDRAWAL. V.S.Belagode, I.L.Rivera, and C.M.Harris,* NYCOM, Old Westbury, NY 11568.

Rats (n=4) were given ethanol liquid diet for 1-5 weeks. After a final dose (4g/kg,po), microdialysis probes were inserted into the caudate n. Samples of perfusate collected during intoxication, withdrawal and reintoxication were analyzed by HPLC with electrochemical detection. Average dopamine, DOPAC and HVA concentrations declined to minimal values by 18-24 hours of withdrawal and increased 30% on reintoxication with ethanol (4g/kg,po). Withdrawal signs included trembling and muscle rigidity. Another group was given ethanol (10%, by gavage, n=7) for 4 days, or isocaloric sucrose (n=3 controls). Withdrawal scores in the ethanol group (24.9+/-0.7) were higher than controls (15.8+/-0.6). L-dopa (20 mg/kg,ip) did not affect these scores, but atropine (3mg/kg,ip) reduced withdrawal scores to the control range, relieved muscle rigidity and tremors and improved performance of a motor task. These data support the hypothesis that abnormal motor function during withdrawal is due to deficient dopaminergic function in basal ganglia which may be relieved by removing a tonic inhibitory cholinergic influence. Supported by DA06873 to CMH.

558.3

DISCRIMINATIVE STIMULUS EFFECTS OF CAFFEINE IN METHAMPHETAMINE-TRAINED SQUIRREL MONKEYS. D. M. Grech*, R. D. Spealman and J. Bergman, New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772-9102.

The behavioral effects of caffeine were studied alone and in combination with methamphetamine (MA) in squirrel monkeys trained to distinguish 0.30 mg/kg MA from saline using conventional drug discrimination procedures. In initial experiments, caffeine (3-56 mg/kg) substituted for MA to varying extents in four of seven monkeys, resulting in 67-100% MA-lever responding. However, the MA-like discriminative stimulus effects of caffeine diminished over successive determinations and could not be reinstated regardless of dose. Pretreatment with caffeine (3 and 10 mg/kg) did not systematically alter the discriminative stimulus effects of MA; on the other hand, pretreatment with a normally ineffective dose of MA did partially restore caffeine's MA-like effects (50-90% MA-lever responding). These findings suggest that, although caffeine and MA generally have dissimilar discriminative stimulus effects, their behavioral actions may overlap to a limited extent. Supported by DA 03774, RR00168, DA 00499 and MH 07658.

558.4

EFFECTS OF α-METHYL-*p*-TYROSINE AND RESERPINE ON DA LEVELS AND AMPHETAMINE-INDUCED BEHAVIORS OF EEDQ-TREATED RATS. C.A. Bolanos, M.A. Duke, S.A. McDougall*, and C.A. Crawford. Department of Psychology, California State University, San Bernardino, CA 92407 and Neuropsychiatric Institute, University of California, Los Angeles, CA 90024

EEDQ irreversibly binds to DA receptors and decreases striatal DA levels. In spite of these actions, EEDQ does not affect the apomorphine-induced behaviors of rat pups, but marginally depresses amphetamine-induced behaviors. To further assess these findings, EEDQ-pretreated rat pups were given the DA synthesis inhibitors α-methyl-*p*-tyrosine (AMPT) or reserpine prior to behavioral testing with amphetamine. More precisely, 16-day-old rat pups were injected with EEDQ (7.5 mg/kg) and then injected, one day later, with either reserpine (5 mg/kg), AMPT (200 mg/kg), or saline. Amphetamine (1.0 mg/kg) and saline-induced activity were then assessed across a 4-day span. An HPLC was used to measure DA levels after EEDQ, AMPT, and reserpine treatment. Results showed that amphetamine's activity enhancing effects were not blocked by EEDQ, but they were attenuated by AMPT and reserpine. EEDQ did not potentiate AMPT's or reserpine's behavioral actions. This was surprising, since EEDQ as well as AMPT and reserpine significantly reduced the DA levels of 17-day-old rats. In general, these results suggest that EEDQ's presynaptic actions are not of great behavioral significance for the preweanling rat.

558.5

EFFECT OF FOURPHIT, AN IRREVERSIBLE INHIBITOR OF STIMULANT BINDING, ON COCAINE-INDUCED BEHAVIORS. M. Schweri*, B. de Costa, and K. Rice. Div. Basic Med. Sciences, Mercer U. Sch. Med., Macon, GA 31207 and NIDDK, Bethesda, MD 20892.

We have shown previously that 20 mg/kg (i.v.) Fourphit HCl (4-isothiocyanato-1-[1-phenylcyclohexyl]piperidine; FPH), an irreversible inhibitor of [^{3}H]methylphenidate binding to the stimulant recognition site on the dopamine transporter, decreases the total, ambulatory, and non-ambulatory activity caused by a low dose (15 mg/kg, i.p.) of cocaine•HCl (COC) 24 hrs after treatment with FPH. The present work examined the effect of the same dose of FPH on the behavioral response to a higher dose of COC (40 mg/kg, i.p.). After overnight acclimatization in individual activity monitor cages, male Sprague-Dawley rats were injected with FPH or vehicle (VEH; 20% EtOH/saline), then challenged 24 hrs later with 40 mg/kg (i.p.) COC. Activity counts recorded after COC injection were analysed by 2-way (time x treatment) ANOVA with repeated measures after square root transformation. Significant time x treatment interactions were obtained for the total ($p<.05$) and ambulatory ($p<.02$) activity counts recorded at three consecutive 25 min intervals, starting at 10 min after COC administration. Analysis by t-test showed that FPH-treated rats were significantly less active than VEH controls during the first measurement interval. Although FPH had no effect on nonambulatory activity (often used as a measure of stereotypy), differences in particular stereotypic behaviors were found: FPH-treated rats exhibited more thigmotaxis ($p<.03$) and less rearing ($p<.04$) after COC than controls. The data show that FPH has a significant effect on behaviors caused by a higher dose of COC. (Supported by NIDA #DA06305 and NINDS #NS28584).

558.6

MODULATION OF DOPAMINE RECEPTOR AGONIST INDUCED ROTATIONAL BEHAVIOUR BY PLG AND ITS NOVEL ANALOGUE PAOPA. D. Whan, R.K. Mishra* & R.L. Johnson[1]. McMaster Univ., Hamilton, Ont. & Dept.[1] of Medicinal Chemistry, Univ. of Minnesota, Minneapolis, Mn.

The interaction of L-prolyl-L-leucylglycinamide (PLG) and its novel synthetic analogue 3-(R)-[N-(L-prolyl)amino]-2-oxo-1-pyrrolidineacetamide (PAOPA) with central dopamine receptors in vivo was investigated in 6-hydroxydopamine lesioned rats. PLG maximally potentiated apomorphine-induced rotations at a dose of 1.0 mg/kg (intraperitoneal injection, i.p.) and 100.0 ng (intrastriatal injection, i.s.). PAOPA was 100 fold more potent than PLG, inducing maximal potentiation at dosages of 10.0 μg/kg (i.p.) and 1.0 ng (i.s.). Co-administered intrastriatal injections of PLG and PAOPA (100.0 ng and 1.0 ng respectively) enhanced the agonist induced rotations by 25%. Concurrent intraperitoneal injections of PLG and PAOPA (1.0 mg/kg and 10.0 μg/kg respectively) similarly increased the rotational response by 50%. PAOPA also potentiated both SK&F 38393 and quinpirole induced contralateral rotations. PAOPA maximally mediated its effects via D_2 dopamine receptors as PAOPA produced an 85% increase in quinpirole (D_2 agonist) induced rotations. In contrast, PAOPA yielded a modest 14% increase in SK&F 38393 (D_1 agonist) induced rotations. Haloperidol blocked these peptide potentiated apomorphine rotations and those induced by quinpirole regardless of the route of administration (i.p. or i.s.). SCH 23390 blocked the SK&F 38393 induced rotations potentiated by PAOPA. Thus PAOPA, with greater potency than PLG to modulate the effects of dopamine agonists, is of therapeutic importance in the treatment of Parkinson's disease and the dyskinetic syndromes. (Supported by NIH Grant NS 20036.)

558.7

DIZOCILPINE (MK-801)-INDUCED BEHAVIOR IN RATS IS INHIBITED BY NEUROLEPTIC DRUGS AND POTENTIATED BY ACIVICIN. P. Andiné*, R. Axelsson and M. Sandberg. Departments of Clinical Neuroscience, Section of Psychiatry, Sahlgrenska University Hospital, and Anatomy and Cell Biology, Institute of Neurobiology, University of Göteborg, S-413 90 Göteborg, Sweden.

N-Methyl-D-aspartate (NMDA) receptor antagonists are psychotomimetics offering the most schizophrenia-like drug-induced syndrome in humans. The NMDA antagonist dizocilpine (MK-801) causes a typical behavior in rats which is used as an experimental model for psychosis. We used adult Sprague-Dawley rats and observed them from 15-75 min after intraperitoneal administration of MK-801 (0.05-1.0 mg/kg). They were scored for locomotion, stereotyped sniffing and ataxia every 5 min throughout the experiment. MK-801 caused increased locomotion and stereotyped sniffing in lower doses and also ataxia in higher doses. Female rats were much more susceptible to MK-801 than males. Neuroleptic drugs, administered in a single-dose 30 min before MK-801, offered a potent reduction of MK-801 behavior, with a total inhibition at doses (haloperidole 1.0 mg/kg, perphenazine 2.0 mg/kg, chlorpromazine 10 mg/kg, remoxipride 20 micromoles/kg) that correspond to their antipsychotic potency in patients. An enhancement of MK-801 behavior was found in male rats pretreated with acivicin (100 mg/kg), an inhibitor of the putative amino acid reuptake enzyme gamma-glutamyl transferase. In vitro, acivicin did not influence extracellular (ec) glutamate, however, it increased ec glutathione. Glutathione may cause NMDA receptor blockade via redox interaction and thereby an enhancement of MK-801 behavior. In conclusion, MK-801-induced behavior in rats is aggravated by gamma-glutamyl transferase inhibition and abolished by neuroleptics.

558.8

THE EFFECTS OF DOPAMINERGIC AGONISTS ON DELAYED MATCHING-TO-SAMPLE (DMTS) PERFORMANCE UNDER THERMAL NEUTRAL AND COLD STRESS CONDITIONS.
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Dopaminergic drugs have been shown to be important in the modulation of working memory. This study examined the role of the D_2/D_3 agonist quinpirol (0.025-0.10 mg/kg) and the D_1 agonist SKF 38393 (1.0-4.0 mg/kg) on working memory in rats using a DMTS procedure. Each DMTS trial required rats to respond to one of two cued levers, which initiated a variable delay of 1-16 sec. Following the delay, rats were reinforced for correctly responding to the previously cued lever. Under thermal neutral conditions (22°C) following saline, rats' matching accuracy showed a characteristic decline as the delay interval increased. Consistent with previous research and relative to 22°C, matching at 2°C (cold) was reduced. Quinpirol at 22°C improved matching following .025 mg/kg but reduced matching accuracy at higher doses. Quinpirol did not prevent the cold-induced decline in matching. SKF 38393 did not reduce matching accuracy at 22°C and did not affect matching accuracy at 2°C. Drug and cold effect results include the analysis of changes in slope (retention) and y-intercept (stimulus encoding) of the matching accuracy gradient. These data suggest that D_2/D_3 receptors are more important in modulating working memory than the D_1 receptor, and these receptors may not be directly involved in cold-induced working memory deficits.

558.9

DIFFERENTIAL REGULATION AND BEHAVIORAL EFFECTS OF THE MODE OF STIMULATION OF DOPAMINE D_1 RECEPTORS IN MPTP-EXPOSED PARKINSONIAN PRIMATES.
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The contribution and optimal mode of stimulation of dopamine (DA) D_1 receptors in the treatment of Parkinson's disease (PD) remain poorly defined. In one experiment, the long-acting, selective D_1 agonist A-77636 ((1R,3S) 3-(1'-adamantyl)-1-aminomethyl-3,4-dihydro-5,6-dihydroxy-1H-2-benzopyran hydrochloride) (Kebabian et al., EJP 1992; 229: 203) was administered once daily for 1 week at doses extending from 0.5 to 10 mg/kg s.c. to 4 MPTP-exposed, parkinsonian cynomolgous monkeys with levodopa-induced dyskinesia (LID). Following the first dose (0.5 mg/kg), all monkeys quickly showed a definite antiparkinson response for at least 8-12 hrs with increased ambulation persisting overnight. Only mild dyskinesia and stereotypies were seen. A second dose the next day was not as effective, and the level of motor activity progressively declined in 3 animals but remained above baseline values during the last 5 days of treatment, in spite of the administration of higher doses. In another paradigm, the short-acting, full D_1 agonist SKF 82958 (6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) was administered to 3 drug-naive, MPTP-exposed, parkinsonian cynomolgous monkeys at a dose of 1 mg/kg s.c. thrice daily for 4 weeks. Parkinson features were quickly relieved in all animals for 35-60 minutes following each dose. However, after several days of treatment and especially in the last 2 weeks of the protocol, the drug response significantly shortened (20-30 min) in all animals and choreiform dyskinesia developed in 2 while peak response was maintained. These data clearly show that selective D_1 receptor stimulation can effectively relieve parkinson features and lead to dyskinesia similar to LID in this primate model of PD. However, this DA receptor subtype seems exquisitely prone to downregulation. Prolonged D_1 receptor occupancy should be avoided. The mechanisms for such pharmacological behavior and ways to prevent it must be explored further. [Supported by FRSQ and MRC (Canada)]

558.10

BEHAVIORAL AND BIOCHEMICAL STUDIES ON THE INTERACTION OF THE NMDA ANTAGONIST MK-801 AND THE D_2 ANTAGONIST HALOPERIDOL.
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Thirty two male rats were assigned to four groups and were given saline, MK-801 (0.3 mg/kg), haloperidol (0.5 mg/kg) and MK-801/haloperidol combined treatments. The behavioral data of locomotor activity showed that the NMDA antagonist MK-801 induced hyperactivity, haloperidol induced hypoactivity but that the activity of the combined MK-801/haloperidol group was identical to the saline animals in both total locomotion distance and the within session habituation pattern. On the other hand, the *Ex vivo* biochemical data revealed no significant effect by MK-801 on dopamine metabolism, nor did MK-801 modify haloperidol effects on dopamine metabolite turnover ratios. None of the drug treatments had an effect on brain serotonin metabolism. Haloperidol, however, significantly increased serum 5-HT level. No drug treatment affected plasma catecholamine or plasma corticosterone levels. Taken together all these results supports the hypothesis that the effect of MK-801 on behavior is independent of biochemical changes in dopaminergic transmission.

558.11

DIFFERENTIAL REINFORCEMENT OF IMMOBILITY IN AMPHETAMINE-TREATED RATS: IMPLICATIONS FOR UNDERSTANDING TOLERANCE.
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The purpose of this study was to determine whether amphetamine-treated rats can learn to suppress stereotyped head movements. Rats implanted with cannulas were reinforced with intraoral infusions of milk for holding their heads stationary within a narrow area defined by intersecting photobeams mounted in the horizontal and vertical planes. Concurrent interruption of these photobeams activated an infusion pump, which delivered milk at the rate of 2 cc/min for the duration of the interruption.

Four of six rats given chronic injections of amphetamine (2 mg/kg) learned to hold their heads within the intersecting photobeams. The amount of milk ingested as a result of these infusions increased over trials at a rate that was comparable to that of bottle-fed rats. Analysis of the temporal distribution of photobeam interruptions revealed a highly fragmented pattern that differed markedly from the sustained pattern found in saline controls. These results support the view that tolerance to amphetamine hypophagia involves an instrumentally learned behavioral adaptation.

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558.12

A HIGH DOSE OF THE SELECTIVE D2 AGONIST QUINPIROLE IS POSTSYNAPTICALLY MEDIATED.
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Utilizing a drug discrimination paradigm, this laboratory demonstrated that the stimulus properties of a low dose (0.05 mg/kg) of the selective D2 agonist quinpirole (QUIN) are mediated by D2 presynaptic autoreceptors (Widzowski and Cory-Slechta, 1993, JPET, 266, 526-534). The current study examined the hypothesis that the stimulus properties of a high dose of QUIN (0.20 mg/kg) would be mediated by postsynaptic D2 receptors, resulting in a pattern of responding to pharmacological treatments opposite to that noted in the previous study. The findings were consistent with that premise. D1 receptor blockade with SCH23390 produced full substitution for 0.05 QUIN, but only saline responding in 0.20 QUIN-trained rats. Also, alpha-methyl-paratyrosine depletion of catecholamines produced drug lever responding (DLR) in the 0.05 QUIN group but not in the 0.20 QUIN group. Low doses of apomorphine (up to 0.167 mg/kg), produced 70% DLR in the 0.05 QUIN group, but only 25% in the 0.20 QUIN group. Higher doses of apomorphine, which substitute for D1 agonists (Cory-Slechta, D.A., 1989, JPET, 250, 800-809), produced only 35-40% DLR in the 0.20 QUIN group. DLR was not produced in either group by the D1 agonist SKF82958. Taken together, these data are consistent with a postsynaptic mediation of the stimulus properties of a high dose (0.20 mg/kg) of QUIN. Moreover, pretreatment with the D1 antagonist SCH23390 decreased DLR in the 0.20 group to 43%, suggesting postsynaptic D1-D2 interactions.

558.13

ASSESSMENT OF THE BEHAVIORAL PROFILES OF CONVENTIONAL AND ATYPICAL ANTIPSYCHOTIC DRUGS IN RODENTS. J.G. Wettstein*, S. Roux and R.D. Porsolt. I.T.E.M.-LABO, 93 ave Fontainebleau, 94276 Le Kremlin-Bicêtre, France.

The effects of a reference neuroleptic, haloperidol, were compared with those of three atypical antipsychotic agents (clozapine, DuP 734 and risperidone) and a 5-HT_2 antagonist, ritanserin, in a number of procedures. Drugs were first examined in mice over a wide dose-range using a primary observation method in order to target the doses for other studies. Haloperidol (0.125-2 mg/kg) was distinctly active in all procedures and produced marked catalepsy. Risperidone was more potent than the other compounds in many of the procedures, often having activity at doses well below 1 mg/kg. Neither clozapine (1.0-16 mg/kg) nor DuP 734 (0.5-8 mg/kg) induced catalepsy in rats yet both drugs appeared to antagonize amphetamine-induced hyperactivity at doses less effective against amphetamine- or apomorphine-induced stereotypies in mice. Clozapine and DuP 734, along with ritanserin, also clearly blocked mescaline-induced scratching. Ritanserin (2-16 mg/kg), in general, did not have a behavioral profile like those of the other four drugs. All compounds also differed with respect to their actions as antagonists of PCP-induced stereotypies and SKF 10047-induced hyperactivity in rats. In view of the clinical findings currently available with these drugs, it appears highly useful to employ a series of methods in order to identify novel antipsychotics that differ from haloperidol. Absence of cataleptogenic activity and selective antagonism of amphetamine-induced hyperactivity would appear to be useful criteria.

558.14

Effect of NO synthase inhibition on behavioral changes induced by a single administration of methamphetamine

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The present study examined the effects of nitric oxide synthesis inhibition on behavioral changes induced by a single administration of methamphetamine(MA). Rats received one subcutaneous injection of MA (3.22 mg free base/kg). Nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester, LNAME(10,30 and 60 mg/kg) were administered intraperitoneally 1 hr prior to injection of MA or saline. MA increased locomotion-stereotypy rating scores and motor activity counts measured by an infrared sensor. LNAME(30 and 60 mg/kg) administerd 1 hr prior to MA significantly decreased the level of locomotion stereotypy rating and motor activity induced by MA. The results suggest that NO synthesis is involved in the full expression of behavioral effects of MA.

558.15

The noncompetitive NMDA receptor antagonist (+)dizocilpine fails to prevent sensitization to apomorphine-induced rotational behavior in rats with nigrostriatal lesions. S Gancher*and A Mayer, Dept of Neurology, Oregon Health Sciences University, Portland, Oregon, 97201.

Sensitization to CNS stimulants is well described and is reportedly prevented by the noncompetitive NMDA receptor antagonist (+)dizocilpine (MK-801). We sought to determine if MK-801 pretreatment prevents sensitization to apomorphine (APO)-induced rotational behavior in rats with unilateral nigrostriatal lesions. Six rats were lesioned with 6-OHDA, and beginning 2 weeks later, received 8 daily doses of MK-801 (0.1 mg/kg, ip) followed 30 min later by APO (0.1 mg/kg, sc). Three and 5 days later, single doses of the D1 agonist SKF38393 (2 mg/kg) & APO (0.1 mg/kg, day 5) were administered without MK-801 pretreatment. Despite MK-801 pretreatment, the rotational behavior increased over the protocol (for doses 1, 4, and 8, total and peak/5 min rotations were 104 and 24, 407 and 92, and 576 and 85, respectively, $p<.05$). The dose of SKF induced 1624 rotations. MK-801 enhanced APO rotations; in comparison with dose 8, APO alone induced 449 rotations ($p=0.05$).

In a separate experiment, 3 rats received MK-801+APO on postlesion day 14, and 3 days later, received 2 mg/kg SKF 38393; as reported by Morelli et al, SKF 38393 failed to induce rotational behavior. These data suggest that in contrast to sensitization in unlesioned animals, lesioned animals sensitize to APO despite NMDA channel blockade.

558.16

QUINOLINIC ACID-INDUCED LESION OF THE SUBSTANTIA NIGRA PARS RETICULATA: BEHAVIOURAL AND NEUROCHEMICAL EFFECTS. B. Zadow*, M. Bubser and W.J. Schmidt. Department of Neuropharmacology, University of Tübingen, D-72074 Tübingen, Germany.

As one of the major output structures of the basal ganglia (BG) the substantia nigra pars reticulata (SNr) is part of the motor loop through the BG and thus is involved in the performance of motor behaviour. The SNr receives information from the striatum and sends inhibitory GABAergic projections to the thalamus and the deeper layers of the superior colliculus. The present study investigated the role of the SNr in mediating motor behaviour in male Sprague Dawley rats. The SNr was bilaterally lesioned with the excitotoxin quinolinic acid (2 x 30 nmol/0.5 µl/side). Locomotor activity and exploration were tested in an open field with holeboard and sniffing stereotypy was tested in an experimental chamber (Schmidt, W.J., 1986, Psychopharmacology 90: 123-130). Both locomotor- and sniffing activity were increased after SNr lesions, whereas exploration was not changed. The histological verification of the SNr lesions demonstrated that in most cases a part of the substantia nigra pars compacta was also lesioned. The levels of dopamine (DA) and its metabolites (DOPAC, HVA) in the anterior and posterior striatum and in the nucleus accumbens were analysed post mortem with HPLC with electrochemical detection. DA and DOPAC were reduced in all three structures, whereas HVA was only reduced in the anterior striatum. The metabolism of dopamine (measured as DOPAC/DA and HVA/DA) was increased in the anterior and posterior striatum. These increases correlated significantly with the increases in locomotor- and sniffing activity. In conclusion, bilateral lesions of the SNr and thus reduced activity of this structure results in an increase of motor activity. This increase may be explained by the deficient inhibitory effects of the SNr on the thalamus and the superior colliculus, but also by the increased dopamine metabolism in the striatum.

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558.17

POTENTIATION OF THE DIHYDREXIDINE DISCRIMINATIVE STIMULUS BY PHOSPOHODIESTERASE INHIBITION AND THE EFFECT OF RECEPTOR RESERVE ON D_1 AGONIST SUBSTITUTION. Q.D. Walker*[1], D.M. Black[1], D.A. Eckerman[1], D.E. Nichols[2] and R.B. Mailman[1]. Curriculum in Toxicol., Univ. of N. Carolina[1], Chapel Hill, NC 27599, and Dept. Med. Chem., Purdue Univ.[2], West Lafayette, IN 47907.

In rats, administration of the full D_1 agonist dihydrexidine (DHX, 2 mg/kg) induces robust discriminative stimulus (DS) effects that are mediated selectively by D_1 receptor stimulation. We investigated whether the DHX cue was also mediated by increased intracellular concentrations of cAMP. The phosphodiesterase inhibitor rolipram (0, 7.5, 15, or 30 µg/kg) was administered either alone or in combination with doses of DHX (0.25 or 0.5 mg/kg) or SKF38393 (1.0 or 2.0 mg/kg) that induce little drug appropriate responding. Rolipram (15 µg/kg) and DHX (0.25 mg/kg) induced 0 and 7% DHX lever responding, respectively, when administered alone, but 50% when coadministered. Rolipram also synergistically increased SKF38393 substitution. Coadministration of rolipram (30 µg/kg) and SKF38393 (2.0 mg/kg) induced 63% drug lever selection, although the compounds induced only 15 and 20% drug lever selection, respectively, when administered alone. The second series of experiments tested the hypothesis that D_1 receptor reserve explains the complete substitution of the partial agonist SKF38393 for the DHX cue. EEDQ (10 mg/kg) was administered with a receptor protectant cocktail (prazosin, ketanserin, remoxipride) to decrease D_1 receptor number by about 50%. Forty-eight hr after EEDQ, rats were administered doses of either DHX (2 mg/kg) or SKF38393 (8 mg/kg) that substituted > 90% prior to EEDQ treatment. Administration of EEDQ significantly decreased the substitution of SKF38393 (93% vs. 26%) but did not affect substitution of DHX (98% vs. 74%). These results suggest that D_1 receptors coupled to adenylate cyclase mediate the DS effects of DHX and that SKF38393 substitutes for DHX by occupying a greater proportion of the receptor population than does DHX.

559.1

ROLE OF NMDA AND NON-NMDA RECEPTORS IN SPINAL TRANSMISSION OF THE EXERCISE PRESSOR REFLEX C.M. Adreani, J.M. Hill, and M.P. Kaufman . C.L. Stebbins*. Cardiovasc. Med., Univ. of Calif., Davis, CA 95616.

Much evidence suggests that glutamate plays a role in the spinal transmission of the reflex autonomic responses to static contraction. Previously we measured the reflex increase in arterial pressure evoked by static contraction of the hindlimb muscles in chloralose-anesthetized cats before and after glutamatergic receptor blockade in the lumbosacral cord. The reflex pressor response to contraction was unaffected by NMDA receptor blockade (AP-5), but was significantly attenuated by non-NMDA receptor blockade (CNQX). Recently, NMDA antagonists have been shown to block the responses of dorsal horn cells to noxious input in decerebrate, but not in chloralose-anesthetized cats (Radhakrishnan and Henry, 1993). This finding raises the possibility that the absence of an effect mediated by NMDA receptors on the pressor response to static contraction could have been due to chloralose. We, therefore, investigated the effect of glutamatergic receptor blockade on the reflex ventilatory and cardiovascular responses to contraction in decerebrate unanesthetized cats. We found that intrathecal injection of 500 μg AP-5, onto the lumbosacral cord attenuated the reflex increase in heart rate due to static contraction (Δ36±4 to Δ21±4 bpm; $p<0.05$), but had no effect on the pressor and ventilatory responses to contraction ($n=10$). Subsequent intrathecal injection of 90 μg CNQX attenuated ($p<0.05$) the pressor (Δ40±6 to Δ14±6 mmHg), heart rate (Δ36±4 to Δ13±3 bpm), and ventilatory (Δ359±116 to Δ105±44 ml/min) responses to contraction. Our results suggest that non-NMDA receptors are involved in the pressor and ventilatory responses to static contraction, and that both NMDA and non-NMDA receptors are involved in the chronotropic response.

559.2

SPINAL RELEASE OF SUBSTANCE P DURING MUSCLE CONTRACTION: EFFECT OF 5-HT$_{1A}$ RECEPTOR ACTIVATION. A.C.L. Nóbrega, A.F. Meintjes, A. Ally, L.B. Wilson*. Harry S. Moss Heart Center, University of Texas Southwestern Medical Center, Dallas, TX 75235-9034

Static muscle contraction increases mean arterial pressure (MAP) and substance P (SP) release in the dorsal horn of the spinal cord. Activation of 5-HT$_{1A}$ receptors in this region attenuates the reflex pressor response to contraction. This study evaluated the effect of microdialysis of 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT; 5-HT$_{1A}$ agonist) into the L_7 dorsal horn on the release of SP during contraction using anesthetized cats. Ipsilateral to the muscle to be contracted, all ventral and dorsal roots from L_5 to S_2 were severed, except the L_7 dorsal root. Static contraction of the triceps surae muscle was induced by alternate electrical stimulation of the distal ends of L_7 and S_1 ventral roots for 5 min (3X motor threshold; 30 Hz; 0.1-0.3 ms). The dialysates from two probes, placed in the L_7 dorsal horn region ipsilateral to the contracting muscle, were analyzed for SP-like immunoreactivity (SP-LI) using a radioimmunoassay. DPAT (10 mM; n= 8) attenuated the MAP response to contraction (control= 38 ± 11; DPAT= 10 ± 3 mmHg; $P < 0.05$). Likewise, the heart rate response was also blunted (control= 13 ± 3; DPAT= 7 ± 3 bpm; $P < 0.05$). However, the SP-LI values during contraction were unaffected by DPAT (control= 0.492 ± 0.026; DPAT= 0.501 ± 0.034 fmol/100 μL; $P > 0.05$). These results suggest that the modulation of the pressor response to contraction by 5-HT$_{1A}$ receptors in the dorsal horn is not mediated through inhibition of SP release.

559.3

SYSTEMIC 2-DEOXY-D-GLUCOSE LEADS TO EXCITATION OF ADRENAL MEDULLARY SYMPATHETIC PREGANGLIONIC NEURONS. S.F. Morrison*. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Adrenal medullary release of epinephrine and the subsequent stimulation of glycogenolysis and gluconeogenesis are important components of the physiologic response to hypoglycemia. To determine the central pathways controlling catecholamine secretion by the adrenal medulla, the responses of adrenal medullary sympathetic preganglionic neurons (AdrSPNs) to 2-deoxy-D-glucose (2-DG) administration (200mg/kg, iv.) were studied in urethane/chloralose-anesthetized, artificially-ventilated rats. AdrSPNs were identified by their antidromic responses to adrenal nerve stimulation and their unique orthodromic responses to stimulation in the rostral ventrolateral medulla (RVLM). Ten minutes after 2-DG injection, the basal discharge rate of AdrSPNs had increased ($p<0.001$) from 5.8 Hz (control) to 9.2 Hz. Subsequent bilateral microinjection of muscimol (25 nmol) into the RVLM eliminated the 2-DG-induced increase in AdrSPN discharge, although a low level of AdrSPN discharge remained. The increase in AdrSPN discharge following 2-DG was not different in animals with transections of the neuraxis rostral to the superior colliculus. These results suggest that activation of AdrSPNs is a brainstem-mediated component of the neural response to the blockade of glucose utilization by 2-DG and that RVLM neurons may be necessary for this response. Supported by NIH HL47196.

559.4

SPINAL NMDA RECEPTOR MEDIATES c-fos EXPRESSION FOLLOWING HYPOTENSION IN THE CONSCIOUS RAT. Huang, W. and West, M.J.* Department of Medicine, University of Queensland, Prince Charles Hospital, Brisbane, QLD 4032, Australia.

Recent studies show that baroreflex induced vasoconstrictor responses following hypotension are attenuated by the administration of NMDA receptor antagonist AP5. We have examined the effect of intrathecal AP5 on spinal cord c-fos expression induced by hypotension in conscious rats. AP5 or saline were administered intrathecally prior to a 90 minute infusion of sodium nitroprusside which lowered blood pressure by 30 mmHg. Animals were subsequently killed under general anaesthesia and the medulla and spinal cord processed for demonstration of c-fos protein using immunocytochem istry. C-fos neuronal immunoreactivity (Fos-IR) was observed in the rostral and caudal ventrolateral medulla and nucleus tractus solitarius and in the intermediolateral cell column (IML) of the spinal cord with the highest segmental concentration in segments T7-T10. At the level of the intrathecal administration of AP5, T9, there was a significant reduction in Fos-IR cells in the IML in animals given AP5 compared to those given saline (125 $\pm$ 6.9/segment (n=3) vs 235 $\pm$ 6.5 (n=2), $p<0.05$). There were no differences between treated and control animals in the medulla or other segments of the spinal cord. The results show that (i) following hypotension, cells in IML of segments T7-T10 show maximal expression of Fos-IR and (ii) AP5 inhibits c-fos expression. We conclude that spinal NMDA receptors in IML of spinal cord are involved in central baroreflex control of the circulation.

559.5

SPINAL NITRIC OXIDE IS INVOLVED IN THE VASOPRESSOR ACTION. S.Y. Kim[1], H.C. Koh[2], K.W. Sung[1] and S.B. Lee*[1]. [1]Dept. Pharmacology, Catholic University Medical College, 505 Banpo-dong, Socho-gu 137-701 and [2]Dept. Pharmacology, Hanyang University Medical College, Seoul, KOREA.

Anatomic studies have localized nitric oxide (NO) synthase activity in the spinal cord intermediolateral column. The present study was designed to investigate a possible role of the spinal NO in the central regulation of blood pressure (BP). Experiments were carried out in urethane-anesthetized, d-tubocurarine-paralyzed and artificially ventilated male Wistar rats. Intrathecal (i.t.) administration of drugs were made using the injector cannula (33 G stainless steel) through the guide cannula (PE 10) which was inserted intrathecally at the thoracic level through a puncture of the atlantooccipital membrane. I.t. injection of a NO donor, sodium nitroprusside (SNP; 1, 5 and 15 nmol) produced dose-dependent increases in BP. The vasopressor effect of SNP (15 nmol, i.t.) was completely blocked by pretreatment with the N-methyl-D-aspartic acid (NMDA) receptor antagonist, D,L-2-amino-5-phosphonopentanoic acid (10 nmol, i.t.). The pretreatment with the non-NMDA receptor antagonist kynurenic acid (250 nmol, i.t.) also attenuated the vasopressor effect of SNP (15 nmol, i.t.). In addition, i.t. injection of an inhibitor of NO synthase, N$^{\omega}$-nitro-L-arginine methyl ester (20, 100 and 1000 nmol) evoked dose-dependent decreases in BP. These results suggest that NO generated in spinal cord plays a role in the facilitatory regulation of BP. This vasopressor action of the spinal NO seems to be tonically active and to be mediated via its interaction with the glutamatergic system in the spinal cord.

559.6

SECOND MESSENGER MODULATION OF MEMBRANE POTENTIAL OSCILLATIONS IN SYMPATHETIC PREGANGLIONIC NEURONS. D. Spanswick, M.L.H.J. Hermes and L.P. Renaud*. Neuroscience Unit, Loeb Reseach Institute, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

The presence of spontaneous oscillations in sympathetic preganglionic neurons (SPNs) endows these neurons with the ability for rhythmogenesis, which can be manifested as burst firing, tonic single spike discharge or combinations of these. This activity is thought to reflect electrotonic coupling between SPNs, the oscillation representing an action potential discharged in an neighbouring neuron. Furthermore, these oscillations and rhythmic activity are subject to modulation by neurotransmitters such as serotonin and noradrenaline (Logan et al., '93). We have utilized whole-cell recording techniques in a spinal cord slice preparation to further investigate factors involved in the regulation of rhythmogenesis in SPNs, in particular the effects of elevating intracellular calcium and cAMP.

Superfusion of the membrane permeable analog of cAMP, 8-Bromo-cAMP (5-25 μM), completely blocked spontaneous oscillations in SPNs. 8-Bromo-cAMP also inhibited oscillations or short latency depolarizations (SLD) evoked by stimulation of the ventral roots. These effects of 8-Bromo-cAMP could be reversed by superfusion of the glutamate metabotropic receptor agonist ACPD (100 μM). The spontaneous oscillations were relatively unaffected by ryanodine (10 μM). Superfusion of caffeine (10-20 μM) induced oscillations and rhythmic activity in previously silent SPNs. Stimulation of the ventral roots in these neurons evoked a SLD which was enhanced in the presence of caffeine.

These data suggest that rhythmogenesis in SPNs is subject to regulation being induced by intracellular calcium release and inhibited by stimulation of cAMP production (Supported by The Heart and Stroke Foundation of Canada).

1. Logan S.D. et al., Soc. Neurosci. Abst. 196.17, '93.

559.7

MEDULLARY STIMULATION CAN EVOKE IPSPs IN SYMPATHETIC PREGANGLIONIC NEURONES IN THE BRAINSTEM-SPINAL CORD PREPARATION. Susan A. Deuchars*, S.F. Morrison, K.M. Spyer and M.P.Gilbey. Royal Free Hospital School of Medicine, Rowland Hill Street, London. NW3 2PF U.K.

In the neonatal rat brainstem-spinal cord preparation using whole cell patch recordings, we have shown that stimulation of the rostral ventrolateral medulla can elicit multiphasic EPSPs in sympathetic preganglionic neurones (SPNs) mediated by both non-NMDA and NMDA receptors (Marks and Morrison, J. Physiol. 473, 155P, 1993). Here we report that medullary stimulation can also evoke IPSPs in SPNs. SPNS were identified on the basis of their antidromic activation following stimulation of the ventral root and their morphology and location within the spinal cord. Stimulation of the ventrolateral medulla (twin, 1ms pulses; 6 ms apart; 50-100 μA) elicited IPSPs in ten SPNs at holding potentials more positive than - 50 mV. The mean latency to onset of these responses was 34.4 ± 4.1 ms (mean ± S.E.M.) while the average amplitude at membrane potentials of - 40 mV was 5.77 ± 1.0 mV. These IPSPs persisted in the presence of the excitatory amino acid receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione and D,L-2-amino-5-phosphonovaleric acid in all but one of the SPNs tested. Indeed, sometimes blockade of the EPSP was necessary to reveal an IPSP. In the presence of these antagonists, hyperpolarising the neurones decreased the amplitude of the IPSPs until reversal occurred at membrane potentials more negative than - 60 mV. On two occasions bath applications of 5 μM bicuculline blocked the evoked IPSP. These results indicate that medullary stimulation can evoke IPSPs in SPNs which involve activation of GABA receptors.

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559.8

CARDIAC AND RESPIRATORY MODULATION OF NEONATAL POSTGANGLIONIC SYMPATHETIC ACTIVITY B.W. Hundley, P.M. Gootman, A.L. Sica*. Dept. of Physiology, SUNY-Hlth. Sci. Ctr., Bklyn, NY 11203.

The major aim of the present investigation was to determine whether sympathetic components of peroneal nerves (**PN**) were modulated by cardiac- and/or respiratory-related rhythms in an age-related manner and whether such relationships were influenced by vagal afferent and/or chemoreceptor inputs. In Saffan-anesthetized, paralyzed and artificially ventilated (100% O_2) piglets aged 1-40 days, different branches of PN were recorded along with C5 phrenic (**PHR**) root activity, AoP, EKG, and EEG. Age-related differences were observed for both types of modulation which were independent of vagal afferent inputs. Piglets < 1 wk old exhibited very little spontaneous PN activity related to either cardiac or respiratory rhythms. However, respiration-related activity in PN discharges of 1 wk old swine was easily elicited by ventilation with a hypoxic gas mixture (10% O_2, balance N_2). Spontaneous PN activity of piglets > 2 wks of age exhibited respiratory-related discharge, which were facilitated by hypoxia. Modulation by cardiac rhythm was revealed by coherence spectral analyses as indicated by significant coherence estimates over the 3-5 Hz range; such coupling was stronger in older piglets. Hypoxia significantly increased coherence regardless of age. The results indicate that cardiac and respiratory modulation of postganglionic sympathetic activity is delayed postnatally compared to preganglionic recordings. (Gootman et al. *Am. J. Physiol.* 1991, 261:R1147). (Supported by NIH grant HD-28931).

559.9

ADULT AND NEONATAL SYMPATHECTOMY WITH ANTI-DBH IMMUNOTOXIN. M.J.Picklo*, J.D.Amlicke, R.G.Wiley, D.A.Lappi, D.M.Roden, D. Robertson. Depts. of Pharmacology, Medicine, and Neurology, Vanderbilt University and DVAMC, Nashville, TN 37232 and Whittier Institute, La Jolla, CA 92037.

The anti-dopamine beta-hydroxylase immunotoxin (DHIT) is a recently developed noradrenergic lesioning tool. In the following study, we examined the degree of sympathectomy achieved in adult and neonatal rats given DHIT peripherally. 30 ug of DHIT was injected i.v. into anesthetized, adult, male Sprague-Dawley rats. Three weeks later animals were anesthetized and cannulas placed into the femoral artery and vein for blood pressure and heart rate recording and drug administration. Plasma was drawn before and after tyramine (300ug/kg) administration to examine basal and tyramine evoked norepinephrine levels. DHIT treated animals had significantly less basal (33%) and tyramine stimulated (20%) plasma norepinephrine levels than controls. Plasma epinephrine was unchanged. Basal blood pressure and heart rate were not significantly changed. One day old neonatal rats were injected subcutaneously with 4ug of DHIT and sacrificed at 1,3,6, and 13 days afterward. Nissl staining of superior cervical ganglia from day three animals showed an almost complete lack of large neuronal cell bodies. Cardiac ventricles and adrenal glands were assayed for catecholamine content. Ventricular norepinephrine levels fell to 60% of controls at day one and dropped to 2% of controls by day 13. Adrenal norepinephrine levels fell to 70% at day one and to 2% by day 13. Adrenal epinephrine levels were 70% of control at day one and fell to 5% by day 13. DHIT is potent in creating adult and neonatal sympathectomy. Sequential doses of DHIT may be necessary to lesion remaining neurons and adrenal chromaffin cells in adults. The ability of a single dose of DHIT to deplete adrenal catecholamines in neonates is an advantage over previous sympathectomy methods.

559.10

INSPIRATORY COMPONENT OF SPLANCHNIC SYMPATHETIC NERVE ACTIVITY IS RESISTANT TO THE SYMPATHOLYTIC DRUG CLONIDINE. N. Koshiya* and P.G. Guyenet. Department of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Splanchnic sympathetic nerve (SND) and phrenic nerve discharges (PND), and arterial pressure (AP) were recorded in urethane-anesthetized, vagotomized, aortic deafferented, paralyzed and artificially ventilated Sprague-Dawley rats (n=7). SND and PND were rectified, analog integrated at 30 ms reset-interval and digitized. Distribution of the SND in the central respiratory cycle was analyzed by means of PND burst-triggered averaging. During control period, mean AP was 106±10 mmHg (mean±SEM); SND distributed throughout the respiratory cycle with increased activity in inspiratory/early-inspiratory phase and peak activity in the post-inspiratory phase.

Clonidine was given i.v. at small (15-30 μg/kg, n=6) and/or large doses (200-250 μg/kg, n=7). Small dose of clonidine produced brief hypertension (<30 sec, 150±9 mmHg peak) followed by moderate hypotension (89±3 mmHg) which was accompanied by a reduction in SND (-63±11% control value). Large dose of clonidine produced sustained hypertension (>10 min, 173±3 mmHg) accompanied by silence of SND. During the sustained hypertension, the vasodilator sodium nitroprusside was administered i.v. (80-150 μg/kg). The nitroprusside-induced hypotension retrieved a component of SND which was barosensitive but resistant to clonidine. During those hypotensive periods (both after small dose of clonidine and during nitroprusside-induced hypotension after large dose of clonidine), SND was largely attenuated in expiratory phase and, in most cases (6 out of 7), SND showed single inspiratory peak with very small activities in the post-inspiratory/expiratory period. In 2 cases, despite the substantial decrease in total SND, the amplitude of the remaining inspiratory peak was virtually unchanged from the control level.

These results suggest that the sympatholytic drug clonidine works on the sympatho-respiratory coupling differentially throughout the respiratory cycle, and the central inspiration-related component of SND is the least sensitive to the drug.

559.11

MEMBRANE PROPERTIES OF SYMPATHETIC GANGLION CELLS IN GENETICALLY HYPERTENSIVE OR HYPERACTIVE RATS. A.P.Gokin*[1,2] and C.J.Forehand[2]. [1]Bogomoletz Inst. of Physiol., Kiev, Ukraine, 252024 [2]Dept. of Anat. and Neurobiol., Univ. of Vermont, Burlington, VT,05405.

Increased sympathetic nerve activity in the spontaneously hypertensive rat (SHR) may be partially due to alteration of the membrane properties of postganglionic neurons. Since the SHR also exhibits significant behavioral hypereactivity to stress, the relationship of neuronal alterations in SHR to the hypertensive phenotype is uncertain. To determine whether the decreased accommodation observed in SHR ganglion cells (Yarowski and Weinrich, Hyperten. 7:268, '85) is associated with hypertension, we examined active and passive membrane properties of cells in four inbred rat strains (WKY, SHR, WKHT and WKHA). The SHR is both hypertensive and hyperactive; WKY is neither. The WKHA is hyperactive, but not hypertensive; the WKHT is hypertensive, but not hyperactive.

Membrane properties were examined in 55 SHR, 44 WKY, 42 WKHT, and 40 WKHA superior cervical ganglion cells. No significant differences were observed in resting membrane potential, input resistance, the after hyperpolarization, or threshold for action potential generation among the four stains. In contrast, the number of action potentials generated during a 1 nA, 400 msec depolarizing pulse was significantly greater in both hypertensive strains (4.3 ± 0.69, SHR; 6.0 ± 0.89, WKHT) relative to the WKY (2.9 ± 0.56); the WKHA ganglion cells generated an intermediate number of action potentials (3.6 ± 0.63). The decreased ganglion cell accommodation in the hypertensive strains reflects an overall difference in the number of neurons in each population that exhibited a non-phasic response (≥4 action potentials) to the 400 msec stimulus: 21% in the WKY, 26% in the WKHA, 33% in the SHR and 48% in the WKHT.

559.12

THE LOCATION AND MORPHOLOGY OF CARDIAC GANGLIA NEURONS IN THE HUMAN HEART. S. Singh*, M. A. Mostafa, R. D. Wurster. Neuroscience Program, Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153.

The parasymapathetic innervation of the human heart consists of preganglionic neurons located in the brain stem that project via the vagus nerve to postganglionic neurons located in the cardiac ganglia. Previous studies on the location and morphology of neurons in the cardiac ganglia have been limited to animal models, to discrete regions of the heart, or to early developmental stages. Therefore, the purpose of this study was to determine the location and morphology of the cardiac ganglia neurons throughout the human heart and at several stages of development. We have mapped the location of the cardiac ganglia neurons using standard histological procedures. Our results indicate that the cardiac ganglia are clustered in two prominent epicardial fat pads in the atria of the heart; one in the right atrium below the pulmonary veins and the other near the junction of the inter-atrial sulcus and atrio-ventricular groove. Smaller clusters of ganglia are interspersed throughout other areas of the atria, including the regions above the coronary sulcus and below the left pulmonary veins.

Image analysis techniques have been used to determine if the morphology of the cardiac ganglia neurons is related to heart size changes as occur during development. Morphometric variables for the nucleolus, nucleus, soma of the neuron, as well as the cardiac ganglia were used to evaluate this possibility. Results indicate that the size of the neuronal soma and cardiac ganglia increases concomitantly with increases in heart size. The ratio of the cytoplasm to the nucleus also increases with heart size. The changes in the size of the neuron and ganglia may reflect changes in the innervation of the developing heart muscle.

559.13

SUBSTANCE P AND CGRP DEPOLARIZE PARASYMPATHETIC POSTGANGLIONIC NEURONS IN THE GUINEA PIG CARDIAC GANGLION. J. C. Hardwick*, G. M. Mawe, and R. L. Parsons. Department of Anatomy & Neurobiology, University of Vermont, Burlington VT 05405.

Increasing evidence suggests that parasympathetic ganglia are sites of complex synaptic integration rather than simple relay stations. The present study was done to establish whether afferent input to parasympathetic neurons of mammalian cardiac ganglia may provide another mode of synaptic modulation. Whole mount preparations from the guinea pig heart were utilized to localize afferent inputs by immunohistochemistry and to record voltage responses of individual neurons *in situ*. Immunohistochemical analysis demonstrated that most parasympathetic neurons were surrounded by fibers immunoreactive for both substance P (SP) and CGRP. Intracellular recordings were obtained from individual parasympathetic neurons maintained in an oxygenated Kreb's solution at 37°C. Action potentials could be elicited both by intracellular current injection and stimulation of interganglionic fiber bundles. In some cells, high frequency (5-10 Hz) interganglionic fiber stimulation produced a slowly developing depolarization of 4-10 mV which lasted for 5-30 sec. These depolarizing events were unchanged in the presence of 100 μM hexamethonium and 1 μM atropine but were significantly diminished by superfusion with calcium-free solution. A prolonged depolarization was also produced by local application of capsaicin (1 mM), which releases both SP and CGRP from afferent terminals. Direct application of either SP or CGRP (100 μM) by pressure ejection depolarized these neurons. This peptide-induced depolarization was often associated with a decrease in input resistance and an increase in excitability. We suggest that the slowly developing depolarization produced by interganglionic fiber stimulation is due to the release of SP and/or CGRP from afferent terminals. Further, we suggest that afferent input to the cardiac ganglia may be involved in local reflex modulation of the parasympathetic cardiac neurons. Supported by NIH grants NS 26995, DK 45410, and NS 23978.

559.14

PHYSIOLOGICAL STUDIES OF MYENTERIC NEURONS FROM HYPERTENSIVE RATS SUGGEST A COMMON PROGENITOR TO SYMPATHO-ADRENAL DERIVED AND SOME ENTERIC NEURONS . B. Jubelin, D.E. Richardson*+and M. D. Gershon, Dept of Anatomy & Cell Biology, P&S, Columbia Univ., NY, NY., 10032, + Dept of Neurosurgery, Tulane Medical Center, New-Orleans, La, 70112.

Our previous studies showed that: 1) sympathetic neurons from genetically hypertensive (SH) rats, unlike their controls (WKY, SD), do not accommodate long-term depolarizing pulses and thus fire repetitively; 2) A sub-population of myenteric neurons shows co-expression of dopamine ß-hydroxylase (DBH) and calbindin, suggesting that enteric and sympathetic neurons may be derived from common precursors. We thus decided to examine the electrical membrane properties of enteric neurons in the SH rats, to see if the lack of accommodative properties was also characteristic of myenteric neurons. Enteric neurons from the myenteric plexus are characterized as S or AH neurons. Besides morphological differences, these neurons are separated on the basis of their electrical membrane properties. AH neurons usually fire one or two action potentials (AP), show a typical Ca^{++} shoulder during repolarization and a characteristic after-hyperpolarization (AHP) following the AP. S neurons fire repetitively and do not show a Ca^{++} shoulder or an AHP. Most myenteric neurons in the SH rats show identical electrical membrane properties to their controls, but a subpopulation (approx 40%) of AH neurons show repetitive firing upon long-duration depolarizing pulses. They still show the Ca^{++} shoulder and the AHP, which is of longer duration since multiple AP allow more Ca^{++} to enter. These neurons were identified during the experiment and labeled for calbindin and DBH: there is no specific label for the repetitively firing sub-population of AH neurons.

In conclusion, similar abnormal electrical behaviors in sympathetic and enteric neurons support the concept of a common progenitor, but the absence of a common cellular markers leaves open the possibility that this fact reflects a default common to all neurons of the SH rats, irrespective of their lineage. Supported by NIH grants NS 12969 and NS 15547 and by awards from the PMAF and the CHF.

559.15

GUANETHIDINE EVOKES VASODILATATION IN MESENTERIC ARTERY BY ACTING ON SENSORY NERVES. Z.L. Zheng, K. Shimamura, T.L. Anthony and D.L. Kreulen*. Department of Physiology, West Virginia University, Morgantown, WV, 26506.

Capsaicin-sensitive sensory nerves mediate an endothelium-independent, NANC vasodilatation in guinea-pig mesenteric arteries. This vasodilatation is potentiated by previous administration of guanethidine (30 µM) presumably through depletion of vasoconstrictor neurotransmitters from sympathetic nerves. Our current hypothesis is that guanethidine also releases transmitter(s) from capsaicin-sensitive sensory nerves and thereby invokes vasodilatation. To test this hypothesis, segments of endothelium-denuded mesenteric arteries (5-8 mm long, 200 µm wide) were suspended in tissue baths (50ml) containing normal Krebs solution and stretched to L_O. Each vessel was precontracted with methoxamine (30 µM). Administration of sodium nitroprusside evoked a 100% relaxation of these vessels. Administration of guanethidine (30 µM) evoked a 73±5% relaxation of the precontracted vessels. Pretreatment with capsaicin (10 µM) reduced the guanethidine-induced vasodilatations to 13±7%. Similarly, 100 µM nitric oxide synthase inhibitor N(1)-Nitro-L-Arginine (NOLA) reduced the guanethidine-induced vasodilatations to 22± 7%. The NOLA-mediated effects could be almost completely reversed with hydroxyguanidine (HOG, 10 µM), an intermediate of nitric oxide, but not L-arginine (1 mM). To determine whether this effect of guanethidine on sensory nerves was dependent on transport, the vessels were pretreated with cocaine (5 µM) for 5 min or ouabain (25 µM) for 15 min. Cocaine decreased the guanethidine-induced vasodilatations to 35±14%. Likewise, ouabain diminished the guanethidine-induced vasodilatation to 9±5%. The guanethidine vasodilatation were not affected by propranolol (100 µM), 6-hydroxydopamine (6-OHDA, 30µM) or tetrodotoxin (TTX, 10 µM). Support: NIH-HL27781.

REGULATION OF AUTONOMIC FUNCTIONS: CARDIOVASCULAR

560.1

POSSIBLE ROLE OF THE FUNDUS STRIATI VP RECEPTORS IN BLOOD PRESSURE (BP) CONTROL. P. Krémarik, K. Cooper* and Q.J.Pittman. Neuroscience Research Group, University of Calgary, Calgary, Alberta, T2N 4N1, CANADA.

Vasopressin, given intracerebrally to rats, can induce motor disturbances, increases in BP and antipyresis. The possibility the highly concentrated vasopressin (VP)-binding sites in the fundus striati (FStr) could participate in these effects was investigated using chronically implanted cannulas into this area. A uni or bilateral injection in the FStr of 100pmol/1µl VP did not induce motor disturbances (scored visually) after the first and even the second injection, 24 hours later. The body temperature recorded simultaneously (i.p. minimitter) was not altered by the VP injections. After a bilateral injection of 100pmol/0.25µl VP in the FStr, the fever induced by an i.p. injection of the lipopolysaccharide of E. Coli (50µg/kg) or by interleukin 1ß (0.1µg/kg) was not affected. In contrast, a bilateral injection of 100pmol/0.25µl VP into urethane anesthetized rats, induced an increase in BP (19.1 +/- 2.24 mmHg, $p < 0.001$) compared with saline controls while the heart rate was not modified ($p > 0.4$). This increase in BP was blocked by a V1 antagonist; OT and a V2 agonist were ineffective.

This study suggests then that the VP receptors in the FStr could intervene in the regulation of the BP together with other regions known to be implicated in such a role.

560.2

AUTONOMIC STRUCTURES IN RAT BRAIN SHOW INCREASED NUMBERS OF C-FOS POSITIVE CELLS DURING CLONIDINE WITHDRAWAL. R.L. Stornetta*, M. Fisher and P.G. Guyenet. Dept. of Pharmacology, University of Virginia, Health Sciences Center, Charlottesville, VA 22908.

Animals and humans become dependent on clonidine (clo) following chronic administration of this α_2-adrenergic receptor (AR) agonist as revealed by either spontaneous or antagonist induced behavioral and autonomic withdrawal symptoms. The present study sought to determine whether specific autonomic areas of brainstem might be involved in clo withdrawal using the presence of c-fos nuclear protein as an indication of cell activation.

17 male Sprague Dawley rats were briefly anesthetized with ether and implanted subcutaneously in the back of the neck with Alzet osmotic minipumps containing either saline(sal) or clo (300 µg/kg/day). Animals were returned to their home cages and after 7-10 days were injected i.p. with either saline or the α_2-AR antagonist atipamezole (at) (1.5 mg/kg) and 2.5 hours later were deeply anesthetized with Nembutal i.p. and perfused with 4% paraformaldehyde. Brains were extracted, cryoprotected for 12-48 hrs and cut on a cryostat in 20 µm sections. Sections were incubated in c-fos antibody (Santa Cruz) and reacted for immunoreactivity using a standard avidin-biotin protocol with VIP as the colorimetric substrate (Vector). Rats were run in the experimental protocol in 4 groups of 4 or 5. The groups consisted of 3 control rats (sal+sal, sal+at, clo+at) and 1 or 2 experimental rats (clo+at).

All experimental rats showed behavioral symptoms of withdrawal such as whole body shakes, paw shaking and increased digging and escape behaviors while none of the control rats exhibited these behaviors. An increase in the number of c-fos positive cells in the clo + at (withdrawing rats) was noted in many brainstem autonomic structures including rostral ventrolateral medulla (RVL), locus coeruleus(LC), nucleus of the solitary tract and raphe pallidus. This increase was quantified for RVL (41.6 ± 4.4 cells/section in clo+at; 5.0±0.8 in clo+sal, 8.2±1.0 in sal+at and 7.3±1.4 in sal+sal) and LC (32.8±4.0 cells/section in clo+at; 3.8±.9 in clo+sal, 4.9±0.7 in sal+at and 4.4±0.7 in sal+sal). The increase in neuronal activity in brainstem autonomic areas could explain the rebound hypertension observed during clonidine withdrawal.

Supported by NIH Grant DA07353

560.3

CARDIOVASCULAR EFFECTS ELICITED BY MICROINJECTIONS OF NEUROPEPTIDE FF (NPFF, MORPHINE MODULATORY PEPTIDE) IN THE NUCLEUS TRACTUS SOLITARIUS. J.H. Jhamandas*, K. Harris and K.H. Jhamandas. Department of Medicine (Neurology) & Division of Neuroscience, University of Alberta, Edmonton, Alberta and Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, Canada.

Nucleus tractus solitarius (NTS), termination site of baroreceptor afferents is enriched in both NPFF immunoreactivity and binding sites. The function of NPFF in this area is unclear. We have examined the potential of NPFF to influence blood pressure in the urethane anesthetized rat following microinjection of the peptide in the NTS. Unilateral injections of NPFF (0.2-1.0 nmols,50-150 nL) produced elevation in blood pressure. The peptide induced response had a finite latency and outlasted the injection. Following a pressor response, higher doses of peptide had to be injected to produce a similar response, suggesting a desensitization to its action. We also observed similar profile of pressor responses following unilateral injections of the NPFF analog (IDME)Y8Fa (0.2-1.0 nmols, 50-150 nL) within the NTS. Naloxone (2-4 mg/kg) administered intravenously failed to influence the effect of NPFF analog. Sites within the NTS where NPFF elicited the most consistent effect included medial and dorsomedial NTS at and just rostral to the level of area postrema. These results suggest that in the NTS, NPFF may serve to modulate cardiovascular function.

Supported by the Medical Research Council of Canada.

560.4

COMPARISON OF HEMORRHAGE- AND NITROPRUSSIDE-INDUCED Fos EXPRESSION IN BRAINSTEM NEURONS THAT PROJECT TO THE PARAVENTRICULAR NUCLEUS (PVN). K.H. Harris*, D. MacTavish, T.L. Krukoff and J.H. Jhamandas. Depts. of Medicine (Neurology); Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2B7

We previously reported that hemorrhage-induced hypotension activates neurons in the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM) that project to the PVN. In the present study, Fos-immunoreactivity (FI) was used to compare the activation of PVN-projecting brainstem neurons in response to hemorrhage- and sodium nitroprusside (SN)-induced hypotension. Under anesthesia, rats received injections of rhodamine-labelled latex microspheres into the PVN and were instrumented with arterial and venous cannulae. One week later, conscious rats (n=6) were hemorrhaged (5-6 ml) to lower and maintain arterial pressure (AP) to 30-50% of resting AP for 1.5 h. A separate group (n=6) received SN intravenously (4 mg/kg,0.5 ml) to lower and maintain the AP to 30-50% of baseline for 1.5 h. A comparable number of neurons with FI were observed at all levels of the NTS and VLM following both hypotensive stimuli. The only exception was the NTS, at the level of area postrema, where greater FI was found following SN-induced hypotension. In sham, controls, few neurons with FI were found. At all levels of the NTS, SN-induced hypotension led to FI in a significantly greater number of retrogradely labelled cells (40-50%) than in hemorrhaged animals (18-30%). These results show that both hemorrhage- and SN-induced hypotension activate medullary neurons in the NTS and VLM which project to the PVN. Moreover, quantitative analysis of FI following the two hypotensive stimuli suggests that a change in blood volume accompanying hemorrhage is not a significant factor in the activation of NTS and VLM neurons.
Supported by the MRC and Heart & Stroke Foundation of Canada.

560.5

CHARACTERIZATION OF BRAINSTEM CIRCUITS FOLLOWING ELECTRICAL STIMULATION OF THE CENTRAL NUCLEUS OF THE AMYGDALA (CNA): A COMBINED C-FOS AND RETROGRADE TRACING STUDY. T. Petrov*, J.H. Jhamandas, T.L. Krukoff. Depts. of Anatomy and Cell Biology and Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

We have previously shown that electrical stimulation of the CNA induces expression of Fos (the protein product of the immediate early gene *c-fos*) in catecholaminergic (CA) neurons in the ventrolateral medulla (VLM) and the nucleus of the solitary tract (NTS). In this study we electrically stimulated the CNA and examined: 1) the connectivity of activated VLM neurons with the NTS, 2) the connectivity of activated NTS neurons with the VLM. 3) the proportion of activated CA neurons that project to the VLM or NTS respectively.

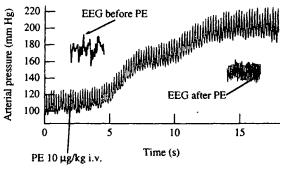
Rhodamine-labelled latex microspheres (R-LLM) were stereotaxically injected into the NTS or VLM of rats. Six days later, the ipsilateral CNA was electrically stimulated for 60 min (25-50μA, 10 sec pulsed trains, 20 Hz, 100 μsec) under urethane anaesthesia. In control animals the electrode was positioned in the CNA without delivery of current. Brains were then processed for Fos and tyrosine hydroxylase (TH) immunocytochemistry. In the experimental animals increased number of profiles that contained R-LLM were Fos immunoreactive (IR) in the ipsilateral VLM and NTS. 1) In the VLM 30% of the Fos IR cells projected to the NTS. 2) In the NTS 12% of the Fos IR cells projected to the VLM. 3) Of the CNA activated, R-LLM containing neurons, 17% in the VLM and 3% in the NTS were also TH IR.

The results indicate that local brainstem circuits between the VLM and NTS may be involved in mediating CNA-induced activation of medullary neurons. CA and non-CA autonomic cells may participate in a reciprocal activation of NTS and VLM neurons.
Supported by MRC and AHFMR.

560.6

ACUTE BLOOD PRESSURE CHANGE ELICITS HIPPOCAMPAL THETA RHYTHM AND BEHAVIOURAL AROUSAL IN THE UNRESTRAINED CONSCIOUS RABBIT Ying-Hui Yu* and W.W. Blessing. Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

Acute changes in arterial blood pressure (AP) cause arousal from sleep in lambs (Fewell and Johnson, Brain Res. 311 1984 259, Horne et al. Am. J. Physiol. 256 1989 H434), a response mediated via arterial baroreceptors. Arousal is accompanied by desynchronization of the neocortical EEG, but no studies have reported the effect of blood pressure changes on hippocampal theta rhythm. In staged operations in New Zealand White rabbits (3-3.2 kg), anesthetised with 1% halothane, we implanted mono-and bipolar EEG electrode in the skull and the dorsal hippocampus, a cuff occluder around the thoracic inferior vena cava, and cannulae in an external jugular vein and a common carotid artery. The lines were keep open by constant infusion of heparnized solution. The rabbit remained in a cage with a swivel on the roof for freely accessing electric and fluid channels. The EEG and AP signals were recorded with Maclab (AD Instruments). The rabbit's behaviour was recorded with a videocamera. AP was reduced by inflation of the IVC cuff, and increased by phenylephrine (3-10 μg/kg i.v.). Changing AP beyond a threshold (approx 60 mmHg) in either direction caused hippocampal theta rhythm (7-10 Hz), sometimes followed by behavioral arousal. Our data suggest that major alteration in baroreceptor inputs affects arousal state.



560.7

ALTERATION OF THE CARDIAC BAROREFLEX APPEARS LATE DURING PREGNANCY IN CONSCIOUS RABBITS. V.L. Brooks* and R.R. Quesnell. Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201.

There is evidence that reflex increases in heart rate (HR) and other effectors are reduced during pregnancy. The present study tested the hypothesis that the change in the baroreflex occurs early in pregnancy, coincident with increases in the steroid hormones estrogen and progesterone. Rabbits were instrumented with abdominal aortic and vena caval catheters. When the animals were nonpregnant (n=8), the relationship between arterial pressure (BP) and HR was determined by first decreasing BP with infusion of increasing doses of nitroprusside (3,6,12,24,48 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and then by increasing BP with phenylephrine (0.5,1,2,4,8 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The cardiac baroreflex was reassessed in the same animals (n=3-7) after about 1,2,3 and 4 wk of pregnancy (term is 31 days). Differences in the baroreflex curves between pregnant and nonpregnant rabbits were determined by comparing with ANOVA parameters generated by logistic analysis. BP was 65 ± 2 mmHg in nonpregnant rabbits and was not significantly different in pregnant rabbits. HR increased ($p < 0.01$) from 142 ±2 in nonpregnant animals to 174±6 and 186±4 bpm after 3 and 4 wk of pregnancy. Baroreflex relationships between BP and HR were not altered after 1,2 and 3 wks of pregnancy. However, maximal reflex gain decreased from -28±5 to -6±1 bpm/mmHg after 4 wk of pregnancy ($p < 0.01$). These results indicate that the cardiac baroreflex is not altered until the end of pregnancy, and suggest that increases in estrogen and progesterone do not mediate the change that occurs. Supported by NIH Grant HL 39923 and MRF of Oregon.

560.8

NITROGLYCERIN-INDUCED FOS-LIKE IMMUNOREACTIVITY AND AP-1 EXPRESSION IN RAT BRAIN. C. Tassorelli*, A.V. Prasad, A.H. Siddiqui and S.A. Joseph. Neurosurgery, University of Rochester, Rochester, NY 14642.

Nitroglycerin (NTG) is a well known vasodilator, commonly used in the treatment of angina pectoris. A less diffuse, but very intriguing clinical application of NTG is the diagnosis of primary vascular headache, as it consistently induces a spontaneous-like migraine attack in predisposed subjects. Recent findings have shown that NTG is capable of inducing the release of different neurotransmitters, such as noradrenaline and calcitonin gene-related peptide, in the central nervous system (CNS). These findings have suggested that NTG may act, at least partially, through a centrally-mediated mechanism, rather than as a pure direct vasodilator.

The proto-oncogene c-*fos* encodes a nuclear protein Fos, whose expression in certain brain regions can be induced by a variety of stimuli. For this reason, the immunohistochemical analysis of Fos-like immunoreactivity has been proposed as a highly sensitive method for the metabolic mapping of polysynaptic pathways in the brain. Moreover, Fos contributes to the formation of the protein activator AP-1, which, in turn, triggers the transcription of specific downstream genes, ultimately cooperating in the regulation of target genes that may underlie cellular adaptive responses.

The aim of the present study was to evaluate, in the rat, the possible CNS effects of systemic NTG by means of the induction of c-*fos* activation and AP-1 expression. The activation of c-*fos* was evaluated by immunocytochemical detection of Fos-like immunoreactive neurons, while AP-1 expression was detected by electromobility-shift assay (EMSA).

The results obtained by a time course study and immunocytochemical analysis reveal the existence of significant differences in both the localization and number of positive neuronal elements expressing Fos.The results obtained by EMSA following NTG injection demonstrate AP-1 expression as early as the second hour post-administration. These data strongly suggest that NTG can induce a selective activation of specific CNS nuclei. (supported by NIH grant NS213323)

560.9

MORPHOLOGY OF SYMPATHETIC GANGLION NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS. C. Cameron* and K.G. Ruit. Department of Anatomy and Cell Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202.

Increases in levels of nerve growth factor (NGF) in the walls of muscular arteries has been hypothesized as a contributing facot in the development of hypertension in genetically hypertensive rats (SHR). Our previous studies of the trophic effects of NGF on sympathetic ganglion neurons prompted us to hypothesize that significant changes in components of the autonomic nervous system may also be contributing to the development of hypertension.

Superior cervical ganglion (SCG) neurons in 6-7 week-old SHR and normotensive Wistar-Kyoto (WKY) rats were intracellularly filled with a 5% solution of Lucifer yellow in order to visualize the dendritic arborizations of these neurons. Each intracellularly filled neuron was drawn by camera lucida, and cell body size, total dendritic length, and number of primary dendrites were determined and compared between groups.

Preliminary experiments show that although SHR ganglion neurons tended to be larger and have greater total dendritic lengths, the differences were not statistically significant. In addition, the number of primary dendrites elaborated by these neurons did not differ. An interesting observation we have made in SHRs, however, is that there is extensive dye-coupling between neurons of the SCG not seen the WKYs. Previous investigators have shown that there is enhanced synaptic transmission through the SCG of the SHR. Our present evidence suggests that this may not be the result of large-scale changes in SCG neuron dendritic architecture and preganglionic innervation, but possibly as a result of the establishment of local ganglionic electrical connections.

560.10

A MODEL OF AUTONOMIC DYSREFLEXIA IN THE RAT. G. Sansone*, R. Bianca, R. Cueva-Rolon, L. Gomez, C. Beyer, B. Whipple, and B.R. Komisaruk, Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

The present study ascertained whether increases in heart rate (HR) and blood pressure (BP) that are produced by vaginocervical stimulation (VS, 500 g) persist after spinal cord transection. Three groups of rats were used: spinal transection at a) T7 (n=13), or b) L5 (n=13), or c) a sham-operated control group (SH, n=10). In the SH group, VS produced significant increases in HR (78.0 ±8.3 [sem] beats per minute [bpm]) and BP (37.8 ±8.1 mmHg), confirming earlier studies. In the L5 group, VS continued to increase HR (20.4 ± 9.8 bpm), but the effect of increasing BP was abolished. By contrast, in the T7 group, while VS significantly increased BP (31.3 mmHg ±8.6), it produced a significant decrease in HR (-42.0 ±11.9 bpm). These results can be accounted for by the human model of autonomic dysreflexia. The T7 transection blocks the reflexive VS-induced increase in heart rate. At the same time, it leaves intact the baroceptor-vagal inhibitory drive to the heart that results from the VS-induced increase in BP. By contrast, the L5 transection permits the stimulatory drive from VS to the heart via the hypogastric nerve, which enters the spinal cord above the transection. However, the low level of transection blocks the ability of VS to increase blood pressure via local reflexes.

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560.11

SPINAL AUTONOMIC REFLEX ARCS. M. Anwar*, K. Otake, E.P. Mtui, and D.A. Ruggiero, Div. Neurobiol., Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Somatovisceral reflex arcs mediate cardiopulmonary adjustments to pain and exercise. The structural underpinnings of short intraspinal reflex pathways were sought using axonal transport techniques in chloral hydrate (0.5mg/kg)-anesthetized adult and 8-12 day-old neonatal Sprague-Dawley rats. ***Study 1. Do first order axial muscle afferents project to spinal autonomic motoneurons?*** Ten 1µl deposits of a 10% solution of Dextran-Lucifer Yellow (DLY) were slowly injected, bilaterally, into the longissimus muscle. Following 6-8 day survival periods, thoracolumbar spinal segments were sectioned and processed immunocytochemically for DLY. Transganglionic transport of DLY labeled first order muscle afferents in the thoracic spinal cord: lamina VII and lamina VIII of ventral horn; bypassing the intermediolateral cell column (IML) in mature (n=6) and young (n=3) animals. ***Study 2. Do neurons in the dorsal horn project to autonomic motor nuclei?*** Iontophoretic deposits of *Phaseolus vulgaris* leucoagglutinin (n=13) centered on lateral loci in upper thoracic (T1-2) or thoraco-lumbar (T11-12 or L1-2) spinal segments transported, intrasegmentally, to the IML and nucleus intercalatus with ipsilateral predominance. A larger medial fiber trajectory decussated in the dorsal gray commissure and terminated within a mirror locus in the dorsal horn on the contralateral side. Deposits confined to medial loci in the dorsal horn terminated in laminae VII and X and the ventral horn, bypassing the IML. We conclude: Intraspinal-autonomic circuits may provide an anatomical substrate for the early reflex component of the exercise pressor reflex. The direct spinal-autonomic reflex arc may be reinforced by way of a multisynaptic commissural pathway. Direct pathways from the dorsal horn to spinal preganglionic motoneurons may explain retention of the capacity for cardiovascular reflex adjustments to peripheral stimulation following spinal cord transection. (HL18974, NS28200)

560.12

NEURONS IN THE INSULAR CORTEX ARE RESPONSIVE TO MUSCULAR CONTRACTION AND HAVE SYMPATHETIC AND/OR CARDIAC-RELATED DISCHARGE T. G. Waldrop* and G.A. Iwamoto Depts. of Physiology & Biophysics, Veterinary Biosciences and Neuroscience Program, University of Illinois, Urbana, IL 61801.

Recent studies have shown that stimulation of the insular cortex elicits cardiovascular responses; this cortical region is known to have connections with several brain sites involved in cardiovascular regulation. The purpose of the present study was to characterize the basal discharge patterns of neurons in the insular cortex and to determine if feedback from contracting muscles impinges upon these neurons. Extracellular recordings of single unit activity were made from insular cortex neurons in anesthetized cats whose lungs were artificially ventilated with a phrenic-triggered ventilator. Static and rhythmic contractions of the hindlimb muscles were elicited by stimulation of the distal cut end of L_7 and S_1 ventral roots. Signal averaging techniques revealed that many of the insular cortex neurons had a basal discharge correlated temporally to the cardiac cycle and/or sympathetic nerve discharge. In addition, contractions of hindlimb muscles altered the discharge frequency of many of the neurons located in the insular cortex; the discharge rate of most of the responsive neurons was decreased by muscular contraction. The activity of most of these neurons was not altered by baroreceptor stimulation elicited by a phenylephrine-induced rise in arterial pressure. The present findings indicate that the peripheral feedback reflex originating in active skeletal muscles during exercise alters the discharge of insular cortex neurons. These results indicate that the insular cortex many be involved in the modulation of cardiovascular responses elicited during exercise. (Support by HL 06296, 37400 & 38726).

560.13

TRIGEMINALLY MEDIATED ALTERATIONS OF NORMAL CARDIO-RESPIRATORY RHYTHMS DURING TRANSNASAL APPLICATION OF CARBON DIOXIDE. P. Yavari, W.M. Panneton, and J.H. Haring*, Dept. of Anatomy & Neurobiol., St. Louis Univ. School of Medicine, St. Louis, MO 63104.

Psychophysical studies in humans have shown that nasal stimulation with carbon dioxide (CO_2) is perceived as painful; physiological studies in the rat have shown that such effects may be mediated through the medullary dorsal horn. Since the medullary dorsal horn is also a probable site for the mediation of the "diving response", we have studied the cardiorespiratory responses in the rat which result from applying CO_2 across the nasal mucosa. The animals were anesthetized with intraperitoneal injections of a mixture of chloralose-urethane. Cannulas were inserted into the femoral vein and artery for the delivery of drugs and recording of blood pressure, respectively. Other tubing was placed in the trachea for breathing and into the nasopharynx for drawing air over the nasal mucosa. The stimulus was 100% CO_2 applied to the nares and slowly drawn through the nose by gentle suction applied to the nasopharyngeal tube. Such stimulation caused an abrupt drop in heart rate, and in the majority of the experiments, apnea and an elevation of blood pressure. In other experiments the application of CO_2 caused deep breathing and hyperventilation (no apnea) or arrhythmic breathing with apneic periods. The bradycardia was not the result of activating baroreceptors since it persisted even in those cases where prazosin, an alpha-1 adrenergic blocker, was used to prevent the increase in blood pressure. The CO_2-induced bradycardia was vagally mediated since it could be completely abolished after intravenous atropine. The nasal application of the local anesthetic procaine resulted in a blockade of up to 90% of the responses, suggesting that the responses are trigeminally mediated. These CO_2 induced responses previously have not been reported and resemble those of the diving response. Supported by NIH grant HL38471.

560.14

A GLUTAMATERGIC RELAY FOR THE DIVING RESPONSE IN THE MEDULLARY DORSAL HORN OF THE MUSKRAT. W. M. Panneton* and P. Yavari, Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Stimulating the upper respiratory tract, including the nasal cavity, can induce powerful cardiorespiratory responses. Such responses include an apnea, bradycardia, and a selective peripheral vasoconstriction; these responses together resemble those of the diving response. In the present experiments, the nasal cavity was stimulated using ammonia vapors to induce the diving response while 100 -150 nanoliter injections of either 2% lidocaine or 40mM kynurenate were made in the medullary dorsal horn (MDH) to block the responses. Muskrats were tranquilized with ketamine hydrochloride and anesthetized with a mixture of alpha chloralose-urethane administered intravenously. The femoral artery was cannulated for the recording of blood pressure, a tube was inserted into the trachea for respiration, and another tube was inserted into the nasopharynx for gently sucking ammonia vapors through the nasal cavity. In animals which showed consistent responses to ammonia vapors either lidocaine or kynurenate injections were made in the MDH to block the response. Effective bilateral lidocaine injections were limited to rostral, ventral parts of the MDH. The distribution of effective kynurenate injections mimicked those using lidocaine. Since kynurenate does not affect fibers of passage, the effective sites probably localize the area where the stimulated primary afferent fibers from the nose synapse upon secondary trigeminal neurons mediating the diving response. Furthermore, the results using kynurenate suggest that a transmitter used at this synapse is glutamate. Supported by NIH grant HL38471.

560.15

CARDIOVASCULAR EFFECTS OF NITRIC OXIDE ON BRAINSTEM NUCLEI OF RATS. C. J. Tseng,* H. Y. Liu, H. C. Lin and C. S. Tung, Dept of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung; Dept of Pharmacology and Physiology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

Nitric oxide (NO) has been reported as second messenger or neuromodulator in vivo. In this study, we evaluated the cardiovascular effects of NO in the nucleus tractus solitarii (NTS), rostral ventrolateral medulla (RVLM) and area postrema (AP) of male Sprague-Dawley (SD), Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) respectively. Unilateral microinjection (60 nl) of L-arginine (L-Arg, 1-100 nmol) into the NTS and RVLM produced a prominent dose-related decrease in heart rate and blood pressure. The depressor and bradycardic effect of L-Arg were significantly attenuated by the pretreatment of NO synthase inhibitor - N^G- monomethyl-L- arginine (L- NMMA). The depressor effect of L-Arg in the NTS of SHR was less sensitive than the WKY. Furthermore, the cardiovascular effects of L-Arg was potentiated via intravenus injection of the lipopolysaccharide (LPS). In addition, a simutaneously inhibition of sympathetic nerve activity was noted during the period of L-Arg induced depressor effect in the NTS. However, there is no significant cardiovascular effect of L-Arg in the AP. These results suggest that NO is involved in the central cardiovascular regulation. The depressor effect of L-Arg in the NTS might be through the inhibition of sympathetic nerve activity.

560.16

CATECHOLAMINE AND NADPH-DIAPHORASE NEURONS ARE SEPARATE IN HUMAN AND RAT MEDULLA AND ARE NOT CO-ACTIVATED. I. L. Smithson, P. J. Zollman, J. D. Schmelzer, P. A. Low and E. E. Benarroch*. Neurophysiology Laboratory, Mayo Clinic, Rochester, MN 55905.

Catecholamine- and nitric oxide (NO)-producing neurons occupy cardiorespiratory regions of the nucleus of the tractus solitarius (NTS) and ventrolateral medulla (VLM). Selective involvement of these neurons may underlie clinical disorders of cardiorespiratory control. We sought to determine 1) the distribution of NADPH-diaphorase (NADPH-d) and tyrosine hydroxylase (TH)-reactive neurons in the NTS and VLM of human medulla and 2) the pattern of activation of these neurons in response to stimuli in the rat. Human medulla obtained within 12-48 hr of death were fixed and sectioned (50 μm). Sections were processed for both TH and NADPH-d. These markers were localized in topographically related but distinct neurons in human NTS and VLM. Rats were anesthetized and ventilated; mean arterial pressure (MAP), heart rate, and arterial blood gases were continuously monitored. The rats were subjected to hypovolemic hypotension or hypoxemia for approximately 60 min. Animals were perfused and 30 μm sections were processed for cFos (antibody provided by Dr F. Sharp) and for TH- or NADPH-d. cFos reactivity overlapped with that of TH- but not NADPH-d labeled neurons in NTS and VLM. Thus, in humans, as well as in rats, TH- and NADPH-d neurons are distinct but interacting populations. Selective involvement of these two neuronal groups may underlie human disorders 'of central cardiorespiratory control.

560.17

Pharmacological characterization of the 10 Hz Sympathetic Nerve Discharge Frequency. ME Clement* and RB McCall. The Upjohn Company, Kalamazoo, MI.

Most of the power in the discharge frequency of sympathetic nerve in baroreceptor denervated decerebrate or urethane-anesthetized cats concentrates in a 2-6 Hz band and a narrower frequency band around the 10 Hz range. Barman and Gebber have shown that chemical inactivation of the midline medullary raphe with muscimol eliminates the 10 Hz peak, while stimulation of this area can induce the 10 Hz rhythm. Our laboratory has recently examined the effect of a number of pharmacologic agents on the frequency distribution of sympathetic activity . We found that systemic administration of the GABA antagonist picrotoxin (0.01-1 mg/kg) selectively increased the power in the 10 Hz band in a dose dependent manner. Incremental doses of picrotoxin also tended to shift the 10 Hz peak to higher frequency. Low doses of 8-OH-DPAT (1-3 µg/kg selectively inhibited the 10 Hz power band in a dose dependent manner. The inhibitory effects of 8-OH-DPAT could be reversed by the 5-HT antagonist spiperone. The putative 5-HT_{1A} antagonist WAY-100135 (0.01-0.1 mg/kg) did not reverse 8-OH-DPAT but did inhibit 10 Hz activity when given alone. The 5-HT antagonist methysergide (0.01-1 mg/kg) caused a dose dependent shift in the 10 Hz band to lower frequencies. Chlorimipramine, a 5-HT uptake inhibitor, also eliminated the 10 Hz peak in SND, but did not block stimulation induced 10 Hz activity. These data suggest that 5-HT and GABA modulate the level of excitability of the central oscillator responsible for the generation of the sympathetic 10 Hz frequency.

REGULATION OF AUTONOMIC FUNCTIONS: CNS PATHWAYS AND TRANSMITTERS

561.1

SUBNUCLEAR DISTRIBUTION OF DOPAMINE RECEPTOR SUBTYPES IN THE HUMAN NUCLEUS OF THE SOLITARY TRACT AND OTHER SELECTED BRAINSTEM NUCLEI. T.M. HYDE*, A.M. MURRAY, M.E. KNABLE, M.M. HERMAN, C.F. SPURNEY, AND J.E. KLEINMAN. Clinical Brain Disorders Branch, IRP, NIMH, Washington, D.C. 20032.

The nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus (DMN), and area postrema (AP) are interrelated structures important in the modulation of visceral function, including those mechanisms underlying nausea and vomiting. Neuroleptics, probably acting on dopamine receptors, have potent antiemetic actions. In order to establish and characterize the role of medullary dopamine receptor subtypes in the neural control of nausea and vomiting, we studied the distribution of D1-D5 receptors. Fresh frozen human brainstem sections from normal controls, n=9, sectioned at 20 µ and slide mounted, were incubated with [^{3}H]raclopride for D2 and D3 receptors, [^{125}I]epidepride for D2, D3, and D4, receptors, [^{3}H]SCH23390 for D1 and D5 receptors, and [^{3}H]emonapride for D2, D3, and D4 receptors, and apposed to ultrafilm for computerized image analysis. Dopamine receptors were most densely distributed over the medial and intermediate subnuclei of the NTS and the DMN. Moderate binding was noted over the dorsal, ventromedial, and substantia gelatinosus subnuclei. The medial region of the NTS receives a dense projection from the subdiaphragmatic vagus. Dopamine receptors in this region of the NTS, as well as in the DMN, probably act as a central site of antiemetic action of neuroleptics.

561.2

LOCAL PROJECTIONS OF THE NUCLEUS OF THE SOLITARY TRACT TO THE DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE PIGEON. M.L. Berk* and K.H. Gerlach. Dept. of Anatomy, Cell and Neurobiology, Marshall Univ. Sch. of Med., Huntington, WV 25755.

The pairing of sensory and motor representation of a specific organ within certain levels of the avian nucleus of the soliatry tract (NTS) and dorsal motor nucleus of the vagus (DMNX) could facilitate vago-vagal reflexes. The present study investigated the existence and peptide content of local NTS projections to DMNX that could subserve esophageal reflexes. Fluorescein-latex beads were injected into an anterior DMNX subnucleus, which innervates the esophagus. Retrogradely labeled NTS cells were observed ipsilaterally and contralaterally and were most dense in medial tier NTS subnuclei, which are recipients of vagal afferents from the alimentary tract. The combination of retrograde labeling and immunofluorescence techniques revealed many bombesin, enkephalin, and neurotensin immunoreactive medial tier NTS cells that were double-labeled. Many of the enkephalin and neurotensin containing double-labeled cells were located in medial tier NTS subnuclei that are the major recipients of esophageal afferents and thereby could subserve as interneurons in esophageal reflexes. Bombesin double-labeled cells were found in medial tier NTS subnuclei that are major targets of gastric afferents. These bombesin containing cells that project to the anterior DMNX may serve to integrate esophageal and gastric functions. On-going experiments will attempt to verify the axonal projections of these medial tier NTS cell groups to esophageal DMNX motoneurons by combining anterograde axonal tracing with retrograde labeling techniques. Support NSF R11-8922106

561.3

DISTRIBUTION OF BOMBESIN-LIKE IMMUNOREACTIVITY IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) AND DORSAL MOTOR NUCLEUS (DMN) IN HUMANS. R.B. Lynn*[1], R.R. Miselis[2], and T.M. Hyde[3]. [1]Dept. of Med., Thomas Jefferson Univ., Phila. PA 19107; [2]Dept. of Animal Biol., Univ. of Penn., Phila. PA, 19104; [3]Clin. Brain Disorders Branch, NIMH,IRP, Wash.,D.C. 20032

Bombesin, also referred to as gastrin releasing peptide (14-27), has important autonomic and behavioral effects when injected into the NTS/DMN complex in rats. Bombesin-like immunoreactive (Bb-LI) labeling is abundant in the rat NTS/DMN complex, but has not been described in the human medulla. Four normal human medulla were flash frozen and subsequently thawed and fixed. Frozen sections (40μ) through the caudal NTS were stained using primary antiserum to bombesin (Incstar) and the ABC technique. The heaviest Bb-LI labeling, probably representing fiber/terminals, was found at the level of the obex, diffusely in the medial and intermediate subnuclei of the NTS and in the medial portion of the DMN. Lighter but significant labeling was noted in the dorsal, interstitial, ventromedial and commissural subnuclei of the NTS and the lateral portion of the DMN. The substantia gelatinosus and the tractus of the NTS appeared void of Bb-LI staining. Rare Bb-LI labeled cell bodies were scattered within the NTS. Bb-LI staining was also noted in the A1 region and the spinal trigeminal nucleus. In controls, all Bb-LI staining was abolished by preabsorption of the primary antiserum with bombesin peptide. This distribution of labeling is very similar to the pattern of labeling in the non-colchicine treated rat. The anatomic similarities between human and rat suggest that bombesin has similar functions in the visceral neuraxis of these two species. Support: KO8 DK02094(1); GM27739(2); IRP-NIMH(3).

561.4

GABA NEURONS IN THE NUCLEI OF THE SOLITARY TRACT RECEIVE SYNAPTIC INPUT FROM TERMINALS OF CENTRAL AMYGDALOID NEURONS. D.M. Cestari*, E.J. Van Bockstaele, J. Chan and V.M. Pickel, Dept. of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021.

Direct applications of GABA agonists within the nuclei of the solitary tracts (NTS) is known to enhance sympathetic activities in a manner similar to that seen following stimulation of the central nucleus of the amygdala (CNA). We examined whether GABA immunoreactive neurons within the NTS received direct synaptic input from efferents derived from the CNA. The anterograde tracers, *phaseolus vulgaris leucoagglutinin* (PHA-L) or biotinylated dextran amine (BDA) were iontophoresed into the CNA of adult rats. These animals were sacrificed after survival periods of 10 - 14 days and single sections through the brainstem were processed for immunoperoxidase and gold silver labeling of the anterograde tracers or GABA, respectively. Anterograde labeling was maximally observed using BDA. Anterogradely labeled varicose processes in the medial and commissural NTS were established as unmyelinated axons and axon terminals by electron microscopy. The terminals formed almost exclusively symmetric synapses with soma and large dendrites, many of which were GABA immunoreactive. Despite the GABA-like symmetry of the anterogradely labeled terminals, the majority were not dually labeled. However, GABA labeling was abundantly seen in other neighboring axon terminals. These also were presynaptic to GABA dendrites. We conclude that stress induced autonomic activation may be mediated through an inhibitory pathway from the CNA to GABA neurons in the NTS. (NIH grants MH48776 and HL18974).

561.5

GLUTAMATE-IMMUNOREACTIVE NEURONES AND AXON TERMINALS OF THE NUCLEUS TRACTUS SOLITARIUS (NTS): DEMONSTRATION OF GLUTAMATE IN VAGAL SENSORY AFFERENTS.

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Although there is a growing body of literature regarding the actions of glutamate in the NTS very little is known about the anatomy and organization of the glutamatergic neuronal structures in this region. Using post-embedding immunocytochemical techniques glutamate-containing neurones and axonal boutons have been examined at both the light and electron microscopic level. Furthermore, by combining this technique with anterograde labelling this study begins to examine the origins of glutamate-immunoreactive axon terminals observed in the NTS.

Light microscopic examination of semi-thin plastic sections revealed glutamate-immunoreactive neurones throughout the NTS and gracile nucleus with a distinctive organization at intermediate levels where groups of immunoreactive perikarya were observed. Axonal boutons were also labelled. These were found throughout all subnuclei providing a very dense and apparently homogeneous innervation.

Ultrastructural examination of glutamate immunogold-labelled sections identified a population of axonal boutons which displayed common morphological features and formed exclusively asymmetric synaptic specializations. The major post-synaptic targets of these boutons were small to medium-sized dendrites. Glutamate immunocytochemistry of sections containing anterograde labelling following the injection of horseradish peroxidase into the nodose ganglion revealed that these axons contribute to the population of glutamate-immunoreactive axonal boutons in the NTS.

Supported by the Wellcome Trust and British Heart Foundation.

561.6

TRANSNEURONAL LABELING OF NEURONS IN THE BRAIN AFTER INJECTION OF PSEUDORABIES VIRUS (PRV) INTO THE RAT PHARYNX. X. Bao, E.B. Wiedner, and S.M. Altschuler*. Children's Hospital of Phila., Univ. of Pa. Sch. of Med., Phila., PA 19104.

The initiation and control of swallowing is dependent on premotor neurons (PMNs) located primarily in NTS. The subnuclear location and connectivity of PMNs innervating the pharynx were determined using a Bartha strain of PRV. In 15 rats, PRV injections were made into the pharynx. Following a 48-96 h survival, brain sections were processed immunocytochemically for PRV. Neuronal labeling was limited to the semicompact formation (sc) of the NA for a survival time of 48 h. At 56-62 h survivals, PRV-labeled 2nd order neurons (PMNs) were localized to the interstitial (ist) and intermediate (int) subnuclei of NTS. At longer survivals, neuronal labeling occurred in adjacent subnuclei of the NTS, raphe nucleus, A5, hypothalamus, and forebrain. Pharyngeal PMNs are localized to the istNTS and intNTS, the sites of termination of superior laryngeal nerve afferents, and have direct synaptic contact with scNA motoneurons. The pattern of labeling at longer survivals suggests widespread CNS control over pharyngeal motility. Supported by NIH grant DK-44487.

561.7

TRANSSYNAPTIC DOUBLE LABELING WITHIN THE VISCERAL NEURAXIS WITH UNIQUELY MARKED STRAINS OF PSEUDO-RABIES VIRUS (PRV). R.R. Miselis*, M.Yang, A. Robbins, M. Whealy and L. Enquist, Animal Biol., Vet. Med., Univ. of Penn., Phila., PA 19104, DuPont-Merck, Wilmington, DE 19880, and Mol. Biol., Princeton Univ., Princeton, NJ 08544

Using recombinant DNA techniques two strains of PRV were made that expressed unique proteins. Cells infected with one or both viruses could be easily distinquished by immunoflorescent techniques. BaBlu was derived from the Bartha strain and expressed E. coli beta-galactosidase. PRV 41/91 was derived from the Becker strain with a gE gene deletion for reduced virulence and expressed a novel protein from a hybrid gene of the PRV gC gene and the Herpes simplex gC gene. The viruses were injected separately into either the esophagus and stomach or stomach and cecum of rats to determine if they would spread trans-synaptically into the same second or third order neurons in the CNS. Double labeling of the same neurons did not occur in the primary motor nuclei for the different viscera but did occur in some neurons of the nucleus of the solitary tract, ventrolateral medulla, parapyramidal area, paragigantocellular reticular formation, raphe magnus and the A5, A6 and sub A6 catecholaminergic areas - known afferent sources to the above primary motor nuclei. The double labeling provides anatomical evidence that some second and third order afferents can simultaneously modulate final common paths to different viscera and thereby serve an integratory or coordinating function. Supported by GM27739 and DuPont-Merck.

561.8

SYMPATHETIC NERVE FIBERS IN THE SUBDIAPHRAGMATIC VAGUS NERVE OF THE RAT SEEN WITH FLUOROGOLD, CT-HRP OR PSEUDORABIES VIRUS. M.Yang*, X. Zhao and R. R. Miselis. Animal Biology, Phila., PA 19104

To identify the source, number and the level of entry of sympathetic nerve fibers in the vagus nerve one of three retrograde tracers was injected into the subdiaphragmatic vagus nerve trunks of the rat. We examined the upper sympathetic ganglia, the nodose ganglion, and the dorsal motor nucleus of the vagus (DMV) in the same animals. We find a small number of labeled postganglionic neurons in the sympathetic trunk ganglia (g): an average of 6 neurons in the superior cervical g. (SCG), 30 in the cervicothoracic g. (CTG) and 25 in the other thoracic g. In addition there are preganglionic parasympathetic catecholaminergic nerve fibers originating from tyrosine hydroxylase containing neurons in the caudal DMV (approximately 324). Rough ratios of postganglionics in the vagus to the total number of postganglionic neurons in the sympathetic g. are 0.12% for the SCG and 0.54% for the CTG. This is a minor contribution by this unusual and perhaps developmentally aberrant path. Many more labeled neurons are seen in the prevertebral g. when the stomach is injected. Since sympathetic fibers enter the vagus as high as the caudal pole of the nodose g. and within the thoracic cavity, it would take a cervical vagotomy at the cranial pole of the nodose g. to ablate all parasympathetic innervation to visceral organs without disrupting sympathetic nerve fibers in the vagus nerve. Supported by GM27739.

561.9

DICHOTOMOUS MODULATION OF VAGAL MOTONEURON ACTIVITY BY THE HYPOTHALAMUS. X. Zhang*, R. Fogel and W.E. Renehan. Div. Gastroenterology, Henry Ford Health Sciences Center, Detroit, MI 48202

Recent evidence suggests that the hypothalamus may play an important role in stress-related gastrointestinal disorders. Stimulation of the paraventricular nucleus of the hypothalamus (PVH) can induce gastric erosion and motility changes that can be eliminated by vagotomy, suggesting that the PVH effect involves neurons in the dorsal motor nucleus of the vagus (DMNV). Little is known, however, about the potentially important PVH influence on DMNV neurons. To address this issue, we used glass micropipettes containing 2% Neurobiotin to record the response of individual gastric- and intestine-sensitive DMNV neurons to PVH stimulation. The stomach and duodenum of 15 rats were separately cannulated to permit distention with normal saline. Stimulating electrodes were placed around the subdiaphragmatic and gastric branches of the vagus nerve. Stimulating electrodes were also placed in the PVH. Following physiological characterization each neuron was injected with the tracer and reconstructed using the Eutectic Neuron Tracing System. A total of 18 DMNV neurons were successfully labeled. Most of these neurons were inhibited by intestinal and/or gastric distention. PVH stimulation, however, inhibited 6 and excited 8 of the distention-sensitive DMNV cells (PVH stimulation did not affect the activity of the remaining 4 neurons). Interestingly, our data suggested that a given DMNV neuron's response to PVH stimulation may be related to certain morphologic features. We noted that those DMNV neurons that were inhibited by PVH stimulation tended to exhibit a higher density of dendritic swellings than the neurons that were excited by this stimulus ($p = 0.068$). These preliminary results suggest that PVH neurons may project to two distinct subsets of gastrointestinal DMNV neurons. These two groups appear to receive similar input from the intestine, but opposing inputs from the hypothalamus. Supported in part by NS30083 and DC01074.

561.10

Pancreatic Polypeptide Increases the Activity of Dorsal Vagal Complex Neurons *in vivo*

Dana M. McTigue* and Richard C. Rogers Dept. of Physiology, College of Medicine, Ohio State University, Columbus, OH 43210

Pancreatic polypeptide (PP), a hormone released during digestion, has been shown to alter vagal control of the stomach by acting at the dorsal vagal complex (DVC) within the brainstem. This area contains fenestrated capillaries through which the circulating hormone gains access. In the present studies, we examined the effect of PP on neuronal activity in the DVC using extracellular recording in anesthetized rats. Cells within the DVC were identified in two ways: vagal stimulation to identify NTS vs DMN neurons or gastric inflation to identify On vs Off cells (neurons that increase or decrease firing rate during inflation, respectively). The results obtained indicate that over 65% of On cells and Off cells were stimulated by PP while none were inhibited. Furthermore, 60% of NTS cells were stimulated by PP. Preliminary studies examining DMN neurons demonstrate that 30% are inhibited while the rest were unaffected. These studies indicate that in general, gastric related DVC neurons are potently stimulated by PP. When the entire population of DVC neurons is studied, more complex effects may be observed. The long-lasting stimulation of gastric related DVC neurons may provide a mechanism for the observed increases in gastric activity following PP administration. (NS 30803 to RCR)

561.11

Dorsal Motor Nucleus Neurons Recorded in Vitro are Activated by Pancreatic Polypeptide Under Conditons of Synaptic Blockade

Richard C. Rogers* and Gerlinda E. Hermann; Dept. of Physiology; The Ohio State University College of Medicine, Columbus, OH 43210

Pancreatic Polypeptide [PP], when injected directly into the dorsal vagal complex, strongly augments gastric acid secretion and gastric motility by increasing vagal cholinergic activation of the stomach [AJP; 265:G1169-1176, 1993]. The adjacent abstract suggests that this increase in vagal excitation of the stomach might come about as the result of direct activation of dorsal vagal complex neurons. To test this hypothesis, brainstem slices of the medulla were prepared for extracellular recording using an interface slice chamber. 400 micron thick slices were incubated in a modified Krebs Solution at 34° C. under a 95% O^2, 5% CO^2 atmosphere. The DMN was easily identified visually and DMN-area neurons were recorded. Recorded cells maintained spontaneous [.5 to 5 Hz] activity under conditions of synaptic blockade [low Ca++/ high Mg++], a feature typical of DMN neurons. The application of a pulse of PP to the perfusion chamber [peak chamber PP concentration of 0.5 micromolar to 50 nanomolar] produced dramatic increases in DMN activity under normal as well as under synaptic blockade conditions. These results support the concept of PP as a peripheral hormone which control digestion by directly altering the excitability of vagal reflex control neurons. Supported by NS 30803.

561.12

2-DEOXY-D-GLUCOSE (2DG) INDUCES C-FOS IN CENTRAL AND PERIPHERAL CATECHOLAMINE (CA) SYSTEMS. S. Ritter* and I. Llewellyn-Smith. College of Veterinary Medicine, Washington State University, Pullman, WA, U.S.A 99164, and Department of Medicine, Flinders Medical Centre, Adelaide, S.A. 5042, Australia.

2DG is a glucose analogue that competitively inhibits glucose utilization. Responses to 2DG include increased feeding and adrenal medullary secretion and induction of Fos-like immunoreactivity (FLI) in the brain and adrenal medulla. Previous studies have shown that 2DG-induced feeding is impaired by lesion of brain CA systems and by pharmacological reduction of CA neurotransmission. In this experiment, 2DG was administered systemically and immunocytochemistry used to determine whether *c-fos* was induced in hindbrain neurons which express tyrosine hydroxylase (TH). Results show that 2DG induces FLI in some, but not all, TH-containing neurons in the A1/C1 and A2/C2 regions and in many neurons not containing TH. 2DG also induced FLI in a subpopulation of sympathetic preganglionic neurons retrogradely labelled by injection of the B subunit of cholera toxin into the adrenal medulla. Results identify specific subpopulations of brain CA and preganglionic neurons activated by glucoprivation.

561.13

EXOGENOUS CCK ACTIVATES CATECHOLAMINERGIC NEURONS IN THE CAUDAL MEDULLA THAT INNERVATE THE HYPOTHALAMUS.

L. Rinaman*, G.E. Hoffman, J. Dohanics, E.M. Stricker, and J.G. Verbalis. Depts. of Neuroscience, Neurobiology, & Medicine, University of Pittsburgh, PA 15260.

Immunocytochemical localization of the protein product of the proto-oncogene cFos allows anatomical identification of physiologically activated neurons. Peripheral administration of CCK octapeptide in rats inhibits gastric motility and feeding, stimulates pituitary secretion of oxytocin, and produces robust expression of cFos immunoreactivity in restricted areas of the caudal medulla and forebrain. The present study used triple-labeling methods to determine whether CCK activates the catecholaminergic neurons in the caudal medulla that innervate the paraventricular nucleus of the hypothalamus (PVN). Male Sprague-Dawley rats were anesthetized, and Fluorogold (FG) retrograde tracer was iontophoresed into the PVN unilaterally under stereotaxic guidance. After 2 weeks, rats were injected with CCK (100 μg/kg, ip), anesthetized 1 hr later, and perfused with fixative containing 4% paraformaldehyde with lysine and sodium meta-periodate. Brain sections were incubated in rabbit anti-cFos (Oncogene Sciences, 1:50K), and blue-black nuclear cFos labeling was produced using Elite Vectastain reagents and a nickel-DAB reaction product. Sections were then incubated in rabbit anti-FG (Chemicon, 1:50K), and green fluorescent FG labeling was produced using Elite Vectastain reagents and BODIPY-streptavidin conjugate (Molecular Probes). Finally, sections were incubated in mouse anti-tyrosine hydroxylase (TH; Incstar, 1:100K), and red fluorescent TH labeling was produced using a modified alkaline phosphatase method (CAS Inc). In rats with precise PVN injections (n=10), approximately 70% of the FG-labeled neurons in the nucleus of the solitary tract and ventrolateral medulla expressed cFos. Of these activated PVN-projecting neurons, approximately 60% were catecholaminergic. These data indicate that catecholaminergic pathways transmit vagal sensory information from the caudal medulla to the hypothalamus, and support the hypothesis that these pathways participate in the neuroendocrine, physiological, and behavioral effects of CCK-induced gastric vagal stimulation. *Supported by NIH grants NS28477 and MH25140 (MERIT award).*

561.14

PANCREATIC POLYPEPTIDE (PP) AND PEPTIDE YY (PYY) mRNA IN THE BRAINSTEM OF RATS DETECTED BY REVERSE-TRANSCRIPTASE PCR (RT-PCR).

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PP and PYY are circulating gut hormones that regulate digestion. Receptors for PP and PYY are present in the brainstem on both sides of the blood-brain barrier (BBB). When PP and PYY are microinjected into certian areas of the dorsal medulla inside the BBB, they produce potent gastrointestinal effects. We hypothesized that PP and PYY are produced by cells within the CNS that project to areas with high densities of receptors, despite the failure to consistently demonstrate these peptides by radioimmunoassay and immunohistochemistry. **Objective**: to determine if PP and PYY mRNA are present in the brain, using RT-PCR **Methods:** Total RNA was extracted from the dorsal pons and medulla of the rat brain, pancreas and distal 10 cm of ileum using the Trisolv method (Biotec, Houston, TX). First strand cDNA was generated using a Moloney Murine Leukemia Virus reverse transcriptase reaction. Five ng of cDNA products were incubated with the PP or PYY primers and Taq DNA polymerase along with controls and standards for 35 cycles. The products were separated directly on Nu-Sieve agarose gels. The identity of the PCR products was tested by Southern blot. **Results:** PP and PYY mRNA were detected in the dorsal medulla of the rat brain using the RT-PCR. The PP primers yielded a single band identical in size to the PP mRNA isolated from the pancreas. For PYY, two bands were amplified from brain mRNA, corresponding to predicted PYY mRNA fragments with alternate splicing. The relative density of the two PYY bands differed between brain and ileum. Southern blots labeled with ^{32}P-PP or ^{32}P-PYY cDNA probes demonstrated that the amplified PCR products hybridized with the appropriate cDNA. **Conclusion**: PP and PYY mRNA are present in the brainstem of rats. This supports the hypothesis that PP and PYY receptors in areas of the brain that are inaccessible to circulating hormones may have physiologic relevance.

561.15

CAUDAL RAPHE STIMULATION EVOKES SEROTONIN (5-HT) RELEASE INTO DIALYSATES OF THE DORSAL VAGAL COMPLEX IN FED, BUT NOT FASTED RATS - GLUTAMATERGIC MEDIATION R.L. Stephens Jr.*, Dept. of Physiology, The Ohio State University., Columbus, Ohio 43210.

The dorsal vagal complex (DVC) is the central relay station coordinating sensory & motor control of autonomic function. The caudal raphe nuclei is the source of the dense serotonergic innervation of the DVC. 5-HT augments the effect of TRH on gastric function at the DVC. Regulatory mechanisms of 5-HT release into regions of the dorsal medulla containing the DVC in anesthetized rats were explored. Basal 5-HT release was enhanced in fed as compared to 24-hr fasted rats. In addition, stimulation of the raphe obscurus with kainic acid produced a marked release of 5-HT into dialysates of the dorsal medulla of fed but not 24-hr fasted rats **[Mean 5-HT release (pgrams/15min; n = 5-8)]:**

	BASAL 5-HT						nRO KAINATE (423 pmoles/10nl)							
FAST	3	8	5	3	2	4	1	2	0	0	0	0	0	0
FED	16	17	15	9	15	15	149	363	106	76	47	84	50	36

Gastric distension of fasted animals did not evoke 5-HT release after nRO-stimulation. However, probe infusion of kynurenic acid (1 mM) in fed animals attenuated nRO-stimulated serotonin release. Given the vagal sensitivity of nRO-stimulated 5-HT release in this system, the results suggest that activation of glutamatergic vagal afferents may facilitate serotonin release into the DVC. Supported by DK 42880

561.16

EFFERENT PROJECTIONS OF THE NUCLEUS PARAGIGANTOCELLULARIS IN THE RAT: A PHA-L TRACING STUDY Y. Zhu*, S. Chen and G. Aston-Jones, Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102.

Previous studies have shown that the nucleus paragigantocellularis lateralis (PGi) strongly innervates the nucleus locus coeruleus (LC) and sympathetic nuclei of the spinal cord. However, other efferents of the PGi have not been systematically examined. In the present study, Phaseolus vulgaris-leucoagglutinin (PHA-L) was used to comprehensively map PGi efferents in the rat. Multiple deposits of PHA-L were iontophoresed into the PGi. After 7-10 days, animals were perfused and processed for PHA-L immunostaining. Dense PHA-L terminal labeling was seen in the intermediolateral cell column and intermediomedial areas of the spinal cord, A1/C1 areas of the ventral medulla, the nucleus of the solitary tract, dorsal motor nucleus of the vagus (X), the hypoglossal nucleus (XII), the LC, medial and lateral parabrachial nuclei, Kolliker-Fuse nucleus , the periaqueductal gray, the interstitial nucleus of the medial longitudinal fasciculus, deep superior colliculus and thalamic intralaminar nuclei. Fewer fibers and terminals were seen in the nucleus prepositus , facial nucleus (VII), A5 area, Barrington's nucleus, dorsal raphe nucleus, lateral hypothalamus and septum. Scattered fibers and terminals were also seen in the hippocampus and subiculum. PHA-L labeling was generally bilateral, but with a ipsilateral predominance. The present study shows that PGi sends divergent efferents to several brain areas, many of which were previously found to project to the PGi. Supported by PHS grant NS 24698.

561.17

TOPOGRAPHY OF SOMATOSENSORY INPUT TO THE RAT PARABRACHIAL COMPLEX: A SITE FOR SOMATO-VISCERAL INTEGRATION? K. Feil* and H. Herbert. Dept. Animal Physiol., Univ. of Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany.

The parabrachial (PB) and Kölliker-Fuse nucleus (KF) receive - in addition to viscerosensory afferents from the solitary nucleus - strong somatosensory inputs from the dorsal horn of the spinal cord and the spinal trigeminal nuclei (Sp5). In the present study we examined, in detail, the spino- and trigeminoparabrachial projections by means of the anterograde transport of PHA-L. Our results show that the organization of spinal and trigeminal pathways to the PB/KF is more complex than previously thought.

Spinal dorsal horn neurons (lamina I-II) of cervical segments project to the ventral portion of the external lateral PB, while those of thoraco-lumbar segments project almost exclusively to the dorsal lateral and central lateral PB. Neurons in lamina V and the lateral reticulated area of all spinal segments project to the interal lateral PB while those of the cervical segments project in addition to the KF.

Trigeminal neurons in the superficial layers of the Sp5, pars caudalis and interpolaris, project strongly to the KF and to the ventral portion of the external lateral PB. In contrast, neurons of the paratrigeminal nucleus project specifically to the ventral lateral PB.

Our data demonstrate that the somatosensory input to the PB/KF is somatotopically organized. We thus conclude that the spino- and trigeminoparabrachial afferents consist of parallel pathways which convey and process either somatosensory information from different parts of the body, or somatosensory information of different qualities, or even both. It is assumed, that the pathways from the spinal cord and the Sp5 through the PB to various forebrain and medullary nuclei represent neural circuits which are involved in somato-visceral processing.
(Supported by DFG He 1842/3-2 and Graduiertenkolleg Neurobiologie Tübingen)

561.18

DESCENDING PATHWAYS FROM THE RAT PARABRACHIAL AND KÖLLIKER-FUSE NUCLEUS: RELATION OF TERMINAL AXONS, AND MEDULLARY MONOAMINERGIC CELL GROUPS. H. Herbert* and F. Weber. Dept. Animal Physiol., Univ. of Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany.

In the present study we analyzed pathways from the parabrachial and Kölliker-Fuse nucleus (PB/KF) to ponto-medullary and spinal nuclei which might participate in mediating the PB/KF cardiovascular, respiratory, and antinociceptive responses. We combined the anterograde transport of PHA-L with immunocytochemistry for noradrenergic (A1-A5), adrenergic (C1-C3), and serotonergic cells (B1-B3).

After large PHA-L injections into the lateroventral aspects of the PB/KF complex a dense plexus of terminal fibers was consistently found in the ventrolateral and caudal commissural solitary nucleus (NTS), along the ventrolateral medulla (VLM), and in the lateral facial nucleus. Smaller numbers of fibers were seen in the gigantocellular reticular nucleus, in the nucleus raphe magnus, and in the spinal trigeminal nuclei. In the spinal cord, terminal axons were found in the intermediolateral and intermediomedial cell column and in the phrenic nucleus.

In the caudal commissural NTS labeled PB/KF axons were codistributed with A2 cells. However, in the light microscope only few potential synaptic contacts could be observed. In the rostral VLM the majority of terminal fibers was located within the pool of C1 cells. Many axonal boutons were seen closely apposed to C1 somata and dendrites, indicating axo-somatic and axo-dendritic contacts. In the caudal VLM, efferent PB/KF fibers were only seen dorsal to the pool of A1 cells where they intermingled with dorsally travelling A1 dendrites. Medullary areas which contain the B1-B3 cells receive only minor projections from the PB/KF. Nevertheless, labeled axonal varicosities were often found in close apposition to serotonergic somata and dendrites.

Our results demonstrate that the PB/KF complex has strong projections to medullary and spinal autonomic, respiratory, and antinociceptive centers. Therefore, these pathways seem to be involved in the central neural control of these functions. *(Supported by DFG He 1842/3-2)*

561.19

EFFECT OF SEPTAL LESIONS IN THE SALIVARY SECRETION INDUCED BY INTRACEREBROVENTRICULAR (ICV) INJECTION OF PILOCARPINE IN RATS. A. Renzi, W.A. Saad*, L.A.A. Camargo, J.E.N. Silveira and J.V. Menani. Dept. of Physiol. Sci., School Odontol., Paulista State University - UNESP, 14801-903, Araraquara, SP, Brazil.

Electrolytic lesions of the lateral hypothalamus or the anteroventral third ventricle reduces the salivary secretion induced by ICV injection of pilocarpine in rats. In this work we investigated the effect of septal (medial + lateral) electrolytic lesion (2 mA x 20 s) on the increase of the salivary secretion induced by ICV (lateral ventricle) injection of pilocarpine in anesthetized rats. The salivary flux was measured by pre-weighed cotton balls inserted in the mouth. Twelve hours or five days (acute lesion) and fifteen or twenty five days (chronic lesion) after the lesion, the saliva was collected 7 min before (control) and 7 min after ICV injection of pilocarpine (120 ug/ul). ICV injection of pilocarpine in sham rats increases the salivary flux (430 ± 26 mg/7 min) compared to control (18 ± 5 mg/7 min). Septal lesion reduces (99 ± 20, 138 ± 17, 210 ± 25 and 327 ± 42 mg/min, respectively) the salivatory effect of ICV pilocarpine, but not the basal salivary flux (20 ± 5 mg/7 min). These results suggests the septal lesion participates in the salivary secretion produced by ICV injection of pilocarpine in rats.
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561.20

CORTICAL EFFERENT PROJECTIONS TO SPINAL CORD AUTONOMIC CENTERS IN THE RAT. **K.G. Ruit* and M. Bennett. Department of Anatomy and Cell Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202.**

Anatomical evidence for the cortical innervation of autonomic preganglionic neurons within the spinal cord is sparse and, in some cases, contradictory because of a lack of systematic approaches to the question and by limitations imposed by tracing techniques. The present study was designed to focus on regions of the cortex known to have a "visceral motor" function and determine their spinal connections using an effective "long-tract" anterograde tracer.

In adult Sprague-Dawley rats, biotinylated dextran (10,000 MW) was injected by iontophoresis into one of three regions of the medial frontal cortex (MFC)--the anterior cingulate (AC), prelimbic (PL), and infralimbic (IL) areas. After a 21 day survival period the brain and spinal cord were removed, sectioned, reacted using ABC histochemistry, and visualized by light microscopy.

Results of preliminary experiments show that injections in more dorsal regions of the MFC (AC and PL) resulted in terminal labelling within ventral laminae of the thoracic dorsal horn and within gray matter adjacent to the central canal. Injections in ventral regions of the MFC (IL) resulted in terminal labelling adjacent to the central canal and within the intermediolateral cell column. Both of these regions of the thoracic spinal gray matter contain sympathetic preganglionic neurons that form the "final common pathway" out of the central nervous system for the innervation of visceral effectors. Cortical connections with spinal autonomic centers may be important for regulating visceral components of emotion or responses to stress.

561.21

GLP-1 AND ITS RECEPTORS IN THE CENTRAL NERVOUS SYSTEM : POWERFULL REGULATORS OF AUTONOMIC FUNCTIONS IN THE RAT BRAIN. M. Tang-Christensen, R. Göke, M. Møller and S. P. Sheikh*.Dept. Clin.Biochem. 7642, National University Hospital, Rigshospitalet Copenhagen and Dept. of Medical Anatomy. MAI-B, Panum Inst., Denmark.

Glucagon like peptide-1(7-36)amide (GLP-1), is a gut hormone known to play an important role as an incretin in the entero-insular axis. Since GLP-1 immunoreactivity and specific receptors are present in the rat brain, we investigated the biological effects following central GLP-1 administration. GLP-1 was injected directly into the lateral ventricle of brain-canulated rats, and food and water intake were measured. In canulated rats with access to food 180 min during a 24 h period, but with free access to water, GLP-1 produced a strong dose-dependent inhibition of both food and water intake. At doses of 1 and 10 μg of GLP-1, food intake was significantly supressed to 46 ± 12 % (n=9) and 23 ± 6% (n=10) of control animals (n=15). To investigate the effect of GLP-1 on water homeostasis, the urine output of freely feeding rats was measured following intracerebral injection of GLP-1. The urine output was considerably increased by GLP-1 in a dose-dependent manner. At doses of 10 and 1 μg of GLP-1 urine output was increased 4.51 ± 0.68 (n=14) times respectively 2.16 ± 0.37 (n=10) times of control (n=34). Measurement of sodium and potassium concentration in the urine-samples revealed GLP-1 as a natriureticum. These significantly effects on both food and water intake and urinary output suggets an important role for GLP-1 or a structurally related peptide as an neurotransmitter in regulation of both ingestive behaviour and salt and water homeostasis.

REGULATION OF AUTONOMIC FUNCTIONS: GASTROINTESTINAL

562.1

CELIAC GANGLIONIC AND VAGAL PROJECTIONS TO THE RAT DUODENUM COMPARED USING DiI/DiA DUAL LABELING AND CONFOCAL MICROSCOPY. Z. Cheng and T.L. Powley*. Purdue University, West Lafayette, IN 47907.

The duodenum receives strong autonomic projections. The patterns of these inputs are still incompletely known. To label both branches of the ANS concurrently, male rats were injected bilaterally in the dorsal motor nucleus of the vagus with DiA and then one to two weeks later in the left celiac ganglion with DiI, after prior sectioning of the abdominal splanchnics. Five days before sacrifice, the animals received i.p. Fluoro-gold injections to label the enteric nervous system. After perfusion, the duodenum was separated into muscularis externa and submucosal wholemounts and processed for confocal microscopy. In contrast to the vagal pattern of dense networks of fibers and varicosities innervating the myenteric ganglia, sympathetic fibers tend to course superficially around the perimeter of the ganglia. These celiac fibers provide fine collaterals within some ganglia but form relatively few varicose contacts. Celiac fibers also form lattices of axons between the longitudinal and circular muscle layers. These lattices are comprised of axons and interstitial cells paralleling the two muscle sheets. Other fibers penetrate into one or the other muscle layers and arborize in varicose endings. These intramuscular efferents parallel the muscle fibers of the respective layers. In the submucosa, the sympathetic efferents form extensive networks of endings both on blood vessels and on interstitial cell complexes. They also supply varicose endings to submucous ganglia. Single vagal varicosities, as well as rings and baskets of vagal endings, occur in apparent contact with those myenteric neurons which retrogradely label from the celiac ganglion. Thus, a novel and particularly direct interaction between the two ANS divisions may be vagal control of the enteric neurons projecting centripetally to the celiac ganglion. NIH DK27627, NIMH MH01023 & Special Initiative Fellowship to Z. C.

562.2

DIFFERENT VAGAL EFFERENT PROJECTION PATTERNS IN STOMACH, DUODENUM AND CECUM. J.B. Kelly*, M-C. Holst and T.L. Powley. Purdue University, West Lafayette, IN 47907.

In a recent survey of vagal motor fibers in the gastrointestinal tract, we observed that vagal efferents collateralize extensively, providing a dense network of endings within enteric plexuses (cf. Boyd et al., Neurosci. Abst. 19:962, 1993). The present inventory of these inputs was designed to characterize and compare efferent patterns in three prominent GI organs distinguished both by projections from different motor neuron pools within the dorsal motor nucleus of the vagus and by their specialized functions. Male rats received one or more iontophoretic injections of PHA-L in the dorsal motor nucleus of the vagus and were perfused 21 days later. Whole mounts of stomachs, small intestines, and ceca, as well as frontal sections of the medullas (56m), were prepared immunohistochemically (Vector ABC Elite Kit). Cuprolinic blue was used to counterstain both enteric neurons and the medulla. Individual motor fibers were identified as they entered the target organs and then traced and digitized in their entirety (Eutectics Neuron Tracing System). In the stomach, vagal efferents enter from the lesser curvature and ramify so extensively, both radially and longitudinally, that single myenteric ganglia (and many individual neurons) receive multiple overlapping, convergent inputs. The proximal duodenum is innervated by two distinctive types of vagal fibers which frequently converge on the same ganglion. One is a longitudinally projecting axon, and the other a radially coursing axon. In the cecum, vagal fibers enter from the mesenteric attachment and fan out through the myenteric plexus to form contiguous radial fields without extensive overlap. Fibers in all three organs have large projection fields, but variations in the amount of ramification and ratio of longitudinal to radial orientation produce differences in the amount of convergence observed in the enteric ganglia of the organs. NIH DK27627 and NIMH MH01023.

562.3

TAXONOMY OF VAGAL AFFERENT PROJECTIONS TO THE GASTROINTESTINAL TRACT. F.B. Wang* and T.L. Powley. Purdue University, West Lafayette, IN 47907.

Vagal afferents have critical roles in the control of gastrointestinal function, but their morphologies and innervation patterns have only been characterized in limited areas. To survey these afferents, including a comparison of their (a) regional morphologies, (b) projection field sizes, and (c) regional densities, male SD rats were given unilateral intracranial motor rootlet rhizotomies (adapted from Walls et al., Neurosci. Abst., 19:816, 1993) and then injected in the ipsilateral nodose ganglion with WGA-HRP or DiI. All rats had i.p. Fluoro-gold injections to counterstain enteric neurons and verify the rhizotomy. Animals were perfused and processed with TMB (WGA-HRP) or for confocal microscopy (DiI). The entire stomach and cecum as well as samples of the duodenum, jejunum, ileum and colon were prepared as whole mounts and/or transverse thick sections. Individual fibers were followed to reconstruct their peripheral arbors. Vagal afferents terminate as free nerve endings which aggregate in polymorphic clusters. These fibers often branch extensively, sometimes distributing separate collaterals to different tissues. Prominent intramuscular and intraganglionic specializations occur, and these vary in distinct regions. Different areas of the GI tract are distinguished by contrasting patterns of intramuscular endings varying (1) in density from almost none to very heavy and (2) in distribution from predominantly in the longitudinal muscle, to predominantly in the circular, to in both layers. Overall, innervation of the muscularis externa and myenteric plexus is densest in the stomach and proximal duodenum and falls progressively along the small intestine. Sensory innervation of the cecum is relatively extensive, and vagal afferents are even found in the distal colon. The polymorphic and polytopic patterns distinguishing different regions may explain the mechano- and chemoreceptive functions of the different organs. NIH DK27627 and NIMH MH01023.

562.4

DISTENSION INDUCED EXPRESSION OF C-FOS IN MYENTERIC PLEXUS OF GUINEA PIG SMALL INTESTINE. R.C. Ritter* and M. Costa. Dept. of VCAPP, Washington State University, Pullman, WA 99164 and Dept. of Human Physiology, School of Medicine, The Flinders University of South Australia, Bedford Park, S.A.

Distension of the small intestine activates enteric reflexes which involve sensory and motor neurons within the myenteric plexus. In order to identify enteric neurons that respond to a distending stimulus, we examined the myenteric plexus for expression of the immediate early gene, c-fos, following distension. Three cm segments of the jejunum or upper ileum were placed in a 37° C Kreb's buffer as open tubes, or closed sacs. Sacs were distended with 0.4 ml Kreb's buffer or left non-distended. Distension resulted widespread expression of fos-like immunoreactivity (Fos-li) in nuclei of myenteric neurons. Neuronal nuclei expressing Fos-li were much less numerous in myenteric plexus of open tubes and non-distended sacs. Application of tetrodotoxin (1μM) in the bath and lumen reduced expression of Fos-li following distension. These results indicate that distension leads to increased expression of c-fos in the in the nuclei of myenteric neurons as a result of increased neuronal activity. *R.C. Ritter was Visiting Professor and Senior International Fellow at Flinders University during the conduct of these experiments.

562.5

ACTIONS OF CALCITONIN GENE-RELATED-PEPTIDE (CGRP) ON GUINEA PIG GALLBLADDER NEURONES. G. M. Mawe*° and A. P. Gokin†. °†Deptartment of Anatomy and Neurobiology, Univ. of Vermont, Burlington, VT,USA 05401; †Bogomoletz Institute of Physiology, Kiev, Ukraine, 252024

CGRP is co-expressed with substance P in varicose, sensory nerve fibers surrounding neurons in gallbladder ganglia. Furthermore, these peptides are released from gallbladder preparations in response to capsaicin. To assess CGRP's action in gallbladder ganglia, intracellular recordings were made from 94 neurons in intact preparations, and CGRP was applied by pressure microejection (0.01-0.1 mM, 20 PSI, 0.1 - 3.0 sec). CGRP caused a 67.0 ± 3.4 sec depolarization with an amplitude of 5.0 ± 0.4 mV that had a reversal potential potential near 0 mV. The response was associated with a 23.2 ± 1.8 % decrease in input resistance. The magnitude of the depolarizing response was dependent on the duration of CGRP application, and was diminished in a low Na^+ bathing solution. During the depolarizing response, neurons had increased excitability, manifested as an increase in number of spikes per current pulse, and activation of anodal break action potentials. These results indicate that CGRP, along with substance P, may mediate slow EPSPs in gallbladder ganglia, and that its depolarizing action is due predominantly to opening of cation channels. Supported by NS26995.

562.6

EXCITATION OF RAPHE PALLIDUS-INDUCED GASTRIC EROSION IS MEDIATED BY TRH IN DORSAL MOTOR NUCLEUS OF VAGUS NERVE (DMN) IN RATS. H. Kaneko, H. Yang, G. Sobue*, and Y. Taché. CURE/VA Wadsworth Medical Center and Department of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073, and Fourth Department of Internal Medicine, Aichi Medical University, Aichi, 480-11, Japan.

Medullary raphe neurons that contain thyrotropin-releasing hormone (TRH) projecting to the dorsal vagal complex are involved in the brain stem regulation of gastric function. We examined whether excitation of raphe pallidus (Rpa)-induced gastric mucosal lesions is mediated by TRH in the DMN in urethane-anesthetized rats. *Methods*: Gastric acid secretion was measured every 10 min after microinjection (30 nl) of vehicle (0.1M phosphate buffer) or kainic acid (KA: 1.5-12 ng) into the Rpa in either vehicle or indomethacin (5 mg/kg, ip, -60 min) pretreated rats. In other groups, indomethacin pretreated rats were killed 4 h after microinjection of KA (12 ng) into the Rpa, and the percentage of corpus mucosal lesions was determined by computerized image analyzer. The effect of TRH-antibody 8964 (1.3 μg) or control antibody (purified IgG from non immune rabbit serum: 1.3 μg) into the bi- or unilateral DMN and atropine (0.3 mg/kg, sc, -30 min) before microinjection of KA into the Rpa on gastric lesions were also studied. *Results*: KA into the Rpa stimulated dose-dependently gastric acid secretion (net output μmol/60 min: 1.5 ng: 50.7 ± 11.5; 3 ng: 60.9 ± 7.6; 12 ng: 82.9 ± 16.6, n=5), which was not modified by indomethacin (μmol/60 min: 1.5 ng: 46.3 ± 7.0; 3 ng: 52.2 ± 9.8; 12 ng: 75.1 ± 15.6, n=5). KA (12 ng) into the Rpa in indomethacin treated rats produced 2.7 ± 0.3% of lesions which were blocked by 99.6%, 93.6%, and 81.1% in atropine, bilateral, and unilateral microinjection of TRH-antibody into the DMN pretreatment, respectively. KA outside of the Rpa and TRH-antibody outside of the DMN had no effect. *Conclusion*: Rpa neurons activated by KA induced gastric erosion through TRH release in the DMN which stimulated vagal cholinergic pathways.

562.7

ACTIVATION OF THE RAPHE PALLIDUS NEURONES INCREASES SERUM INSULIN LEVELS THROUGH VAGAL PATHWAYS. **H.Yang*, V.L.W.Go, and Y. Taché. CURE, VA Med. Cter., Brain Res. Inst. and Dept Medicine, UCLA. Los Angeles, CA 90073**

Chemical stimulation of medullary raphe neurons induces gastric secretion, motility, cytoprotection and ulcer formation through vagal pathways in urethane-anesthetized rats. The effect of excitation of raphe pallidus (Rpa) neurons on the endocrine pancreatic secretion was examined. Methods: Rats (280-350 g) were anesthetized with pentobarbital, a PE-50 cannula was inserted into the jugular vein for taking blood samples and a double-lumen cannula was placed into the forestomach for measuring gastric acid secretion. Serum insulin and glucagon levels were tested using Linco RIA kits. Results: Microinjection of kainic acid (10 ng/50 nl) into the Rpa induced a increase in serum insulin level. Values before and 30, 60 and 90 min after microinjection were 0.34 ± 0.02, 0.54 ± 0.06, 0.60 ± 0.06 and 0.99 ± 0.13 ng/ml respectively (n=8). All values after Rpa stimulation were statistically significant from the basal level. In the same rats, stimulated gastric acid secretion was also observed (basal: 2.3 ± 0.55; 30 min after Rpa stimulation: 26.1 ± 8.6 μmol/15 min). Microinjection outside of the Rpa had no effect. Serum glucagon level (basal: 48.8 ± 4.3 pg/ml) significantly increased only 90 min after the Rpa stimulation (86.6 ± 11.2 pg/ml). The increases of gastric acid secretion and serum insulin levels were completely prevented by bilateral cervical vagotomy whereas the increase in glucagon levels was only slightly but not significantly reduced. Conclusion: Chemical stimulation of Rpa stimulates not only gastric function but also endocrine pancreatic secretion through vagal pathways.

562.8

EXTRINSIC DENERVATION INCREASES FOS-LIKE IMMUNOREACTIVITY IN THE SUBMUCOSAL AND MYENTERIC GANGLIA OF GUINEA PIG ILEUM. A.M. Yunker* and J.J. Galligan Dept. Pharmacology and Toxicology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824

Gastrointestinal functions are normally controlled by interactions of enteric neurons found within the wall of the gut, and by extrinsic sympathetic, parasympathetic, and sensory nerves. However, normal digestion can occur after extrinsic denervation, suggesting enteric neurons can assume functions previously regulated by extrinsic nerves. Antiserum raised against Fos protein was used to determine if specific enteric neurons are altered following loss of extrinsic nerves. As Fos protein is related to neuronal activity, increased Fos protein-like immunoreactivity (Fos-ir) would be indicative of changes in nuclear events following denervation. One week after denervation of a loop of ileum, 9 ± 1 Fos-ir nuclei/submucosal ganglion and 110 ± 8 Fos-ir nuclei/myenteric ganglion were observed. These values were significantly greater ($p < 0.05$) than those from unoperated segments from the same animals, as 3 ± 0.3 Fos-ir nuclei/submucosal ganglia and 12 ± 6 Fos-ir nuclei/myenteric ganglia were observed. This difference was unlikely due to surgical trauma, as there was no difference ($p > 0.05$) in the number of Fos-ir nuclei in the submucosal plexus (2 ± 1 Fos-ir nuclei/ganglion) or myenteric plexus (17 ± 6 Fos-ir nuclei/ganglion) in sham-denervated segments as compared to controls (2 ± 1 Fos-ir nuclei/submucosal ganglion, 17 ± 7 Fos-ir nuclei/myenteric ganglion). Although significantly more ($p < 0.05$) nuclei contained Fos-ir 7 and 10 weeks after surgical denervation as compared to preparations from unoperated segments, the number of nuclei/ganglion decreased in both submucosal and myenteric ganglia, possibly as a result of regrowth of extrinsic nerves. Finally, no differences were observed in Fos-ir in control and denervated preparations 24 weeks after surgery. These data suggest that most, if not all, enteric neurons are altered following loss of extrinsic nerves. Some of these changes may be part of the adaptive process that allows enteric neurons to function in the absence of extrinsic innervation. (Supported by DK 40210 and NS 07279)

562.9

SEROTONIN INHIBITS, BUT 8-OH-DPAT STIMULATES, GASTRIC ACID SECRETION THROUGH VAGAL-INDEPENDENT MECHANISMS. K.J.LePard*, S.Gidener and R.L.Stephens Jr., Department of Physiology, The Ohio State University, Columbus, OH 43210.

Serotonin (5HT) is a neuroendocrine component of the gastrointestinal tract. $5HT_{1A}$ receptors have been demonstrated autoradiographically in high density in the rat stomach. However, the physiologic role of $5HT_{1A}$ receptors in the stomach is not known. Thus, the effect of the selective $5HT_{1A}$ agonist, 8-OH-DPAT, on gastric secretory function was compared to 5HT. In acute, urethan-anesthetized rats with gastric fistula, acid secretion was stimulated by pentagastrin (24 μg/kg/hr, i.v.). After 90 minutes, either 5HT or 8-OH-DPAT (3.5 μmoles/kg, i.v.) was given. 5HT inhibited, but 8-OH-DPAT stimulated, gastric acid secretion [mean ± SEM, % change: 5HT (n=8), -47 ± 11; 8-OH-DPAT (n=7), +71 ± 42; $p<0.05$]. Bilateral, cervical vagotomy did not reverse the effect of either 5HT or 8-OH-DPAT on acid secretion. In addition, the enhancement of acid by 8-OH-DPAT was spiperone-sensitive. Hence, the data suggests that selective activation of $5\text{-}HT_{1A}$ receptors may augment gastric secretory function. Supported by NIH DK 42880.

562.10

GASTRIC MUCOSAL EROSIONS PRODUCED BY NMDA INFUSIONS INTO THE LATERAL HYPOTHALAMUS:EFFECTS OF SELECTIVE KNIFE CUTS. C.V. Grijalva*, J. Landeira-Fernandez and Stacey Nuccion. Department of Psychology, Univ. of California, Los Angeles 90024.

Bilateral infusions of N-methyl-D-aspartate (NMDA) into the lateral hypothalamus (LH), which interrupt intrinsic neurons, produce gastric erosions in rats (Grijalva, Rios-Jimenes & Landeira-Fernandez. Soc. Neurosci. Abst. 19, part 2: 1993, 959). The present study attempted to determine the pathways mediating this effect. In order to interrupt axonal transmission, knife cuts (KC) were made in different planes adjacent to the LH. In separate groups of rats, KC were made anterior, posterior or lateral to the LH just prior to bilateral NMDA infusions (20 μg/μl). The incidence of gastric erosions was measured 24 h after NMDA infusions. Animals receiving Sham KC and infused with NMDA exhibited significantly more gastric erosions than those infused with PBS control ($p<.05$). Lateral parasagittal KC blocked the occurrence of gastric erosions produced by NMDA, whereas anterior coronal KC significantly increased the incidence of erosions produced by NMDA ($p<.005$). Posterior coronal KC did not significantly alter the incidence of gastric erosions produced by NMDA. These results suggest that intrinsic LH neurons with gastric function project axons laterally and probably descend through the internal capsule to brainstem medullary nuclei. The results of the anterior KC suggest that the LH sends and/or receives inhibitory projections from neural structures (possibly the amygdaloid complex) anterior to the plane of the KC. (supported by UCLA research grant PZ-06 and CNPq)

562.11

EFFECTS OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) ON GASTRIC MOTOR FUNCTION IN THE BRAINSTEM OF THE RAT. P.J. Hornby[1*], G. Légrádi[2], A. Arimura[2], and Z.K. Krowicki[1], [1]Dept. of Pharmacology, LSU Medical Center, New Orleans, LA 70112 and [2]U.S.-Japan Biomedical Research Laboratories, Tulane Univ. Hebert Ctr., Belle Chasse, LA 70037.

PACAP-like immunoreactive cell bodies and fibers exist in the brainstem nuclei known for regulatory effects upon autonomic functions (Légrádi and Arimura, Soc. Neurosci. Abstr., 732, 1993). These findings prompted us to investigate the effect of PACAP38 (1, 10, and 100 pmol; 30 nl) and saline (30 nl) in the dorsal vagal complex (DVC), the nucleus raphe obscurus (NRO) and in the vicinity of the nucleus ambiguus (NA) of alpha-chloralose-anesthetized rats on intragastric pressure (IGP), pyloric and greater curvature motility, heart rate, and blood pressure. Increases in gastric motility upon microinjection of L-glutamate (15 nmol; 30 nl) were used as an indicator of the location of the tip of the micropipette. Overall, PACAP dose-dependently increased the indices of gastric motor function; however, this attained statistical significance only for the increases in IGP at the highest dose of PACAP (100 pmol), as follows: in the DVC (N=5), the increase in IGP was 1.08 ± 0.35 cm^2 ($P<0.01$ vs. saline); in the NRO (N=5), the increase in IGP was 0.54 ± 0.12 cm^2 ($P<0.001$ vs. saline); in the region of NA (N=5), the increase in IGP was 0.5 ± 0.2 cm^2 ($P<0.05$ vs. saline). In the region of NA, PACAP (100 pmol) decreased heart rate by 6 ± 3 bpm ($P<0.05$ vs. saline). These data indicate that PACAP is a stimulator of IGP in the brainstem nuclei of the rat. Supported by PHS grant DK42714.

562.12

CONSTITUTIVE AND INDUCIBLE NUCLEAR ONCOPROTEINS IN THE ENTERIC NERVOUS SYSTEM OF THE GUINEA PIG ILEUM. E.J. Parr and K.A. Sharkey*. Neuroscience Research Group, The University of Calgary, Calgary, AB, Canada T2N 4N1.

Constitutive c-Myc and c-Fos antigens are detectable in the nuclei of all enteric neurons of the guinea pig ileum. We have demonstrated that they make suitable markers to count enteric neurons in double-labelled preparations as well as providing a method to distinguish neurons from glia.

We have also investigated inducible nuclear oncoproteins in the guinea pig enteric nervous system. Inducible nuclear c-Fos, JunB and c-Jun immunoreactivity was found to be elicited in neurons by *in vitro* incubation of ileal segments in media containing 50mM K^+ or 20μM veratridine as depolarizing stimuli. The neurons expressing activity-related c-Fos antigens included all major immunohistochemically defined subgroups of the submucous plexus. However, in incubated ileal segments, smooth muscle cells and substantial numbers of glia as well as submucous neurons containing VIP-immunoreactivity expressed nuclear c-Fos-immunoreactivity in the absence of a depolarizing stimulus. We conclude that these immediate early genes appear to be regulated by neuronal activity in most or all subgroups of neurons but *in vitro* isolation of ileal segments is sufficient to induce expression of these genes in many cell types by as yet unidentified stimuli.
Supported by the Medical Research Council of Canada.

562.13

MUCOSAL EXCITATORY INFLUENCE ON ANATOMICALLY IDENTIFIED NEURONS IN THE MYENTERIC PLEXUS OF THE GUINEA-PIG ILEUM. W.A.A. Kunze, J.C. Bornstein, J.B. Furness and H.M. Young.* Departments of Physiology and Anatomy & Cell Biology, University of Melbourne, Parkville, Victoria, Australia 3052.

To examine the influence of input from the mucosa on S neurons of the myenteric plexus, we compared the discharge properties of neurons very close to the mucosa with those whose somata lay further away. Opened segments of guinea-pig ileum were superfused with oxygenated physiological saline at 37°C; contractions were minimised by addition of 3 µM nicardipine. The myenteric plexus was exposed for half the area of each segment by removal of overlying mucosa, submucosa, and circular smooth muscle. Intact mucosa was circumferentially adjacent to the exposed plexus. Neurons were impaled with conventional microelectrodes filled with 1M KCl and 4% neurobiotin™ (Vector) and subsequently visualised using Texas Red fluorescence. Twenty-three neurons were recorded adjacent (<500 µm) to, and 13 far (>500 µm) from the mucosa. Twelve adjacent neurons, but only one far neuron discharged tonically throughout 500 ms depolarising current pulses, whereas 11 adjacent and 12 far neurons fired phasically. None of the tonic neurons projected orally (6 of 7 had anal projections), but 3 of 8 phasic neurons impaled >500 µm from the mucosa projected orally. The results indicate that input from the mucosa may preferentially enhance the excitability of anally projecting S neurons.

562.14

NEURAL CONTROL OF GASTRIC ACID SECRETION: ANATOMICAL DEMONSTRATION OF VAGAL INPUT TO GASTRIN RELEASING PEPTIDE (GRP) CONTAINING ENTERIC NEURONS. H.-R. Berthoud* and Q. Lin. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808.

The prevailing theory of vagal control of gastric acid secretion suggests the presence of at least two stimulatory pathways. One is a direct cholinergic input to the parietal cells, the other involves the release of gastrin from mucosal endocrine cells that is partially driven by GRP containing vagal postganglionic enteric neurons. Additionally, there may be vagal inhibitory input mediated by somatostatin cells. In order to anatomically and neurochemically characterize this vago-enteric interface, we started to examine the relationship between DiA labeled vagal preganglionic terminals and immunocytochemically characterized enteric neurons and their projections.

The percentage of GRP-positive myenteric neurons is gradually decreasing from about 40% in the fundus or forestomach, to 25% in the corpus and only about 10% in the pyloric antrum. Dense networks of GRP-positive varicose terminal fibers were found around gastric glands, in myenteric ganglia, and more sparsely within the external smooth muscle layers. In the corpus and antrum, 34% and 35%, respectively, of the GRP-positive neurons receive vagal efferent contacts. Conversely, of all the vagally contacted neurons, 33% and 16% were GRP-positive in the corpus and antrum, respectively. Vagal contacts with enteric neurons of other neurochemical phenotypes and with somatostatin cells, as well as possible contacts of DiI-labeled vagal afferent fibers within such neurons, are currently being analyzed. In conclusion, there is a significant number of GRP-positive enteric neurons that receive vagal preganglionic input, supporting a role of GRP in vagally controlled gastrin and gastric acid secretion and possibly other functions. Supported by NIH grant DK47348.

562.15

DUAL ANTAGONISTIC EFFECTS OF VAGAL AFFERENTS AND EFFERENTS ON JEJUNAL ALANINE ABSORPTION. C.F. Nassar, W.S. Hamdan, K.A. Barada, A.M. Taha, S.F. Atweh*, S.J. Jabbur and N.E. Saadé. Fac. Med. American University of Beirut, Lebanon.

The role of the vagus nerve in the regulation of water and electrolyte transport across the small intestine is well documented. However, there is lack of adequate information on its role in amino acid absorption. This study was thus undertaken to characterize the role of the vagus nerve in intestinal amino acid absorption, using selective chemical stimulation or block of various groups of vagal afferents and efferents, and the single-pass perfusion technique. Application of capsaicin (cp) to the isolated intact cervical vagii produced a 14% decrease of alanine absorption while similar application to the distal parts of cut vagii produced a 28% inhibition of alanine absorption. Chronic destruction of the cp sensitive vagal afferents resulted in 40% increase of alanine absorption. On the other hand selective activation of preganglionic vagal neurons by microinjection of kainic acid into the dorsal motor nucleus of the vagus produced a 48% increase in jejunal alanine absorption; this increase was abolished by previous treatment of the injected rats by atropine.

These results suggest that cp sensitive vagal afferents exert an inhibitory effect (direct and reflex) upon jejunal alanine absorption while cholinergic muscarinic vagal efferents exert an opposite facilitatory effect. (Supported by grants from the L.N.C.S.R. and U.R.B.).

562.16

ELECTROPHYSIOLOGICAL EFFECTS AND IMMUNOCYTOCHEMICAL LOCALIZATION OF PACAP IN GUINEA PIG PANCREATIC GANGLIA. M.-T. Liu, A. Arimura, M.D. Gershon, and A. Kirchgessner*. Dept. of Anat.& Cell Biol., Columbia University, New York, NY 10032.

Intracellular recordings were used to examine the actions of pituitary adenylate cyclase-activating peptide (PACAP) on morphologically identified neurons in the guinea pig pancreas. In addition, the distribution of PACAP immunoreactivity in the pancreas was examined using an antibody that recognizes PACAP-27 and -38. Pancreatic neurons were classified as tonic, phasic, or nonspiking based on their physiological activity. PACAP-38 applied by pressure microejection evoked excitatory responses in phasic pancreatic neurons. The responses consisted of membrane depolarization in association with increased input resistance and repetitive spike discharge. Nonspiking neurons became excitable after application of PACAP. Most of the phasic pancreatic neurons that responded to PACAP had filamentous processes that were densely surrounded by VIP-immunoreactive terminals. PACAP immunoreactivity was observed in nerve fibers and in varicose terminals that covered a subset of pancreatic neurons. PACAP immunoreactive pancreatic neurons were not observed; however, they were observed in myenteric ganglia in the adjacent duodenum. The effects of PACAP and distribution of PACAP immunoreactive nerve fibers on pancreatic neurons suggest that PACAP is a neuromodulator. The possibility that PACAP immunoreactive axons are of enteric origin is being studied. Supported by grants NS27645 and NS01582.

563.1

DETECTION OF INSPIRATORY RESISTIVE LOADS IN HUMANS TRAINED TO BREATHE WITH DIAPHRAGM OR INTERCOSTAL MUSCLES. S.M. Reynolds*, W.R. Revelette, and D.T. Frazier. Department of Physiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

We recently recorded cerebral evoked potentials in response to inspiratory occlusion in humans trained to breathe with their diaphragm or intercostal muscles (*Neurosci. Abst.19:764,1993*). The amplitude of the evoked signal during intercostal breathing was significantly greater than that for any other condition. One explanation for this could be that the greater amplitude is related to the greater number of group I afferents in intercostal muscles compared with that of the diaphragm. The objective of the present study was to determine whether differences for the detection threshold of added resistive loads during free breathing (FB), diaphragm breathing (DB), and intercostal breathing (IB) exist. Assuming a correlation between perception of added loads to breathing and evoked potential amplitude exists, we hypothesized that the threshold for detection during intercostal breathing would be lower than that for diaphragm breathing. Respitrace was used to monitor abdominal and rib cage motion. Subjects were provided visual feedback by watching the output of each band on an oscilloscope. Nineteen inspiratory resistive loads (.26-3.54 cmH2O/L/sec) were presented to ten subjects for a single breath under three conditions (FB, DB, IB). The detection threshold (mean ± SEM) was 0.53 ± 0.09 for FB, 0.62 ± 0.11 for DB, and 0.54 ± 0.09 for IB. There was no statistically significant difference between these means. There appears to be no significant correlation between evoked potential amplitude elicited by inspiratory occlusion and detection threshold for resistive loads across the breathing conditions. (Supported by NIH Grant # PO1 40369)

563.2

ACTIVE AND PASSIVE CHEST INFLATION DIFFERENTIALLY MODIFY ABDOMINAL EXPIRATORY ACTIVITY. B. Bishop*, F. Cerny, L. Puckree, J. Darbee, D. Allen. Depts. of Physiology, and of PT and Exercise Science. State Univ. of NY at Buffalo, Buffalo, NY 14214

When supine, abdominal muscles are silent. However, they are active in expiration during an expiratory threshold load (ETL) or during continuous positive airway pressure (CPAP). We questioned how the sensory inputs of these two loads are integrated. Both loads change end-expiratory lung volume (EELV), and hence, vagal-volume feedback, but CPAP modifies feedback from chest wall receptors as well. EELV was passively increased to 25% and 50% of inspiratory capacity (IC) by rapidly reducing pressure in a full chest cuirass. EELV was actively increased by impeding expiration with a water column. We measured changes in breathing pattern and in expiratory activity in surface EMGs from external oblique (EO) and internal oblique (IO) abdominal muscles during ETLs of 5, 10 and 15 cm H_2O in 5 supine subjects (Ss). We used a "Respitrace" to record EELV changes; and a pneumotach to assess V_t, T_i, and T_e. An increase in ETL evoked deeper, slower breathing, and higher peak expiratory activity in IO and EO, with minimal change in burst duration. Breathing was not altered by inflation to 25% IC. Inflation to 50% IC increased V_t without changing timing, and in 2 Ss evoked expiratory activity in IO. Combined ETL and CPAP evoked no additional changes in breathing pattern or burst duration, but did elevate the EO and IO expiratory peaks more than either load alone. Thus, feedback during the combined loads had additive effects on the output of the central pattern generator, but not on that of the central rhythm generator.

563.3

METABOLIC AND RESPIRATORY RESPONSES TO MILD EXERCISE IN PATIENTS WITH CFS DL Cordero*, JG Pareja, MT Bergen, WN Tapp and BH Natelson. Neurobehavioral Unit, DVA, East Orange, NJ 07018.

To identify patients with Chronic Fatigue Syndrome (CFS), our lab has been studying the physiological responses that CFS patients exhibit during and after treadmill exercise. Seven non-medicated CFS patients and seven sex-matched controls screened for their level of fitness were recruited for this study. Subjects walked on a motorized treadmill at 2.5 mph for 4 minutes and then sat for 4 minutes, this pattern repeated 4 times. Physiological variables were collected from an ECG machine and a metabolic breath analyzer. Although initial baseline heart rate, tidal volume, minute volume, respiratory rate, VO2 and RQ were not different between groups, there was a significant difference in end-tidal CO2 during the initial resting stage (CFS: 32.61±.88 mmHg, controls: 35.73±.50, mean ± SEM, $p<.01$). For the entire test, ANOVA revealed significant group differences for respiratory rate and ventilation, which were higher for CFS patients, and end-tidal CO2, which was lower for CFS patients (all $p<.05$). The decreased end-tidal CO2 at rest does not correlate with hyperventilation and so may reflect either a physiological abnormality or pre-test hyperventilation.

Supported by NIH #AI32247 & VA Medical Research Funds

563.4

SCALP TOPOGRAPHY OF RREP ELICITED BY EXPIRATORY OCCLUSION IN ADULTS. C.A. Smith-Hammond*, C. Sapienza, D. Dalziel and P.W. Davenport. Dept. of Physiol. Sci., JHMHC, University of Florida, Gainesville, Florida, 32610.

Respiratory related evoked potentials (RREP) was previously only recorded with inspiratory loads. It was hypothesized that a RREP can be recorded using expiratory occlusion. Adults were studied semi-reclined at rest, screened from the experimenter and apparatus. They respired through a mouthpiece and non-rebreathing valve. The expiratory occlusion was produced using a pressure activated valve with a 2 msec closure time. Early expiration was interrupted with an occlusion for 350 msec. EEG activity was recorded from F_3, F_4, C_3, C_4, C_3', C_4', T_3, T_4, P_3, P_4, T_5, T_6, C_Z and P_Z referenced to the joined earlobes. Two occlusion trials of 80 occlusions were separated by a control trial on 80 no-load presentations. The EEG activity was computer averaged on-line and saved on disk. Expiratory occlusion elicited an evoked potential was found in all subjects. An initial positive peak (P_1) was the only consistent peak observed in all subjects and was present in electrodes C_3, C_4, C_3', C_4', P_3, P_4, C_Z and P_Z. This evoked potential was reproducible in both occlusion trials. The mean P1 peak latency was measured 42 msec. No activity was consistently observed in the frontal electrodes. No peaks were observed with control R_0. These results support the hypothesis that a RREP can be recorded with an expiratory load. The distribution of the P_1 peak is consistent a bilateral sensory cortex origin. The positive peak is similar to the P_1 peak found with inspiratory occlusion suggesting that this expiratory positive peak is the initial respiratory mechanoreceptor mediated activation of the sensory cortex. Supported by NIH/NHLBI grant HL48792.

563.5

IDENTIFICATION OF HUMAN BRAIN REGIONS UNDERLYING RESPONSES TO INSPIRATORY LOADING WITH FUNCTIONAL MAGNETIC RESONANCE IMAGING. O. Omidvar, D. Gozal, K.A.T. Kirlew, G.M. Hathout, R. Hamilton, J. Zhang*, R.B. Lufkin and R.M. Harper. Depts. of Anatomy & Cell Biology and Radiology, UCLA School of Medicine, Los Angeles, CA 90024-1763.

In man, locations of putative brain sites mediating responses to respiratory loading are unknown. To examine this issue, we employed a 1.5 Tesla MR scanner, and used SPGR pulse sequences (TR: 72 msec; TE: 45 msec; flip angle: 30°; FOV: 26 cm; slice thickness: 5 mm; 128 x 256 x 1 NEX). Mid-sagittal and axial images across the rostral pons were acquired in 8 healthy volunteers during two-way valve unloaded breathing, and while applying a 30 cm H_2O resistor to the inspiratory port. Digital image subtraction and ROI analysis revealed significant increases in image intensity in discrete regions of the dorsal medulla, pons, and cerebellum, as well as bilaterally, in the inferior colliculi, putamen, and internal capsule. With continuous loading, signal increases progressively diminished over time. Upon cessation of stimulus, ROI signal gradually returned to baseline. We conclude that inspiratory loading elicits consistent regional activation of discrete brain locations. We speculate that temporal changes in signal intensity may indicate respiratory afterdischarge and habituation phenomena.

(Supported by HL-22418, HD-22695 & Parker B. Francis Foundation)

563.6

LOCALIZATION OF POTENTIAL HUMAN BRAIN REGIONS MEDIATING RESPONSES TO EXPIRATORY LOADING USING FUNCTIONAL MAGNETIC RESONANCE IMAGING. D. Gozal*, O. Omidvar, G.M. Hathout, K.A.T. Kirlew, R. Hamilton, R.B. Lufkin, and R.M. Harper. Depts. of Anatomy & Cell Biology and Radiology, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Functional MRI provides a noninvasive tool to assess spatial and temporal characteristics of neural response to respiratory stimuli. We attempted to identify human brain regions which may underlie the ventilatory response to increased expiratory resistance. To study this issue, we employed a 1.5 Tesla MR scanner, and used SPGR pulse sequences (TR: 72 msec; TE: 45 msec; flip angle: 30°; FOV: 26 cm; slice thickness: 5 mm; 128 x 256 x 1 NEX). Mid-sagittal and axial images across the rostral pons were acquired in 5 healthy volunteers during two-way valve unloaded breathing, and while applying a 30 cm H_2O resistor to the expiratory port. Digital image subtraction and ROI analysis revealed significant increases in image intensity in discrete regions of dorsal medulla, and the ventral and dorsal rostral pons. With continuous loading, increases in signal progressively diminished over time. Upon cessation of the stimulus, ROI signals immediately returned to baseline. We conclude that expiratory loading elicits consistent regional activation of discrete brain locations which differ from those which uniformly activate during inspiratory loading. We speculate that temporal changes in signal intensity may indicate either a switch to oxidative neural metabolism or habituation phenomena.

(Supported by HL-22418, HD-22695 & Parker B. Francis Foundation)

563.7

NUCLEUS AMBIGUUS AND THE VENTRAL RESPIRATORY GROUP: A CORRELATIVE ANATOMICAL STUDY OF THE RAT AND HUMAN VENTROLATERAL MEDULLA OBLONGATA. H. H. Ellenberger* and D. A. Hopkins, Department of Anatomy and Neurobiology, Dalhousie University, Halifax N.S., Canada, B3H 4H7.

The nucleus ambiguus (NA) and ventral respiratory group (VRG) in the ventrolateral medulla of the human brainstem were characterized by an anatomical comparison with homologous neuron populations in the rat ventrolateral medulla oblongata. To identify these neuron populations, we performed parallel immunohistochemical labeling studies on rat and human brainstem tissue using antibodies to choline acetyltransferase (ChAT) to label NA motoneurons, and parvalbumin (PV) to label presumptive bulbospinal VRG neurons (*Cox & Halliday, Neurosci Lett., 160:101-105, 1993*). Anatomical subdivisions of NA in the rat were identified in transverse and sagittal sections of ChAT-immunoreacted tissue. ChAT-immunoreactive neurons in the ventrolateral medulla of the human brainstem were distributed in locations and patterns that were comparable to those in the rat NA. In particular, the compact division of NA formed a distinct dorsal column in both species. By combining PV immunohistochemistry with retrograde labeling after Fluoro-Gold injections into the C4 spinal cord, we have shown that most bulbospinal VRG neurons are PV-immunoreactive in rats. Our comparison of the location and distribution of PV-immunoreactive neurons in the ventrolateral medulla of the human and rat brainstem suggests the presence of a human homologue of the rat bulbospinal VRG neuronal population.

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SOMATIC AND VISCERAL AFFERENTS: THERMORECEPTION

564.1

RESPONSES OF PRIMATE CUTANEOUS NOCICEPTORS EVOKED BY NOXIOUS COLD. D.A. Simone[1]*, H.D. Gilchrist[1] and K.C. Kajander[2]. [1]Neurosci. Res. in Psychiatry and [2]Depts. of Oral Science and Cell Biology & Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Unlike heat pain, the peripheral neural mechanisms underlying sensation of cold pain are poorly understood. Although many studies have examined responses of cutaneous nociceptors to noxious heat and stimulus-response functions have been well described, responses of nociceptors to cold stimuli have not been well characterized. We have examined responses of cutaneous nociceptors to controlled noxious cold stimuli. Single fiber recordings were made from the saphenous nerve in anesthetized monkeys. Nociceptors were located by mildly pinching the skin and receptive fields mapped using von Frey filaments. Conduction velocity, mechanical threshold (von Frey), and responses to thermal stimuli were assessed. Heat stimuli of 39-51° C (5 sec duration) and cold stimuli of 30 to -14° C (5 or 10 sec duration) were applied from a base temperature of 32° C and delivered in ascending or descending order.

Recordings were made from 9 Aδ and 6 C-fiber nociceptors. Mechanical thresholds for Aδ and C-fibers ranged from 5.5-149.3 mN and from 0.61-19.6 mN, respectively. Under normal conditions only one A-fiber was excited by noxious heat. In contrast, all were excited by noxious cold stimuli with a mean response threshold of -4.2° C (range: 8° to -12° C). 7 of 9 A-fibers had a response threshold below 0° C. All C-fiber nociceptors were excited by both noxious heat and cold stimuli. The mean response threshold to cold was 15.3° C and thresholds ranged from 30° to -2° C. Unlike Aδ fibers, 5 of 6 C-fibers exhibited response thresholds ≥ 0° C. Responses of both A- and C-fibers typically increased monotonically as stimulus temperature decreased. It is concluded that: 1) Aδ and C "mechanonociceptors" are excited by noxious cold and 2) C nociceptors contribute to cold pain at temperatures above 0° C, while both Aδ and C nociceptors contribute to pain sensation evoked by temperatures below 0° C. Supported by NS31223 and NS29567.

564.2

THERMOSENSITIVITY OF CULTURED TRIGEMINAL NEURONS T.K. Baumann*[1,2] and M.E. Martenson[1], Division of Neurosurgery[1] & Department of Pharmacology[2], Oregon Health Sciences University, Portland, OR 97201

Little is known about the membrane mechanisms involved in thermal transduction because the sensory endings are inaccessible for direct study *in vivo* or in isolated tissues. However, thermo-sensitive membranes may be exposed in culture. To examine the thermal response properties of cultured somatosensory neurons we have built a miniature heat stimulator (Baumann et al., Biophys. J. 66: 442, 1994) which can stimulate individual cells. Whole-cell, patch-clamp recording techniques were used to study the thermosensitivity of cultured adult rat, rabbit, and cat trigeminal ganglion neurons. Triangular or rectangular heat stimuli (up to 20°C in amplitude, 3 to 10 s in duration) were applied every 10 to 30 s from a baseline temperature of 25 to 29°C. Responses were non-uniform, but largely reproducible. A proportion of neurons responded to heating with a depolarization and action potential discharge. Others showed the opposite behavior; they were inhibited by heating and discharged action potentials upon heat removal. Some neurons showed no overt response to changes in temperature. Following exposure to high temperatures, some neurons developed spontaneous action potential discharge, some became inexcitable. We conclude that cultured trigeminal ganglion neurons show features of thermoreceptors and heat nociceptors and may provide a useful model for studying thermal transduction mechanisms. (Supported by NSF grant IBN 92-11545.)

564.3

RESPONSE CHARACTERISTICS OF REGENERATING COLD-SENSITIVE CORNEAL C-FIBERS. S.Monroe* & D.L. Tanelian, Dept. of Anesthesiology and Pain Mgt, UT Southwestern Med. Ctr., Dallas, TX 75235-9068.

To date, there has been no quantitative, systematic, study of the electrophysiology of regenerating cold receptors. This study, therefore, examines the changes in cold-receptor neural activity following a circular wound (5 mm dia, 200 um deep) on the surface of the rabbit cornea. This is a well studied wounding model, in which neural regeneration has been anatomically quantified. Extracellular recordings were obtained from a total of 90 single cold fibers, at 1, 3, 10, 20, or 30 days following wounding. Conduction velocities for regenerating cold-fibers were similar to those recorded from normal fibers, 0.65 +/-.03 and 0.96 +/-0.22 M/sec, respectively. The adapting temperature was 35°C in all experiments. Thermal sensitivity for each fiber was determined using a series of temperature steps, 0.2 °C ranging from 35 to 34°C, and 2 °C steps ranging from 35 to 24°C. The rate of temperature change ranged from 0.2 to 1°C/sec. At the adapting temperature, the tonic activity of the regenerating cold-fibers was not significantly different from normals. On day 1, regenerating fibers showed decreased responsiveness to cooling ($p<.05$). At days 3 and 10 post wounding, action potential rates in response to cooling were enhanced by 180 - 200% of normal ($p < .05$) and returned to pre-injury values by 20 to 30 days post wounding. There was no difference in the slope of the rate of cooling vs action potential frequency at any time point from pre-injury through the 30 day regeneration period, suggesting that the cold transduction mechanism itself was not altered during regeneration. Beuerman & Rozsa (1984), using the same experimental wound in rabbit corneas, reported elevated neurite density between 3 to 14 days post wounding. This data, in combination with our electrophysiological study, suggest that the elevated responsiveness of regenerating fibers may be a result of increased transducer numbers, as opposed to alteration in the transduction mechanism. Supported by NIH grant NS28646 and Sid W. Richardson Foundation.

564.4

RATE DEPENDENCE OF COLD TRANSDUCTION IN CORNEAL C-FIBER COLD RECEPTORS. D.L. Tanelian*& S.Monroe, Dept. of Anesthesiology and Pain Mgt, UT Southwestern Med. Ctr., Dallas, TX 75235-9068.

The component of thermal energy change (i.e. rate, magnitude) which the cold receptor tranducer responds to remains in question. In this study ramps and sinusoidal temperature changes were used to investigate the dependency of neural activation and adaptation on the rate and magnitude of temperature change. Single unit extracellular recordings were obtained from 54 cold units in 22 rabbits. The adapting temperature was 35°C in all experiments. Thermal sensitivity for each fiber was determined using a series of temperature steps, 0.2 °C ranging from 35 to 34°C, and 2 °C steps ranging from 35 to 24°C. The rate of temperature change (dT/dt) ranged from 0.2 to 1°C/sec for ramps. For sinusoids the frequency ranged from 0.07 to 0.005 Hz at a median temperature of 35°C with a +/-5°C change in amplitude. Action potential frequency (AP) was proportional to the magnitude (ΔT, 0 to -9°C) and rate (dT/dt, .2 to 1 °C/sec) of temperature change. The action potential acceleration (dAP/dt) and rate of change of adaptation were proportional to dT/dt and independent of ΔT. Sinusoidal stimulation showed that AP always peaked prior to reaching maximal ΔT, indicating that the thermal transducer mechanism does not depend on ΔT. Conversely, and more logically, AP and dAP/dt always peaked just following maximal dT/dt. Additionally, phase analysis of the sinusoidal ΔT vs AP showed the best phase correlation, at all frequencies, to be between dT/dt and dAP/dt, suggesting that it is the rate of change in temperature that is being transduced in cold receptors. We hypothesize that dAP/dt correlates better than AP with dT/dt because dAP/dt may be a reflection of cold receptor generator potential. Further studies are being done to investigate the effects of adapting temperature upon these receptor processes. Supported by NIH NS28646 and the Sid W. Richardson Foundation.

564.5

SPATIAL LOCALIZATION OF THERMAL STIMULI, AND ITS RELATIONSHIP TO DERMATOMIC ORGANIZATION. D.K. Lee, S.L.B. McGillis, and J.D. Greenspan*. Dept. of Neurosurgery, SUNY Health Science Center, Syracuse, NY 13210, USA.

Fourteen healthy subjects (4 males, 10 females) were asked to localize a thermal stimulus applied to their left distal forearm. For these experiments, two thermal stimulating probes (1.2cm diameter each) were strapped to the forearm, separated by approximately 8cm. Each trial consisted of a tone, followed by a temperature change at one of the probes. The subject then indicated at which of the two probes he/she felt the temperature change. Stimulators were oriented in one of three ways: 1) longitudinally within the C6 dermatome, 2) longitudinally within the region overlapping the C8/T1 dermatomes, and 3) transversely with one probe on the C6 and one probe on the C8/T1 dermatomes. Every day, all orientations were used, in different orders on different days. For each orientation, one block of 20 trials provided 10 stimuli at either probe. For six days, warming stimuli were used (change from 35° to 40°C), and for three days, cooling stimuli were used (change from 32° to 27°C).

For any given stimulus orientation, cooling stimuli were localized significantly better than warming stimuli. For both warming and cooling, the transversely oriented probes (across dermatomes) provided significantly better localization than the longitudinally oriented probes. Thus, the anisotropy that is well described for tactile spatial acuity is also present for thermal localization. These results suggest that cutaneous spatial information is integrated similarly for both tactile and thermal stimuli, but differently within vs. across dermatomes.

564.6

TONGUE ADAPTATION TEMPERATURE INFLUENCES THE DYNAMIC AND STATIC RESPONSES OF LINGUAL NERVE FIBERS TO THERMAL STIMULATION Robert F. Lundy Jr* and Robert J. Contreras, The Florida State University, Program in Neuroscience, Tallahassee, FL., 32306-1051.

Previous studies have investigated the response characteristics of thermal-sensitive neurons to small temperature changes at varying adaptation temperatures in the cutaneous skin of many species including humans. The present study repeated as well as extended this line of investigation to the oral cavity. Phase 1 examined the effects of tongue adaptation temperature (35 vs. 25 °C) on the neural responses of 32 lingual nerve fibers to: (1) a 1 °C/s temperature decrease until reaching a plateau of 10 °C, and (2) menthol stimulation for 40 s. In Phase 1, two groups of fibers were identified on the basis of response latency, peak sensitivity, and range of sensitivity after adaptation to 35 °C water. In addition, the effect of menthol in the oral cavity is highly dependent upon adaptation temperature. All menthol concentrations were effective when adaptation and stimulus temperature was 35 °C. Phase 2 examined the response of 8 thermal-sensitive fibers to a 2 °C temperature decrease (at 1 °C/s) after adaptation to several different tongue temperatures. Similar to other cutaneous areas, the mean change in response rate of thermal-sensitive neurons as a function of adaptation temperature resembled a bell shaped curve with a maximal discharge at approximately 29 °C. In the present study, the change in response rate seemed to be linearly related to baseline rate since large response rates were associated with high baseline activity.

SOMATIC AND VISCERAL AFFERENTS: ANATOMY AND PHYSIOLOGY OF MECHANORECEPTION

565.1

SYNAPTIC CONTACTS BETWEEN JAW-MUSCLE SPINDLE AFFERENTS AND TRIGEMINOTHALAMIC NEURONS: A COMBINED RETROGRADE HRP AND INTRACELLULAR BIOTINAMIDE STUDY. Pifu Luo and Dean Dessem*, U. Maryland Dental Sch., Baltimore, MD 21201

Trigeminothalamic neurons were identified by retrograde HRP labeling from the VPM of the thalamus in rats. HRP-labeled neurons were found contralaterally in the supratrigeminal region (Vsup), the trigeminal principal sensory nucleus, the ventrolateral part of the trigeminal subnucleus oralis, the spinal trigeminal subnuclei interpolaris and caudalis, the reticular formation and an area ventral to the trigeminal motor nucleus (Vmo) and medial to the trigeminal principal sensory nucleus (AVM). Jaw-muscle spindle afferents were physiologically identified and intracellularly injected with biotinamide. Axon collaterals and boutons from jaw-muscle spindle afferents were found in: Vmo; Vsup; the dorsomedial part of the trigeminal principal sensory nucleus (Vpdm); the dorsomedial part of the spinal trigeminal subnuclei oralis, interpolaris (Vidm) and caudalis; the parvicellular reticular formation (PCRt) and the mesencephalic trigeminal nucleus. Trigeminothalamic neurons in Vsup, Vpdm, Vidm, PCRt and AVM were associated with boutons from intracellularly-stained spindle afferents. Trigeminothalamic neurons in Vsup, Vpdm, Vidm, and PCRt were closely apposed by up to 14 intracellularly-labeled boutons from jaw-muscle spindle afferents suggesting a powerful input to some trigeminothalamic neurons. Ultrastructural examination of Vpdm and the caudolateral part of Vsup demonstrate that the dendrites and somata of retrogradely labeled trigeminothalamic neurons are contacted by intracellularly labelled boutons in addition to unlabeled type "S" and "F" boutons. These data demonstrate that muscle spindle sensory feedback projects to the contralateral thalamus via multiple regions of the trigeminal system. Supported by NIH DE10132.

565.2

A CIRCUIT MODEL FOR WHISKER RECEPTIVE FIELDS IN NUCLEUS PRINCIPALIS. M.F. Jacquin*, J.J.A. Arends, P.J. Shortland, D.W. Doherty, J.A. DeMaro & T.A. Woolsey. Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis MO 63104; Neurology & Neurological Surgery, Washington Univ. Sch. Med., St. Louis MO 63110.

There is a paucity of data on the brainstem link in the whisker-barrel neuraxis: trigeminal nucleus principalis (PrV). A circuit model has been developed to guide research aimed at explaining PrV cell responses. We view the primary afferent inputs to PrV, and the dendrites in PrV, as a complementary set of somatotopic and overlapping columns. Regions of overlap are not revealed by histochemical or glucose uptake methods due to their low sensitivity or processing that suppresses transmission at border zones. Available data suggest that: adjacent whisker primary afferents interdigitate in PrV's transverse plane, dendrites from thalamic-projecting and GABAergic cells in PrV span multiple columns, spinal V and cortical inputs to PrV are point-to-point, and most PrV cells have 1-whisker receptive fields. The model must then include local and higher-order modulatory mechanisms. One possible scenario is that the B1 whisker receptive field, say, is produced in the B1 column by neighboring (e.g. B2) whisker activation of GABAergic cells in the B2 column via primary afferent, spinal V and cortico-V projections, which then inhibit transmission of B2 primary afferent inputs to more distal B1 dendrites extending into the B2 column. Spinal V and cortical inputs to the B1 column end on more proximal dendrites to amplify responses to the B1 whisker. Many predictions follow; for example: cells in the B1 column should receive synaptic inputs from B1 and B2 primary afferents, as well as from spinal V's B1 column via local circuit axons, and from the B1 barrel via the cortico-V pathway. GABA cells in PrV's B2 column should terminate on distal dendrites of B1 cells and receive inputs from B2 primary afferents, as well as from spinal V's B2 column and from the B2 barrel. Support: NIH DE07662, DE07734, NS17763, NS29885.

565.3

QUANTITATIVE ANALYSES OF WHISKER AND NON-WHISKER PRIMARY AFFERENTS STAINED IN THEIR ENTIRETY. P.J. Shortland*, P.M.E. Waite, J.A. DeMaro & M.F. Jacquin. Anatomy, St. Louis Univ., St. Louis MO 63104; Anat., Univ. New South Wales, Sydney 2033 Australia; Neurol., Washington Univ. Sch. Med., St. Louis MO 63110.

Prior studies suggest that some categories of A-β primary afferents have distinct morphologies in the brainstem, and that single fibers give rise to collaterals with different shapes in different trigeminal subnuclei. However, conclusions are based on relatively few and incompletely stained fibers and limited statistical rigor. Here, 86 fibers were stained with Neurobiotin in rats to provide repeated within-animal inter-subnucleus comparisons, as well as between-animal intra-subnucleus comparisons, of numeric features of collaterals associated with a vibrissa, guard hairs, hairy skin or oral structures. Qualitative features were as described before. Multiple analysis of variance and post-hoc group comparisons (Scheffe´) indicated that fibers differed between subnuclei on 3 measures: collaterals were most numerous in interpolaris, arbors were largest in caudalis, and arbors were less circular in caudalis than in oralis and principalis. When subnucleus and peripheral receptor association were compared, the only reliable difference pertained to interpolaris, where vibrissa fibers gave rise to more collaterals than guard hair or oral afferents. There were no indications that a particular fiber category was disproportionately represented at particular sites along the rostrocaudal axis, although most fibers adhered to an onion-leaf topography in caudalis. Moreover, when analyses were restricted to individual subnuclei, no differences were revealed between fiber categories. Peripheral receptor association and adaptation rate failed to predict arbor dimensions, arbor circularity and bouton number in every subnucleus. We confirm that arbor location is predicted by receptive field location, but that collateral number and structure are predicted only to a limited extent by subnucleus and never by peripheral receptor. Support: DE07662, DE07734, NS29885, NH, MRC.

565.4

THE POSSIBLE INPUTS OF GABA-IMMUNOREACTIVE NEURONS IN THE CUNEATE NUCLEUS OF THE RAT. C.Y. Wen*, J.H. Lue, K.N. Chen and J.Y. Shieh. Dept. of Anat., College of Med., Nat'l Taiwan Univ., R.O.C.

After chloral hydrate anaesthesia, the rat were injected 0.2ul of 2% of wheat germ agglutinin conjugated with horseradish peroxidase (Sigma, WGA-HRP) into the forelimb area of the primary somatosensory cortex in six rats or into the brachial plexus in another six rats to label respectively the corticocuneate terminals (CCTs) and the primary afferent terminals (PATs). 24 to 36 h survival, the animals were perfusion fixed with a mixture of aldehydes (containing 0.5% glutaraldehyde and 4 % paraformaldehyde in 0.5M phosphate buffer) under slightly overdose of chloral hydrate. Vibratome sections of the cuneate region were cut and incubated with anti-GABA antiserum (Incstar) to label the GABA-immunoreactive (GABA-IR) neurons. Some of ultrathin sections, which were proved to have definite synapse between PAT and GABA-IR terminals, with anti-glutamate antiserum (Sigma). The results showed that: (1) the labeled CCTs made predominantly contact with dendrites of the GABA immuno-negative neurons. On the other hand, the PATs made direct contact to the profiles of the GABA-IR neurons, (2) some GABA-IR boutons were found to form axosomatic and axodendritic synapse other GABA-IR neurons, (3) triple labeling of the same sections for the GABA, the PAT and the glutamate-IR terminals revealed that PATs, which were glutamate positive, were presynaptic to the dendrites of GABA-IR neurons, while a few none-PAT glutamate-IR terminals, which were unlabeled by WGA-HRP, synapsed with the dendrites and the somata of the GABA-IR neurons. (Supported by NSC-80-0412-B-002-157, R.O.C.).

565.5

MECHANISTIC MODELS FOR TACTILE NEURAL CODING OF SHAPE.
M. A. Srinivasan* and K. Dandekar, Dept. of Mechanical Engineering and Research Lab. of Electronics, MIT, Cambridge, MA 02139.

The relationship between the biomechanics of primate fingertip and the responses of slowly adapting mechanoreceptors (SA-I) in the tactile sensing of object shape was investigated. Finite element analysis was performed on a progressively realistic series of mechanistic models of the primate fingertip under a variety of shape stimuli. The external shape of the models ranged from a circular cylinder of about the same diameter as typical primate fingertips to realistic 3-dimensional fingertips. The latter were generated from video images of accurate epoxy copies of monkey and human fingertips at various known orientations, obtained by using a videomicroscopy system. The internal geometry consisted of a central rigid bone enclosed by either a homogeneous or a layered material. The stresses, strains and deformations calculated from the models were correlated with data from both biomechanical experiments on fingertip deformations and neurophysiological experiments on the responses of SA-Is.

In all the models, the strain energy density and the maximum compressive strain were the leading contenders to be the *relevant stimulus* for SA-I. Because the models took into account the curvature and finiteness of the primate finger, the predictions of SA-I population responses to rectangular gratings indented into the fingerpad were more realistic than the predictions from previously proposed flat, semi-infinite model (Phillips and Johnson, J. Neurophys., 46(6), 1981). Although these population responses were radically different from the previous predictions, the spatial response profiles matched the neural data as well as before. In addition, the new models predicted that the spatial response profile of a receptor was strongly dependent on its location with respect to the center of contact region on the fingerpad. Analysis of the nonlinear mechanics of contact between the models and cylindrical indentors of various radii demonstrated good correlation between strain measures at the receptor site and corresponding neural response. (Supported by NIH Grant DC00625 and ONR URI Grant N00014-92-J-1814)

565.6

RESPONSES OF CUTANEOUS MECHANORECEPTOR AFFERENTS TO STEP AND SINUSOIDAL SKIN DISPLACEMENTS.
J. C. Makous*[1] and S. J. Bolanowski[1,2]. [1]Institute for Sensory Research and [2]Department of Bioengineering and Neuroscience, Syracuse University, Syracuse, NY 13244-5290

Neural activity was recorded from more than 100 nerve fibers innervating the glabrous skin of the cat hind-paw. Action potentials from individual nerve fibers of the medial plantar nerve were monitored in response to sinusoidal displacements of the skin ranging in frequency from 1 to 500 Hz and in amplitude from threshold to 250 μm. The same fibers were also characterized in response to step indentations (2 ms rise-time) of the skin over a similar range of intensities. In addition, receptive fields were mapped with von Frey hairs, and conduction velocities were measured. Fibers were characterized on the basis of these responses and were classified as slowly adapting (SAI, SAII), rapidly adapting (RA) or Pacinian corpuscle (PC) afferents. Vibratory stimulation revealed frequency characteristics of the RA and SA fibers that often spanned the range of 1 to 200 Hz. The PC fibers were typically tuned with a peak sensitivity near 250 Hz. The SA fibers showed a transient and a sustained response to steps, whereas the RA and PC fibers showed only transient responses to steps. Peri-stimulus-time histograms revealed that, at the highest amplitudes of stimulation (> 25 dB above threshold), the step-responses showed periodicity of firing for the majority of fibers studied. Interval histograms demonstrated that the periodicity ranged from about 3 to 35 ms depending upon the fiber type. The PC and RA fibers tended to have shorter inter-spike intervals in response to the steps than the SA fibers. Supported by NINCDS RO1-DC00380.

565.7

A VARIABLE THRESHOLD MECHANORECEPTOR MODEL.
F. J. Looft*, S.S. Hsiao[1], and K.O. Johnson[1]. *Dept. of Elec. and Comp. Eng., Worcester Polytechnic Institute, Worcester, MA 01609 and [1]Krieger Mind/Brain Inst. and Dept of Neurosciences, Johns Hopkins University, Baltimore, MD 21205.

The responses of primary mechanoreceptive (SA, RA and PC) afferents in macaque monkeys were studied using a sinusoidal vibratory stimulus and a two-pulse condition-test paradigm (CT). The CT experiments consisted of presenting either a mechanical or electrical conditioning pulse followed by a mechanical test pulse. For sub-threshold conditioning pulses and CT delays 2msec and greater, the mechanical test pulse amplitude required to elicit an action potential was essentially invariant - a result that would occur if there was no residual membrane depolarization. By comparison, the use of a supra-threshold conditioning pulse required a significantly increased test pulse amplitude to generate an action potential. Supra-threshold CT experiments showed that threshold recovery following an action potential is closely fit by a two-exponent decay function that has fast (1-5msec) and slow (15-40msec) time constants. These results suggest that a previous mechanoreceptive model [J. Physiol. 323:43-64, 1982] was flawed since these authors had assumed that threshold is reset immediately following an action potential and that there is a substantial residual membrane depolarization following sub-threshold stimuli. A revised mechanoreceptive model in which threshold recovery was modeled by an exponential decay resulted in significant improvements in fits to the responses of mechanoreceptive afferents to sinusoidal stimuli. In addition to effectively modeling the statistical regularity of the impulse trains, the variable threshold model also properly predicts the initial increase followed by a subsequent decrease in impulse phase retardation with increasing stimulus frequency. Finally, the model predicts that the neural responses are only slightly affected by noise bandwidth or absolute refractory period and are moderately sensitive to the shape of the generator potential.

565.8

ADAPTATION OF MECHANORECEPTIVE AFFERENTS TO CONTINUOUS SINUSOIDAL VIBRATION.
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The effects of stimulus duration, amplitude and frequency of an adapting sinusoidal stimulus on thresholds of rapidly adapting (RA), slowly adapting (SA) and Pacinian (PC) mechanoreceptive afferents were studied. Recordings were made from median and ulnar nerves of 3 anaesthetized monkeys while vibration was delivered via a 1 mm diameter probe glued to the skin. Experimental trials consisted of an adaptation period containing the adapting vibratory stimulus and a recovery period in which no adapting stimulus is applied. During both periods, a sinusoidal test stimulus was presented once every 4 secs to track changes in absolute and entrainment thresholds. This testing rate was determined to have minimal effects on thresholds. The amplitude of the test stimulus was systematically varied between tests to evoke between 0 and 1 impulse per stimulus cycle for the duration of each test stimulus. Adapting and test stimulus frequencies of 10, 30, and 60 Hz were used for SAs; 30, 60 and 100 Hz for RAs; and 60, 100, and 300 Hz for PCs for a total of 9 combinations of test and adapting frequencies for each afferent type. Amplitudes of the adapting stimulus ranged from 2 to 30 times absolute threshold and were applied for durations of 1, 2, 4 or 8 min.

All afferents (6 SAs, 8 RAs, and 5 PCs) exhibit increases in thresholds with increases in either the adapting stimulus amplitude or frequency: there is about a 1 to 10 elevation in threshold over the range of frequencies and amplitudes studied. SA and RA thresholds increase and recover rapidly (τ=10-30 sec) during both the adapting and recovery periods with stimulus durations greater than 1 min having no effect on the time constants nor on the level of adaptation. PC afferents adapt slowly (τ>5 min) with the thresholds of some PCs still increasing after 8 min of stimulation. The PCs exhibit variable recovery time constants (30 sec to 6 min) and often have a small residual elevation in thresholds 50 min after the adapting stimulus was discontinued.

565.9

ACTIVITY-DEPENDENT CHANGES IN CONDUCTION LATENCY: A PROBE OF MODAL SPECIFICITY OF MECHANOSENSITIVE AFFERENTS IN RAT.
S.S. Waikar, J.G. Thalhammer*, S.A. Raymond, D.S. Chang, and G.R. Strichartz. Anesthesia Research Laboratories, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115.

Impulse activity in axons generates aftereffects on membrane excitability that can alter the conduction velocity of subsequently conducted impulses. We used computerized stimulus patterns to assess *in vivo* activity-dependent changes in conduction latency of functionally identified rat cutaneous afferents from the posterior tibial branch of the sciatic nerve. We measured several different parameters of activity dependence: super- and subnormality, a decrease or increase in conduction latency following conditioning with a single preceding impulse; depression, an increase in conduction latency during tetani; and recovery, the time to return to baseline conduction latency following these tetani. Taken together, these parameters generate a "profile" of the activity dependence of a fiber's conduction latency. We have shown previously that such profiles of nociceptor and cold receptor afferents correlate with modality; our current work extends this investigation to mechanosensitive afferents. We wished to determine whether diameter-matched afferents (inferred from matched resting conduction velocity) of two different types of functionally identified mechanoreceptors differed in activity dependence of conduction latency.

Axons of Pacinian corpuscles (PC; n=3) and rapid adapting mechanoreceptors (RA; n=6) of the glabrous skin were examined for activity-dependent changes in conduction latency using a standardized stimulus protocol. Five of the six isolated RAs exhibited subnormality, i.e., the second of two impulses initiated within 5 msec of another conducted less rapidly than the first. By contrast, only one PC exhibited subnormality. PC and RA latency changes differed significantly during 20 separated bursts of 10 stimuli at 200 Hz in the mean normalized difference between the first and second impulse latency: in all three PCs the latency of the second impulse of the burst was supernormal, while in six of seven RAs the latency was subnormal ($p<.05$). Our data suggest that differences in function among mechanoreceptors in the glabrous skin may extend into their axons' conduction properties. (NIH USPHS GM 35647)

565.10

TACTILE SIGNALS GENERATED BY A TOOL USED TO DISCRIMINATE THE SOFTNESS OF OBJECTS.
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Subjects were 90-100% correct in ranking the softness of 9 rubber disks that differed in compliance (1.8 to 10.9 um/g) either by indenting each specimen with the fingerpad, or by tapping each with a stylus held between two fingers. In order to study the role of tactile signals, the back of a finger was rigidly mounted to a load cell. One end of the stylus indented the volar distal pad with a base force of 20 g-wt, maintained by a servocontrolled motor, and the other end tapped with a specimen held by the experimenter. Subjects were able to discriminate between hard ($\leq$1.8 um/g-wt) vs. soft ($\geq$7.7) specimens and a "medium soft" standard of 4.0 um/g-wt when the velocity of tapping was held approximately constant. The perceptions of softness felt "natural" as did a tap-evoked sensation produced when a previously recorded force trace from a specimen was "played back" through the motor. Subjects were unable to discern whether a "live" or a "played-back" trace was delivered. It was found that perceived softness depended primarily on the slope of the initial segment of the rising phase of the force trace which, for hard specimens, changed little with moderate variations in tapping force and velocity in comparison with that for softer specimens. The perceived softness of softer specimens decreased with increasing tap velocity suggesting that, for these specimens, passive touch without independent information about velocity may not be sufficient for reliable discriminations of softness. It is hypothesized that the greater the initial force rate, the more likely that rapidly-adapting as opposed to slowly-adapting mechanoreceptors will be engaged and convey the tactile signals associated with the perceived softness of objects. (Supported by ONR URI grant# N00014-92-J-1814)

565.11

INTERLAMELLAR FLUID DYNAMICS OF THE PACINIAN CORPUSCLE (PC). B. Pietras* and S.J. Bolanowski. Institute for Sensory Research and Bioengineering and Neuroscience, Syracuse University, Syracuse, NY 13244.

Receptor potentials recorded from isolated PCs in response to sinusoidal vibrations have been shown to have non-linear asymmetrical input-output functions and U-shaped frequency characteristics. The PC's accessory capsule was studied both experi-mentally and theoretically to determine its mechanical contribution to the observed non-linear receptor potential. During compression of the accessory capsule, a shear stress is created and inter-lamellar fluid flows with velocity components orthogonal to the direction of displacement. The viscosity of the fluid imparts a resistance to the shear stress which arises as a force parallel to the compression. Inter-lamellar fluid is considered to be incompressible and, at the velocities considered here, to have a resistive force proportional to its velocity. Measurements of reactive force in response to sinusoidal displacements have shown that the reactive force is linear and increases with increasing frequency and amplitude. Furthermore, the reactive force can be described by a first-order linear differential equation of the form $f(x,t) = kx + c\,dx/dt$. A series of first-order linear differential equations was used to compute the inter-lamellar fluid velocities and lamellar displacements for a range of frequencies, stimulus amplitudes, and probe sizes. Computational simulations of the inter-lamellar fluid flow in response to sinusoidal displacement of the surface of the capsule shows that the multi-layered structure linearizes the inter-lamellar fluid velocity, thus linearizing the inter-lamellar compressive force. Video analysis of PCs subjected to a static stress shows that the stress load is carried by the outermost lamellae. Indeed, it is only during a highly time-dependent displacement of the surface (frequencies above 40 Hz) that a strain is observed below the outermost lamellae. Video analysis and computational simulations also show that the multi-layered capsule acts as a mechanical high-pass filter. Furthermore, the compressive strain and reactive forces increase with stimulus frequency. Thus the non-linear asymmetrical input-output function and the U-shaped frequency response cannot be mechanical in origin and must arise from intrinsic neural properties of the neurite. Work supported by NSF, IBN-9211561.

565.12

STRETCH SENSITIVITY OF SA2 MECHANORECEPTORS IN ISOLATED RAT SKIN Peter Grigg * Department of Physiology University of Massachusetts Medical School, Worcester MA 01655

SA2 mechanoreceptors were recorded in a preparation of skin (appx. 20 mm square) isolated from the hindlimb of rats and studied in vitro. Responses of afferents were recorded from filaments of the femoral nerve. The skin was subjected to pure in-plane stretching, by pulling on 12 tabs that were cut along its edges (3 tabs / side). Each tab was coupled to a miniature tensile load cell, and was actuated with a miniature linear actuator. Loads measured at the tabs were used to calculate tensile and shear stresses in the skin. Deformations of the skin were measured by optically tracking a set of opaque markers that were fixed to the skin surface. While there were many afferents with rapidly adapting responses to in-plane skin stretching, relatively few SA2s were observed in these preparations. The SA2s were readily activated by stretching the skin. Their responses were strongly determined by uniaxial stresses or strains. There was no strong relationship with shear stresses or strains. Biaxial stresses and strains were ineffective stimuli. Supported by NIH grant NS-10783

565.13

A SECOND SYSTEM FOR INNOCOUS MECHANORECEPTION IN THE HUMAN SKIN. H. Olausson, N. Kakuda, J. Wessberg and Å.B. Vallbo. (SPON: European Neuroscience Association). Department of Physiology, Göteborg University, Medicinaregatan 11, S-413 90 Göteborg, Sweden.

In various mammals many unmyelinated (C) afferents respond to light touch. In man on the other hand, C-fibers with sensory functions have been identified almost exclusively with the senses of pain and temperature. For that reason it has been suggested that an older and slowly conducting system for innocous mechanoreception has faded away during the evolution.

Using the microneurography technique we have now found low threshold mechanoreceptors with conduction velocities within the unmyelinated region to be frequent in the hairy skin of the human forearm. The impulse rate associated with clearly innocous mechanical stimulus amounted to 100 imp/s, leaving no doubt that light touch was the adequate stimulus. The thresholds to v Frey hairs ranged between 0.01 and 8 g with a median value of 0.25 g. The conduction velocity assessed using electrical or mechanical stimulation ranged between 0.7 and 1.0 m/s. For some units the receptive fields were explored in detail using a scanning method. The fields were found to be fairly large (10 mm x 5 mm) and composed of multiple areas of different sensitivity.

Our study showed that low threshold mechanoreceptors are common in the hairy skin of the human forearm. It suggests that the human body is provided with a widespread system for innocous mechanoreception subserved by C-fibers.

565.14

A NOVEL METHOD FOR SELECTIVE ELECTRICAL STIMULATION OF SMALL DIAMETER NERVE FIBERS. J.C. Petruska* and R.D. Johnson. Departments of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

There is a good deal of evidence that the small diameter nerve fibers in peripheral nerves, the thinly myelinated A-delta fibers and unmyelinated C fibers, display a high degree of central and peripheral plasticity in response to injury to the nervous system. The electrophysiological investigation of specific spinal cord input from these fibers is difficult because standard electrical stimuli recruit large myelinated fibers before small fibers. We have designed the following method for selectively stimulating small fibers.

In urethane-anesthetized rats, the caudal cutaneous sural and sciatic nerves were surgically isolated. A lumbar laminectomy was performed to isolate dorsal root filaments for recording. A bipolar Ag/AgCl electrode was placed around the sural nerve for stimulation of various fiber groups. Specially fabricated Ag/AgCl ring electrodes producing differential current densities were placed around the sciatic nerve. Using an arbitrary-waveform constant-current stimulus isolator and a modified triangular stimulus waveform, an anodal polarization block of sural-activated large A fibers was achieved at the sciatic site, allowing only the smallest A-delta and/or C fibers to conduct through to the dorsal root filaments.

This method could be used to study the input from small fibers into the spinal cord and/or the peripheral target tissue without the contamination by large myelinated fibers. Supported by NS 27511.

565.15

INHERENT FIRING PATTERNS OF MUSCLE AFFERENT AXON TERMINALS ARE GRADUALLY LOST IN CHRONIC NEUROMAS. J.B. Munson* and R.D. Johnson. Depts. of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

We have previously shown that following transection and cross-regeneration of cat medial gastrocnemius (MG) muscle nerve into the sural nerve skin territory, large diameter muscle afferent fibers retained their stretch-sensitive and slowly-adapting firing patterns despite the presence of a foreign target devoid of muscle receptors. We have also shown that in short-term 6-day neuromas, even the sprouts of axotomized muscle afferents retained these native firing patterns. Since the characteristic patterns were evident for at least 2 years in skin, we designed the present study to determine if these firing patterns could be obtained from muscle afferent sprouts in long-term neuromas.

In adult cats, the MG nerve was transected and the proximal end sutured into a blind-ending Gore-Tex sleeve. Following a recovery period of 3-16 months, the mechanosensitivity of sprouted afferents was tested by recording from dorsal root filaments while mechanically stimulating the neuroma. Compared to afferents from 6-day neuromas, afferents from long-term neuromas were significantly more difficult to mechanically activate and showed a progressive decrease in conduction velocity, stretch-sensitivity, and slow-adaptation properties.

We suggest that the rescue of these physiological properties may be dependent on trophic factors present in peripheral targets such as muscle and skin but absent in chronic neuromas. Supported by NS15913 and NS27511.

566.1

SHORT-TERM CHANGES IN EFFICACY OF CORTICOCORTICAL EPSPs IN RACCOON PRIMARY SOMATOSENSORY CORTEX. P. Zarzecki* and P. Istvan. MRC Group in Sensory-Motor Physiology, Depart. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Neurons in the cortical representation of glabrous skin of raccoon digits receive input from their "on-focus" digit by direct thalamocortical connections. Weaker and less common inputs from "off-focus" digits are probably relayed by a corticocortical pathway from the heterogenous zone a few mm rostral to the glabrous zone. "Off-focus" inputs quickly become more effective during short-term cortical plasticity, a change that could come about from altered function of corticocortical synapses.

Our purpose was to test for short-term plasticity in the corticocortical pathway from the heterogeneous zone to the glabrous zone. Neurons were recorded intracellularly in the representation of glabrous skin in primary somatosensory cortex of raccoons anesthetized with Nembutal. Short latency corticocortical EPSPs were recorded following electrical stimulation (5 Hz) of the heterogenous zone. Corticocortical synaptic responses were paired with simultaneous depolarizing current steps (up to 3 nA, 100 ms duration) injected intracellularly. Pairings of EPSPs with depolarizing current steps (50 pairings at 5 Hz) caused enhancement of corticocortical EPSPs lasting beyond the period of pairing. Enhanced EPSPs that were subthreshold before pairing usually evoked spike discharges after pairing. The effect was short-term (1 to 2 min), reversible, and reproducible in repeated pairing cycles. Thus, corticocortical EPSPs that could be relaying "off-focus" somatosensory inputs can be enhanced by occurring during depolarization of the target neuron by another input. Remaining questions are whether somatosensory EPSPs are also enhanced after corticocortical EPSPs are paired with current steps, and whether any such effects on somatosensory responses are restricted to "off-focus" inputs.

Supported by the Medical Research Council of Canada.

566.2

QUANTITATIVE ANALYSIS OF DEVELOPING INTRINSIC CONNECTIONS IN THE CAT SOMATOSENSORY CORTEX. R.V. Sonty* and S.L. Juliano. Depts of Anatomy & Neuroscience, USUHS, Bethesda, MD 20814.

Clustered intrinsic connections in the cat somatosensory cortex (SSC) develop from an initially diffuse network in the early postnatal period. Although the shift from relative homogeneity to intermittent clusters can be observed visually, the transition is not easy to define. As a result we found it important to quantify the developmental sequence of events to more clearly identify the changes that occur in intrinsic cortical development. To do this, confocal images were obtained from coronal or horizontal sections taken through DiI-labeled kitten SSC at various postnatal ages. Several statistical tests were conducted in order to analyze the clustering of retrogradely labeled neurons and the distribution of tangential fibers emanating from the injection site. For some tests, individual sections were drawn to indicate labeled neurons. We analyzed neuronal clustering using spatial point process analysis. The null hypotheses stated that the distribution of labeled neurons at each age, did not differ from CSR (complete spatial randomness). Quadrat counts showed a change from a random distribution on PND3 ($p=0.786$) to a clustered one on PND6 ($p=0.001$). A refined nearest neighbor analysis confirmed conversion from a random distribution on PND3 ($p=0.43$) to a clustered distribution on PND6 ($p<0.01$). Other tests (dispersion indices, density recovery profiles, etc.) verified the transformation of a diffuse organization of neurons to a clustered one. Coronal sections on PND15 confirmed a patchy distribution of intrinsic connections resembling the adult distribution. The numbers of labeled neurons were significantly higher in clusters than in the intercluster zones ($p<0.001$) on PND6. Profile plots (reflecting axonal distributions) revealed a significant periodicity (center-to-center ~ 750μm) on PND6. We demonstrate that the study of local circuit development in the cerebral cortex is amenable to formal statistical analyses. Support: PHS NS24014.

566.3

SEQUENCE OF EVENTS IN THE DEVELOPMENT OF LAMINATION, INTRINSIC AND EXTRINSIC CONNECTIONS IN FERRET SOMATOSENSORY CORTEX. S.L. Juliano*, R.V. Sonty, S. Palmer, P. Awenowicz. Depts. of Anatomy & Neuroscience, USUHS, Bethesda, MD.

Despite the relative immaturity of ferret nervous system at birth, we previously demonstrated that axons of thalamic neurons reach the somatosensory cortex by PND 1, and are organized into distinct clusters by PND 5. Horizontal connections, in contrast, do not display a clustered organization until PND 28. To more precisely define the development of intrinsic and extrinsic connections in relation to laminar development, we used slices of ferret brains aged PND 1-28. After injections of fluoroscein-labeled dextran the slices were maintained in an oxygentated chamber to allow for transport of the tracer. On PND 1-3, injections into the deeper layers of cortex, or subcortical white matter, led to a slim radial column of labeled cells that extended from the upper portion of the cortical plate to the base of the ventricular zone. The labeled radial column contained both radial glia and neurons migrating to their proper positions within the neocortex. The cells within the column did not extend axons into the cortex, although horizontally-running fibers were seen beneath layer 6. In contrast, injections into the superficial cortical plate led to labeled neurons that extended their axons tangentially. Comparison with the locations of cells labeled with BRDU and born from E22-E38, indicate that neurons destined to occupy all cortical layers are present in the cortex by PND 1. At PND 1, injections into the white matter labeled thalamocortical axons that terminated in crude clusters within the neocortex. With increasing age, the deeper injections lose their radial character and by PND 14 many axons extend into all cortical layers. After injections into more superficial cortical sites, a continuous band of labeled cells can be seen in layers 2-3. By PND 28, injections into the upper layers results in discrete clusters of labeled cells in layers 2-3. Supported by NS24014.

566.4

DYNAMIC PATTERNS IN COLUMNAR DISTRIBUTION OF SYNAPTIC ZINC IN DEVELOPING RAT SOMATOSENSORY CORTEX. R.H. Dyck*, N.L. Ruff, and D.D.M. O'Leary. Molecular Neurobiology Lab, The Salk Institute, La Jolla, CA 92037.

A subset of excitatory synapses in the mammalian telencephalon contain zinc within synaptic vesicles of their terminal boutons. In this study we describe the normal distribution of zinc in the somatosensory cortex of rats during postnatal development.

In the adult rat, specific staining for zinc is lowest in lamina 4 but demarcates the barrel pattern by staining at highest levels in the barrel septa. This pattern of zinc staining gradually arises during postnatal development as a result of complex tangential and laminar redistributions during the first 4 weeks. Zinc staining in somatosensory cortex was minimal at birth (P0). From P0 to P4, levels of zinc gradually increased across cortical laminae in a deep to superficial gradient, but a barrel-related pattern was not evident. Subsequently, the increase in the density of zinc staining within layer 4 was heterogeneous across its tangential extent. By P7 the highest levels of zinc clearly demarcated the barrel fields, but by heavily staining the barrel hollow and lightly staining the barrel septa. During the second and third weeks, zinc staining was reduced within barrel hollows with a distinct spatiotemporal pattern. For example, an adult-like pattern of zinc staining was attained by P12 in the posteromedial barrel subfield but not until P21 in the anterolateral division.

Thus, the distribution of excitatory inputs, defined by synaptic zinc, undergoes distinct spatiotemporal changes during the first 3 postnatal weeks. This process may reflect a differential maturation of the functional organization within barrel cortex.

566.5

FACTORS THAT INFLUENCE NEURONAL RESPONSE VARIABILITY IN SOMATOSENSORY CORTEX: THE ROLE OF NMDA RECEPTORS. D. Prince, B. Whitsel, M. Tommerdahl, O. Favorov. Departments of Biomedical Engineering and Physiology, University of North Carolina, Chapel Hill, NC 27599.

Potentials evoked in rat sensorimotor cortical slices by single-site stimulation of the white matter were examined (i) to determine the effects of stimulus frequency and intensity on intertrial response variability (hereafter referred to as response variability) in SI neurons, and (ii) to evaluate the prediction that response variability is an indicator of the degree of cortical NMDA receptor activation (Lee and Whitsel, 1992). Evoked potentials (EP's) were recorded for 50 to 100 consecutive stimulus presentations at a given stimulus frequency and intensity (frequencies between 0.2 - 5 Hz and intensities between 1.0 - 3.5 times the minimum intensity required to evoke a response). Magnitude of the EP for each trial was determined by integrating the EP over the duration of its post-synaptic component; response variability was calculated as the coefficient of variation of the EP magnitude for all responses recorded for each stimulus condition. To ascertain the contribution of the NMDA receptor population to response variability, some experiments were performed following application of ketamine, a selective NMDA receptor antagonist. A direct relationship was found between response variability and stimulus intensity. As intensity was increased, variability decreased linearly, and the slope increased over the range 0.2 to 5 Hz. The relationship between response variability and stimulus intensity and the effects of stimulus frequency on this relationship were observed more clearly at more superficial sites in SI cortex (those containing the highest density of NMDA receptors). Moreover, blocking NMDA receptors with a selective antagonist decreased the dependency of response variability on stimulus intensity in a dose-dependent manner. This work was supported in part by NIMH RO1 MH48654 and the Whitehall Foundation.

566.6

NMDA RECEPTOR EXPRESSION IN THE RAT TRIGEMINAL PATHWAY TO BARREL FIELD CORTEX DURING DEVELOPMENT. V. Rema* and F.F. Ebner. Institute for Developmental Neuroscience, Kennedy Center, Vanderbilt University, Nashville, TN 37203

Polyclonal antibodies against the C-terminal and N-terminal domains of the NMDAR1 subunit were produced to analyze changes in expression of NMDA receptors during normal postnatal development of the trigeminal pathway relays to rat barrel field cortex. At P-0 there is already substantial expression of NMDAR1 subunit in the trigeminal (V) complex, the VPM, POm + intralaminar thalamic nuclei, and in barrel field cortex. From birth to P-180 there is relatively high level of NMDAR1 expression in the V complex, with slight variations in the intensity of staining detectable as a peak at P-30 and a gradual slight reduction from P-30 to P-180. Around P-40 a single row of intensely immunoreactive cells is noteworthy along the line between the spinal V tract and nucleus. The VPM nucleus is uniquely outlined by its intense staining from P-3 to P-40 when it blends with the moderate subunit density seen throughout the mature thalamus. POm has a more gradual onset to peak densities at P-21 that are always less intense than VPM. Intra-laminar nuclei, especially CL, peak even later at P-35, retaining scattered cells that express high levels of NMDAR1 for up to 12 months. Overall staining intensity in the barrel cortex increases from P-3 to P-21, when staining apprears more diffuse due to a transient expression of NMDAR1 by astrocytes, which appear vividly in fiber tracts such as the corpus callosum and fornix. At P-30, NMDAR1 is only marginally lower than P-21, but at P-35 & P-40 neurons in layers IV + VI show markedly decreased expression. Analysis of the most intensely labeled pyramidal cells in layer V of the barrel cortex shows very intense staining at P-3 & P-7 which reduces through P-14, P-21 & P-30. The expression in these cells again goes up at P-35 where they remain for as long as 12 months. These differences are currently being quantified by Western & Northern blots + *in situ* hybridization. (supported by NS-13031)

566.7

LOCALIZATION OF NMDAR1 SUBUNIT IN RAT LAYER IV BARREL CELLS. F.F. Ebner*, S.-M. Lu, V. Rema, W. Huang, M. Armstrong-James & A. Banerjee, Inst. for Dev. Neuroscience, Kennedy Center, Vanderbilt University, Nashville, TN 37203.

NMDAR1 subunits were localized with immunocytochemical methods in sections cut tangential to the cortical surface. In layer IV barrels, a striking pattern of NMDAR1 immunoreactivity was created by the presence of a small number of densely labeled, large somata in the periphery of the barrels whose dendrites point toward the center of the barrel. These cells have intensely staining puncta, roughly 1 µm in diameter, on their soma and dendrites, plus diffuse cytoplasmic labeling. Since NMDAR1 can form functional NMDA channels, the aggregates on the cell membrane suggest that the puncta are active glutamate receptor sites. These cells exhibit multipolar dendritic morphology and few spines suggesting that they are aspinous, GABAergic, stellate cells. The distribution of these cells and their high content of NMDAR1 further suggest that they may be extraordinarily sensitive to changes in sensory activity levels arising from the whiskers and from other barrels. These cells are surrounded by many other cells containing far lower levels of reaction product that is seen almost exclusively in their cell body. In addition to layer IV cells, each barrel contained a few clusters of densely labeled, small, circular profiles that we interpreted as cross-sections through some of the apical dendrite bundles of layer V cells. (supported by NS-25907)

566.8

CHARACTERIZATION OF A FUNCTIONAL WHISKER REPRESENTATION IN RAT BARREL CORTEX: OPTICAL IMAGING OF INTRINSIC SIGNALS VS. SINGLE-UNIT RECORDINGS. R. D. Frostig*, S. A. Masino, M. C. Kwon, Dept. of Psychobiology, University of California, Irvine, CA 92717.

The whisker-to-barrel system provides the opportunity to stimulate an independent sensory unit in the periphery and measure the extent of its cortical representation. To achieve this goal, we compared the functional representation of a whisker obtained with optical imaging of intrinsic signals to that obtained with single-unit recording. Specifically, we compared the area which exhibits stimulus-related intrinsic signal responses to the area which exhibits stimulus-related neuronal responses.

We first determined the functional representation of the whisker in barrel cortex by imaging through a thinned skull during stimulation of an individual whisker for one second at 5 Hz. After imaging, we removed the skull and proceeded with closely spaced single-unit recordings guided by the imaged representation of the whisker while delivering identical whisker stimulation.

Although the largest change in the intrinsic signals are found within a localized area which we defined as the functional representation of the whisker (Masino et al, PNAS, 90: 9998-10002, 1993), there is a widespread area beyond the functional representation which exhibits a stimulus-related change in intrinsic signals. When we compared the area of the stimulus-related neuronal responses to the area of the stimulus-related intrinsic signal responses we found that the two were similar. Both response areas spread over a radial distance of approximately 2 mm from the center of the imaged representation. However, the spread of single-unit activity was not uniform: there was a significant decrease in single-unit responses starting at and extending beyond the border of the localized functional representation determined with imaging. These results demonstrate an extensive response area activated by stimulation of a single whisker as assessed by both techniques, and highlight the correspondence between intrinsic signals and underlying single-unit response characteristics. Supported by the Beckman Foundation and NIH grant MH-50362.

566.9

Stimulus-Dependent Expression of the Immediate-Early Genes C-*fos* and *Zif268* in the Whisker-to-Barrel Pathway of the Rat.
P. Melzer* and H. Steiner. Laboratory of Cerebral Metabolism (P.M.) and Section of Neuroanatomy (H.S.), NIMH, Bethesda, MD 20892.

Recent studies in the rat whisker-to-barrel pathway have shown that the expression of the immediate-early genes c-*fos* and *zif268* is decreased in barrel cortex, when the corresponding whiskers had been clipped (Steiner & Gerfen, in press). Here we report that stimulation of one whisker in the freely moving albino rat increases the expression of the two genes in the appropriate barrel, and that the magnitude of *zif268* expression is dependent on the amplitude of whisker deflection. The rats were anesthetized with halothane, and a metal piece (6.0 mm long, 0.4 mm in diameter) was glued on left whisker C2 about 15 mm above the skin; all other whiskers on that side were clipped. The whiskers on the right side were left intact. Thirty minutes later, each rat was exposed to a pulsating magnetic field at one of nine strengths from 0 to 15.7 mT for 10 minutes. After an additional 10 minutes, the rats were sacrificed, and the brains were removed and frozen at -55° C. Twelve µm-thick sections, cut either coronally or, with cortical flat mounts, parallel to the pia, were hybridized with [^{35}S]cDNA probes for the mRNAs of c-*fos* and *zif268*. Superimposing autoradiograms on sections stained for cytochrome oxidase activity showed a localized increase in the expression of the two genes in the C2 barrel contralateral to stimulation. The expression of *zif268* in barrel C2 was increased above control levels at field strengths higher than 8.0 mT. The increase in immediate-early gene expression in the barrel representing the stimulated whisker follicle and its dependence on stimulus intensity suggest that the products of these genes are associated with neuronal processing of sensory information in the whisker-to-barrel pathway.

566.10

EFFECTS OF WHISKER TRIMMING ON GABA$_A$ RECEPTOR BINDING IN DEVELOPING RAT WHISKER BARREL CORTEX. E. Salazar, S.C. Carpenter, R.L. Miller, T.A. Austin and J.L. Fuchs*. Dept. Biological Sciences, University of North Texas, Denton TX 76203.

Sensory deprivation affects GABAergic systems in adult neocortex (e.g., Hendry et al. '90; Akhtar & Land '91), but little is known about the role of this predominant inhibitory system in shaping the developing neocortex. The present study investigates developmental effects of sensory deprivation on GABA$_A$ receptors in rat whisker barrel cortex. Either the middle row or the outer rows of vibrissae were kept trimmed. In [^{3}H]muscimol autoradiographs of tangential sections from rats trimmed from birth (P0) to 6 wks, [^{3}H]muscimol binding declined in deprived relative to nondeprived barrel rows (10.3%; $p<0.028$, $n=6$), confirming a previous report (Fuchs '93). This deprivation effect was not yet present by P10 ($n=7$), indicating that neonatal deprivation did not interfere with the transient developmental peak in [^{3}H]muscimol binding. In rats trimmed as adults (6-12 wks; $n=4$), the decrease was notably smaller than in rats trimmed from P0 to 6 wks, suggesting the presence of a developmental "sensitive" period. Preliminary observations suggest that if whiskers are trimmed from P0 to 6 wks followed by 10 wks of recovery, the deprivation effect persists, compatible with electrophysiological evidence for long-lasting decreases in inhibition following a similar period of whisker trimming (Simons & Land '87).

Our observations extend previous reports that sensory deprivation can lead to a decline in GABAergic systems, and suggest that some of these effects may have a developmental sensitive period. GABAergic down-regulation might be compensatory, disinhibiting the reduced excitatory sensory input. Such a role could be particularly important during the refinement of synaptic circuitry. Supported by MH41865.

566.11

THE ROLE OF RAT SUPRAGRANULAR CORTEX IN LAYER IV PLASTICITY AFTER WHISKER-PAIRING. W. Huang*, M. Armstrong-James, S.-M. Lu, M.E. Diamond, F.F. Ebner Inst. for Dev. Neurosci., Kennedy Ctr., Vanderbilt Univ., Nashville, TN 37203

Neurons in rat SI barrel cortex have been reported to develop biased receptive fields (RF) after days to weeks of experience with all but 2 whiskers trimmed on one side of the face ("whisker-pairing"). This type of adult plasticity or "bias" occurred in the superficial and deep layer neurons after only 24 hours of whisker pairing, a time prior to any change in layer IV neuron RF's. These results raised the question of whether early-altered neurons in the superficial layers are required for the later layer IV plasticity. Epidural application of NMDA (150 mM for 15 m) selectively destroyed neurons in layers II and III over the barrel field cortex. Fourteen days after the lesion, neurons of layers IV, V, VI still exhibit center (D2 whisker) and surround (D1, D3, C2 and E2 whiskers) RFs under urethane anesthesia. We started whisker pairing 7 days after the cortical lesion, when all but 2 whiskers were cut on one side (leaving either D2 + D1 or D2 + D3). The trimming was repeated every other day for 5 days. On day 7 layer IV neurons in the D2 barrel failed to show the expected bias toward the paired (ie., intact) whisker. In contrast, deep layer neurons in the same radial penetrations developed the bias. These results indicate that the superficial layers of SI cortex must be intact for normal plasticity in layer IV. We are studying now whether this apparent delay in layer IV plasticity is permanent after long periods of whisker pairing. (supported by NS-25907)

566.12

NONSPECIFIC SHORT-TERM ELECTRICAL STIMULATION OF A SINGLE FOREPAW DIGIT INCREASES THE PHYSIOLOGICAL REPRESENTATION OF THAT DIGIT IN LAYER IV OF RAT BARREL FIELD CORTEX: AN EXTRACELLULAR RECORDING STUDY. C.X. Li*, R.S. Waters, C.A. McCandlish, E.J. Johnson. Dept. of Anatomy and Neurobiology, UT, Memphis, Col. of Medicine, Memphis, TN 38163

Limited differential sensory experience can alter the physiological organization of the glabrous representation of the forepaw in the forepaw barrel subfield (FBS) cortex in rat layer IV (see companion abstract, Waters et al.). To determine whether nonspecific short-term electrical stimulation can also alter the physiological representation of the forepaw, we examined barrel cortex prior to and immediately after a short period of electrical stimulation.

Adult rats were anesthetized with Nembutal, head was stabilized, skull was opened over SI, dura was removed, and cortex was covered with warmed silicon fluid. Carbon fiber electrodes were used to map the representation of digit two (D2), D3, D4 prior to and immediately after electrical stimulation of the D3. Pulses of direct current (amplitude: 80-120 µA, duration: 100 µsec, interval: 1 Hz) were applied to the tip of D3 for 2 hr. Following the period of electrical stimulation, the cortex was immediately remapped. The following results were obtained:

1. Following electrical stimulation, the area of cortex served by D3 increased by approximately 10%.
2. These results suggest that brief periods of electrical stimulation are sufficient to immediately alter the physiological representation of the stimulated digits. These results bear directly on the role of afferent input on cortical organization.

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566.13

DIFFERENTIAL SENSORY EXPERIENCE ALTERS THE REPRESENTATION OF THE FOREPAW IN FOREPAW BARREL SUBFIELD (FBS) CORTEX IN RAT. R.S. Waters*, C.X. Li, S.C. Fowler[1], C.A. McCandlish. Dept. of Anatomy and Neurobiology, UT, Memphis, Col. of Medicine, Memphis, TN 38163; [1] Dept. of Psychology, Uni. of Mississippi, Oxford, MS 38677.

The relationship between the representation of the forepaw and the barrel-like structures in the forepaw barrel subfield (FBS) in rat SI cortex offer an excellent model system to study the differential effects of sensory experience on cortical structure and function. We describe results of physiological mapping and morphological analysis of FBS in rats trained on a sensory-motor task.

Adult rats, water deprived, were trained to depress a 9mm disk to receive simultaneous liquid reinforcement. Training lasted approximately 15 min per/day for a period time of 18-30 days. Following training, animals were anesthetized with Nembutal, the head was stabilized, the skull was opened over SI, dura was removed, and the cortex was covered with warmed silicon fluid. Carbon fiber electrodes were used to map the forepaw representation in SI cortex in both hemispheres. Lesions were made to identify specific recording sites and to relate the electrophysiological map with the morphological map in FBS. Following physiological mapping, the cortex was removed, hemispheres were flattened and cut tangentially. Tissue was stained with cytochrome oxidase (CO). The following results were obtained:

1. Sensory-motor training resulted in an average increase of 24% in the area of cortex represented by glabrous digit tips in trained as compared to untrained hemispheres.
2. No morphological differences within the FBS were observed between trained and untrained hemispheres.
3. Limited sensory-motor experience is sufficient to alter the physiological map without a noticeable change in the morphological map in FBS.

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566.14

EARLY TACTILE EXPERIENCE ALTERS THE CUTANEOUS FOREPAW REPRESENTATION IN THE PRIMARY SOMATOSENSORY CORTEX OF THE RAT.

O. Coq, Y. Zennou-Azogui, C. Xerri*. *Lab. Neurobiology of Functional Restorations, University of Provence, 13397 Marseilles, France.*

Earlier anatomical, biochemical and behavioral studies have shown that subject-environment interactions have significant effects on brain structure and learning capabilities. Moreover, it is well established that the cutaneous representations of the primary somatosensory (SI) cortex display a remarkable plasticity and can be reshaped through an experience-dependent process involving spatio-temporal coactivation of inputs. We have designed experiments to evaluate the influence of early differential tactile experience on the organization of the cutaneous "maps" of the SI cortex in the rat. Long-Evans female rats were housed under different tactile environments (standard, impoverished, enriched) for 1 month from the time of weaning. Their forepaw representation within SI was then reconstructed by using microelectrode mapping. The "standard" rats were housed in groups of 3, in a conventional environment. The "impoverished" rats were also exposed to a conventional environment, but each animal was housed in a separate cage. The "enriched" animals were housed in larger cages, in groups of 12 and were provided with objects of different shapes, sizes and textures. Preliminary results suggest that: 1) the skin territories more likely to be stimulated (protuberant skin surfaces) are best represented within the SI cortex; 2) early tactile enrichment through object manipulation and contact with mates improves the spatial resolution (smaller cortical receptive fields, RFs) of the cutaneous forepaw representation; 3) tactile impoverishment results in a degradation of the forepaw representation that is characterized by larger RFs, many double RFs, and by the emergence of non-cutaneous small "islands" within the cutaneous map. These results indicate that early tactile experience plays a critical role in the development and maintenance of the cutaneous cortical representations.

566.15

ACUTE CHANGES OF THE RECEPTIVE FIELDS AND NEURONAL ACTIVITIES OF THE VPL THALAMIC NEURONS FOLLOWING EITHER TEMPORARY OR PERMANENT DEAFFERENTATION OF SINGLE DIGITS OF ANESTHETIZED RATS. J.H. Sohn*, J.O. Son*, H.C. Shin*, S.A. Raymond* & H.J. Park*. *Dept. Psychol., Chungnam Natl. Univ. Taejun, Korea, Dept. Physiol.,* College. Med., Hallym Univ. Chunchon, Korea #200-702, Dept. Anesthesia Res* Labs. Brigham & Women's Hospital, Harvard Med. School, Boston, MA. USA.

To investigate whether either temporary (TD) or permanent (PD) deafferentation produces reorgaization of sensory maps in the forepaw area of the ventral posterior thalamus, we made single or multiple unit recordings from neurons in VPL thalamus of urethane anesthetized rats. Neuronal responses to subcutaneous electrical stimulation to the receptive field (RF), a forepaw digit, and to other digits were quantitated by analyzing post-stimulus time histograms. After determining stable RF and evoked unit responses for 30 min of control period, either TD or PD of a RF (single digit) was carried out by lidocaine injection (2%) or amputation, respectively. Ten of 12 VPL units showed a reversible block of original RF (ORF) after TD and exhibited a new RF (NRF) located neighbouring digits. NRFs were initially observed at 23.8±3.1 min after TD and they disappeared at 57.3 ±6.1 min after TD. The NRF showed much weaker responsiveness to the peripheral activation than the ORF did (maximum 43.5±13.6% of the ORF (133.8 Hz/stimulus, n=8), at 28.0±4.4 min after TD). Onset response latencies from the NRF (9.3±0.7-15.4±0.8 ms) were slightly longer than those from the ORF (7.9±0.4-15.4±1.0 ms). Seven VPL units were subject to PD study and they exhibited NRF digits located next to the ORF digits. NRF appeared at 14.7±6.2 min after PD and lasted up to 40-80 min of experimental period (58.6±6.7 min). The NRF showed weaker responsiveness to the peripheral activation than the ORF did (maximum 66.8±22.5% of the ORF (119.7±26.1 Hz/stimulus), at 34.4±9.1 min after PD). Response latencies from the NRF (10.5±0.8-20.6 ±1.8 ms) were slightly longer than those from the ORF (8.9±0.7-18.6± 2.3 ms). These results demonstrate that either temporary or permanent deafferentation of single digit induces a marked reorganization of the somatosensory map within minutes in the VPL thalamus. **(KRF 93)**

566.16

DEAFFERENTATION CHANGES THE DISTRIBUTION AND ORGANIZATION OF THE BURSTING PATTERNS OF NEURONS IN SOMATOSENSORY CORTEX OF AWAKE CATS. R.W. Dykes,*H.H. Webster, A.A. Myasnikov and I. Salimi. Dept de physiologie and Centre de recherche, Dept de psychiatrie, Hôpital Sacré-Côeur, Université de Montréal, Montréal, Québec H3C 3J7.

After identifying specific classes of neurons in the cat somatosensory cortex based on the structure of their spontaneous activity, these same classes were sought in somatosensory cortex that had undergone a large deafferentation by transection of the radial, median and ulnar nerves. Recordings made from several days to two weeks after the surgery showed an increased probability of encountering neurons with a pattern of short bursts and a decreased probability of encountering neurons with long bursts. This change suggests that deafferentation leads to less structure in the ongoing activity within deprived cortex. We hypothesize that this results from a reduction of the strength of corticocortical and corticothalamic feedback loops impinging on the deprived area.

(supported by the Medical Research Council of Canada)

566.17

DISTINCTIVE PATTERNS OF BURSTING IN NEURONAL ACTIVITY FROM SOMATOSENSORY CORTEX OF AWAKE CATS. A.A. Myasnikov,* H.H. Webster and R.W. Dykes. Departement de physiologie, Université De Montréal, Montréal, Québec H3C 3J7.

Recent studies show that differences in the membrane characteristics of cortical neurons allow cortical neurons to be distinguished on the basis of the shape and temporal pattern of their action potentials. These characteristics may allow their identification in behaving animals. To examine this possibility in the somatosensory cortex, we obtained extracellular digital records from neurons in awake, quietly resting cats using tungsten-in-glass microelectrodes. Neurons were characterized by their action potential waveforms, receptive field characteristics and other electrophysiological attributes. Analysis of their spontaneous activity showed some cells to have distinctive bursting patterns. Cells of one class had multiple peaks in their autocorrelograms, many impulses per burst and nearly constant interburst intervals. A second had only one peak in their autocorrelograms, short bursts, and increasingly longer intervals within the burst. Other cells lacked any temporal dependence between spikes.

(funded by the Med. Res. Council of Canada)

566.18

NEOCORTICAL AND THALAMIC PLASTICITY FOLLOWING NEONATAL DEAFFERENTATION IN RATS D.M. Liu*, D.T. Ross, J. Nissanov, and P.J. Hand. Institute of Neurological Science, University of Pennsylvania, PA 19104.

One critical issue involving activity-dependent neocortical plasticity is the relative contribution of intracortical and subcortical structures to representational changes. Our previous study showed that neonatal removal of all whiskers except C3 produced a prominent enlargement of 2DG functional representation of spared C3 (SC3) in SI cortex. In this study, we used the same model to investigate the plasticity at the cortical and subcortical level. SC3 preparation resulted in a significant expansion of 2DG functional representation in both SI cortex (303.3±35.8%) and ventrobasal thalamus (112.2±35.2%) but not in the brainstem trigeminal complex. However, we only found a 99.5±17.6% enlargement of barrel size as revealed by CO staining. Presumably the striking representational enlargement was only partly due to a structural changes in SI cortex. To test the hypothesis that GABAergic disinhibition may involve in this additional expansion, we performed GAD immunocytochemistry in the SC3 model. Preliminary results showed that the pattern of GAD positive labeling in control barrel cortex is well organized and homogenous. In contrast, the boundary of each barrel in deafferented barrel cortex is disorganized and the GAD positive cell density of adjacent barrels is significant attenuated by 39.5±6.0% as compared with SC3 region. These results suggest the GAD expression may be activity-dependent and its down regulation following deafferentation may reflect a decreased lateral GABAergic inhibition that contributes to the observed neocortical plasticity(Supported by NIH-2P41RR01638).

567.1

FUNCTIONAL MAPPING OF HUMAN SOMATOSENSORY CORTEX WITH ECHO PLANAR MAGNETIC RESONANCE IMAGING. K. Sakai[1*], E. Watanabe[2], Y. Onodera[3], H. Itagaki[3], E. Yamamoto[3], H. Koizumi[3] and Y. Miyashita[1]. [1]Dept. Physiol., Sch. Med., Univ. Tokyo, Tokyo 113, [2]Dept. Neurosurg., Tokyo Metro. Police Hospital, Tokyo 102, and [3]Central Research Lab., Hitachi, Ltd., Tokyo 185, Japan.

Somatotopical organization of the human somatosensory cortex was analyzed with echo planar imaging (EPI) at 1.5 T, utilizing deoxyhemoglobin as an endogenous contrast medium. Scrubbing stimulation at a frequency of 3 Hz was applied to one of three cutaneous areas: toes, fingertips, and tongue tip. Each measuring session consisted of resting (40 s), stimulation (30 s), and resting (40 s) periods. Sagittal EPI slices of 10- or 15-mm thickness (data acquisition time, 60 ms per slice; in-plane resolution, 2 x 4 mm^2) were obtained every 2 s continuously in a single session. A spin echo image of the corresponding sagittal slice was taken just before and after several sessions. Statistical significance of signal changes was assessed with Student's t-test for each voxel. We found focal bands of increased signals (1.5 to 8 %) during stimulation periods, with rise time of 2 to 6 s. These activated bands were identified on the contralateral postcentral gyrus. The cortical responses from the three stimulation sites were anatomically distinct and organized medially-to-laterally in the order of toes, fingertips, and tongue tip.

567.2

PUTATIVE HUMAN SOMATOSENSORY AREA III (SIII) AND ITS MODULATION BY ATTENTION. J.V. Pardo*, J. T. Lee. Psychiatry PET Unit, Psychiatry Service, VAMC, and the Division of Neuroscience Research, Department of Psychiatry, University of Minnesota, Minneapolis, MN.

PET and MEG have recently helped to define human SI (along the Rolandic Sulcus) and SII (parietal operculum). By analogy to the organization of non-human somatosensory cortices, Caselli has posited the existence of human SIII and SIV. Human SIII and SIV are hypothesized to compose a ventrolateral somatosensory associative cortex whose damage causes tactile agnosia.

Ten healthy volunteers participated in a somatosensory processing task while cerebral blood flow was measured with the $H_2{}^{15}O$ bolus autoradiographic technique and PET. In the somatosensory attention condition, subjects monitored their finger, lip, or great toe for pauses in a series of taps (3 Hz) from a von Frey hair and maintained passive visual fixation. In the visual attention condition, subjects fixated attentively looking for rare, transient dimmings of the fixation mark while ignoring the taps on their finger, lip, or toe. The somatosensory attention condition produced activation in a region of the contralateral supramarginal gyrus (Brodmann 40) consistent with the predicted location of human SIII. Finger, lip, and toe responses in SIII were closely mapped together and were modulated significantly by attention.

567.3

MOTOROTOPIC ORGANIZATION OF HUMAN CORTICAL 20-Hz OSCILLATIONS. R. Salmelin*, R. Hari, M. Hämäläinen, and M. Kajola. Low Temp. Lab., Helsinki Univ. of Technology, 02150 Espoo, and Neuromag Ltd., 00510 Helsinki, Finland.

In humans, the spectral distribution of cortical spontaneous rhythms ('alpha', 'mu', 'tau') is dominated by frequencies around 10 and 20 Hz. The reactivity and source areas of cortical somatomotor rhythms associated with self-paced left- and right-sided index finger, toe, and mouth movements were studied non-invasively in eight healthy subjects employing a 122-channel whole-head neuromagnetometer.

Following the complete suppression during movements, the rhythms manifested a marked increase above the base level, particularly in the 20-Hz range. Sources of the enhanced 20-Hz activity were found in mesial cortex for toe movements and about 5 cm and 8 cm from vertex (measured along the cortex) for hand and mouth movements, respectively, apparently tracking the representation of the moving body parts in the motor cortex. The reactive 10-Hz rhythms, on the contrary, concentrated at about 6 cm from vertex, close to the hand area in the somatosensory cortex, for all tasks.

The 10- and 20-Hz somatomotor rhythms seem to have separate spatial distribution and distinct functional roles in motor tasks, with the 20-Hz range manifesting motorotopic organization.

567.4

FUNCTIONAL MAGNETIC RESONANCE IMAGING OF HUMAN CORTEX ACTIVATED DURING SOMATOSENSORY AND MOTOR STIMULATION. K.D. Davis*[1], M.L. Wood[2], C.A. Stewart[4], E.T. Kiriakopoulos[2], J.J.S. Barton[3] and D.J. Mikulis[2]. [1]Division of Neurosurgery, [2]Department of Radiology, and the [3]Division of Neurology, University of Toronto, and the [4]Eye Research Institute of Canada, The Toronto Hospital, Toronto, Ontario, Canada M5T 2S8.

Functional magnetic resonance imaging (fMRI) techniques have recently been developed to identify cortical areas activated during specific tasks. We have further modified these techniques to identify regions in the cortex activated during simple somatosensory and/or motor tasks.

All data were collected from normal subjects. Images were obtained with a 1.5T MRI scanner. Each protocol typically consisted of alternating sets of 6 images during a task and 6 images at rest or during a control task for a total of 72 images. Typical imaging parameters were: 4mm axial cortical slice, TR=68ms, TE=40ms, bandwidth=4.92KHz, ~6sec/image, FOV=30x22cm, 256x128 matrix, 45° flip angle. Somatosensory-related activity was evoked with either 1) electrical stimuli applied to the median nerve with a TENS-type stimulator to evoke strong paraesthesia in the distribution of the nerve, or 2) natural tactile stimuli (eg, light brush) of the skin. For comparative purposes, scans were also obtained during a sequential motor/sensory task (e.g., thumb to finger opposition with contact). Activated regions were determined based on a pixel by pixel statistical analysis (t-test) of the mean intensity signal differences during the task and controls.

Natural tactile stimuli activated large areas of S1 and M1. Electrical stimuli that evoked paraesthesias activated discrete areas of S1. During simple motor tasks, S1, M1 and MII were activated. Within activated areas, the mean intensity signal followed the expected sawtooth pattern indicative of cortical activation. These data indicate that fMRI can be used to identify and confirm the functioning of sensory and motor cortical areas active during simple tasks.

567.5

RELATIONSHIP BETWEEN RREP AMPLITUDE AND RESISTIVE LOAD MAGNITUDE IN CHILDREN. P.W. Davenport*, C. Smith-Hammond, D. Dalziel and I. Colrain. Dept. of Physiol. Sci., JHMHC, University of Florida, Gainesville, Florida, 32610

Respiratory related evoked potentials (RREP) recorded from C_Z-C_3 and C_Z-C_4 have been elicited by occlusion of inspiration. The initial positive peak (P_1) is believed to represent the sensory cortical response to the activation of the afferents transducing the inspiratory load. It was hypothesized that the P_1 peak amplitude is proportional to the inspiratory load magnitude in children. Children were studied semi-reclined at rest. They respired through a mouthpiece and non-rebreathing valve connected to a loading manifold. EEG activity was recorded from C_Z-C_3 and C_Z-C_4. The resistive (R) load magnitudes were 2.0, 9.0, 21.0 cm H_2O/lsec^{-1} and occlusion. Control evoked potentials are obtained by interruption of inspiration with the no-load, R_0, port open. The EEG activity was averaged on-line and stored with computer system. Each trial consisted of 80 load presentations. Each load magnitude and the control no-load were applied 20 times each in an trial. There were 4 trials. The P_1 peak amplitude and latency was measured. No P_1 peak was observed with control R_0. The P_1 peak was found with all R loads and occlusion. The mean P_1 latencies were C_Z-C_3=38.2, C_Z-C_4=38.4 msec and there were no significant differences between R load magnitudes. The P_1 amplitude increased with increasing R load magnitude for both electrode pairs. Inspiratory occlusion and resistive loads produce mechanical changes to inspiratory transduced by respiratory mechanoreceptors and project to the sensory cortex. These results support the hypothesis that graded increases in inspiratory load are correlated with increased activation on sensory cortical neurons. Supported by ALAF grant UF #92092907.

567.6

SOMATOSENSORY CORTICAL REPRESENTATION OF EACH FINGERTIP IS DISTINGUISHABLE IN INDIVIDUAL SUBJECTS, USING MULTI-SLICE FUNCTIONAL MRI. B.R. Krauss[1], P.A. Gelnar[1], N.M. Szeverenyi[2], C.J. Hodge[1*], A.V. Apkarian[1], Depts. Neurosurgery[1] and Radiology[2], SUNY HSC; Syracuse, NY.

Functional magnetic resonance imaging (fMRI) has the potential to achieve greater spatial resolution than PET and SPECT. We tested the ability of fMRI to resolve individual finger representations within the somatosensory cortices (SI and SII) by applying a vibratory stimulus to single digits during one imaging session.

A 1.5 Tesla MRI system with a within slice pixel size of 1.6 X 1.6 mm, a single surface coil, and an echo planar acquisition sequence were used to image changes in activity in the middle third of the cerebral cortex contralateral to experimentally applied stimuli. In each session vibratory and proprioceptive stimuli were applied to D1, D2, and D5, the control was rest. Eight 6.0 mm slices were imaged during 6 stimulus-control cycles in each session. Five images/slice were obtained during stimulus or control. T-maps were superimposed on high resolution MR images.

The vibratory cortical activation maps for all three digits exhibited significant activation (t-map clusters containing 9 or more pixels all with $p<0.05$) in SI and SII. The regions activated by D1, D2 and D5 in SI were spatially distinct when measured both by the center of gravity and by the pixel which exhibited the largest increase in intensity within the activated cluster. In SI, the activated regions for D1 and D2 were spatially closest while the activated regions for D1 and D5 were more distant. The proprioceptive cortical activations were less localized than the vibratory activations.

This demonstrates that fMRI can distinguish the cortical representation of individual fingers in single subjects. Therefore, the spatial resolution of fMRI is higher than that of PET or SPECT.

567.7

SPATIAL MAPPING OF PAIN AND MOTOR ACTIVATION IN HUMAN CEREBRAL CORTEX WITH fMRI AT 4 TESLA. Michael J. Iadarola*, Han Wen, Robert C. Coghill, Steven D. Wolff and Robert S. Balaban. Neurobiology and Anesthesiology Branch, NIDR and Laboratory of Cardiac Energetics, NHLBI, NIH, Bethesda, MD USA.

A high resolution gradient recalled echo fMRI method was developed to visualize neuronal activity induced by thermal nociceptive stimuli and motor tasks. Changes in neural activity reflected by blood flow and blood oxygenation level differences (in flow effects and T2* effect, respectively) were obtained in 4, 5 or 8 mm thick transaxial slices with repetitive 13 sec scans that provided good contrast and were registered to high resolution anatomical scan. In both cases, activated voxels were discretely organized such that the activity generally traced the gyral borders and overlapped somewhat into the sulcus. The appearance of the signal was consistent with it originating from marginal or central gyral veins of medium caliber and possibly their collaterals. Multislice acquisitions indicated that the nociceptive signal (from heating the tip of the thumb) occurred over 4-8 mm in the axial dimension and was contiguous between slices. The motor task (repetitive flexion of the last joint of the thumb) yielded a more extensive signal. For example, in 9 successive 5 mm slices starting from the superficial cerebral cortex to the level of the corpus callosum, contiguous activity (associated with the posterior bank of the precentral gyrus/central sulcus) could be traced through adjacent 3 slices. One slice contained the major locus of activity. In the coronal plane the activity was very discretely distributed as well. These data suggest that a prominent portion of the signal increase arises from the venous compartment and that the signal provides functionally relevant and anatomically discrete information.

567.8

NEURAL BASIS FOR TACTILE ROUGHNESS PERCEPTION: THE RELATIVE CONTRIBUTIONS OF SLOWLY ADAPTING AND RAPIDLY ADAPTING AFFERENTS. D.T. Blake, S.S. Hsiao*, and K.O. Johnson, Krieger Mind/Brain Inst. and Dept. of Neuroscience, Johns Hopkins Univ. Baltimore, MD. 21205

Psychophysical and neurophysiological studies were done to assess the relative contributions of slowly adapting (SA) and rapidly adapting (RA) peripheral afferents to tactile roughness perception. The stimuli consist of 18 embossed surfaces composed of tetragonal dot patterns with constant, 3.5 mm center-to-center spacings, variable diameters (250, 700, 1050, 1600, 2050 and 2500 um) and variable heights (280, 350, 680 um). Dot diameter and height are shown to have differential effects on the responses of SA and RA afferents. It is postulated that differentially stimulating these afferents will allow us to determine the relative contributions of SA and RA afferents to roughness perception.

Psychophysical reports from 15 subjects show that both dot diameter and dot height have significant effects on roughness perception. Decreases in dot diameter and increases in dot height both cause increases in perceived roughness with dot height having a larger effect at small diameters: narrow dots feel more than two times as rough at 680 than at 280 um, while wide dots feel equally smooth at all dot heights. Neurophysiological recordings from 12 SA and 14 RA afferents from the ulnar and median nerves of 3 Macaque monkeys were made using the same dot patterns. The neural responses, which are plotted as two-dimensional spatial event plots, are analyzed using a measure of spatial variation in firing rates (*J.Neurosci.* 12:3414-3426, 1992.). In accordance with previous results (Can. J. Physio. and Pharm, in press), only the SA afferents systematically match the psychophysics. While both SA and RA spatial variation measures are mildly affected by dot diameter, only the SAs are affected by dot height. These results strongly suggest that RA afferents do not contribute to tactile roughness and that SA afferents alone form the neural basis for tactile roughness.

567.9

TRANSFORMATION OF TACTILE SPATIAL FORM WITHIN A CORTICAL COLUMN IN AREA 3b OF THE MACAQUE. J.J. DiCarlo*, S.S. Hsiao, and K.O. Johnson. Krieger Mind/Brain Inst. and Dept. of Neuroscience, Johns Hopkins Univ., Baltimore, MD 21205.

Multi-electrode (7 elect., 400 μm spacing) recordings were made in area 3b from both hemispheres of an awake Macaque monkey. The electrodes were positioned such that isolated neurons had receptive field centers on the same spatial location of the monkey's distal finger pad. Typically this allowed simultaneous recording from 3-5 neurons located along a perpendicular to the cortical surface, which were assumed to be within a single cortical column. The monkey performed a visual task while embossed letters and oriented bars were repeatedly scanned at a controlled force (30 gm) and velocity (40 mm/sec) across the distal finger pad. The locations of the penetrations were confirmed using standard histological techniques.

Two-hundred and forty-four neurons (65 recording sets) were studied using the letter and bar stimuli. The neural responses to the embossed letters were used to construct two-dimensional spatial event plots (SEPs). A structural analysis of the SEPs shows neurons recorded in the central layers tend to be more isomorphic (similar in structure to the stimulus letters) and more responsive (higher impulse rates) than neurons in both supra- and infragranular layers. Cross-correlation analysis on 225 simultaneously recorded pairs of neurons suggests monosynaptic excitatory connections between 34% and common input to 16% of the pairs. In general, among pairs exhibiting monosynaptic excitatory connections, the SEPs of the "sender" neuron are more isomorphic than those of the "receiver" neuron. The overall pattern of functional connectivity is consistent with anatomical studies; there are monosynaptic connections from the central layers of cortex to supragranular and infragranular layers and monosynaptic connections from supra- to infragranular layers. Together, these results support the hypothesis that local, columnar cortical processing of spatial form proceeds from layer 4 to supra- and infragranular layers and that this processing results in a significant transformation of spatial form.

567.10

SHORT- AND LONG-LATENCY SOMATOSENSORY EVOKED FIELDS REFLECT DIFFERENT ASPECTS OF TACTILE INFORMATION PROCESSING. N. Forss, R. Salmelin, V. Jousmäki and R. Hari*. Low Temperature Laboratory, Helsinki University. of Technology., 02150 Espoo, Finland.

We recorded whole-head somatosensory evoked fields (SEFs) from 7 healthy subjects to airpuff (dorsum of the middle finger) and electric (median nerve at the wrist) stimuli; the interstimulus interval (ISI) was 3 s. The early (20 – 60 ms) SEFs to both stimuli originated in the contralateral first somatosensory cortex (SI) and the late (> 85 ms) SEFs bilaterally in the second somatosensory cortices (SII). An additional source was active at 70 – 110 ms in the contralateral parietal posterior cortex (PPC) in the wall of the postcentral fissure. Only SI responses were markedly smaller in amplitude and longer in latency to airpuffs than to electric stimuli.

In an odd-ball paradigm, 'standard' electric stimuli were delivered to the thumb and 'deviants' (15%) to the little finger, or *vice versa* (ISI 0.5 s). In SI, the deviants elicited similar responses irrespective of whether they were presented alone or among standards. In contrast, SEFs from SII, and especially those from PPC, were clearly larger to deviants presented without the intervening standards.

The early and late SEFs apparently reflect different aspects of tactile information processing. SII and PPC seem to be more integrative and less stimulus-specific in function than the SI cortex.

567.11

VIBROTACTILE SENSITIVITY IN PATIENTS WITH CARPAL TUNNEL SYNDROME (CTS). C.M. Checkosky* and S.J. Bolanowski. Institute for Sensory Research and Department of Bioengineering and Neuroscience, Syracuse University, Syracuse, NY 13244.

CTS is commonly diagnosed by neurologic examination and electrophysiologic evaluation. However, many investigators have recently advocated the use of vibrotactile testing as an early diagnostic tool. The rationale for this stems from the idea that the large-diameter sensory fibers are affected first in nerve compression, possibly resulting in elevated vibrotactile thresholds. More recently, however, it was shown that vibrotactile thresholds are elevated during coincident pain (Apkarian et al., Soc Neurosci Abstr: 1992). The purpose of this study was to assess vibrotactile sensitivity in CTS quantitatively using strictly controlled laboratory conditions and psychophysical techniques, and to determine whether elevated thresholds correlate with the presence of pain. Detection thresholds were measured on 20 patients (36 hands) clinically diagnosed with CTS. Bursts of vibratory stimuli were delivered to the thenar eminence of the affected hands (stimulus frequencies, 1, 10 and 300 Hz; contactor sizes, 0.008 cm^2 and 2.9 cm^2). The subjects also rated the amount of pain that was present at the beginning of each testing session. Thresholds were compared to normative data in the literature and to thresholds obtained on a within-subject control site, the hypothenar. No elevations in threshold were found on affected areas in 19 hands during all testing sessions when compared to controls. Thus, the testing of vibrotactile thresholds for CTS may not be warranted. In 11 testing sessions elevated thresholds were obtained on affected areas where the subjects reported the presence of pain. These results indicate that elevated thresholds in CTS may actually be the result of the presence of pain. Supported by NIH, DC00098.

567.12

HUMAN PERFORMANCE ON DELAYED TACTILE DISCRIMINATION TASKS. R. Sinclair*, J. Pruett, and H. Burton. Department of Anatomy & Neurobiology, Washington University Sch. Med., St. Louis, MO 63110.

Human subjects identified either the higher of two vibrotactile stimuli differing in frequency or the rougher of two tactile gratings differing in groove width. Stimuli were presented under computer control by a feedback regulated vibrator or a servo operated surface stimulator previously described. On each trial stimulus pairs were separated by a ~0.5 to 30 second delay interval. For both vibrotactile and textured stimuli, percent correct decreased and reaction times increased as a function of increased delay interval length. Engagement in a distracting backwards counting task impaired performance relative to unfilled intervals, for delays greater than 5 seconds. For delays of 0.5 and 5 seconds, performance was unaffected by interference. Results are consistent with initial reliance on a fading sensory "trace" and, then, use of coded stimulus information at longer delays. Similar performance decay functions have been reported for visual and auditory stimuli. Preliminary analysis suggests that performance depended on order of stimulus presentation, e.g., rough-smooth grating presentation order was better discriminated than smooth-rough. A possible neural correlate of this order effect has been previously reported (Sinclair & Burton, 1988). These results establish parameters of vibrotactile and textured stimuli for use in animal studies of the neurophysiological basis of tactile memory. (Supported by NS 31005)

567.13

RESPONSES IN AREAS SII AND 7B RECORDED DURING A SPATIAL AND CROSS-MODAL TACTILE ATTENTION TASK. H. Burton*, K. C. Whang, and R. J. Sinclair. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Two rhesus macaques performed a task that manipulated attention across tactile space and between tactile and auditory modalities. The task involved three concurrent stimuli: two tactile vibrations presented to the hands and an analogous auditory tone. The monkeys were cued in a blockwise fashion to discriminate between target and distractor amplitude patterns at one of the three stimulus positions at a time. This enabled us to measure neural responses to physically identical stimulus arrays under conditions differing only by cue. An asynchrony between stimuli allowed us to separate components of the response. Psychophysical tests using 80% valid probability cues verified that the monkeys used the cue to direct their attentive resources selectively.

We surveyed 371 cells in areas SII and 7b. Of these, 170 were held long enough for us to record replicate blocks under each attentional condition (for a total of 420 trials per cell), and 85 satisfied an additional *F*-ratio criterion representing task-relatedness and stability of response. Excitatory responses to the transient phases of the tactile stimuli were the most common response pattern, but there were notable exceptions. In SII, a subset of cells was excited by the sustained phases of the stimuli but inhibited by the transients. In 7b, bimodal tactile and visual responses were common, and a few cells showed consistent directional selectivity across modalities. Bilateral tactile receptive fields were found in both areas. In many cases, the response to the first stimulus was followed by a diminished response to the second. These temporal order effects may be related to the phenomenon of tactile masking. *(Supported by NS 31005)*

567.14

RANDOMIZATION ANALYSIS OF BLOCKWISE VARIABILITY AND THE APPEARANCE OF ATTENTION EFFECTS. K. C. Whang* and H. Burton. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Cells in areas SII and 7b were observed under three different attention treatments, two cued to tactile stimuli and one cued to an auditory stimulus, with each treatment presented in replicated blocks of trials. 40% of the cells showed firing rate differences of 20% or greater between left and right tactile treatments or between tactile and auditory treatments. There was no consistency, however, in the directions of these effects. Across the population that we sampled, both spatial and cross-modal attention effects averaged to zero.

Randomization analyses were applied to determine whether the magnitudes of these observed effects were greater than would be expected by chance alone. The partition of our data set based on our three true treatments was compared to reference distributions generated by random partitioning by block or by trial of the same data set. These analyses showed that the observed attention effects were not significantly greater than would be expected by chance and, furthermore, that there was a significant serial correlation between observations within blocks. The assumption of independent observations made by conventional analyses of variance or *t*-tests is therefore inappropriate.

Analyses of variance using hierarchical error terms corroborated these findings. While a conventional analysis found 75% of the cells to show attention effects significant to $p < .05$, an analysis that made no assumption of independence within blocks found only 6% to be significant. Time series plots reveal random drifts in activity underlying the serial correlation effect. *(Supported by NS 31005)*

567.15

RESPONSES OF NEURONS IN PRIMARY SOMATOSENSORY CORTEX TO PREHENSON OF OBJECTS OF DEFINED GEOMETRY. E.P. Gardner*, S. Ghosh, and C.E. Kops. Dept. of Physiology and Biophysics, NYU Medical Center, New York, NY 10016

These experiments measure the responses of cortical neurons when monkeys actively grasp and manipulate objects of defined geometry. The monkey is presented with a set of knobs of specified shape (large and small spheres, cylinders, cubes and rectangular blocks) which he is trained to grasp and lift. Visual cues delivered on the screen of a video monitor indicate which knob should be grasped. The monkey's hand movements are videotaped using two genlocked cameras, and stored together with extracellular spike records of cortical neurons on a VHS recorder. QuickTime movies are made from JPEG compressed video and electrophysiological data on a Macintosh Quadra computer, to allow frame-by-frame correlation of hand position with neuronal firing patterns. These experiments indicate differences in information processing in anterior and posterior portions of S-I cortex. In anterior S-I, where neurons have small receptive fields on single digits, the cell's firing patterns correlate with hand displacement across the knob. These neurons appear most strongly activated when the edges of the cylindrical or rectangular knobs contact the receptive field, and are less responsive when the hand moves over the gently curved spherical surfaces. In posterior S-I, where receptive fields cover multiple digits, some neurons appear to differentiate the surface curvature of individual objects. Other neurons are silenced upon hand contact with the knobs, regardless of shape, and resume tonic activity when the hand is removed. We postulate that neurons in posterior S-I may play a role in global appreciation of the entire object, rather than in perception of specific features of single surfaces. *(Supported by NIH: NS11862).*

567.16

TWO TYPES OF VIBRATORY RESPONSIVE PACINIAN-LIKE NEURONS IN MONKEY PRIMARY SOMATOSENSORY CORTEX (SI) STUDIED DURING ACTIVE HAND MOVEMENTS. M.A. Lebedev* and R.J. Nelson
Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Some SI neurons respond to vibrotactile stimulation of their receptive fields with vibration-entrained responses (Mountcastle et al., *J. Neurophysiol.* 32:452, 1969). Previously, we described changes in entrained activity that occurred prior to voluntary movements (Lebedev and Nelson, *Neurosci. Abst.* 18:503, 1992). In this study, we examined vibration-induced activity of two types of Pacinian-like SI neurons. They were recorded extracellularly from four monkeys that made ballistic wrist flexions and extensions in response to palmar vibration (27, 57, or 127 Hz) that served as the go-cue. Animals held a steady position for a period (≥ 500 ms) preceding the go-cue. The paradigm met NIH animal utilization guidelines. To date, 31 SI neurons (2, 6, 20 and 3 from areas 3a, 3b, 1 and 2) have been considered to be Pacinian-like because each responded better to the highest of the three vibratory stimulus frequencies.

Two types of Pacinian-like SI neurons were found: (1) those that were strongly vibration-entrained (N=19), and (2) those that were not (N=12). Entrainment was evident by peaks at multiples of the vibratory period in interspike interval (ISI) and expectation density histograms. Joint interval analyses (Perkel et al., *Biophys. J.* 7:391, 1967) revealed negative serial correlations of ISIs for the entrained but not for non-entrained neurons. Negative serial correlations are typical for driven neurons. Type 1 neurons often had short ISIs (2-3 ms) that corresponded to discharge douplets and triplets. The spike trains of Type 2 neurons had unique temporal properties (e.g., the shape of ISI distribution). However, these attributes were not related to the temporal characteristics of the vibration. For both types, the activity patterns resulting from vibratory stimulation of the neurons' receptive fields changed prior to movement onset although the stimulus remained on and no change in hand position was detected.

These two classes of SI Pacinian-like neurons may represent different stages of cortical processing of the information coming from Pacinian corpuscles. Supported by **USAF Gr AFOSR 91-0333.**

567.17

DIVERGENCE OF THALAMIC INNERVATION TO AREA3b OF THE MONKEY SOMATOSENSORY CORTEX E. Rausell[1*], A. Viñuela[2], M. Molinari[3] and E.G. Jones[4]. Autonoma University, 28029 Madrid, Spain[1,2]; Catholic University, 00168 Roma, Italy[3], and University of California Irvine, CA 92717,[4].

In the monkey somatosensory system, the extent of divergence and overlap in the projections of individual thalamic cells with the same or different receptive fields has not been established.

Pairs of non-overlapping small injections of fast blue (FB) and diamidino yellow (DY) were made in area 3b at sites where neurons had overlapping (four cases) or non overlapping (five cases) receptive fields. Double labeled neurons were found when the injections were 600 µm or less apart. The probability for ventrobasal (VB) neurons to be double labeled while varying the distance between injections was calculated. Linear regression analysis of the relative percentage of double labeled neurons with respect to separation between the dyes gave a figure of 627 µm. This can be taken as an approximation to the diameter of the area of arborization of thalamocortical axons in layer IV.

In VB, there was much intermingling of FB and DY labeled cells even with injections separated by greater than 1,000 µm. A variable number of clusters of neurons were retrogradely labeled. The number of cells retrogradely labeled with a single dye within a cluster was proportional to the extent of the cortical injection. Separation of injections by less than 600 µm resulted in intermingled FB, DY and double labeled neurons within the same cluster. Separation greater than 600 µm resulted in less intermingling, a tendency for neurons labeled with different dyes to be segregated in neighboring clusters, and no double labeled neurons.

These findings have implications for somatosensory cortical mapping and as a potential basis for activity dependent plasticity of representational maps.

567.18

MODULATION OF REACTION TIME BY STIMULUS INTENSITY AND DELAY DURING DIFFERENT ATTENTIONAL STATES. J. Shenasa* and C.E. Chapman. CRSN, Université de Montréal, Canada

As part of a long range goal to investigate the influence of attention on central neural responses to tactile stimuli, a monkey was trained to perform two discrimination tasks: to discriminate either an increase in light intensity (visual), or an increase in the roughness of textured surfaces presented to the immobile digit tips (tactile). The standard textured surface consisted of raised dots with a 2 mm spatial period (SP). The increase in roughness was achieved by increasing the SP to 4.1 or 5.1 mm. Instructional cues indicated whether the animal should direct its attention towards one or both modalities. Trial types were presented in a quasi-random order via the laboratory computer. Results from psychophysical data show that, for the visual task, the animal's mean reaction time (RT) decreased with an increase in either stimulus intensity or delay ($p<0.01$), with the latter effects being most prominent with the smallest increment in light intensity ($p<0.001$). The influence of the change in intensity on RT was mostly observed at the shortest visual delay (< 1 sec.). In the texture discrimination task, RT also decreased with increasing stimulus "intensity" (4.1 vs 5.1 mm SP, $p<0.01$). In contrast to the visual task, however, tactile RT increased with an increase in the stimulus delay ($p<0.05$). These trends were present for both the directed and the divided attention conditions. It is suggested that the RT for discriminating a change in the visual stimulus is a monotonically increasing saturating function of the stimulus delay. The additive effect of stimulus intensity is most pronounced at shorter delays, i.e. far from the region of saturation. The differential modulation of RT by stimulus delay in the visual and tactile tasks is likely related to the different states of the animal's attention towards these tasks. Supported by the MRC, the FRSQ and the GRSNC (FCAR).

567.19

INFLUENCE OF SCANNING VELOCITY ON TEXTURE-RELATED RESPONSES IN THE PRIMARY SOMATOSENSORY CORTEX (SI) DURING THE PERFORMANCE OF A PASSIVE TEXTURE DISCRIMINATION TASK. F. Tremblay* and C.E. Chapman. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Canada.

Psychophysical studies have shown that the ability to scale and discriminate textures by touch is little affected by large variations in scanning velocity. Yet, the signal carried by the cutaneous afferents that subserve texture is strongly modified by changes in scanning velocity. To maintain perceptual constancy, the brain needs to interpret, or perhaps even recode, the peripheral neural input to take into account the peripheral conditions. In the present study, we have addressed this issue by looking at how changes in velocity influence the discharge of texture-related units in SI of monkeys trained to perform a passive texture discrimination task. The task required the monkey to stay immobile while surfaces (smooth or smooth/rough) were displaced under its digit tips using a rotating drum activated by a DC motor. Selected units were tested using two to four different motor speeds. Out of a total of 180 cells with a digital cutaneous field, 56 (31%) were classified as texture-related (significant change in discharge over the second half of the surface, rough vs. smooth, at the slowest speed). Correlations performed on 21/ 56 units, revealed that 48 % (n=10) showed a positive linear relationship between unit discharge and velocity ($p<.01$), while 52 % (n =11) showed no relationship. An additional 5 units showed a texture-related response that was only apparent at faster motor speeds. These results suggest that there are some SI cells that do reflect changes in velocity in their texture-related discharge, but that a significant proportion of SI cells signal texture independently of scanning velocity. The latter cell type might play an important role in maintaining perceptual constancy of texture when scanning conditions are changing. (funded by the MRC and the FRSQ).

567.20

DISCHARGE PROPERTIES OF NEURONES IN THE SOMATOSENSORY FIELDS OF THE LATERAL SULCUS DURING PASSIVE TEXTURE DISCRIMINATION IN THE AWAKE MONKEY. W. Jiang* and C. E. Chapman. CRSN, Université de Montréal, Canada.

We investigated the role of neurones in the upper bank of the lateral sulcus, tentatively the second somatosensory cortex, in discriminating a change in texture of surfaces passively applied to the fingertips of the monkey. Unitary discharge was recorded from 1 monkey trained to discriminate a standard surface (raised dots, 2 mm spatial period (SP) over the entire length) from 3 other surfaces in which, over the second half, the SP was proportionally increased (3, 4 or 5 mm; surfaces physically continuous). The monkey indicated the presence or absence of the change of each surface by, respectively, pulling or pushing a lever with the opposite hand to obtain a juice reward. 54 out of 64 neurones tested had a cutaneous receptive field (RF) that included the scanning digit tips; no RF was found in 10 units although 6 of these discharged vigorously during active hand movements. The discharge of 51/64 cells was modified during the presentation of the texture surfaces (44 increased, 7 decreased). Texture-related modulation of discharge was observed in 15 (28%) of the cutaneous units: 9 displayed proportional changes in their discharge frequency to the SP increase, while the mean discharge rate of the other 6 changed in a single step fashion and so signalled the difference but not the SP. A further 15 cells with varied RF properties (10 cutaneous, 5 no RF) showed a change in discharge at the time that the fingertips contacted the mid-point of the surface irrespective of whether or not a change occurred. While it is evident that some neurones in this area encode specific details of peripheral tactile stimuli (e.g., SP), others are less tightly coupled to the periphery. Together, the results suggest that the somatosensory fields of the lateral sulcus are involved in a higher level of the signal processing hierarchy underlying the sensory decision process required in the present discrimination task. Supported by the MRC and FRSQ.

PAIN MODULATION: PHARMACOLOGY VI

568.1

INTRATHECAL TREATMENT WITH ANTISENSE OLIGODEOXY-NUCLEOTIDES TO THE NMDA-RECEPTOR SHOW EFFECTS IN NOCICEPTIVE TESTS IN RAT. M. Rydh-Rinder, L. Alari , M.P. Sandberg, O.-G. Berge*, C.-J. Dalsgaard, C. Wahlestedt. Astra Pain Control AB, Novum Unit, S-141 57 HUDDINGE, SWEDEN

The NMDA receptor has been shown to be involved in transmission and modulation of nociceptive information in the spinal cord. Molecular cloning has recently revealed the existance of five different receptor subtypes termed NR1 and NR2A-D. Functional NMDA-receptors can be reconstituted using the NR1 subunit in combination with any one of the four NR2 subunits. The different combinations differ in expression patterns in the CNS and in their pharmacological properties. We have studied the effect in nociceptive tests of administration of intrathecal antisense oligodeoxynucleotides (ODN) to the NR1 subunit in rats. **METHOD:** Rats (n=18) were divided into three groups:1) NR1-antisense 2) Mismatch probe 3) Saline 0.9%. Three injections were made on day one, three and five. On day six, tail flick, hot plate and formalin tests were performed. The behaviour was scored by means of a computer program with five behavioural categories where time was continously recorded 60 minutes after injection; rest and activity with/without paw protection and active pain behaviour (licking, biting). Number of paw jerks were also registered. After testing the animals were killed and the spinal cords were collected on dry ice for radioligand binding experiments. **RESULTS:** In the hot plate test the time latency was significantly prolonged in the antisense-treated group compaired to controls. In the tail flick test the tendency was the same although no significance was obtained. In the formalin test the total number of paw jerks were markedly reduced in the antisensegroup and active pain behaviour was also affected. No motor disturbances were observed in any of the groups. Autoradiography with CGP39653 binding in spinal cord sections from all three groups was undertaken. **CONCLUSION:** This study shows that antisense ODN may be a useful tool in understanding the role of different receptors and their subtypes in complex events such as spinal nociceptive transmission.

568.2

ROLE FOR NMDA RECEPTORS IN THE DEVELOPMENT OF MECHANICAL HYPERALGESIA PRODUCED BY CAPSAICIN IN RATS. H.D. Gilchrist* and D.A. Simone. Neuroscience Research in Psychiatry, University of Minnesota, Minneapolis, MN 55455.

Intradermal injection of capsaicin (CAP) has been used as a model for cutaneous hyperalgesia in humans. When injected into the skin, CAP produced hyperalgesia to heat and mechanical stimuli. Furthermore, it was shown that hyperalgesia following CAP correlated with an enhanced excitability of dorsal horn neurons. In order to study underlying pharmacological mechanisms of CAP mediated hyperalgesia, a similar model was developed in rat and discussed previously (Soc. Neurosci. Abst., 396.19, 1993). Briefly, rats received one intraplantar injection of CAP into the plantar surface of one hindpaw. CAP (1-30 μg) produced a dose-dependent decrease in withdrawal latency to heat and increased the frequency of withdrawal responses to mechanical stimuli (von Frey monofilaments). In the present study, we investigated the role of spinal NMDA receptors in the development of hyperalgesia following CAP.

A chronic intrathecal (I.T.) catheter was surgically placed at the level of the lumbar enlargement. I.T. pretreatment with the competitive NMDA receptor antagonist AP-5 (2, 6, or 8 μg in 5 μl), administered 10 min prior to intraplantar CAP (10 μg in 10 μl), dose-dependently attenuated the normally enhanced withdrawal responses evoked by mechanical stimuli without altering hyperalgesia to heat stimuli. This suggests that spinal NMDA receptors are necessary for the development of mechanical hyperalgesia following CAP in rat. It is also concluded that in this model, hyperalgesia to heat may involve alternate mechanisms, such as sensitization of peripheral nociceptors. Data will be discussed in terms of the contributions of spinal NMDA as well as non-NMDA receptors to the development of hyperalgesia produced by CAP. Supported by NRSA DA05592 and the Minnesota Medical Foundation.

568.3

THALAMIC N-METHYL-D-ASPARTATE (NMDA) RECEPTORS ARE INVOLVED IN THE DEVELOPMENT AND MAINTENANCE OF HYPERALGESIA FOLLOWING UNILATERAL HINDPAW INFLAMMATION. R. Kolhekar* and G.F. Gebhart, Dept. of Pharmacology, Univ. of Iowa, Iowa City, 52242.

Effects of inter-thalamic injection of an NMDA receptor antagonist, D,2-amino-5-phosphonovaleric acid (D-APV) on the thermal and mechanical hyperalgesia induced by hindlimb intraplantar injection of carrageenan in the rat were studied in the "acute phase" (within 3 hrs) and the "subacute phase" (24 hrs) after carrageenan administration.

Male Sprague-Dawley rats were injected in the left hindpaw with 2mg carrageenan. Carrageenan produced inflammation and thermal and mechanical hyperalgesia within 3 hours of injection. Two consecutive doses spaced 30 min apart of either D-APV (5 nmol) or saline were injected into the hindlimb representation area in the thalamus (0.5 μl). Treatment with D-APV, but not saline, in the contralateral but not in the ipsilateral thalamus significantly reduced both the acute mechanical and thermal hyperalgesia. There was no effect on the non-inflamed hindpaw. Rats treated with D-APV in the acute phase failed to develop thermal and mechanical hyperalgesia in the subacute phase. In contrast, rats treated with saline or with D-APV in the ipsilateral thalamus in the acute phase demonstrated significant thermal and mechanical hyperalgesia in the subacute phase.

Taken together, these results suggest an involvement of thalamic NMDA receptors in the development and maintenance of hyperalgesia associated with inflammation in a model of chronic pain.

568.4

NMDA AND NK1 RECEPTOR ANTAGONISTS ACT ADDITIVELY TO REDUCE BEHAVIORAL HYPERALGESIA IN RATS WITH UNILATERAL INFLAMMATION. K. Ren*, and R. Dubner, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies suggest that both N-methyl-D-aspartate (NMDA) and neurokinin 1 (NK1) receptors are involved in spinal nociceptive transmission. To assess the interaction between NMDA and NK1 receptor systems on nociceptive responses of rats with complete Freund's adjuvant-induced hindpaw inflammation/hyperalgesia, an isobolographic analysis was performed using selective antagonists against NMDA or NK1 receptors. Thermal hyperalgesia, determined by a reduction of paw withdrawal latency to a heat stimulus, was induced by a single injection of complete Freund's adjuvant (1:1 emulsion, 100 μg *Mycobacterium*) into the hindpaw of rats. The drugs were given intrathecally. Dextrorphan, a non-competitive NMDA receptor antagonist, attenuated hyperalgesia dose-dependently with a maximal ≈80% reversal of hyperalgesia. WIN-51,708 and CP-96,345, nonpeptide NK1 receptor antagonists, also attenuated hyperalgesia when they were given alone. WIN-51,708 appeared to be about 8-fold more potent than CP-96,345. A combination of Dextrorphan and WIN-51,708 was then given at a fixed ratio. The ED_{50} value obtained from co-administration of the drugs was found to be not significantly different from the isobolographic line generated by individual ED_{50} values of the two receptor antagonists. These results suggest that although both NMDA and NK1 receptor systems play a role in inflammation-induced hyperalgesia, the two systems appear to act independently.

568.5

NMDA AND MUSCARINIC ANTAGONISTS IN THE ROSTRO-VENTRAL MEDULLA INHIBIT MORPHINE ANALGESIA IN THE PERIAQUEDUCTAL GRAY IN RATS. M. Spinella*, L.A. Schaefer, M.L. Cooper and R.J. Bodnar. Dept. of Psychology and Neuropsychology Doctoral Program, Queens Col., CUNY, Flushing, NY 11367.

Supraspinal morphine analgesia in the peri-aqueductal gray (PAG) is modulated by mu and delta opioid receptors and by $5HT_2$ and $5HT_3$ receptors in the rostro-ventral medulla (RVM). Since NMDA and muscarinic receptors in the RVM have been implicated in nociception, the present study evaluated whether NMDA (MK-801, AP7) or muscarinic (scopolamine, pirenzepine) antagonists in the RVM altered PAG morphine analgesia on the tail-flick and jump tests. PAG morphine (2.5 ug) analgesia was dose-dependently reduced by MK-801 (0.03-3 ug: 50-100%) and AP7 (0.01-1 ug: 30-70%) in the RVM on both tests. RVM injections of MK-801, but not AP7 reduced basal nociception. PAG morphine analgesia was dose-dependently reduced by the muscarinic antagonist, scopolamine (0.5-5 ug: 70%) and less so by the M_1 antagonist, pirenzepine (0.05-5 ug: 35-50%). Both muscarinic antagonists reduced basal latencies, but not jump thresholds. These data implicate NMDA and muscarinic receptors in the complex modulation of PAG morphine analgesia by the RVM.

568.6

SELECTIVITY OF NMDA AND NON-NMDA EXCITATORY AMINO ACID ANTAGONISTS FOR NOCICEPTION EVOKED BY ACTIVATION OF C OR Aδ NOCICEPTORS IN RATS. D. C. Yeomans*, J. Youngwerth, and H. K. Proudfit. Dept. of Pharmacology, U. Illinois at Chicago. Chicago, Illinois 60612.

Excitatory Amino Acids (EAAs) are contained in and released from nociceptive primary afferents. Although EAAs are apparently contained in all nociceptive afferent types, the EAA receptors that mediate the nociceptive responses produced by activating these afferents have not been determined.

In this experiment, we determined the effects of intrathecal (i.t.) administration of the NMDA antagonist AP5 and the AMPA/Kainate antagonist DNQX on nociception produced by activation of Aδ or C nociceptors. Administration of AP5 produced a selective, dose-dependent increase in latencies to foot withdrawal responses evoked by low noxious skin heating rates, but did not affect responses produced by high skin heating rates in doses that did not alter motor activity. These results indicate that activation of C-nociceptors produces nociception mediated in part by NMDA receptors. In contrast, intrathecal DNQX increased latencies for both high and low heating rates. These results suggest that EAAs released from both Aδ and C nociceptors act on non-NMDA receptors to produce nociception.
Supported by USPHS Grants DA08256 (DCY) and DA03980 (HKP).

568.7

AGE-RELATED CHANGES IN THE NEUROCHEMICAL MEDIATION AND THE MAGNITUDE OF SWIM STRESS-INDUCED ANALGESIA IN MICE W.F.Sternberg* and J.C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

Organismic variables have been shown to play a modulatory role in the expression of endogenous pain control mechanisms. This study assessed lifespan changes in the magnitude and neurochemical mediation of cold-water swim stress-induced analgesia (SSIA) in male and female young and old Swiss-Webster mice. We have previously shown that this swim stressor produces non-opioid analgesia on the hot-plate test in both male and female mice, and this analgesia is mediated by the NMDA receptor in young adult males, but not in similarly aged females (Mogil et al., Pain, 53, 17-25, 1993). In the present study, male and female young (32 days of age) and old (approximately 15 months of age) mice were tested for their analgesic response to a 3-min swim in water maintained at 15°C. In 32 day old animals, male and female mice exhibited analgesia following the swim stressor that was antagonized by MK-801 (0.075 mg/kg; i.p.), and was therefore NMDA-mediated, in both sexes. Thus, prepubescent females display primarily NMDA-mediated SSIA, whereas we previously reported that sexually mature females do not (Mogil et al., ibid). In the 15-month old animals, MK-801 did not antagonize SSIA in either sex. Thus, a qualitative difference in SSIA mediation is noted across the lifespan in male mice, as well as females. Analgesic magnitude increased significantly in male mice with aging, but not in females. These findings indicate that ontogenetic stage can contribute significantly to the expression of SSIA, and should be taken into consideration in future studies on SSIA mechanisms. This research was supported by NIH grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

568.8

PERIPHERAL ADMINISTRATION OF EXCITATORY AMINO ACIDS MODULATE NOCICEPTIVE BEHAVIOR IN RATS. D.L. Jackson*, J.D. Richardson, K.M. Hargreaves. Depts. of Rest. Sciences and Pharmacology, Minneapolis, MN.

While it has been demonstrated that certain primary afferent neurons in a neonatal rat spinal cord-tail preparation are activated by peripherally administered glutamate (Ault and Hildebrand, Agents Actions 1993; 39:C142-144), relatively little is known about the peripheral actions of EAAs in the intact adult animal. To evaluate possible peripheral mechanisms of nociceptive modulation by EAAs, male Sprague-Dawley rats were tested for thermal hyperalgesia by placing them in a clear plastic chamber with an infrared heat source aimed through the glass floor of the chamber at the treated hindpaw (Pain 1988; 32:77-88). Paw withdrawal latencies were detected by a photocell and recorded by an observer blinded to treatment allocation. Following the collection of baseline withdrawal latencies, rats received 100 µl ipl injections of L- or D-glutamate (10 nmol), aspartate (100 nmol), or saline vehicle. Post-injection latencies were measured at 15 and 60 minutes. Data were analyzed by ANOVA and Duncan's test and expressed as mean±s.e.m. L-glutamate reduced latencies at 15 minutes relative to the saline control (-19.9±5.6% vs. +9.5±8.3%; p<0.05). The stereoisomer, D-glutamate, had no effect on latencies at this time point (+10.8±12.7%). This is in contrast to the observed increase in latency at both 15 and 60 minutes following the ipl injection of aspartate relative to control (15 min = +42.1±14.1% vs. -4.4±11.2%; p<0.05; and 60 min = +54.0±15.0% vs. +1.8±14.2%; p<0.05). Collectively, these results support the hypothesis that the EAAs glutamate and aspartate can differentially modulate nociceptive behavior when injected into normal tissue. **This research was funded by K16-DE0027, DE09860, DE10093, and a Howard Hughes Medical Institute Predoctoral Fellowship (JDR).**

568.9

SPINAL ACTIONS OF MEDETOMIDINE AND XYLAZINE ON RESPONSES TO EXCITATORY AMINO ACIDS AND PERIPHERAL STIMULATION. J. F. Herrero and P. M. Headley. The Medical School, Bristol BS8 1TD, U.K. SPON: BRAIN RESEARCH ASSOCIATION.

α_2-adrenoceptor agonists, including medetomidine (Med) and xylazine (Xyl), are analgesic in animals and man (for review see Pertovaara, A. 1993, Prog. Neurobiol. 40, 691). They affect spinal nociception, but the underlying mechanisms are not clear. We have now examined whether this effect is related to an interaction with excitatory amino acids (EAA) and is selective for nociceptive responses. Experiments were performed on spinalized Wistar rats anaesthetized with α-chloralose. Multibarrel pipettes were used for recording single neuron activity. Xyl (10 to 160µg/kg) and Med (1.25 to 10µg/kg) were injected i.v. in a $\log_2$ regime, and their effects tested on responses elicited by cycles of 2 EAA (NMDA, AMPA or kainate) and 2 peripheral stimuli (noxious pinch, noxious heat or innocuous vibration) in different combinations.

Xyl significantly depressed responses to kainate (ID_{50}=60µg/kg), whereas NMDA and AMPA were very little affected. Med, however, produced a non-selective depression of all three EAA, being more potent on kainate and NMDA (ID_{50}=6µg/kg) than on AMPA (35% reduction with 10µg/kg). Both Xyl and Med depressed nociceptive pinch and heat responses, leaving innocuous vibration responses unaffected. The ID_{50} for Xyl was 70µg/kg for pinch and 170µg/kg for heat, and, for Med, 10µg/kg for pinch and 2µg/kg for heat. Spontaneous activity was not modified by Xyl and was only depressed with 10µg/kg of Med. All these effects were reversed or prevented by Atipamezole (80µg/kg). The results indicate that at doses of Med and Xyl that cause spinal antinociception, EAA responses are reduced. The pattern of EAA reduction does not, however, correlate clearly with the antinociception.
Supported by the Wellcome Trust.

568.10

ANTAGONISTS AT THE GLYCINE MODULATORY SITE OF THE NMDA RECEPTOR COMPLEX REVERSE INFLAMMATION-INDUCED MECHANICAL HYPERALGESIA IN THE RAT.
J.M.A. Laird*, G.S. Mason, R.J. Hargreaves & R.G. Hill Dept. of Pharmacology, Merck, Sharp & Dohme Research Labs., Harlow, CM20 2QR, U.K.

Intraplantar injection of carrageenan (CARRA) in one hind paw in the rat produces inflammation associated with decreased thresholds to noxious mechanical and thermal stimuli applied to the inflamed paw (hyperalgesia). The effects on this mechanical hyperalgesia of L-695,902 and L-701,324, structurally distinct antagonists at the glycine site on the NMDA receptor, and of L-687,414, a low efficacy partial agonist that acts as a functional antagonist at the NMDA/glycine site, were compared.

Mechanical thresholds were measured in both hind paws of male SD rats using an Ugo-Basile algesiometer 1 hr before and 3 hrs after CARRA or saline was injected into one hind paw. Test compound or vehicle was given *i.p.* 1 hr before the final test. Dose-response curves for each compound were constructed in separate experiments. All 3 compounds produced dose-dependent and statistically significant ($p < 0.05$) reversal of mechanical hyperalgesia in the CARRA injected paw with minimum effective doses (M.E.D.s) as follows: L-695,902 = 50, L-701,324 = 3 and L-687,414 = 100 mg/kg. L-695,902 and L-701,324 also produced a dose-dependent and statistically significant ($p < 0.05$) increase in mechanical threshold in the non-injected paw (analgesia) with M.E.D.s of 20 and 10 mg/kg respectively. In contrast, L-687,414 had no effect on the threshold of the non-injected paw.

We conclude that both antagonists and partial agonists at the NMDA receptor glycine site reverse inflammation-induced mechanical hyperalgesia, but that NMDA receptor glycine site antagonists also affect the response to noxious stimuli applied to normal tissue.

568.11

PAIN REDUCTION BY INTRATHECAL ADMINISTRATION OF GLYCINE.
R.K. SIMPSON*, M.M. GONDO, C.S. ROBERTSON, AND J.C. GOODMAN.
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Glycine, the primary inhibitory neurotransmitter of the spinal cord, is found in high concentration within segmental interneuron pools. Glycine is released during epidural spinal cord stimulation, an effective method of treating chronic pain. We hypothesize that the pain is attenuated by glycine.

Neuropathic rats were created by unilateral partial ligation of the proximal sciatic nerve with 4 absorbable chromic sutures place 1 mm apart. A PE-10 catheter was placed into the cisterna magna and directed toward the lumbar enlargement. Twelve days after ligation these rats were treated with intrathecal infusion of glycine, strychnine, MK-801, and 5,7-DKA, at a concentration of 0.1 µmol for 2 hours and at a rate of 5-10 µl/min. Pain scores were calculated using the Randall-Selitto technique of mechanonociceptive stimulation and compared to rats receiving artificial CSF. Significant differences, as calculated by ANOVA, were defined as $p<0.05$. Each spinal cord and nerve was evaluated using standard neurohistopathologic techniques.

Our results showed that the force necessary to produce a pain response, or movement of the neuropathic limb, was increased from 70 gr to approximately 120 gr. In contrast, strychnine lowered the threshold from approximately 70 gr to 40 gr. Neither MK-801 or 5,7-DKA alone had significant influence on the pain response. When glycine and strychnine were administered together, the effect of glycine on pain was blocked. When administered with glycine, MK-801 or 5,7-DKA did not block the reduction in the pain response to glycine.

We suggest that the amelioration of mechanonociceptor induced pain is mediated, in part, by glycine primarily at the strychnine sensitive glycine receptor.

568.12

ROLE OF NMDA RECEPTOR AND NITRIC OXIDE (NO) IN THE FACILITATION BY INTRATHECAL (IT) NMDA OF LIMB WITHDRAWALS EVOKED BY GRADED NOXIOUS HEAT IN CONSCIOUS RATS. E. Carstens*.
Neurobiology, Physiology & Behavior, Univ. of California, Davis, CA 95616.

Secondary hyperalgesia is thought to be mediated by hyperexcitability of nociceptive spinal neurons via (a) glutamate release from nociceptors to (b) activate postsynaptic NMDA receptors to (c) allow Ca++ influx to (d) effect persistent cellular changes. In behavioral studies, hyperalgesia is seen as a reduced threshold for nocifensive responses such as the tail-flick. We investigated if IT NMDA enhances suprathreshold and threshold responses using a quantitative measure of limb withdrawal response (WR) magnitude, and if this is prevented by NMDA receptor/channel blockers (APV, MK801, respectively) or the alternate substrate for NO synthase (L-NAME).

One wk after implanting IT catheters, the ventral hindpaw was fixed to a thermode to deliver an ascending series of 5 s heat pulses (40-52°C, 2° steps; 2 min interval). The normalized area of integrated limb flexor EMGs gave a measure of WR magnitude across temperatures (stimulus-response function=SRF). Two paradigms were used: (1) One SRF was generated with (a) no drug (control), (b) 3 ul NMDA (100 pmol) given IT prior to each heat stimulus, or (c) 10 ul bolus of IT APV (pmol-umol), MK801 (umol) or L-NAME (umol), followed by (b). (2) Two SRFs were generated, the first with no drug (control) and the second 20 min later with (b), (c), (d) each heat stimulus preceded by IT NaCl (0.9%), or (e) APV, MK801 or L-NAME followed by (d).

In the 2-SRF paradigm, NMDA significantly increased WR magnitude at 40-48°C vs. controls, reducing the SRF threshold and slope. This was not prevented by APV, MK801 or L-NAME. In these cases or when NaCl or MK801 + NaCl were given, the second SRF was shifted toward significantly higher temperatures. Thus, WRs appear to be sensitized during the second SRF. This sensitization may confound any effect of NMDA on the second SRF.

In the 1-SRF paradigm, NMDA significantly reduced the threshold of the SRF compared to controls. This was prevented in rats pretreated with APV, MK801 and L-NAME. These data support the idea that the NMDA receptor and NO are involved in the development of hyperexcitability in spinal neurons mediating the WR.

568.13

SPINAL STRYCHNINE INCREASES RESPONSES OF NOCICEPTIVE DORSAL HORN NEURONS TO LOW THRESHOLD INPUT AND LENGTHENS AFTERDISCHARGE. L.S. Sorkin* and S. Puig.
Anesthesiol. Research Lab, Univ. of Calif. @ San Diego, La Jolla CA 92093-0818

Hyperesthesia following many neurogenic injuries is associated with inhibitory interneuronal loss. Pharmacologic blockade of glycine receptors results in tactile evoked allodynia. It has been proposed that this is due to loss of "auto-inhibition" of low threshold (LT) input to wide dynamic range (WDR) cells via an inhibitory interneuron. This study determined if spinal strychnine (STR) results in excessive responsivity of dorsal horn neurons.

Dialysis probes were placed in the dorsal horn of chloralose-anesthetized cats and extracellular recordings made from WDR cells in close proximity to the probes. Artificial CSF was perfused and baseline responses to a range of mechanical stimuli measured. The perfusate was changed to STR (1 mM), STR + an NMDA antagonist (2 mM AP7) or STR + a non-NMDA antagonist (1 mM CNQX) and the receptive field properties redetermined.

Exposure to STR resulted in a greatly enhanced high frequency response to LT input (especially hair). The firing pattern was usually one of irregular bursting. The response to high threshold input also increased, but to a lesser degree. The high threshold response was usually followed by a prolonged afterdischarge (several min). This was seen less frequently after LT input. Receptive field size also increased substantially. In most cases, basal firing rates were unaffected. Preliminary results indicate that this enhanced responsiveness was blocked by co-administration of AP7 and that CNQX reduced evoked activity while leaving the afterdischarge relatively intact.

These results support the hypothesis that removal of tonic glycine inhibition leads to excessive activity in WDR neurons which is mediated by activity at a local NMDA receptor. [This study was supported by NS 11255]

568.14

ADRENAL MEDULLARY TRANSPLANTS ATTENUATE NMDA-INDUCED HYPERALGESIA AND ALLODYNIA. J. Siegan*, A.T. Hama, J. Sagen. Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612

Excitatory amino acids, via activation of the NMDA receptor, are implicated in the persistence of pathological pain. Recent work in our laboratory indicates that the transplantation of adrenal medullary chromaffin cells into the spinal cord subarachnoid space can alleviate chronic pain syndromes in animal models. The purpose of this study was to determine whether adrenal medullary transplants act via modifying spinal NMDA hyperexcitability. For this study intrathecal catheters were implanted into the spinal subarachnoid space of rats. Rats also received either adrenal medullary transplants or control striated muscle transplants via laminectomy at the level of the lumbar enlargement. Abnormal pain responses to several doses of intrathecally administered NMDA were determined using behavioral tests for tactile allodynia and mechanical and thermal hyperalgesia. Results indicated that NMDA produced allodynia and hyperalgesia in control transplanted animals in a dose related fashion. In contrast, the dose response to NMDA was significantly shifted rightward in animals with adrenal medullary transplants, and hyperalgesia and allodynia were completely alleviated in the lower dose ranges of NMDA in these animals. The beneficial effects of the transplants were not decreased by naloxone, but were partially attenuated by phentolamine, indicating a limited role for catecholamines released by the transplanted cells. However, this did not completely account for the effects of the transplants. In summary, these results suggest that adrenal medullary transplants may intervene in the cascade of hyperexcitability initiated by the activation of NMDA receptors in pathological pain syndromes. Supported by NIH grant NS25054.

568.15

DEXTROMETHORPHAN SUPPRESSES THE FORMALIN-INDUCED INCREASE IN SPINAL CORD C-FOS mRNA. K.J. Elliott*, M. Brodsky, A. Hynansky, K.M. Foley and C.E. Inturrisi. Dept. Pharmacology, Cornell U. Med. Coll., NY, NY and Memorial Sloan-Kettering Cancer Center, NY, NY 10021.

Dextromethorphan (DM) is a clinically available oral antitussive and NMDA receptor antagonist with the ability to attenuate neuronal "wind-up" (Dickenson et al., 1991). We have found that pretreatment with DM (6, 20 or 60 mg/kg sc) suppresses formalin-induced nociceptive behaviors in a dose-dependent manner. Nociception turns on the expression of the immediate early genes (IEG) including c-fos proto-oncogene. Formalin injection (20 ul) to the right hindpaw of BALB/c mice produces a reliable induction of c-fos mRNA of almost 100% in the right spinal dorsal horn (SDH) as assessed with quantitative solution hybridization at 30 minutes post injection. No change in c-fos mRNA was detected in the contralateral SDH, nucleus raphe magnus, periaqueductal grey, medial thalamus, or sensori-motor cortex. Pretreatment with DM at 60 mg/kg sc thirty minutes prior to formalin resulted in a suppression of c-fos induction, so that c-fos mRNA levels in SDH of animals receiving DM prior to Formalin did not differ from controls. These data indicate that DM suppresses formalin nociceptive behavior and one of the biochemical consequences of formalin nociception, i.e., induction of c-fos mRNA. Supported by the VZV Research Foundation (KJE) and DA01457, DA07274, DA00198 and CA32897.

568.16

THE N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST DEXTROMETHORPHAN SELECTIVELY REDUCES TEMPORAL SUMMATION OF SECOND PAIN IN MAN D.D. Price*, J. Mao, H. Frenk and D.J. Mayer, Dept. Anesthesiology, Medical College of Virginia, Richmond, VA 23298

Slow temporal summation of second pain is thought to be a psychophysical correlate of temporal summation of C afferent-mediated responses of dorsal horn nociceptive neurons, termed *windup*. Windup has been shown to be mediated by N-methyl-D-aspartate (NMDA) receptor activation within the spinal cord of experimental animals. We thus tested whether a NMDA receptor antagonist would reduce slow temporal summation of second pain in man. Oral doses of dextromethorphan (DM), a common cough suppressant and NMDA receptor antagonist, and their vehicle control were given on a double blind basis to normal volunteer human subjects who rated intensities of first and second pain in response to repeated painful electric shocks and repeated 52° C heat pulses. Doses of 30 and 45 mg but not 15 mg (in a single bolus) were effective in attenuating temporal summation of second pain as compared to vehicle controls (Wilcoxon signed-ranks tests, $P< 0.03$). By contrast, neither first pain nor second pain evoked by the first stimulus in a train of stimuli were affected by any of these doses of DM ($P> 0.05$). These results further confirm temporal summation of second pain as a psychophysical correlate of windup by providing evidence that DM selectively reduces temporal summation of second pain, as has been shown for windup.

568.17

NMDA MEDIATED EXCITABILITY CHANGES OF SOMATIC AND VISCERO-SOMATIC NOCICEPTIVE REFLEXES IN THE SPINAL CORD. F. Cervero*, J.M.A. Laird & P. Gª de la Rubia. Dept. Fisiología y Farmacología, Fac. Medicina, Univ. Alcalá de Henares, Madrid, Spain.

Repetitive stimulation of somatic nociceptors evokes progressive increases in the excitability of spinal neurones ("wind up"). Some forms of nociceptive stimulation are also reported to evoke long lasting potentiation of somatic reflexes. These changes are believed to be mediated by the activation of NMDA receptors on spinal neurones. In this study we have investigated afferent-induced excitability changes of nociceptive viscero-somatic reflexes and the role of spinal NMDA receptors in their generation.

Experiments were conducted on decerebrated and spinalised rabbits. Reflex activity was recorded from the first lumbar nerve in response to supramaximal electrical stimulation of the ipsilateral second lumbar nerve (somatic reflex) or of the splanchnic nerves (viscero-somatic reflex). Changes in the excitability of these reflexes during repetitive afferent stimulation at 1Hz ("wind up") and up to 5 minutes after (reflex potentiation) were studied as were the effects of intravenous administration of the NMDA receptor antagonist ketamine at doses between 1 and 30 mg/kg.

"Wind up" was consistently evoked in somatic reflexes but less so in viscero-somatic reflexes. Potentiation of viscero-somatic reflexes was rarely seen and often these reflexes were depressed by repetitive stimulation of visceral afferents. Ketamine caused a dose-dependant decrease of both somatic and viscero-somatic reflexes and a reduction in the magnitude of "wind up". We conclude that there are important differences in the spinal organization of somatic and viscero-somatic nociceptive reflexes and that NMDA receptors influence the baseline levels of reflex excitability in the spinal cord as well as the dynamic components of the reflexes.

568.18

BEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF MEMANTINE IN A PRIMATE MODEL OF PERIPHERAL NEUROPATHY S.M. Carlton*, H. Rees, K. Gondesen, M. Tsuruoka and W.D. Willis. Dept. of Anatomy and Neurosciences, Marine Biomedical Inst. UTMB, Galveston, TX 77555

The development of aminal models mimicking painful peripheral neuropathies allows investigation of underlying mechanisms with the expectation that this will lead to improved clinical treatment. NMDA receptor activation is an important mechanism contributing to neuropathic pain. The present study addresses the efficacy of Memantine (MEM), a non-competitive NMDA angatonist, in attenuating behavioral and electrophysiological abnormalities previously documented in a monkey model of peripheral neuropathy.

In an anesthetized monkey (Macaca fascicularis), one L7 spinal nerve was tightly ligated, a sham operation was performed on the contralateral side. Compared to pre-surgery levels, the animal demonstrated an increased sensitivity to mechanical stimulation (allodynia) with von Frey hairs and brushing with a camel hair brush 5 days post-surgery. On subsequent days, MEM was delivered in a bolus either IM (5 and 10mg), subcutaneous (15mg) or orally (30 and 50mg). The first test period of each day established the baseline; following administration of MEM, testing was repeated at hourly intervals. The most effective route in attenuating allodynia in the EXP foot was IM or oral administration; the subcutaneous injection did not have any effect. Responses on the control foot were not affected by any route.

In 3 normal primates, 100mM MEM administered through a dialysis fiber in the dorsal horn did not change spontaneous activity of STT cells (n=3), but resulted in decreased responses to brush (↓14%), press (↓55%), pinch (↓40%) and heat (↓49%). 10mM MEM administered to 1 neuropathic animal resulted in a similar decrease in 2 STT cells: brush (↓11%), press (↓26%), pinch (↓35%) and heat (↓53%). Responses to von frey filaments were also decreased by 27%. These results suggest MEM affects processing by STT cells and may be a suitable therapeutic drug for the treatment of neuropathic pain. (Supported by NS11255 and NS27910).

568.19

SYSTEMIC TREATMENT WITH MEMANTINE REDUCES MECHANICAL ALLODYNIA IN A RAT MODEL OF PERIPHERAL NEUROPATHY. G. Hargett*, A. Friedman and S.M. Carlton. Dept. of Anatomy and Neurosciences, Marine Biomedical Inst. UTMB, Galveston, TX 77555

A recently developed animal model of peripheral neuropathy is characterized by mechanical allodynia which develops within days and lasts for 3 months following the injury. The goal of the present study was to determine whether treatment with the non-competitive NMDA antagonist Memantine (MEM) had therapeutic and/or prophylactic affects in relieving mechanical allodynia observed in this animal model.

Acute delivery: In 5 groups of rats (Sprague-Dawley, 125-175gm) a dose-response curve was established. Following baseline testing with von Frey hairs on the ventral surface of each foot and performance on a rotowheel, animals were anesthetized and the L5 and L6 spinal nerves tightly ligated with 6-0 silk. Animals were retested on days 1,3,5,7; on day 7, they (n = 5 to 9 per group) were injected IP with saline or either 0.1, 5.0, 10 or 20mg/kg of MEM. The animals were retested for mechanical allodynia and motor performance at 1,2,4,6,8 and 24 hrs post-injection.

Chronic delivery: To determine therapeutic effects, MEM was chronically administered via IP Alzet pumps (#2ML1, delivers 10μl/hr/7 days), implanted on day 7 postsurgery. The pumps delivered MEM at a continuous rate of 4mg/kg/hr for 1 week. To determine prophylactic effects, rats were implanted with IP pumps 2 days prior to surgery for the nerve ligation. All animals were retested every day (7-14 day survival) for withdrawal responses to von frey hairs and rotowheel performance.

Acute IP injections of MEM demonstrated that saline and 0.1mg/kg MEM had no effect on behavior. 5.0mg/kg MEM significantly attenuated mechanical allodynia at 1hr post-injection with no motor impairment. Although 10 and 20mg/kg effectively attenuated mechanical allodynia at 1,2 and 4hrs, the animals displayed hyperactivity. No effect was observed on the contralateral control paw. Preliminary studies concerning chronic treatment with 4mg/kg/hr via pumps (n=3) demonstrated a therapeutic effect with no abnormal motor signs. However, this same dose had no obvious prophylactic effect. Results indicate that NMDA receptors play an important role in the expression of allodynia and MEM may be used as a successful treatment for neuropathic pain. (Supported by NS11255 and NS27910.)

568.20

MODULATION OF ALTERED NMDA RECEPTOR BINDING BY SPINAL ADRENAL MEDULLARY TRANSPLANTS IN ANIMALS WITH PAINFUL PERIPHERAL NEUROPATHY **A.T. Hama*, J.R. Unnerstall and J. Sagen. Dept. Anatomy and Cell Biol., Univ. IL at Chicago, Chicago, IL 60612.**

The sequence of events that leads to neuropathic pain may include activation of the *N*-methyl-D-aspartate (NMDA) receptor-ion channel complex since pretreatment with competitive as well as noncompetitive NMDA receptor antagonists prevents nerve injury- induced abnormal pain. Earlier studies in our laboratory have shown that spinal adrenal medullary tissue transplants reduce neuropathic pain over an extended period of time. The effects of adrenal tissue transplants on spinal NMDA receptor binding was investigated using a new high-affinity glutamate binding site antagonist, CGP-39653. Saturation studies in spinal cord revealed that [^{3}H]CGP-39653 binding affinity was similar to cerebral cortex but B_{max} was lower. Neuropathic pain was induced in rats by loose ligation of the right sciatic nerve (Bennett and Xie, 1988). Two weeks following nerve surgery, either adrenal medullary or control striated muscle tissue pieces were implanted in the subarachnoid space above lumbar spinal cord. One week following implantation, membrane preparations were made from spinal lumbar regions L_4-L_5, the spinal cord spilt in half down the dorsoventral axis and homogenized separately. Animals that received control transplants showed decreased [^{3}H]CGP-39653 specific binding ipsilateral to nerve ligation, compared to the contralateral side and unoperated controls. In contrast, adrenal medullary transplants bilaterally increased [^{3}H]CGP-39653 binding, bringing binding on the nerve-injured side back to near normal control levels. These results suggest that adrenal medullary transplants reduce neuropathic pain by equilibrating abnormal excitatory amino acid transmission in the spinal cord. Supported by NIH grant NS25054.

RETINA AND PHOTORECEPTORS VI

569.1

DO GLIA-NEURONAL INTERACTIONS GENERATE SENSORY EVOKED POTENTIALS? R. Galambos* and G. Juhász. Eötvös Loránd Univ., 1088-N, Budapest, Hungary

We will state and discuss the proposition that cortical evoked potentials are generated when neurons interact with glial cells across extracellular space. The evidence is as follows. First, glial research by Kuffler, Nichols, Dowling, Newman, and dozens of others validates the claim that the major evoked potential of the eye, the retinal b-wave, is produced this way. Second, the b-wave of freely-moving rats (stimulated through a light emitting diode implanted above an eye) approximately doubles in amplitude during slow wave sleep; similar plastic changes takes place in the visual cortical evoked potential recorded at the same time. Third, when the rat receives paired flashes, the refractory periods measured for b-wave and for cortical responses are both prolonged, but not identically. b-waves begin when synapses activated by rods and cones perturb $[K^+]_o$ and initiate potassium currents within Müller (glial) cell cytoplasm. The evidence that cortical astrocytes similarly optimize $[K^+]_o$ is still indirect, but nothing uncovered so far seems to rule the possibility out.

569.2

SCLERAL DISTRIBUTION OF ERG B-WAVE AND OSCILLATORY POTENTIALS (OPs). P. Lachapelle*[1], J. Benoit[1] & C. Casanova[2]. Depts of Ophthalmology, [1]McGill University - Montreal Children's Hospital, Montréal, Qué., Canada H3H 1P3 and [2]Université de Sherbrooke, Sherbrooke, Qué., Canada J1H 5N4.

Previous studies reporting the scleral distribution of the ERG made use of a bipolar recording approach a method which could have masked the existence of localised ERG dipoles. We investigated this issue in (n=10) anesthetized, paralyzed and artificially ventilated New Zealand rabbits . Scleral ERGs (1-1000 Hz) and OPs (100-1000 Hz) were recorded with a 0.5 mm tungsten electrode with reference and ground electrodes positioned in the mouth and neck muscles respectively. The anterior portion of the globe was dominated by a positive b-wave whose amplitude was maximal at the cornea. It was gradually replaced by a slow negative wave (inversion at 5-6 mm posterior to the limbus) onto which were superimposed large oscillations. These new ERG components, never reported in bipolar recordings, reached a maximum at 7-8 mm posterior to the limbus. FFT analysis performed at this scleral location indicated that: 1- the negative wave is not an inverted b-wave and 2- the large oscillations are, given their frequency domain, enhanced OPs. This was confirmed with the analysis of the tracings obtained with the OPs specific bandwidth (100-1000Hz). It showed that the OPs reached their maximal amplitude (85% larger than corneal OPs) some 7-8 mm posterior to the limbus. Our results indicate that the OPs are the most consistent retinal potentials since they are found at all scleral locations while the b-wave appears to be limited to the anterior portion of the eye. Furthermore in showing that maximal OPs are obtained in b-wave free locations suggests that the two retinal generators (b-wave and OPs) may interact in an inhibitory fashion. Funded by MRC MT12153.

569.3

STIMULATION OF RETINAL DOPAMINE D2 RECEPTORS AFFECTS AMPLITUDES OF THE B BUT NOT A WAVES OF THE RABBIT ELECTRORETINOGRAM.(ERG)

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The two classical sub-types of dopamine receptors , D1 and D2, are present in the retina. We have shown that stimulation of both receptors with the mixed D1 and D2 agonist apomorphine results in marked reductions in amplitudes of both a and b waves of the rabbit ERG. In order to better delineate the subtype of dopamine receptor responsible for these findings, we have examined the effects of R-norpropylapomorphine (NPA), a purported selective D-2 agonist, on retinal electrophysiology in rabbits. Experiments were carried out in anesthetized adult pigmented rabbits. A corneal electrode was inserted near the limbus to record the ERG. Responses were evoked by a diffuse flash (Grass PS2) and averaged 25 times. Three intensity levels were tested. NPA was injected intravitreally at various concentrations (0.01-10 µg in 100 µl). Our results indicate that the intravitreal injection of NPA reduced, in a dose related fashion, the amplitude of the ERG b wave (up to 40%) in both scotopic and photopic conditions without significant changes in the implicit time. No effects were seen at the level of the a-wave. These results suggest that the D2 receptor is implicated in modulation of the b-wave, whereas the D1 receptor is involved in the modulation of the a-wave.

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569.4

MULTIPLE SITES OF S-LAMININ SYNTHESIS IN THE MATURE RAT RETINA.

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Components of the extracellular matrix (ECM) have been implicated in such diverse phenomena as differentiation, neurite outgrowth, and synapse formation. We have previously shown that one such ECM component--s-laminin--is present in the retina during development, and that s-laminin may be involved in photoreceptor differentiation. S-laminin expression remains in the mature retina, especially in the interphotoreceptor matrix (IPM); however, the function and sites of synthesis of s-laminin in the mature retina remain unclear. We have examined the expression of s-laminin RNA and protein in the mature retina to begin to address these issues.

In situ hybridization--using both ^{35}S- and digoxigenin-labeled cRNA probes--demonstrates several sites of synthesis for s-laminin RNA in the mature rat retina. One, the inner nuclear layer, may represent the site of synthesis of s-laminin that is eventually deposited in the IPM. A second prominent site of s-laminin RNA, the retinal ganglion cell layer (GCL), is somewhat surprising: we had previously found little s-laminin protein in this layer when we examined unfixed sections of mature retina. In the absence of fixation, our antibodies detect largely the ECM-associated s-laminin (including in the IPM); notably, these same antibodies, when used on fixed tissue, reveal cellular s-laminin immunoreactivity in the GCL. The presence of s-laminin immunoreactivity under these conditions supports our hypothesis that the GCL is one site for s-laminin synthesis. We are currently testing whether cells in the GCL deposit the s-laminin that they produce in the retina, and whether they ship this s-laminin to their synaptic targets.

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569.5

EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR mRNAs IN ADULT RAT RETINA.

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Glutamate is an important transmitter in the vertebrate retina and recently a series of ionotropic and metabotropic glutamate receptors has been cloned and characterized. Whereas most of the glutamatergic neurotransmission in the retina seems to be mediated by ionotropic receptors, the extent of involvement of metabotropic receptors in retinal function is unclear. We have therefore investigated the expression patterns of six metabotropic glutamate receptors (mGluR1-mGluR6) by *in situ* hybridization. Retina sections were hybridized with ^{35}S-labeled oligonucleotide probes and thereafter exposed to photo-sensitive emulsion for up to 12 weeks.

mGluR1: expressed by virtually all cells in the ganglion cell layer (GCL) and by some cells with their somata in the inner third of the inner nuclear layer (INL), most likely amacrine cells. *mGluR2:* expressed by some cells in the GCL and by some amacrine cells in the INL. *mGluR3:* not expressed at detectable levels. *mGluR4:* expressed by cells in the GCL and the INL, similar pattern to that seen for mGluR1. *mGluR5:* expressed by cells with their somata in the outer third of the INL, possibly horizontal cells. *mGluR6:* expressed by cells with their somata located in the outer half of the INL, consistent with the recent suggestion by Nakajima et al. (1993) that this receptor corresponds to the physiologically and pharmacologically defined APB-receptor that mediates synaptic input from photoreceptors to ON-bipolar cells. Taken together, metabotropic glutamate receptors show a considerably more widespread pattern of expression in the retina than suggested from physiological experiments. We are currently extending this investigation by examing the expression of the recently cloned mGluR7 and improving the cellular resolution by *in situ* hybridization of dissociated cells. Supported by AvH-foundation (EH) and SFB-269/B4.

569.6

IMMUNOLOCALIZATION OF THE INOSITOL TRISPHOSPHATE RECEPTOR IN THE CATFISH RETINA.

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Inositol 1,4,5-trisphosphate ($InsP_3$) mobilizes calcium from intracellular stores by binding to specific receptors. In the brain, $InsP_3$ receptors are concentrated in cerebellar Purkinje cells where they have been biochemically characterized.

Immunocytochemical localization in retinas from rat, turtle and goldfish reveals a characteristic distribution of the $InsP_3$ receptor at the synaptic layer. For this reason, it has been proposed that the $InsP_3$ sensitive calcium store may be involved in transmitter release within the retina.

Here, we used an affinity-purified rabbit polyclonal antibody against the 19-mer C-terminal of the mouse cerebellar $InsP_3$ receptor to determine: 1) whether the antibody to the mammalian $InsP_3$ receptor can bind the fish receptor in the cerebellum and retina; and 2) the distribution of the receptor in this retina. Since the distribution of the $InsP_3$ receptor has been described in the rat retina, we used this retina as a control for our antibody.

Observation of catfish retina frozen sections in the confocal microscope revealed that the $InsP_3$ receptor is localized to the inner nuclear layer with some positive staining also visible at the outer and inner limiting membrane. Immunocytochemistry on dissociated retina cells further demonstrated that the receptor is mainly localized in the cone horizontal cells and in the Müller glia cells.

These data reveal a distribution of the $InsP_3$ receptor in the catfish retina different from that observed in other vertebrates that may be suggestive of a different function of $InsP_3$-sensitive calcium stores in this retina.

We have previously demonstrated that a rise in intracellular calcium can occur via influx through voltage-sensitive calcium channels and glutamate-gated channels. In addition, catfish cone horizontal cells contain a calcium store sensitive to ryanodine and caffeine. The presence of $InsP_3$ provides for yet another source of calcium that may be important for the regulation of calcium-dependent metabolic pathways. Supported by grant NEI-01897.

569.7

ONTOGENESIS OF ACETYLCHOLINESTERASE (AChE) IN THE DEVELOPING OPOSSUM RETINA.

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We have previously shown that the differentiation of cholinergic neurons in the opossum retina was detected around 15 postnatal days (P15). In this work, we follow the pattern of expression of AChE, the hydrolyzing enzyme for acetylcholine, during the development of the opossum retina, applying the Hedreen method (1985). The onset of AChE activity was seen in the P10 opossum, in cell bodies present in the ganglion cell layer (GCL). This activity is detected before the emergence of the inner plexiform layer (IPL) and before the appearance of cholinergic neurons, much earlier than the onset of synaptogenesis in the IPL (P22). Between P25-P33, the histochemistry reaction becomes more intense, but is still restricted to cell bodies in the GCL. Only after P45 AChE positive bands in the IPL (strata 1, 3 and 4/5) and somata in the inner nuclear layer (INL) could be seen. In P50, when the retina matures, the AChE-histochemistry reveals the adult pattern, i.e., cell bodies in the GCL and INL, labeled bands in the IPL and OPL. Since AChE could be detected so early during opossum retinal development, a question arises whether AChE takes part on cellular proliferation and/or differentiation in the retina.

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569.8

IDENTIFICATION OF CELL TYPES AND CELLULAR COMPARTMENTS CONTAINING NITRIC OXIDE SYNTHASE IN THE TIGER SALAMANDER RETINA.

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NADPH-diaphorase histochemistry in retinal sections revealed staining within rod and cone photoreceptor ellipsoids, in pockets external to the photoreceptor cell bodies and in the outer plexiform layer (OPL). NADPH-diaphorase positive puncta were found also throughout the inner plexiform layer (IPL), possibly representing synaptic terminals of bipolar, amacrine or ganglion cells. Lighter staining was associated with retinal glial cells (Müller cells; counterstained for GFAP) and the axon hillocks and surface of the somata of ganglion cells. NADPH-diaphorase histochemistry performed on enzymatically isolated retinal cells indicated that the distal portions of Müller cells and bipolar cell Landolt clubs account for at least part of the NADPH-diaphorase activity surrounding the photoreceptors. No reaction product was observed when NAD or NADP were used in place of NADPH showing that the reaction required NADPH-dependent electron transfer. NO-synthase localization was detected with polyclonal antibodies directed against rat brain NO-synthase and was consistent with NADPH-diaphorase activity. Labelling was detected in photoreceptor ellipsoids and around their inner segments, in the OPL, Müller cells, a few inner nuclear layer cells, and within the IPL but primarily at the border between sublamina a and b. These results suggest that NO may have several roles in visual processing in the retina.

Anti-NOS antibodies were kindly provided by B. Mayer, Universität Graz. *Supported by the Medical Research Council and the Alberta Heritage Foundation for Medical Research.*

569.9

GABA RAISES INTRACELLULAR CALCIUM IN NEURONS OF DEVELOPING RABBIT RETINA. B. Huang* D. Redburn
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Previous studies in this laboratory have shown that horizontal cells of the rabbit retina are transiently GABAergic during early post-natal development and that lesioning these horizontal cells or blocking GABA receptors with antagonists interrupt cone synaptogenesis. In order to determine the mechanism by which GABA might exert these effects, we have used a Ca^{2+} fluorescence assay to explore the effects of GABA on calcium flux in developing retinal neurons.

Cells from neonatal rabbit retina were freshly isolated and then loaded with fluo-3 AM. When challenged with 100 μM GABA, intracellular Ca^{2+} increased in certain populations of retinal neurons. This GABA-induced Ca^{2+} response began to appear in retinal cells of postnatal day 3 and was most prominent on day 5. Both picrotoxin and bicuculline blocked the response, indicating that $GABA_A$ receptors were involved. Based on these results, we propose that during postnatal retinal development GABA may exert its trophic action on retinal synaptogenesis through membrane depolarization and a rise in intracellular Ca^{2+}.

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569.10

CIRCADIAN RHYTHMS IN THE QUAIL RETINA: MODULATION BY LIGHT AND DOPAMINE

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The Japanese quail exhibits a circadian rhythm in retinal sensitivity. [1] Rhythmic shifting of rod-cone dominance with time of day appears to be intensity dependent. Circadian rhythms in dark-adapted eyes are readily detectable for photopic stimuli: rods dominate retinal sensitivity at night while cones dominate during the day. Suprisingly, for scotopic stimuli, rods dominate retinal sensitivity regardless of time of day, thus abolishing circadian rhythms of sensitivity.

How does the circadian clock shift rod-cone dominance during the day but not at night? We hypothesize that dopamine blocks rod signals to intense stimuli during the day and thereby shifts the retina to the cone-dominated state. Retinal dopamine levels are high during the day and low at night in constant dark making dopamine an obvious candidate for modulating retinal sensitivity.[2] Dopamine agonists and antagonists alter the rhythms of retinal sensitivity, and light adaptation can disrupt the normal rod dominance at night.[1,2] Preliminary results suggest light and dopamine are independent modulators of the ocular clock in the Japanese quail.

[1] Uchiyama, H, *et al*. 1990 *Neurosci. Abstr.* **16**:1333.
[2] Buelow, NF, *et al.* 1992 *Neurosci. Abstr.* **18**:138.

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MOTOR CORTEX: BIOPHYSICS, MODELS, PLASTICITY

570.1

RAPID CHANGES IN THE SIZE OF NERVE DOMINANCE AGGREGATES IN PRIMATE SOMATOSENSORY CORTEX AFTER NERVE INJURY. A. Silva, S.K. Rasey, and J.T. Wall*. Department of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The area 3b cortex of primates contains band- and patch-like aggregates of neurons that are dominantly activated by cutaneous inputs from the ulnar, radial, and median nerves to the hand. The ulnar nerve innervates about 43% of the hand surface, and normally provides dominant inputs to about 38% of the area 3b hand cortex. This study evaluated whether ulnar nerve dominance aggregates change in size over the first several minutes to hours after wrist level transection of the radial and median nerves. Acutely after injury, cortical aggregates of the ulnar nerve rapidly expand from a normal mean area of about 4.9 mm^2 to a mean area of about 7.1 mm^2. Due to this change, ulnar inputs rapidly gain access to about 59% of the area 3b hand cortex, or about 1.5 times the normal cortical space. These size changes are initiated within minutes after injury. The extent and spatial distribution of these changes provide an image of central substrates that were available for decompression of ulnar nerve dominance aggregates at the time of injury. Previous evaluations of radial nerve dominance aggregates after acute transection of the ulnar and median nerves show that these aggregates rapidly gain access to a cortical space that is about 3 times the normal cortical space. Thus, dominance aggregates of different nerves undergo rapid size changes of variable extents. It is suggested that this variability is dependent on differences in the patterns of central connections that are rapidly impacted by the different injuries, and on differences in the patterns of central substrates that can be rapidly accessed by the uninjured nerve.

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570.2

REORGANIZATION OF MOVEMENT REPRESENTATIONS IN PRIMARY MOTOR CORTEX OF ADULT SQUIRREL MONKEYS FOLLOWING DISTAL FORELIMB RESTRICTION. G.W. Milliken*, E.J. Plautz, G.A. Gardner, R. Raiszadeh, & R.J. Nudo. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030.

The consequences of distal forelimb restriction on the functional topography of primary motor cortex (area 4) were examined in normal adult squirrel monkeys. Using intracortical microstimulation techniques, a detailed map of the movement representations of the hand, wrist, and arm contralateral to the preferred hand was derived (Nudo, et al., *J. Neurosci.*, 12:2918, 1992). After a post operative recovery period the preferred forelimb was placed in a cast that effectively restricted movements of the hand and wrist. Movement maps of the distal forelimb representation were re-derived at 3 month intervals.

In each case, post-restriction maps revealed significant changes in movement representations. The results show that within 6 months, finger representations decreased while wrist representations increased in total area extent. In contrast, the movement representations of control animals that did not undergo forelimb restriction remained relatively unchanged. These results demonstrate that distal forelimb movement representations are alterable through *disuse* and that the changes associated with forelimb restriction are progressive over several months. Taken together with our previous findings that forelimb representations are alterable through *increased* use, these studies lend further support to the idea that movement representations in primary motor cortex are shaped by experiential factors related to use.

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570.3

MENTAL MOVEMENT EXERCISES PREVENT MOTOR DISTURBANCES AFTER IMMOBILIZATION OF A LIMB.
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We studied the effec ts of mental movements and isometric contractions on motor consequences of complete immobilization of forearm, hand, and fingers in a cast for 5 days.

We divided randomly 12 volunteers into group 1 who simply wore the cast, group 2 who performed isometric muscle contractions, and group 3 who performed mental finger exercises while wearing the cast. We tested subjects before cast placement and 1 hr. and 4-8 hrs after cast removal. We recorded maximal voluntary force for grip and thumb-index oppositioin, serial choice reaction time with individual digits, performance of a sequence of finger movements, Grooved Pegboard performance, H-reflex in flexor carpi radialis (FCR), and cortical output maps to abductor pollicis brevis, first dorcal interosseus, and FCR gemerated by transcranial magnetic stimulation. After cast removal, group 1 showed poorer performance in all tasks. This effect was less pronounced in group 2 and absent in group 3. Cortical output maps decreased in size in group 1 but remained unchanged in groups 2 and 3. H-reflexes were stable in all subjects.

Isometric contractions and specially mental exercises decrease the motor disability after limb immobilization by preventing the modulation of cortical outputs to immobilized muscles.

570.4

ROLE OF SPONTANEOUS ELECTRICAL ACTIVITY IN MOTOR CORTEX REORGANIZATION INDUCED BY FACIAL NERVE TRANSECTION IN ADULT RATS. S. S. Suzuki*, T. Shitama, Y. Harada1 and N. Nagamura. Biosignaling Dept., Natnl Inst. Biosci. and Human-Technol., Tsukuba 305, and [1] Dept. Physiol., Nippon Med. Sch., Tokyo 113, Japan

The motor cortical maps in adult mammals are reported to reorganize following various manipulations including peripheral denervation (Donoghue et al. 1990), repetitive intracortical microstimulation (ICMS) (Nudo et al. 1990) and local disinhibition by intracortical bicuculline infusion (Jacobs and Donoghue 1991). These studies suggest that differential activities in competing regions within the primary motor cortex (MI) mediate the reorganization of motor representations. The present experiment examined this hypothesis by recording multiple unit activity (MUA) from vibrissal and other regions of MI before and after facial nerve transection.

Three to seven microelectrodes were placed at low threshold depths of the right MI in ketamine-anesthetized rats. The same microelectrodes were used for ICMS and MUA recording. Multisite MUA, ICMS-evoked movements and electromyograms of left facial, neck and forelimb muscles were recorded before and after transection of the buccal and mandibular branches of the left facial nerve. The MUA at sites where ICMS had evoked vibrissal movements was greatly reduced, compared with other sites, immediately after the transection and remained so up to several hours. This result suggests that the differential neural activities in the vibrissal and other MI regions might play a role in MI map reorganization. The MUA reduction in the vibrissal regions of MI may be due to a decrease in vibrissal (trigeminal) afferent tone resulting from the motor nerve transection.

570.5

CORRELATION DIMENSION AND LYAPUNOV EXPONENT CALCULATIONS FOR NEURAL ACTIVITY RECORDED IN PRIMATES DURING VOLUNTARY TWO-DIMENSIONAL HAND MOVEMENT WITH INSTRUCTED DELAY Gyöngyi Gaál * Dept of Neuroscience, Brown University, Providence, RI 02912

In addition to linear methods, such as Fourier transforms, autocorrelations and crosscorrelations, nonlinear dynamical analysis methods were used to examine multichannel cortical activity in order to characterize the spatio-temporal dynamics of the cortical network during movement performance (Gaál et al., 1994, submitted to J. Neurophysiology). Oscillatory local field potentials (LFP) proved to be nondirectional in a planar directional hand movement task using instructed delay, while units showed directional selectivity (Donoghue et al., 1994, this volume). Therefore both LFP and extracellular unit activity were subjected to correlation dimension (CD) and largest Lyapunov exponent (LE) calculations using MTRCHAOS and MTRLYAP Version 1.0 developed by M. Rosenstein (Rosenstein et al., 1993; Physica D 65) as well as Chaos Data Analyzer distributed by the American Physics Institute. Both LFP and unit data turned out to be higher dimensional than results of previous relevant calculations performed by other researchers. Two methods were used to verify the reliability of our methods: 1) phase-randomized surrogate data served as controls for brain-derived data; and 2) data from Lorenz and Rössler systems of equations and extracellular activity of four point spiking neurons driven by Lorenz and Rössler signals were used to test the CD and LE algorithms. The high correlation dimension values obtained indicate that no simple set of equations can be derived to model the system. New methods, such as complexity calculations (Rapp et al., J. Neurosci, 1994) have to be used to further investigate extracellular spiking activity.

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570.6

QUANTITATIVE HOMUNCULUS: GRAPHICAL REPRESENTATION OF DENSITY INFORMATION ON A SURFACE. D.C. Fain, D. Rosenbluth, A.H. Barr, and J.M. Allman*. Computation and Neural Systems, Caltech 139-74, Pasadena CA 91125

In many situations, the data collected in experiments is in the form of scalar data taken at many points distributed on a 2D surface in 3-space. Two familiar examples are two-point discrimination data on the body surface and estimates of the relative representation area on the sensory and motor cortex of various parts of the body. The visualization of such data is problematic and in many situations researchers have resorted to artists' renditions of the data as in the classic picture of the sensorimotor homunculus. We describe a method which takes density information on a surface and produces a deformed surface whose characteristics accurately represent the density data on the original surface. This technique yields a 3D model which may then be projected into the plane from various viewing angles using standard computer graphical rendering techniques.

We use constrained optimization techniques to arrive at a new surface which has uniform density (a hard constraint) and is as close in curvature to the original surface as possible (a soft constraint).

We would like to stress the widespread applicability of this tool within neuroscience as an aid in the visualization of increasingly complex data. One particularly promising application is to the representation of plasticity of the sensory cortex using animation of deformations of a surface.

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570.7

THE POTENTIAL FUNCTIONAL ROLE OF REVERBERATING SYNFIRE CHAINS DEVELOPING IN RANDOMLY CONNECTED NEURAL NETWORKS

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Over the past few years, several types of synchronized neural activities have been observed in different cortical areas. This gave rise to a theoretical debate on the potential role of such synchronizations for cortical function. Experimental results on the abundance and behavioral dependence of precise spatio-temporal spike patterns in the prefrontal cortex of awake behaving monkey (Abeles et al. 1993) support the hypothesis, that 'synfire volleys' propagate through the cortex in 'reverberating synfire chains' (RSC): feedforward networks with additional feedback connections.

To get insight into the possible functional role of such RSCs, we investigated their development in a model neural network (see also Bienenstock, 1991). An initially randomly connected network of excitatory and inhibitory neurons with poisson-distributed coupling strength was repeatedly stimulated by a single synchronized spike event, adjusted to activate only a small fraction of the neurons in the network. The global dynamics of the resulting activity spread through the network can be described analytically following Anninos et al. (1970). Concomitantly, the synaptic connectivity changes gradually as a result of interaction with a 'competitive learning rule'. This development leads eventually to the formation of RSCs. Moreover, interconnections between different RSCs develop, depending on the spatio-temporal structure of the corresponding stimuli. The path through the network defined by the RSC, may be interpreted functionally as a trace of auto- and hetero-associations. Thus, the space-time composition of the RSC's and their interconnections provide a natural implementation of stimulus context memory.

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570.8

THEORETICAL IMPLICATION ON JOINT MOVEMENTS REPRESENTATION BY POPULATIONS OF MOTOR CORTICAL NEURONS. S. Tanaka*, Dept. Electrical Engineering, Sophia Univ., Tokyo 102, Japan.

It has been shown that the discharge rate of neurons in the arm area of the monkey motor cortex varies with the movement direction of the arm (e.g. Georgopoulos et al. 1988). Further, the discharge rate has been shown to be modulated by the position of the arm (Caminiti et al. 1990, 1991). Although these results suggest that the motor cortex represents certain aspects of the arm movement, it is still unclear whether a neuronal population codes the movement of the whole arm, which requires the synergistic activation of the muscles of the arm.

In this study the coding of the voluntary movement in the motor cortex and the transmission of the command through the corticospinal system are analyzed theoretically and numerically without using the population vector hypothesis. It is shown that the amount of the shift of the preferred direction of the motor cortical neurons, which has been observed in the experiments by Caminiti et al. (1990a, b, 1991), can be estimated as a function of the posture of a simplified model arm. In the estimation, it requires several populations in the arm area of the motor cortex, which represent the movements of the different joints or agonist-antagonist pairs of the muscles of the arm. The present model shows further that, if the motor cortex receives the feedback information on the rotation of the joint angles, the populations can make the appropriate coding of the joint movements.

These results suggest that there are several populations of neurons in the motor cortex arm area and that each population codes the movement of the corresponding joint of the arm.

570.9

THE PROFILE OF VOLTAGE-DEPENDENT CONDUCTANCES IN NEOCORTICAL LAYER I NEURONS. J.A. White*, T. Budde, and A.R. Kay, Dept. of Biological Sciences, Univ. of Iowa, Iowa City, IA 52242 U.S.A.

Layer I of the mammalian neocortex contains >90% GABAergic interneurons and a very low neuronal density. Presumably because of this low density, it has proven to be extremely difficult to record from layer I cells. As a consequence, nothing is known about these neurons' intrinsic firing properties or the complement of ion channels underlying these properties.

We studied membrane currents in acutely dissociated layer I neurons obtained from mature Long-Evans rats by trimming layer I from neocortical slices and subjecting the pieces to enzymatic digestion. Inward currents exhibited by layer I neurons included a fast-inactivating Na^+ current and a high-threshold Ca^{2+} current. No evidence was found of a persistent Na^+ current, TTX-resistant Na^+ current, or low-threshold Ca^{2+} current. Outward currents included a delayed K^+ current, an A-current, and a Ca^{2+}-activated K^+ current.

The ion channel profile in layer I cells differs from those reported previously in pyramidal or nonpyramidal neurons from lower layers. Unlike nonpyramidal cells, mature layer I cells express an A-current. Unlike pyramidal cells, layer I cells do not exhibit a low-threshold Ca^{2+} current. Possible consequences of this particular complement of ion channels will be discussed.

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570.10

PHYSIOLOGICAL SUBGROUPS OF NONPYRAMIDAL CELLS IN LAYER II/III OF RAT FRONTAL CORTEX. Y. Kawaguchi*. Lab. for Neural Circuits, Bio-Mimetic Control Research Center, RIKEN, Rokuban, Atsuta-ku, Nagoya 456 Japan.

Physiological and morphological properties of nonpyramidal cells in layer II/III of frontal cortex of young rats were studied in vitro by whole cell recording and intracellular staining of biocytin. Layer II/III nonpyramidal cells could be divided into four subgroups by their firing patterns in response to depolarizing pulses and patterns of dendritic and axonal arborizations. (1) Fast-spiking (FS) cells had shorter-duration action potentials and constant spike frequencies during an episode of discharges. FS cells had local or horizontal axonal arbors which did not enter layer I. FS cells contained chandelier cells and GABAergic parvalbumin cells. (2) Late-spiking (LS) cells exhibited slowly-developing ramp depolarizations near threshold. LS cells were neurogliaform cells. (3) Low-threshold spike (LTS) cells had low-threshold spikes with larger amplitude and longer duration. The main axons of LTS cells ascended, and the collaterals entered into layer I. (4) Although the remaining cells (regular-spiking (RS) nonpyramidal cells) did not have clearly distinguishable electrophysiological properties, RS cells are tentatively classified into two types. (4a) RS type 1 (RS-1) cells induced depolarizing fast notches with smaller amplitude by depolarization from hyperpolarized potentials. RS-1 cells had vertically elongated axonal fields, extending from layer I to V, sometimes to layer VI. This type contained bipolar cells. (4b) RS type 2 (RS-2) cells extended axonal branches vertically, ascending to layer I. RS-2 cells also contained a chandelier cell. These suggest that each subgroup may differentially contribute to the laminar and columnar circuitry of cortex.

570.11

PYRAMIDAL MOTOR EVOKED POTENTIALS EVOKED BY CORTICAL STIMULATION IN RATS. Y.G. Park*, J. W. Chang, J.H. Kim and S.S. Chung. Dept. of Neurosurgery, Yonsei Univ. College of Med. Seoul, Korea

Motor evoked potential(MEP) produced by cortical surface stimulation has recently evolved as a new clinical and experimental tool to monitor the integrity of motor pathways. The original concept using MEPs was based on the assumption that synchronized compound action potentials evoked by electrical stimulation of pyramidal neurons in the motor cortex were conducted along the corticospinal tracts in the spinal cord. It was, however, found that MEPs recorded in the spinal cord were mainly produced by activation of reticular systems in the brain stem due to spread of stimulus current applied on the motor cortex, especially in non-primates with small brain.

The purpose of this study was, therefore, to selectively monitor MEPs evoked by cortical neurons in the rat. A small surface electrode with a diameter of 1.5mm and another electrode protruded 1.5mm from the surface electrode were used as an anode and cathode, respectively. The evoked responses were recorded on the spinal cord using bipolar electrodes. The potentials monitored were cerebral cortex in their origin since the potentials were abolished when the pyramidal tract was selectively lesioned at the internal capsule. The intracord recording of these potentials revealed that the amplitude was highest at the ventralmost area of the dorsal column where the corticospinal tract was located. Latencies of these potentials were much longer than the latencies of the non-pyramidal MEP. Amplitudes, latencies and wave forms of these potentials were studied following intravenous injection of various general anesthetics to examine the effect of these drugs on MEPs.

MOTOR CORTEX: HUMAN STUDIES

571.1

MULTIPLE MOVEMENTS MAY BE RELIABLY DIFFERENTIATED USING fMRI, PROVIDING ORGANIZATIONAL PERSPECTIVES ON BRAIN FUNCTION. T.W. Kjaer*, J.A. Hertz, P. Jezzard, T.P. Pons, B.J. Richmond. Lab. of Neuropsychology, NIMH/NIH, Bethesda MD, USA; Nordita, Copenhagen, Denmark; Lab. of Cardiac Energetics, NHLBI/NIH, Bethesda, MD, USA.

We have investigated the differentiability of activation in the human brain during several randomly-interleaved self-paced motor tasks, as revealed in functional magnetic resonance imaging (fMRI). We used a neural network as a distribution-free technique to classify the tasks on the basis of the fMR signal. This approach directly estimates probabilities of multiple conditions from an fMRI picture (or any area within it). It does not make a priori assumptions about the distribution of the signal or the relative signal strength for different conditions. Discriminability is computed as the information carried in the image about the condition set.

Different sets of fMRI-amplitude data from 4 motor tasks were analyzed. The analyses were carried out at 6 spatial scales, from individual voxels to the full picture. In some cases, the data were preprocessed using principal components. In general, information was found to be encoded across many different voxels. However some voxels were found to be completely independent of their neighbors. Hence spatial smearing of fMRI data (by intentional smoothing) appears undesirable.

To determine whether the overall discriminability of a set of conditions was due only to the discriminability of a few of them, we repeated the analyses for subsets of the conditions and compared the information carried about these subsets with that conveyed about the entire condition set. In this way we found areas in the lower part of the frontal lobe where the information in the signal was larger for 4 conditions than for any subset of 2 conditions. Another area at the fronto-parietal junction only permits differentiation between pairs of conditions. For these areas, there is no information in the pictures about added conditions. On the basis of these results, we conjecture that the first set of areas could be involved in planning movements and the second in specific movement components.

571.2

Functional MRI of the Lateral Premotor Area during Simple and Complex Finger Movements. S.-G. Kim, G. Tagaris, X. Hu, R. Menon, J. Strupp, A. Golub*, K. Ugurbil, J. Ashe Ctr. for Magn. Reson. Res. Univ. of Minnesota, Minneapolis, MN 55455 and Brain Sciences Center, VAMC, Minneapolis, MN 55417.

Imaging studies in human subjects and single cell recording in animals have shown that the premotor motor is activated during visuomotor tasks. We tested the hypothesis that the activation of the premotor cortex is modulated by the complexity of the motor output, using functional magnetic resonance imaging at 4 Tesla.

Five healthy human subjects participated in these experiments. They performed visually instructed finger-thumb opposition movements in two different tasks. In task I ("simple") fingers were moved sequentially, while in task II ("complex") the order in which the fingers were moved was randomized. The rate of finger movement (1.25 Hz) and fingers to be used were instructed by a flashing light placed in front of the subject. Three-slice high speed gradient echo planar images (30 ms echo time and 3.1x3.1x10.0 mm^3 resolution) were acquired before, during, after each of tasks.

All subjects showed activation in the contralateral premotor area during both tasks. However, the area of activation was significantly greater during the complex task. This suggests that when the premotor cortex is activated during visuomotor tasks, the area of activation is related to the complexity of the motor behavior.

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571.3

NEUROMAGNETIC STUDY OF SOMATOSENSORY GATING. Kristeva-Feige, R.[1]*, Rossi, S.[2], Pizzella, V.[3], Lopez, L.[4], Tecchio, F.[5], Erne, S.[1-4], Edrich, J.[1] and Rossini, P.-M.[5-6]. SPON: European Brain and Behaviour Society [1]Inst. Biomed. Engin., 89081 Ulm, Germany; [2]Ist. Clin. Mal. Nervose e Mentali, Univ. Siena; Italy [3]Ist. Elettronica Stato Solido, CNR, 00156 Roma, Italy; [4]Ist. Tecnologie Avanzate Biomediche, 66100 Chieti; Italy, [5]Div. Neurol., Ospedale "Fatebenefratelli", Roma; [6]I.R.C.C.S., "Santa Lucia", Roma, Italy

Neuromagnetic fields elicited from the left hemisphere of five healthy right-handed subjects were investigated under three different experimental conditions run in random order every 5-8 s: electrical stimulation of the right index finger (task S), voluntary movement of the right index finger (M), and "interference" condition (M+S). The M+S condition consisted of voluntary movements triggering the S at the very beginning of the electromyogram of the active muscle. The activity of the sources corresponding to the main components of the task SEFs and movement-related fields was mapped and localized by means of a moving dipole model. The gating effect was measured by S-((M+S)-M).

In all the subjects investigated the gating effect was observed starting at approx. 30 ms after movement onset and lasting for the whole period of the ongoing movement. The site of the early gating effect (mostly centrifugal) was found to be more anteriorly located than the later (mostly centripetal) gating effect . The task SEFs were found to be larger (significantly after 40 ms) than the control SEFs elicited in a basal condition.

The results are discussed in terms of the timing, the mechanism (centrifugal and centripetal), locus and selectivity of the somatosensory gating. The interesting corollary finding about larger task SEFs as compared to the control SEFs is interpreted in terms of specific attentional influences upon the components after 40 ms.

571.4

CORONAL FMRI STUDY OF CEREBELLAR AND CEREBRAL ACTIVATION BY FINGER TAPPING. 1,3 T Nordahl, 2 M Buonocore, 2 L Gao, 3 J Eberling*, 1 R Maddock. 1Department of Psychiatry and 2Radiology, UC Davis Medical Center, Sacramento, CA 95817, 3Lawrence Berkeley Laboratory, Berkeley, CA 94720.

Several fMRI studies have noted motor and sensory cortical blood flow changes with finger tapping contralateral to the side of the tapping hand. Sabatini et al. 1993 using 133 Xenon blood flow found contralateral primary sensorimotor area , supplementary motor area, and ipsilateral cerebellum were activated during right and left finger movements performed at fast frequency. We thus sought to study similar fast finger tapping with coronal multi-slice echo-planar fMRI (EPI) as this can image cerebellar and cerebral regions simultaneously and detect subtle temporal differences in flow. High resolution fast spin echo images were obtained to more precisely identify the location of activation. 3D phase contrast angiography was performed to locate vessels. Echo planar flash (TR=2000, TE=40) and spin echo (TR=2000, TE=95) images were acquired as multislice, multirepetition acquisition. Scan parameters were 64X64 matrix, 22 cm FOV, 6 mm slice thickness, and 2 mm gap. Following baseline scans (16 images), subjects performed sequential finger tapping at their highest rate. Alternation period was 16 seconds (8 images), repeated 8 full cycles. Ten coronal slices were taken. Correlation maps were obtained using box car response template with temporal offsets of 0, TR, ...5TR. Threshold value for display of correlation was set at 0.2 or 0.3. Talairach atlas was used to identify regions. Activation was detected in the contralateral cerebrum (precentral gyrus, postcentral gyrus) and matched homunculus localization of the hand. Cerebellar activation on the dominant side had a corelation greater than 0.3 while the nondominant side had 0.2 to 0.3 typically. Cerebellar activation peak preceded the cortical peak by 1000±500 ms. This data is consistent with known findings and may give temporal distinction in regional activation.

571.5

DOES THE CORTEX PROCESS POSTURAL ADJUSTMENTS ASSOCIATED WITH BALLISTIC MOVEMENTS? E. Palmer*, E. Cafarelli, P. Ashby. Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Canada.

When seated human subjects rapidly abduct one arm in response to a tone, EMG activity occurs in the deltoid of that arm, and, almost simultaneously, in the contralateral latissimus dorsi. The contralateral activity is assumed to be an "associated postural adjustment". When subjects abducted the left arm rapidly, magnetic stimulation over the left motor cortex delayed the onset of the EMG burst in the right latissimus dorsi relative to the initial burst in the left deltoid. When subjects abducted the right arm rapidly, magnetic stimulation over the left motor cortex delayed the onset of the initial EMG burst in the right deltoid relative to the burst in the left latissimus dorsi. In each case, the delay was greatest when the stimulus was given just before the burst was expected to occur. The inhibition of voluntary movements by transcranial stimulation was not associated with a reduction in the excitability of spinal motoneurons. We postulate that both the focal ballistic movement and the associated postural adjustment are preprogrammed, held in memory until the "go" signal and then released through both motor cortices to spinal neurons.

(Supported by MRC 6727)

571.6

MOTOR EVOKED POTENTIALS (MEPS) TO PAIRED TRANSCRANIAL MAGNETIC STIMULI SHOW DIFFERENCES DURING REST, ACTIVATION AND POST-EXERCISE FACILITATION. A. Samii*, M. Hallett, S. Grill and E.M. Wassermann, Human Motor Control Section, NINDS, Bethesda, MD 20892

A TMS pulse to the motor cortex at an intensity of1.1 x threshold inhibits the MEP produced by a second pulse at intervals (ISIs) from about 40 to 90 ms (Valls-Solé et al., 1992). Both voluntary muscle activation and the post-exercise state in resting muscles increase MEP amplitude and might be expected to reduce this inhibition.

We studied paired stimuli under conditions of rest and activation and after exercise in the wrist extensors of 2 subjects. 10 stimulus pairs at 1.1 x resting motor threshold were given at ISIs from 20 to 150 ms under conditions of rest and 10% maximal force. 8-10 stimulus pairs were given at ISIs of 90 ms in 1 subject and at 70 ms in the other, 500 ms after 10 sec periods of 20% maximal isometric exercise.

In both subjects activation caused a large increase in the amplitude of the first MEP and a progressive decrease of inhibition at ISIs greater than about 100 ms which was not seen at rest. Post-exercise facilitation caused a significantly smaller increase in the amplitude of the first MEP in both subjects, but complete reversal of the inhibition of the second MEP in one subject and frank facilitation of the second MEP in the other. Post-excercise facilitation has a weaker facilitatory effect on the first MEP, but it can cause facilitation or a greater reduction in inhibition after the MEP than voluntary activation.

571.7

RESPONSES TO TRANSCRANIAL MAGNETIC STIMULATION DURING FATIGUING MAXIMUM VOLUNTARY CONTRACTION W.B. McKay*, S.M. Tuel, A.M. Sherwood, M.R. Dimitrijevic, Div. Restorative Neurology and Human Neurobiology Baylor College of Medicine, Houston, Texas

Sustained maximum voluntary contraction (MVC) is a widely used paradigm for the study of fatigue mechanisms. Cortical motor output was measured by surface recorded motor evoked potentials (MEPs) resulting from transcranial magnetic stimulation (TMS) of the motor cortex. TMS at 80% of maximum (2.0 Tesla) was delivered over the Cz scalp EEG location. MEPs and M-waves from supramaximal peroneal nerve stimulation (PNS) were recorded at 15 second intervals before and during MVC of ankle dorsiflexors in six healthy human subjects. The subjects were asked to maintain MVC of the right ankle dorsiflexors until the force developed decreased to 50% of its initial value. During ankle dorsiflexor MVC, MEP amplitudes in right and left quadriceps, hamstring, anterior tibial and triceps surae muscles increased compared to pre-contraction values with the largest increase occurring in the exercised anterior tibial muscle ($p < 0.05$). Also, MEP amplitudes in the contracted anterior tibial muscle were maintained while its voluntary activity amplitude and median EMG frequency and force of contraction decreased. Anterior tibial muscle M-wave amplitudes did not change but twitch forces decreased during the contraction. The results show that motor cortex output for single TMS delivery does not decrease while voluntary force generation and EMG do decrease during sustained MVC.

571.8

LOCALIZATION OF rCBF INCREASES IN HUMAN FRONTAL CORTEX AND CEREBELLUM IN VOLUNTARY INDEX FLEXION MOVEMENTS. R. J. Seitz*, J. Missimer, G. Schlaug, R. P. Maguire, U. Knorr, K. L. Leenders. Dept. of Neurology, University of Düsseldorf, Germany, Paul-Scherrer-Institute, Villigen, Switzerland.

Controversy in human mapping studies of unilateral movements relates to bilaterality, quantitation, and spatial extent of the activations. We used quantitative measurements of regional cerebral blood flow (rCBF) with [15O]-butanol and positron emission tomography for mapping of simple finger movements. Ten healthy, right-handed volunteers performed self-paced index flexions with their right hand at 1.9 +/- 0.9 (SD) Hz. The rCBF images (image resolution: 9 mm FWHM) were analyzed by use of the CBA (Seitz et al., J Cereb Blood Flow Metab 10: 443, 1990) and the SPM (Friston et al., J Cereb Blood Flow Metab 11: 690, 1991).

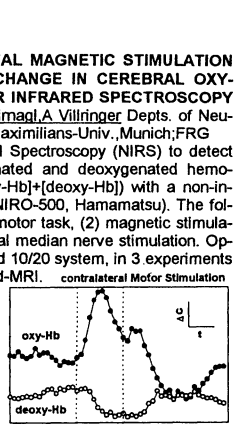
CBA analysis showed significant mean rCBF increases ($p < 0.01$, uncorrected) in the left motor cortex (44 %) and the right parasagittal part of the anterior neocerebellum (38%). At an uncorrected $p < 0.05$ there was also a mean rCBF increase (30%) in the left frontomesial cortex. Smoothing of the images with 10 mm resulted in a three-fold increase of the activation areas and a reduction of the mean rCBF increases that was maximal (17%) in the frontomesial cortex. After smoothing of the images with 20 mm and using SPM significant mean rCBF increases ($p < 0.001$, uncorrected) occurred in the left motor cortex, left frontomesial cortex, and in the right parasagittal part of the anterior neocerebellum.

We demonstrated that ballistic index flexions of the dominant hand induce significant rCBF increases contralateral in frontal cortex and ipsilateral in cerebellum. Quantitation depended on the methods used.

571.9

VIBRATORY, MOTOR AND TRANSCRANIAL MAGNETIC STIMULATION IN HUMANS PRODUCE A LOCALIZED CHANGE IN CEREBRAL OXYGENATION DEMONSTRATED WITH NEAR INFRARED SPECTROSCOPY H Obrig,S Brandt,B Meyer,GD Borasio*,U Dimagl,A Villringer Depts. of Neurology Humboldt-Univ., Berlin and *Ludwig-Maximilians-Univ.,Munich;FRG

We examined the potential of Near Infrared Spectroscopy (NIRS) to detect localized concentration changes of oxygenated and deoxygenated hemoglobin ([oxy-Hb], [deoxy-Hb], [total-Hb]=[oxy-Hb]+[deoxy-Hb]) with a non-invasive near infrared spectroscopy monitor (NIRO-500, Hamamatsu). The following stimuli were tested: (1) a sequential motor task, (2) magnetic stimulation, (3) vibratory stimulation and (4) electrical median nerve stimulation. Optodes were positioned according to a modified 10/20 system, in 3 experiments optode position was assessed by T1-weighted-MRI.



(1) In 20 subjects a finger opposition task ipsi- and contralateral to optode position over left primary motor cortex was repeatedly performed and averaged time-locked to movement onset. A clear rise of [oxy-Hb] and [total-Hb] accompanied by a decrease of [deoxy-Hb] was detected exhibiting a pronounced contralateral predominance.
(2) In 5 subjects transcranial magnetic stimulation was applied over the midpoint between the optodes resulting in a contralateral finger movement. Voluntary movement of the same finger was performed in the same experiment. Magnetic stimulation and voluntary finger movement resulted in similar changes of NIRS-parameters. (3) A localized response to a vibratory stimulus was elicited over primary somatosensory cortex, whereas (4) electrical stimulation of median nerve did not show a clear response. The experiments show that NIRS is able to detect rather small changes of local cerebral oxygenation, though optode positioning remains a problem.Supported by the DFG Vi93/7-1

571.10

MEG EVALUATION OF MOTOR FUNCTION: A COMPARISON OF EVENT-RELATED FIELDS AND EVENT-RELATED DESYNCHRONIZATIONS J.T. Davis*, J.D. Lewine, C.C. Wood[1], C. Edgar, R. Thoma, and W.W. Orrison, MSI Facility, VAMC, 2100 Ridgecrest Drive, SE, Albuquerque, NM, 87108, and [1]Los Alamos National Laboratory, M-715, Los Alamos, NM, 87545.

Pfurtscheller and colleagues have previously demonstrated that movements of the digits of the hand induce changes in the power spectra of electroencephalographic data recorded over contralateral parietal regions. In particular, an event related desynchronization (ERD) (and associated reduction in power) is seen in the mu-band of the power spectra. Similar changes are observable in the magnetoencephalogram (MEG). Via signal averaging of continuous data epochs time-locked to movement onset, it is possible to extract an alternative type of information in the form of an average event-related field. Dipole modeling of these data provide for identificationof the primary motor cortical area associated with digit movements. In this study we sought to compare spatial patterns of MEG event-related de-synchronization and average event-related fields associated with movement. Available data indicate that ERDs are most prominent at the locations of the maxima and minima of the event-related field. This implies common neural generators for the two types of signals.Pfurtscheller and colleagues have demonstrated that neural network analysis of neuroelectric ERD data allows for good single-trial classification of contra- versus ipsilateral movements. Our preliminary data indicate that neuromagnetic ERD data provide for better network classification than that reported by Pfurtscheller for neuroelectric data. Interestingly, even better results are obtained via examination of single trial waveforms, as opposed to ERD parameters.

571.11

SPATIAL AND TEMPORAL DISTURBANCES IN PATIENTS WITH PARIETAL LESIONS WITH AND WITHOUT IDEOMOTOR APRAXIA.

H. Hefter*, P. Weiss, S. Meermagen, C. Dohle, H. Kuhlmann and H.-J. Freund
Department of Neurology, Univ. of Duesseldorf, 40225 Duesseldorf, Germany.

Lesions of the parietal cortex lead to servere motor disorders, known as apraxia for left and hemineglect for right hemispheric lesions. In a prospective study, 6 patients with left parietal lesions (4 with and 2 without ideomotor apraxia) and 6 patients with right parietal lesions were tested for spatial and temporal errors and the presence of a neglect component during arm movements. Movement trajectories were recorded by a Selspot II® system and a video camera. During the first set of experiments patients had to produce horizontal, vertical, and circular arm movement components in their personal space, out of which 5 simple movement sequences (SMS) were composed. These 5 SMS had to be produced after reading aloud the instructions ('reading'), after verbal instructions ('instruction'), and after the experimenter had demonstrated the movement ('imitation'). The 5 SMS and the 3 modes of instruction were randomized for each side. For all patients the same sequence was used. In patients with left parietal lesions, significantly more spatial (direction reversions) and sequencing errors (wrong order of movement components) of the movement trajectory were observed in comparison to the right parietal lesioned patients. More omissions or repetitions of movement components revealed aspects of motor neglect or perservation in the patients with right parietal lesions. During the second set of experiments, the patients had to grasp a 10 cm long stylus (lying horizontally in front of them) after eye opening without previous visual feedback. Whether hand inversion (HI) or hand eversion (HE) is used for grasping depends on the angle of the stylus in relation to the sagital plane resulting in a (HI/HE) probability curve. In normals, the HI/HE-probability varied within a small range of 20 degrees only. In both parietal groups, especially in the left hemispheric patients with apraxia, this angle range was significantly enlarged up to more than 100 degrees in comparison to the controls.

We conclude, that the right parietal cortex is mainly involved in the decision "what to do" and the left parietal cortex in the problem "how to do" a motor task. Thus, normal motor behavior affords an intensive interaction between both parietal cortices.

571.12

AN EEG-BASED BRAIN-COMPUTER INTERFACE: USE BY INDIVIDUALS WITH ALS. D.J. McFarland*, L. McCane, T. Vaughan, and J.R. Wolpaw, Wadsworth Labs, NY State Dept Health and SUNY, Albany, NY 12201.

Humans can learn to control the amplitude of EEG activity recorded over sensorimotor cortex (e.g., the 8-12 Hz mu rhythm) and use it to move a cursor to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991 and in press). Brain-computer interface technology may provide a new communication channel for those with severe motor disabilities. We are using high-resolution EEG frequency and topographic analysis to improve this technology and are investigating its applicability to individuals with specific disorders.

After initial evaluation of the EEG and its relationships to sensorimotor activity, subjects learn to control cursor movement while 64 channels of EEG are stored for offline analysis aimed at improving the algorithm that converts EEG activity into cursor movement.

In initial studies, individuals with early-stage amyotrophic lateral sclerosis (ALS) were able to learn to control cursor movement. If this control persists as the disease progresses, EEG-based communication could prove of considerable value in the later stages, when useful voluntary movement is largely or totally gone. (Supported by the National Center for Medical Rehabilitation Research (NIH grant HD 30146))

OCULOMOTOR: BRAINSTEM AND MUSCLE

572.1

DOWNSTREAM AND UPSTREAM MODELS OF IBN ACTIVITY DURING GAZE SHIFTS IN THE HEAD-FREE CAT AND MONKEY. K.E.Cullen*, C.G. Rey and D. Guitton, Montreal Neurological Inst. McGill Univ., Montreal, Canada.

Our recent studies in the cat (Cullen et al. 1993) have demonstrated that IBN discharges are best correlated, not with the metrics of the movement of the eye in the head but, with the movement of the visual axis in space (gaze) during rapid orienting coordinated eye and head movements. To further address whether cat and monkey IBNs carry gaze or eye related signals, we carried out a dynamic analysis of IBN discharge. The hypothesis that the discharge of IBNs reflects an upstream input signal which encodes a non-linear representation of motor error (Van Gisbergen et al. 1981) was first tested. For our neurons, discharges head-free or head-fixed were significantly less well predicted by a quasi-linear expression of eye or gaze motor-error, than by simple downstream dynamic models which incorporated eye or gaze velocity and a firing rate bias term. For downstream models, we found that gaze velocity was better at predicting IBN activity than eye velocity. The estimated firing rate based on a model using gaze velocity produced significantly better fits of the actual firing rate than that which was based on eye velocity (≈ 30% less RMS). Adding a bias term to the gaze and eye velocity models improved the fit of both models. Interestingly, the value of the bias estimated for the gaze model was comparable to that which we determined for head-fixed saccades, while the value estimated for the eye model was significantly larger. Since the bias term could reflect the physiological "resting discharge" of the IBN, the necessity of increasing the bias value to predict IBN firing with eye velocity as an input further suggests that gaze velocity is more relevant than eye velocity in describing the discharge of IBNs during gaze shifts. We also tried more complex downstream models. The best downstream fits were obtained using a quasi-linear expression of eye or gaze velocity that included a bias and a pole term for which initial conditions were fitted as parameters. For a significant number of the primate neurons studied, these estimated initial conditions were correlated with the metrics of the eye and/or gaze movements.

572.2

HISTOLOGICAL IDENTIFICATION OF PREMOTOR NEURONS FOR HORIZONTAL SACCADES IN MONKEY AND MAN BY PARV-ALBUMIN STAINING. A.K.E. Horn* and J.A. Büttner-Ennever, Inst. Neuropathology, LMU Munich, 80337 Munich, F.R.G.

The premotor excitatory (EBN) and inhibitory burst neurons (IBN) are essential for horizontal saccades. In the monkey, EBNs are located in the ipsilateral paramedian pontine reticular formation (PPRF), the IBNs more caudally in the contralateral dorsal paragigantocellular nucleus (pgd). For a neuropathological analysis of degenerative changes in saccadic disorders of patients the histological identification of EBN- and IBN-areas in man is important. Here we show that this is possible by using parvalbumin (PAV) as a histological marker. First, in monkeys EBNs and IBNs were retrogradely filled by injections of WGA-HRP or cholera-genoid into the abducens nucleus (VI) or tetanus toxin fragment C into the lateral rectus muscle and shown by double-labelling to contain PAV. Then, human brainstem sections were immunoreacted for PAV, and by comparing the PAV-staining pattern to that in the monkey the homologue burst neuron areas were defined in man. In the PPRF EBNs were confined to the nucleus reticularis pontis caudalis (nrpc), and did not extent further rostrally into nucleus reticularis pontis oralis (nrpo). All retrogradely labelled cells in the nrpc and pgd were PAV-positive, and ca. 70% of the PAV-positive cells were retrogradely labelled. Both, the EBN-area and IBN-area, were high-lighted by their PAV-content and could be outlined in man as well. EBN: the medial part (2.5mm width) of the nrpc immediate rostrally (250μm) to the omnipause neurons extending 2.2mm rostrally, IBN: the medial part of the pgd immediate ventrocaudally to VI extending 1.8mm caudally. The location of the burst neuron areas, and probably even the EBNs and IBNs themselves, with PAV will help the analysis of clinical cases of slow saccades.
This work was supported by the DFG (SFB 220).

572.3

INTERACTION BETWEEN VERTICAL EYE POSITION-RELATED NEURONS IN AND AROUND THE INTERSTITIAL NUCLEUS OF CAJAL ON BOTH SIDES. Y. Iwamoto*, S. Chimoto, E. Nambu, K. Yoshida Dpt. Physiology, Inst. Basic Med. Sci., Univ. of Tsukuba, Tsukuba, 305 Japan

We have recently shown that many neurons in and around the interstitial nucleus of Cajal (INC) that project contralaterally carry vertical eye position signals. In the present study, we recorded activity of vertical eye position-related INC neurons and examined electrophysiologically their axonal course and synaptic nature in the alert cat. For 11 downward-on and 5 upward-on neurons, depth profiles of threshold and latency for antidromic activation were studied by tracking in and around the contralateral (c-) INC. These neurons were activated from not only c-INC but a region 1-2 mm dorsal to c-INC. The latency became longer as the stimulation site moved ventrally, showing that the stem axon coursed ventrally toward c-INC. Double or triple low-threshold peaks and latency variation were observed in 4 downward-on and 2 upward-on neurons when stimuli were applied to c-INC, indicating the existence of branches. Stimulation of the midline region suggested that axons of these neurons projected through the posterior commissure (PC). Stimulation of c-INC and the more dorsal region inhibited spike generation of 7 downward-on and 2 upward-on neurons that were not antidromically activated. The latency of inhibition ranged from 0.7 to 1.4 msec. Effective stimulation sites seemed to be distributed along the course of fibers from PC. Results suggest that these non-commissural INC neurons receive monosynaptic inhibitory connections from commissural INC neurons on the contralateral side, most likely from those having an opposite on-direction.

572.4

'EYE-NECK' RETICULAR NEURONS CONTROL ORIENTING GAZE SHIFTS BUT NOT GAZE-STABILIZING VESTIBULAR REFLEXES. T.Kitama, A.Grantyn*, A.Berthoz, LPPA, C.N.R.S.- Collège de France, Paris, France.

Previously we defined a class of reticular formation neurons (RFN) controlling eye-neck synergies during orienting. Studies by others in decerebrate cats suggested that some RFNs can be involved in the control of both active orienting head movements and of gaze-stabilizing vestibulo-collic reflex (VCR). In the present work a possibility that one and the same RFN can participate in these two antagonistic functions has been evaluated in alert animals.

RFNs, some of them identified as reticulospinal neurons, were recorded in the caudal pontine and rostral bulbar RF. Orienting-related 'eye-neck' neurons (EN-RFNs) were identified by their phasic-sustained or quasi-tonic discharges correlated with visually triggered eye movements and with activation of ipsilateral neck muscle EMG. They were tested during horizontal sinusoidal rotation (0.2-1.0 Hz; 2.0-21.5°) in dark and in light. None of EN-RFNs showed any modulation during VCR or VOR. They were active only during ipsilaterally directed anticompensatory movements: quick phases, shifts of the ocular beating field and associated EMG activity. Firing rate correlations with these motor events were qualitatively the same as during visually triggered gaze shifts. In the control sample of RFNs 'unrelated' to orienting movements the fraction of cells modulated by passive rotation (15%) was similar to that reported in decerebrate cats.

Synaptic inputs to EN-RFNs and to RFNs 'unrelated' to orienting were tested by stimulation of the superior colliculi (SC) and the vestibular nerves (VN). Monosynantic excitatory input from the contralateral SC was found in 92% of EN-RFNs *vs* 75% of 'unrelated' RFNs. Only 2 out of 17 tested EN-RFNs showed early excitatory responses to VN (latencies 1.25-10.0 ms), in contrast to 30/42 (71%) 'unrelated' neurons. The tecto-vestibular convergence was therefore very rare in EN-RFNs.

The study points to a segregation of reticular neurons controlling orienting eye and head movements from those mediating compensatory reflexes during natural vestibular stimulation. Rhombencephalic vestibulo-reticular input to orienting-related EN-RFNs appears to be negligible, as compared to tectal input. Activity of these neurons during rotation-induced anticompensatory gaze shifts must therefore require contributions of excitatory pathways from higher order structures.

Supported by Human Frontier Science Program Organization.

572.5

MOVEMENT FIELD PROPERTIES OF SACCADE-RELATED BURST CELLS IN THE MESENCEPHALIC RETICULAR FORMATION (MRF). V.L. Silakov* and D. M. Waitzman. Newington VAMC, 555 Willard Ave, Newington, CT 06111.

The movement field properties and the discharge dynamics of 206 MRF neurons located lateral to the oculomotor complex have been studied in 2 awake behaving monkeys performing visually guided saccades. At least two major classes of neurons can be distinguished. One group, the long lead burst neurons, has a build up of activity which begins 150 msec before saccades, an accelerated burst just before the saccade, this burst declines during the saccade, and the tail of activity may continue for 50 msec after saccade end. The optimal saccade vector for these cells is primarily contraversive with a vertical component. The movement fields for these cells are large and open-ended. These cells are located in the more ventral and caudal portions of the MRF. A second group of cells is distinguished by a tightly coupled burst of activity which starts 20-30 msec before, peaks just prior to, and declines sharply during visually guided saccades with little post saccadic activity (i.e. clipped cells). These tightly coupled cells can have either large (open-ended) or small (closed) movement fields. While many of these cells have omnidirectional discharges, the optimal saccade vector for these cells is again contralateral with a pronounced vertical component, These cells are located in the more dorsal and rostral portions of the MRF. The correlation of cell type with histologic location suggests at least two physiologically distinct regions of the MRF.

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572.6

EFFECTS OF REVERSIBLE LESIONS OF THE CENTRAL MESENCEPHALIC RETICULAR FORMATION (cMRF) ON PRIMATE SACCADIC EYE MOVEMENTS. D. M. Waitzman* and V. L. Silakov. Newington VAMC, 555 Willard Avenue, Newington, CT 06111.

Recent studies suggest that the cMRF participates in the generation of saccades, but its exact role remains unclear (Waitzman, *Soc. Neurosci. Abst.*, 1992). Visually guided saccadic eye movements of one awake rhesus monkey were recorded using a scleral search coil before, during and after the injection of 2% lidocaine (1 - 5 µl) and muscimol (1 - 3 µg) into the cMRF. Fifteen injections of lidocaine, 5 injections of muscimol, and 4 injections of saline were placed at a series of sites (containing saccade associated, multi-unit activity) which were at 1 mm increments along the rostral-caudal axis, 2 mm lateral to the oculomotor complex. Within 2 minutes of injection of either lidocaine or muscimol, contraversive horizontal and vertical saccades were hypometric, while ipsiversive saccades were hypermetric. The primary position of gaze was shifted 3° down and to the ipsilateral side. The trajectories of vertical and oblique saccades were markedly curved toward the ipsilateral side. Injections 2 mm above or below the cMRF produced no effects on saccades. The effects on saccade trajectory suggest that the cMRF participates in the descending control of the horizontal component of movement, while changes in saccadic accuracy suggest the cMRF participates in the feedback control of saccade amplitude. The shift in the primary position of the eyes implies that activity of cMRF neurons is used in the maintenance of the primary position of the eyes.

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572.7

THE TIMING OF SACCADE-RELATED RESPONSES OF BRAINSTEM OMNIPAUSE NEURONS IN CAT. M. Paré* and D. Guitton. Montreal Neurological Institute and McGill Univ., Montreal H3A 2B4, Canada.

Recent evidence (Paré and Guitton 1994) suggests that fixation neurons (FNs) in the superior colliculus project onto brainstem omnipause neurons (OPNs). Both cell types pause for eye saccades in monkey and gaze saccades in cat and it is of interest to determine whether they share other discharge properties. We recorded OPNs during visually-guided horizontal gaze shifts in the head-fixed and head-free cat and quantitatively analysed the temporal relation of their discharge relative to parameters of eye or gaze saccades. **1.** Cat OPNs ceased firing, on average, 25ms before saccade start. They resumed firing, on average, 6ms before saccade end. Some cells were reactivated after saccade end. Consequently, as already reported, the pause duration was well correlated with saccade duration ($r>0.9$) but the slope of this relation was always less than one - a similar relation holds for monkey FNs (Munoz and Wurtz 1993). The slope increased linearly with the associated correlation coefficient, indicating that OPNs whose pauses are temporally well correlated to saccades have equal onset and end times. **2.** Monkey FNs are reactivated sooner for contraversive saccades compared to ipsiversive ones. The timing of the majority of our cat OPNs also depended on saccade direction; two thirds of the cells had significant left-right differences in pause end time. **3.** Pause end time was twice as variable as pause onset time; the inverse has been observed for monkey FNs. **4.** OPN tonic discharge rate influenced pause timing: the higher a neuron's firing rate, the closer to saccade start it stopped discharging. **5.** Pause end time did not depend on any saccade parameters. On average, OPNs were reactivated at either ~1deg of gaze error or ~100deg/s. In **1** and **2**, OPNs and FNs have similar discharge characteristics. This suggests that OPNs are segregated into two lateralized populations of cells each receiving inputs from contralateral FNs. However, the timing characteristics of OPNs are also clearly influenced by extra-FN inputs.

572.8

EFFECTS OF BOTULINUM NEUROTOXIN TYPE A ON MUSCLE PROPERTIES AND OCULAR MOTONEURON DISCHARGE CHARACTERISTICS IN THE ALERT CAT. B. Moreno-López, R.R. de la Cruz*, A.M. Pastor and J.M. Delgado-García. Lab. de Neurociencia, Fac. de Biología, Univ. de Sevilla, E-41012, Spain.

Botulinum neurotoxin A (BoNt) is an useful tool for the study of motoneuron-muscle interactions, since it produces a functional disconnection by blocking the release of acetylcholine at the neuromuscular junction. Are there any retrograde effects on the discharge characteristics of motoneurons when their target muscle is paralysed with BoNt? To address this question, adult cats were prepared for the chronic recording of eye movements, lateral rectus muscle potentials and abducens motoneuron electrical activity. After control recordings, BoNt was injected into the lateral rectus muscle at either 3 or 0.3 ng/Kg. Both doses produced in a few hours the paralysis of abducting eye movements that paralleled the disappearance of muscle field potentials induced by the electrical stimulation of the VIth nerve. The time course of these changes was shorter for the high BoNt dose and the recovery lasted longer. On the contrary, only the high dose produced significant alterations in the discharge characteristics of abducens motoneurons. Thus, motoneurons lost their typical tonic-burst firing that accompanies eye fixations and saccades. Instead they showed a sustained low firing rate (15-50 spikes/s) and barely entered into cut-off even for the fastest eye movements in the off-direction. Firing modulation for both spontaneous and vestibularly-induced eye movements was extremely weak yielding low sensitivities to both eye position and velocity. The signalling deficits of motoneurons for off-directed eye movements were deeper than those for the on-direction. Accordingly, the scatterplots of firing rate versus eye position or velocity showed that two rather than one regression line fitted better the data and corresponded to the data partition into on- and off-directions of movement. The low dose of BoNt produced only subtle changes in the firing of some motoneurons despite total muscle paralysis. These data suggest that the firing alterations found result of a direct action of BoNt on motoneurons and not only due to the target disconnection.

572.9

MOTONEURONS OF EXTRAOCULAR MUSCLES CODE DIRECTIONS IN VESTIBULAR COORDINATES. V Henn*, Y Suzuki, D Straumann, BJM Hess, K Hepp. Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland

Extracellular activity of motoneurons was recorded in alert rhesus monkeys. They were put into different static roll positions to induce ocular counterrolling, while they made spontaneous eye movements. This provided a database of eye positions of about +/- 40 deg in the horizontal, +/- 30 deg in the vertical, and +/- 8 deg in the torsional direction. Three-dimensional eye positions during fixation periods were related to steady-state firing rates of neurons, and on-directions were calculated by multiple linear regression analysis. On-directions were compared to anatomically defined pulling directions of extraocular muscles and to orientations of semicircular canals. In the rhesus monkey only the lateral canals show an approximate correspondance with lateral recti muscle action planes. Average on-directions of 52 motoneurons were more closely related to canal than to muscle coordinates.

Results support the notion that vestibular coordinates provide a common reference frame for directional coding also in the oculomotor system (see Suzuki et al, this vol).

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572.10

THREE-DIMENSIONAL EYE MOVEMENTS EVOKED BY ELECTRICAL MICRO-STIMULATION OF THE TROCHLER NERVE IN ALERT RHESUS MONKEYS. Y. Suzuki*, D. Straumann, K. Hepp, V. Henn. Neurology Department University Hospital Zürich, CH-8091 Switzerland.

In the rhesus monkey, anatomical coordinates of vertical and oblique eye muscles show major deviations from orthogonality and are therefore not well aligned with semicircular canals. We electrically stimulated the trochlear nerve (0.25ms negative pulses, 20-80µA, 0.5-1kHz over 9-70ms), while the alert animal made spontaneous eye movements covering the whole oculomotor range, and measured the evoked eye movements in 3 dimensions. This allowed to test the following hypotheses:

(A) The eye moves in alignment with the anatomical pulling direction of the superior oblique muscle, if the eye plant is organized as an isotropic passive element, where muscle force translates into movement independent of other muscles.

(B) The eye moves along a direction taking into account the equilibrium direction of all other eye muscles.

Results show that movements occurred along a trajectory approximately 45 deg between vertical and torsional planes, thus closely corresponding to the ipsi-lateral anterior canal sensitivity vector, but not to the muscle pulling direction of the superior oblique muscle. This falsifies hypothesis (A). We conclude that extraocular motoneurons code movement in vestibular, not in muscle coordinates in alert condition (see Henn et al, this vol).

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572.11

SYNAPTIC CONNECTIONS OF ASCENDING TRACT OF DEITERS AND ABDUCENS INTERNUCLEAR INPUTS TO MEDIAL RECTUS MOTONEURONES IN THE CAT OCULOMOTOR NUCLEUS. L.T. Nguyen* and R.F. Spencer. Dept. of Anatomy, Med. Coll. of Virginia, Richmond, VA 23298.

The synaptic connections of abducens internuclear (Abd IN) and ascending tract of Deiters (ATD) neurones with medial rectus (MR) motoneurones in the cat oculomotor nucleus have been examined by light and electron microscopy following injections of biocytin into the abducens and vestibular nuclei, respectively. Both Abd IN and ATD endings contained spheroidal synaptic vesicles and established asymmetrical synaptic contacts with single postsynaptic profiles. Abd IN and ATD synaptic endings were similar in size (1.68 μm^2 and 1.62 μm^2, respectively). The majority (54%) of Abd IN synaptic endings, however, established synaptic connections with distal dendrites, whereas most ATD synaptic endings contacted proximal dendrites (49%) or somata (30%). In contrast to Abd IN synaptic endings, which typically were associated with only a modest postsynaptic densification at sites of synaptic contacts, ATD synaptic endings frequently exhibited either subjunctional dense bodies in association with axodendritic synaptic endings or subsurface cistern that were associated with axosomatic synaptic endings. The more proximal location of ATD synaptic endings is consistent with the faster rise time and earlier reversal to polarizing currents of ATD EPSPs in comparison to those evoked by the Abd IN pathway as determined electrophysiologically. Given that the ATD pathway conveys only a weak eye position signal, the findings in this study suggest that the ATD pathway may function to reduce the threshold for the activation of MR motoneurones by the Abd IN input related to conjugate horizontal eye movements, possibly as a mean of counteracting the effects of other inputs that are related to vergence eye movements.

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572.12

BILATERAL PROJECTION OF SINGLE MOTOR NEURONS INVOLVED IN VISUOMOTOR CONTROL, A DOUBLE LABELLING STUDY IN THE RAT. P.M.Young **(SPON : Brain Research Association). Department of Psychology, University of Central Lancashire, Preston PR1 2HE, UK.**

Preliminary retrograde double labelling studies have identified oculomotor nucleus motoneurons projecting bilateral axonal collaterals through the retrorubral fields. This study was designed to combine retrograde / anterograde tracers in delineating these projections. Twenty male Wistar rats (220 - 330g b. wt.) were anaesthetised with tribromoethanol, and individual extraocular muscles, bilaterally, were injected (0.05 - 0.2 µl) through 50 µm tip diameter glass micropipettes connected to a 1 µl Hamilton syringe, with either Fluorogold (FG), DiI, Fast Blue (FB), PHA-L, Biotinylated Dextran Amine (BDA) or choleratoxin - horseradish peroxidase (CTB). Survival times ranged from 48-96 hours, and following perfusion 80 µm frozen sections were appropriately processed. Tetramethylbenzidine diaminobenzidine and Vector VIP were used as chromogens with PHA-L, BDA and CTB. Fluorescent tracers were viewed with a Leitz Diaplan. These studies showed only very occasional bilateral innervation of functional pairs of extraocular muscles. A more common configuration would be, for example, for a ventromedially and rostrally located oculomotor neuron, retrogradely labelled following an injection to the ipsilateral left inferior rectus, to also send an axon collateral to terminate in association with the contralateral third nerve fascicles retrogradely labelled in turn following an injection of tracer to the right inferior rectus muscle. The results demonstrate the anatomical bases of muscle linkages carried out at the simplest level of individual neurons. A primitive system overlain and increased in sophistication by the complex interconnections of premotor afferents.

572.13

TRANSNEURONAL TRANSPORT OF BIOCYTIN THROUGH ABDUCENS MOTONEURONS OUTLINES THE INTERNUCLEAR AND VESTIBULAR PATHWAYS IN TELEOST FISH. R. Baker, E. Marsh and R. F. Spencer*, Dept. of Physiology and Biophysics, NYU Medical Center, New York, NY 10016 and Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298.

Selective application of biocytin crystals to the VIth nerve in divergent species of teleosts reveals rostral and caudal subgroups of abducens motoneurons with intensely labeled somadendritic trees spanning the entire mediolateral extent of the hindbrain. Located immediately dorso-medial to each sub-group and in about a 1:2 ratio are internuclear neurons whose axons ascend dorsally in the medulla, pass through the ipsi-MLF to cross the midline, and then project rostrally in the dorsomedial quadrant of the contra-MLF where they terminate onto and occasionally also label medial rectus motoneurons. In those species with rhythmically conjugate horizontal scanning eye movements (e.g. goldfish, catfish vs. sculpin, sunfish) the bipartite motoneuronal/internuclear somata position is observed medial in the hindbrain and this location appears to be independent of either the optic axis or location of the eye in the orbit. However, even when the abducens complex is located most laterally, adjacent to the vestibular nucleus (eg. midshipman), the medially directed dendrites not only reach but also cross the midline. In all cases, labeled dendrites from each motoneuronal subgroup extensively interdigitate with those of the adjacent internuclear populations suggesting a similar embryonic origin of all neurons in rhombomeres 5 and 6. Transneuronal biocytin transport also marks the pathway and location of contralateral excitatory vestibular neurons in the descending octaval nucleus including occasionally the axons of vestibular neurons that project to medial rectus motoneurons. Electron microscopy of the oculomotor and abducens motor nuclei in goldfish reveals numerous axosomatic and axodendritic synaptic endings showing membrane specializations characterizing chemical and gap junctions. Therefore, the extensive electrotonic coupling and transneuronal transport of biocytin prevalent in some teleosts, like toadfish and midshipman, suggest the possibility of fully describing both the visual and vestibular pathways specific to each extraocular subdivison.

572.14

SIMULATION OF MOTOR UNIT RECRUITMENT IN EXTRA-OCULAR MUSCLE. Paul Dean*, Dept. of Psychology, University of Sheffield, Sheffield S10 2TN, England.

Understanding the recruitment pattern of extraocular motor units is important for constructing distributed models of oculomotor control. Recruitment in the abducens nucleus was simulated using data relating steady-state horizontal eye position to (a) the firing rates of oculomotoneurons (eg Robinson and Keller, Biblio. Ophthal. 82: 7, 1972), and (b) the total active force developed in the lateral rectus muscle (e.g. Miller and Robinson, Comp. Biomed. Res. 17: 436, 1984). The purpose was to determine the distribution of motor unit strengths that would fit both sets of data over the oculomotor range.

The lateral rectus was represented as 100 motor units, initially of identical strength. For a given position of the eye, the force developed by each unit was calculated, and the sum of unit forces compared with the active force in the entire muscle as measured experimentally. The properties of the active units were then adjusted to reduce the size of any resultant error, in a manner related to gradient descent methods for neural-net training. The simulated eye muscle was 'trained' in this fashion for a series of eye positions drawn at random from the oculomotor range until performance stabilised. Plots of motor unit strength against oculomotoneuron threshold revealed a U-shaped pattern, with the strongest units being recruited at both extremes of the oculomotor range, and the weakest units recruited in the middle. The pattern remained qualitatively unaltered over a range of assumptions about the distribution of oculomotoneuron parameters, and the relation between motoneuron firing rate and unit force.

One part of the U-shaped pattern is similar to that observed in spinal motoneurons, where stronger units tend to have higher recruitment thresholds, whereas the other part is consistent with evidence from single motor-unit stimulation studies (Shall and Goldberg, Brain Res. 587: 291, 1992) suggesting that in eye muscles the stronger units are recruited first. The pattern as a whole may reflect the functional need for precise control of eye position in the middle of the oculomotor range.

OCULOMOTOR: PERFORMANCE AND MODELING OF SACCADES

573.1

CHARACTERISTICS OF ANTI-SACCADES IN MONKEY. H.Sanchez, N. Amador, C. Salumbides, M.Schlag-Rey* & J.Schlag. Brain Research Institute, UCLA, Los Angeles, Ca. 90024.

The performance of anti-saccades (AS) - saccades directed opposite to a visual target - entails the inhibition of reflexive foveation and the coordinate computation of a goal specified by instruction, i.e. not pointed to by the visual event. Normal humans (with intact frontal lobe) can readily do it. How do monkeys compare to humans? Long-term behavioral data were obtained from 2 monkeys trained in a task interleaving AS with normal, pro-saccade (PS) trials. The instruction to make AS or PS was given on each trial by the shape of the initial fixation point (respectively a square or a point) lasting 400 - 900ms.Upon its offset, the directional cue (always a point) was briefly flashed (10, 20, or 200ms) in one of 8 directions, at a distance of 10° to 30° from the fixation point.

Compared to PS, AS were slower, less accurate saccades, affected by an upward drift in darkness, but not more so than ordinary memory saccades. Mean latencies of AS were in the same range for both monkeys (~210 ms), greater than PS latencies for one monkey, as expected, but shorter for the other one.This latter result may be related to the prior training experience of that monkey.

As in humans, directional errors were unrepressed PS, but many were followed by corrective AS, some with almost no intersaccadic delay. Back-to-back saccades subsided as performance improved.

Thus, monkeys can be taught to repress their natural tendency to look at any visual stimulus flashed on a screen. They can also learn a rule for directing gaze to a blank site radially opposite to a visible target. *(Supported by USPHS grants EY02305 and EY05879).*

573.2

IMPROVED TEMPORAL DISCRIMINATION OF TWO BRIEF FLASHES AT THE SAME RETINAL LOCATION JUST BEFORE A SACCADE. R.H.D.Cai*, M.Schlag-Rey & J.Schlag. Brain Research Institute, UCLA, Los Angeles, Ca. 90024.

Just before a saccade, there is a period (about 100 ms) when the timing of an incoming visual signal and the current internal representation of eye position seem mismatched. As a result, 2 flashes at the same retinal localization appear to be at different places. Could this illusory spatial mislocalization improve the temporal discrimination of 2 stimuli flashed in rapid succession?

Horizontal eye movements were recorded with an Ober2 eye-orbit scanner in 2 human subjects, head fixed in the dark. Subjects were instructed to make a saccade to a 10-ms light spot flashed 10° rightward from the point of fixation. During the latency period, another 4-ms light spot was flashed twice at 12° to the right of the point of fixation. Flash separation varied from 10 to 100 ms. Subjects were to report after each trial, whether they saw 1 or 2 flashes, and when seeing 2, whether they were at the same or different places. The frequency of reporting 2 flashes increased as the pair of flashes occurred closer to the saccade onset. The frequency of reporting two flashes at different locations increased in parallel. We suggest that the improvement in temporal discrimination was due to the impact of the eye position signal on visual input.*(Supported by USHHS grant T32 GM08375 and USPHS grant EY05879)*

573.3

BEHAVIORAL INHIBITION OF SACCADES: A STUDY USING THE COUNTERMANDING PARADIGM. D.P. Hanes* & J.D. Schall. Dept. Psychology, Vanderbilt University, Nashville, TN 37240

How the brain regulates saccade initiation is not known. For useful neural correlates to be identified, novel behavioral methods are needed that systematically manipulate saccade production. To manipulate the initiation of saccades, we implemented a countermanding paradigm (Lappin and Eriksen, 1966, *J Exp Psych*, 72:805) in two rhesus monkeys. After fixation of a central spot, a target appeared at 1 of 2 locations. At the same time the fixation spot disappeared, signaling the monkeys to generate a saccade to the target. On 25% of the trials the fixation spot reappeared after a delay, signalling the monkey to withhold the saccade. The stop-signal delay ranged from 25-225 ms. With short stop-signal delays monkeys withheld saccades to the peripheral target, and with longer stop-signal delays monkeys increasingly failed to withhold the saccade. We observed a few trials in which monkeys generated a saccade falling short of the target, and on those trials a return saccade was usually made. The probability of saccade generation as a function of stop-signal delay increased monotonically with stop-signal delay in a sigmoid fashion (best fit logistic equation, $r^2 = 0.97$). The hypothesis that generation of the saccade is determined by a race between a go and a stop process provides a means of analyzing this inhibition function to estimate the covert latency of response to the stop signal (Logan and Cowan, 1984, *Psych Rev*, 91:295). For each stop-signal delay the stop-signal response time was estimated as the point on the cumulative distribution of go trial saccade latencies corresponding to the proportion of stop-signal trials in which a saccade was generated for that stop-signal delay. Stop-signal response time decreased with increasing stop-signal delay from 140 ms at a 25 ms delay to 30 ms at a 225 ms delay. The average stop-signal reaction time for both monkeys was 80 ms. This countermanding paradigm will be useful to study the neural mechanisms that regulate saccade initiation. (Supported by R01-EY08890 and T32-EY07135)

573.4

INFLUENCE OF FAST SACCADIC GAIN ADAPTION AND FATIQUE ON SACCADE METRICS IN THE MONKEY. A. Straube, S. Usher, F. R. Robinson, and A. F. Fuchs*, Neurologische Klinik, Ludwig-Maximillians University, 81377 Munich, Germany and Dept. of Physiology and Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, WA.98195, USA.

To provide accurate saccades, the oculomotor system must be able to compensate for changes in its neural elements. Such changes can be simulated by altering saccade accuracy optically. We increased or decreased the gain of visually-guided saccades by electronically detecting a targeting saccade and then causing the target to step either forward or backward by 30 or 50%. After about 1500 trials, this paradigm caused either a decrease or increase of saccadic gain to the initial target step by an average of 0.28 and 0.24 to 30% step, respectively in 3 monkeys. There was no difference in the adaptation in vertical or horizontal direction. The time constant for the readaptation towards the normal gain was always smaller than the time constant of the primary adaptation. For 30% backadaptation, the gain decrease was still 18% after 20 hours in the dark.

Associated with the gain change, however, there also was a change in saccade metrics. For both forward and backward adaptation, saccades were slower with longer durations. The percentage decrease in peak velocity was larger for larger saccades. It also varied from monkey to monkey and became less if saccades were adapted with a background. Saccade slowing could not be explained solely by fatigue because the same number of visually-guided saccades without a artifical target step caused smaller changes in duration and peak velocity.

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573.5

BIMODAL LATENCY DISTRIBUTION OF AVERAGING SACCADES IN MONKEY. I. Chou*, M.A. Sommer and P.H. Schiller, Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Saccades made to a visual target can have a bimodal latency distribution, the first mode of which is termed "express". If more than one target is presented, express saccades are suppressed (Schiller *et al.*, 1987). It has also been reported that saccades occurring during the simultaneous presentation of two targets can be "averaging" and terminate between the targets. In this study, we examined whether averaging saccades can exhibit bimodal latency distributions. Two rhesus monkeys with implanted scleral search coils were used. At the start of a trial, a fixation spot was presented. After a randomized amount of time, this spot was extinguished and either one (80% of trials) or two (20%) targets were turned on at 6 deg. ecc. For the two target condition, separations varied from 3 to 8.5 deg. of arc. Saccadic latencies and their endpoints in space were analyzed. Whenever a distinct averaging saccade population appeared, these saccades had bimodal latencies. As the separation of the paired targets was increased, the probability of averaging saccades declined and saccadic accuracy improved. Also, as saccades became less "averaging" and more "target directed", the express mode was markedly reduced. This study shows that express saccades can be made when two nearby targets are presented simultaneously, but they tend to land at averaged positions where there is no actual visual stimulus.

573.6

DO SACCADES SHOW TORSIONAL ADAPTATION? B.J.M. Melis and J.A.M. Van Gisbergen *
Department of Medical Physics and Biophysics, University of Nijmegen, P.O.Box 9101, 6500 HB NIJMEGEN, The Netherlands.

The torsional components of saccades and fixations are relatively small (Listing's law). From an oculomotor point of view this reduces position signals to 2D. On the other hand, Listing's law could serve the visual purpose of providing orientational invariance of the retinal images. In contrast to torsional eye position, the horizontal (H) and vertical (V) components of fixation are under accurate and voluntary control. In addition, H-V saccadic components can be adapted, which obviously serves the visual function of foveation. If the torsional component could also be changed in a short-term adaptation paradigm, this would provide a possible visual basis for Listing's law.

Binocular human eye movements were recorded using the 3D magnetic search coil technique. Subjects made leftward saccades between two points on a large, grey-scaled checkerboard pattern. During these saccades the whole stimulus was rotated about the left target. To check classical H-V adaptability, in some experiments this rotation was combined with a horizontal translation.

Translation of the stimulus induced rapid adaptation of horizontal saccade amplitude. By contrast, rotation of the visual field did not elicit a significant adaptation in torsion. Thus, our results do not support the hypothesis that Listing's law serves a visual purpose.
This work was supported by NWO and ESPRIT II Mucom 6615.

573.7

EXPRESS AND REGULAR SACCADIC REACTION TIMES GENERATED BY CONTINUOUS, STOCHASTIC DYNAMICS. K. Kopecz and C.C.A.M. Gielen*. Lab. Med. Phys. & Biophys. Univ. Nijmegen. The Netherlands.

The existence of an *express* (E) and a *regular* (R) mode in distributions of saccadic reaction times (SRT) is now a widely accepted fact (review, Fischer & Weber, Beh.Brain.Sci.:16, 1993). The E-mode is often observed in the gap paradigm, where the offset of an attention attractor precedes target onset. These results led to the notion of "engaged" and "disengaged" attention. Without inducing E-saccades, gap conditions also lead to a reduction of mean regular SRTs (Saslow, J.Opt.Soc.Am.:57, 1967).

We have put the intuitions about the relation of SRTs to states of attention into a quantitative model. We assume that: (1) The attentional state can be mapped onto one, continuous variable, $a(t)$. (2) Aside from conduction delays, the total SRT is determined by a "processing time", which is mapped onto a relaxation of $a(t)$, governed by stochastic dynamics of the form $\dot{a} = f(a) + \xi(t)$, down to a threshold. $\xi(t)$ is a Gaussian white-noise. (3) Two behaviorally relevant "signals" act parametrically onto the attentional system: a *warning*- and a *go*-signal. Without these signals the dynamics is *bistable*, reflecting the states of engaged and disengaged attention. Due to noise, the system switches spontaneously between the two states, so that they are visited with a certain probability. During the gap interval the system is still bistable, but now the disengaged state is more stable than the engaged state. Thus, the probability of being disengaged increases with time after the warning event. The go-signal changes the system to be monostable. During the relaxation to its equilibrium, $a(t)$ crosses the threshold after a time which depends on the state at go signal onset.

Simulations show the basic phenomenology of gap paradigms, including skewed SRT distributions and the dichotomy of E- and R-saccades as well as the reduction of regular SRTs with increasing gap interval.

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573.8

EYE SACCADES GUIDED BY VISUAL, TACTILE AND PROPRIOCEPTIVE STIMULI IN MAN. O. Blanke, O.-J. Grüsser and W.O. Guldin*, Department of Physiology, Freie Universität, 14195 Berlin, Germany.

Horizontal visual saccades (binocular stimulation with light-emitting diodes, distance up to ± 40 degrees horizontal eccentricity) were used to calibrate eye position recordings (DC-electrooculography or infrared reflection method). In addition to "visual" saccades the subjects performed saccades in the dark directed to the left and right index fingers positioned along a perimeter circle at eye level but different horizontal eccentricities. A short (<10 ms) mechanical stimulus was applied to the fingertips. The subjects were asked to look at the stimulated fingertip (alternation rate 0.7-7 per sec). The subjects were also asked without tactile stimulation to look at the index fingers (same position as in the preceding paradigm), whereby a short auditory klick determined the alternation frequency.

At an alternation rate of 0.7-4 per sec the "tactile" and "proprioceptive" saccades were significantly larger than the visually guided saccades in all but one subject, i.e. there was a considerable overshoot, indicating that the efference copy signals controlling eye position in the dark were operating at a gain below 1. This "error" in saccade amplitude increased with crossed hands. When visual saccades were suddenly changed into tactile or proprioceptive saccades, the increase in saccade amplitude occurred gradually with a time constant between 3 and 5 sec. The same was true when saccades were guided 20 times by two alternately lit diodes and thereafter by spatial memory. (Supported in part by a grant from the Bundesministerium für Wissenschaft und Technologie, Germany).

573.9

DYNAMICAL PROPERTIES OF AUDITORY EVOKED SACCADES M.A. Frens and A.J. Van Opstal * Department of Medical Physics and Biophysics, University of Nijmegen, P.O. Box 9101, 6500 HB NIJMEGEN, The Netherlands.

We measured saccadic eye movements of human subjects towards visual and auditory targets throughout the oculomotor range. It was noted that auditory-evoked saccades had trajectories and velocity profiles that were less stereotyped than visually-elicited saccades. This often resulted in curved trajectories for saccades driven to auditory targets in oblique directions. Close inspection of the velocity profiles showed that the main sequence properties of the horizontal and vertical components of auditory-evoked saccades seem to be **independent** of saccade direction. This phenomenon may reflect the fact that the auditory system derives azimuth and elevation of a sound through different neural pathways. Therefore, convergence of these pathways in a late stage of the audio-oculomotor pathway may give rise to these effects.

This is in strong contrast to visually-driven saccades, the dynamics of which seem to be **vectorially encoded**, resulting in approximately straight saccades.

In order to further study these apparent differences, we are currently recording the activity of saccade related burst neurons in the deep layers of the monkey Superior Colliculus during saccades towards auditory and visual targets.

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573.10

EFFECTS OF AUDITORY STIMULI ON HUMAN GAZE SHIFTS TO VISUAL TARGETS. B.D. Corneil and D.P. Munoz* MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

We investigated the effects of presentation of auditory stimuli on gaze shifts (eye-head movements) to visual targets in human subjects. Subjects were instructed to look as fast as possible from a central LED to an eccentric LED, either 40° to the right or left of center. An auditory stimulus was presented at either the same location as the eccentric LED (enhancer), on the opposite side (distracter), or not at all (control). The auditory stimuli had predictable effects on gaze shifts to the visual targets. Reaction times to acquire the eccentric LED were reduced significantly in all subjects during enhancer trials and increased significantly in about half of the subjects during distracter trials. These experiments were also performed in a gap condition in which the central LED was turned off 200ms before onset of the eccentric LED. In this gap condition, subjects frequently produced direction errors in the distracter condition, incorrectly looking to the auditory stimulus. The proportion of direction errors made by each subject was tightly correlated to their reaction time during control trials. Subjects with the fastest reaction times produced the most direction errors, while those with the slowest reaction times produced no direction errors. Thus, subjects able to disengage fixation during the gap and produce the shortest latency (express) movements, reacted to the first perceived stimulus. In multimodal trials, the auditory cue is perceived first due to its shorter afferent pathway. We varied the temporal disparities between presentation of the auditory and visual cues ±200ms. Presentation of the auditory cue more than 50ms after onset of the visual target eliminated all direction errors and did not modify reaction times. Auditory cues presented prior to onset of the visual target strongly influenced reaction time and the number of direction errors. Supported by MRC Canada and the Sloan Foundation.

573.11

TRANSCRANIAL MAGNETIC STIMULATION OF THE CEREBELLUM ELICITED DYSMETRIC SACCADES IN MAN. M. Hashimoto, K. Ohtsuka and M. Sawa*. Dept. of Ophthalmology, Sapporo Medical Univ. Sch. of Med., Sapporo, Hokkaido 060, Japan.

Previous neurophysiological studies in the monkey have shown that lobules VIc and VII of the vermis (oculomotor vermis) are involved in the control of saccades. Microstimulation of the oculomotor vermis evokes ipsilaterally directed saccades. When the oculomotor vermis is stimulated before the onset of visually guided saccades, ipsilateral saccades overshoot the target, and contralateral saccades undershoot the target. The oculomotor vermis, especially the lobule VII is located just under the skull bone in man. Therefore, it will be possible to stimulate the lobule VII with a transcranial magnetic stimulation (TMS) device in man.

In this study, single TMS pulses were applied systematically at various locations over the occipital area during visually guided saccades using TMS device (NIHON KODEN, SMN-1100, 1.5 Tesla). The experiment was conducted on four normal volunteers. The effective area was marked on the head by a marker which produces high signal intensity on T1 image of MRI. The stimulation site was identified by parasagittal sections of MRI in each subject. Dysmetric saccades were elicited by TMS pulses near the external occipital protuberance before the onset of visually guided saccades. By TMS of right side of the cerebellum, rightward saccades overshot the target, and leftward saccades undershot the target. These findings suggest that the posterior vermis in man has the same role in the control of saccades as shown in the monkey.

573.12

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION OVER THE PREFRONTAL LOBE DISTURBS MEMORY-GUIDED SACCADES. S.A. Brandt, B.-U. Meyer, C. Ploner, A. Villringer and P. Stoerig*. Laboratory of Neurophysiology, Department of Neurology, Charité, 10098 Berlin, Germany.

Repetitive, rapid-rate transcranial magnetic stimulation (rTMS) was used to study the role of the dorsolateral prefrontal cortex (DLPFC) for memory guided saccades. Precision of horizontal memory guided saccades, made to remembered targets after a 3 and 10 seconds delay were measured in 5 volunteers. We compared precision of saccades after stimulation (10 pulses at 10 Hz) of the prefrontal cortex (PFC), after stimulation of the posterior parietal cortex (PPC) and without stimulation. In all conditions the subjects made saccades towards the correct side of the remembered target. Without stimulation the average error was 9% of saccade amplitude. Presaccade stimulation over the PPC increased error to 14%, over the PFC to 18% of saccade amplitude. Saccades were predominantly hypometric but were unchanged in velocity and latency. Results show that stimulation over the PPC and the PFC does not extinct but significantly alters the ability to execute memory-guided saccades. Since elementary saccade parameters remain unchanged it can be speculated that rTMS unspecificly affects the premotor planing of memory guided saccades by disturbing parietal and prefrontal visuospatial representations of extrapersonal space.

573.13

FUNCTIONAL MRI OF COOPERATIVE CORTICAL ACTIVATION PATTERNS DURING EYE MOVEMENTS. A. Kleinschmidt*, K.-D. Merboldt, M. Requardt, W. Hänicke, J. Frahm. Biomedizinische NMR, Pf. 2841, 37018 Göttingen, Germany.

Gradient-echo MRI is sensitive to regional changes in cerebral blood oxygenation occurring during brain activation. Using this property for functional neuroimaging we analysed MRI signal changes during various oculomotor tasks comprising mainly smooth pursuit and saccades along the horizontal meridian.

Five healthy subjects (23-32 years, written informed consent) were studied on a conventional MRI system (2 T, Siemens Magnetom, standard headcoil). Dynamic MR recording (rf-spoiled FLASH, TR/TE/α=62.5ms/30ms/10°, measured in-plane resolution 0.8x1.6 mm^2 interpolated to 0.8^2 mm^2, section thickness 4 mm, 6 s/image) serially monitored task-associated signal changes in several sections mostly parallel to the bicommissural plane. Data evaluation was based on correlation maps calculated with reference to the stimulation protocol and individually corrected. for noise.

Eye movements were associated with signal changes in the precentral and posterior median frontal gyrus ("frontal eye field"), the medial part of the superior frontal gyrus ("supplementary eye field") and the anterior cingulate. Responses were stronger in the saccadic condition but did not depend on visual cueing. Further foci were detected in dorsal parietal and dorsal visual associative cortex and depended to differing degrees on visual cues. Activation in primary visual cortex was studied in cued conditions and reflected retinotopic positioning of the stimuli (prior to eye movement). Additional sections covering the flocculus showed its activation during smooth pursuit while basal ganglia and thalamus were not included in the volume studied and activation in the superior colliculus could not be detected.

Overall, these results are in line with what has been inferred from electrophysiology in monkey and positron emission tomography in man. Additionally, the data suggest that MRI may even resolve activation in small subcortical structures that participate in the distributed network for gaze control.

573.14

SACCADE ADAPTATION WITH A NON-VISUAL CUE. M. FUJITA* and T. OGAWA. Communications Res. Lab., Koganei-shi, Tokyo 184, Japan.

Repetitive intrasaccadic target step induces saccade adaptation (Deubel, 1987). After several hundred trials, subjects make first saccades to a second target position. The cerebellum seems to be responsible for the adaptation (Goldberg et al., 1993). In amplitude lengthening adaptation, changes in saccade velocity profile suggests a modification of the saccadic motor command (FitzGibbon et al., 1986). To elucidate the adaptation inducing factors, we made adaptation experiments with an auditory cue which taught subjects, 500 msec posterior to a saccade onset, whether the primary saccade was hypometric, normometric, or hypermetric to an expected saccade, in place of target step. Subjects, instructed to make a saccade to a briefly flashed target, made corrective saccades to the expected position, and after several hundred trials became to make, for example, an 8 deg saccade to an instantaneously illuminated 10 deg target. This excludes a case that the adaptation may take place with coupling of the estimate of the saccade performance and the motor command of a preceding primary saccade, because of the sufficiently long latency between them. In another experiment, subjects, instructed to make a saccade not to a flashed target but to an expected position by the sound, did not show any sign of adaptation. Simple association of a flashed target position to a saccade of preset size could not be acquired reflexively. Inevitability of making corrective saccades, not excluded by the second experiment, remained unresolved.

A new model is proposed that the adaptive modification of voluntary motor command can be realized either by strengthening or by weakening the feature of the command signal automatically by the cerebellum, if the accumulation of repetitive error estimation is responsible.

574.1

A SYSTEM FOR NAKED-EYE NEAR-FIELD DEPTH PERCEPTION AND LOCAL VIRTUAL SPACE SCANNING OF TYPE II FORMATTED STEREO PAIRS USING OCULOMOTOR LEARNING R.E. Winter[1]*, T.C.Chisnell[1], and S. W. McLeskey[2], [1]The Winter Co., Falls Church VA 22044, and [2]Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC 20007.

The invention of the stereoscope in 1830 (Wheatstone) and the synthetic stereoscope in 1969 (Chisnell and Winter), made possible depth perception using type I formatted stereo-paired images. These images supply each eye with a picture appropriate to its field of vision. Type II formatted stereo-paired images are viewed at a normal distance and must be integrated by the brain for the perception of field depth. Unlike the task of real fixation, this task requires a prefixation on two displaced fixation points, translation of the two fixation points, merging them, and fusing them into one integrated perception in the fixation plane. Each eye must view the member of of the stereo pair which is diagonally opposite it so that the target image impinges on the fovia in a way that it can be integrated with the image from the other member of the pair on the opposite fovia upon merging of the target images. Because of this translation requirement, motor relearning is necessary to achieve naked-eye depth-of-field perception using type II formatted stereo pairs. We submitted *Begonia Rosaflora* - Winter, 93, an 8"X10" type II formatted stereo pair to the *Green Springs Gardens* photo contest in August, 1993. Of about 400 persons who viewed it, none spontaneously acquired an integrated image with depth-of-field perception. Approximately 50 people volunteered for one-on-one training in visualization of the integrated image. Twenty percent (10 people) were able to achieve integration of the image with depth-of-field perception. Training time was limited to 30 minutes. *Begonia Rosaflora* in type I format in a stereoscope was perceived by all viewers without formal training. Thus, although it is not a spontaneously acquired skill, motor learning necessary for viewing of type II formatted stereo pairs can easily be taught. Persons with such training could view type II formatted stereo pairs supplied from virtual reality or television monitors in real or delayed time, or photographed or printed pairs. This technology could be used for 3-dimensional viewing of arthroscopic or endoscopic surgery, low power microscopy, macro-imaging, televised scenes or printed stereo pairs.

574.2

VERGENCE FOLLOWING VERTICAL SACCADES IS UNDER ADAPTIVE CONTROL. Z. Kapoula* T. Eggert and F. Fioravanti[1]. Lab Physiol. de la Perception et de l'Action, CNRS-Collège de France, Paris, France. [1]DEEI, Univ. Trieste, Italy.

Prior studies (Collewijn et al. J. Physiol. 1988; Enright J. Physiol. 1989; Kapoula et al. J. Neurophysiol. 1993) described horizontal vergence movements during and after vertical saccades. Such movements could be due to mechanical and/or innervational coupling of the vertical and horizontal muscles. The present study tests whether vergence movements following vertical saccades are under adaptive control.

Identical patterns were projected, one to each eye (dichoptic viewing). At first the patterns were superimposed; subjects fused them. At the end of each vertical saccade the patterns drifted exponentially, one left the other right, by the same amplitude, 10% of the antecedent saccade. The resulting disparate retinal slip was divergent after up saccades, convergent after down saccades. Four subjects were trained for 1 to 3 hrs. Binocular recordings were done with search coils. Prior to training, up saccades showed a rapid intrasaccadic divergence time locked to the onset of the saccade (group mean: 1.48°); this was followed by postsaccadic convergence (0.34°). Down saccades showed a slower convergence that started around the peak velocity of the saccade (0.73°); postsaccadic vergence was also convergent (0.2°). Adaptation was assessed from saccades in an open loop condition precluding visual following systems. Changes occurred in the intrasaccadic vergence but they were idiosyncratic: the group mean was still divergent for up saccades (1.18°) and convergent for down saccades (0.6°). In contrast, the change in postsaccadic vergence was always in the direction of the former pattern drift: up saccades decreased their baseline convergence (0.19°); down saccades increased their convergence (0.46°).

We conclude that the vergence coupled with vertical saccades is under adaptive control. Separate adaptive controllers exist for the intrasaccadic and postsaccadic components. The postsaccadic vergence controller stimulated in this study may be responsible as well for the disconjugate drifts we induced after horizontal saccades (Kapoula et al. Neurosci. Abstr. 1993). Supported by SCI*-CT91-0747 and CRAMIF.

574.3

SHORT-LATENCY DISPARITY VERGENCE RESPONSES IN HUMANS. C. Busettini*, F. A. Miles, and R. J. Krauzlis. Lab. Sensorimotor Research, National Eye Institute, Bethesda, MD 20892 and Dipartimento di Elettrotecnica, Elettronica ed Informatica, University of Trieste, 34100 Trieste, Italy.

The latency of disparity vergence has generally been estimated to be ≅ 160 ms. Using large stimuli, we now report latencies approximately one-half this value.

Subjects faced a tangent screen onto which two identical, densely textured images subtending 40°x40° were back-projected. Orthogonal polarizing filters in the two projection paths ensured that each of the two eyes saw only one of the two patterns, and their horizontal binocular disparity was controlled by mirror galvanometers. Positions of both eyes were recorded using the search coil technique. Stimuli were disparity steps [0.5, 1 and 2 meter angles (MA), 300ms duration] applied in the wake of 10° leftward saccades into the center of the pattern.

Vergence responses were highly repeatable with very short latencies. For example, the mean latency (±SD) for 5 subjects using 0.5 MA steps applied 50 ms after the centering saccade was 79 (±5) ms. The largest initial vergence accelerations were generated by the smallest disparity steps (0.5MA) and were always in the correct direction: convergent with crossed steps and divergent with uncrossed. However, with the largest uncrossed steps (2MA), responses started in the wrong (convergent) direction in 2 of the 3 subjects tested and only later reverted to the correct direction.

In all 5 subjects, small disparity steps (0.5MA) applied 50 ms after the centering saccade resulted in higher initial vergence accelerations than when applied 500 ms after the saccade. This transient enhancement of disparity vergence after a saccade is reminiscent of the post-saccadic enhancement of initial ocular following responses to sudden (conjugate) drifts of the visual scene (Gellman et al. *Vis. Neurosci.* 5:107,1990). We suggest that this enhancement will help to speed the realignment of the two eyes when gaze is redirected to (large?) objects in a different depth plane.

574.4

BINOCULAR COORDINATION, STEREO VISION AND LISTING'S LAW. D. Tweed*. Departments of Physiology and Ophthalmology, University of Western Ontario, London, N6A 5C1, Canada.

Recently Mok et al. and van Gisbergen et al. have shown that when the eyes converge on near targets, the Listing's plane of each eye rotates temporally through about half the vergence angle. The aim of the present study was to use computer simulations to identify the functional purpose of this behaviour. The first hypothesis, suggested by a paper by Howard, was that the brain strives to avoid binocular disparity of images of horizontal lines; but simulations of binocular coordination strategies that eliminated this disparity did not match the observed pattern of Listing's plane rotations. The hypothesis that the system minimizes pointwise disparity over the horopter fared no better. Van Rijn and van den Berg have shown that the pattern reported by Mok et al. is consistent with the brain trying to eliminate binocular disparity of images of lines orthogonal to the plane of regard. However, there are many different strategies that eliminate this disparity, so some additional performance criterion must be introduced to uniquely determine the observed behaviour. Adding the criterion that the rotational displacements of the eyes from their centre positions be minimized yielded a model that matched the observed behaviour. The motivation behind this second condition was that it would reduce extraocular muscle work, and it would keep the eyes near the centres of their motor ranges, for fast responses to new events. This combined strategy reduces to Listing's law for targets at optical infinity, and yields the correct temporal rotations of Listing's planes for near targets. On this view, the neural circuits transforming 3-D target locations into eye positions embody a mixed strategy with a sensory constraint and a motor cost function.

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574.5

EFFECTS OF PRISMATIC VERGENCE ON LISTING'S PLANE. S. Mikhael, T. Villa*, D. Nicolle, Department of Ophthalmology and Physiology, University of Western Ontario, London, Ont., N6A-5C1

During convergence on near targets Listing's plane (LP) is preserved but rotated temporally in the two eyes (Mok et al, *Vis Res*, 1992). Here we examined the changes in LP resulting from prismatically induced vergence. The three dimensional rotations of the two eyes were compared in five normal subjects wearing search coils and gazing at targets 1.9 m away with and without prisms. For horizontal base out prisms, the results were similar to gaze at near targets without prisms. Each degree of convergence yielded +0.83° (r^2 0.70) in LP. The results from vertical prisms were not what might be expected from the horizontal results. A base up prism on the right eye induced a downward, rather than the expected upward, rotation of LP. For each degree of vertical vergence LP rotated +1.27° (r^2 0.63). In addition the eyes rotated temporally +0.72° (r^2 0.43). A base down prism on the right eye induced an upward and nasal rotation of LP. The effects of oblique prisms were qualitatively those expected from combining the effects of horizontal and vertical prisms. For each degree of vertical vergence, LP rotated by +0.67° (r^2 0.73) in the same direction. The temporal rotation was greater with the base up and out than with the base down and out. In summary, horizontal, vertical and oblique prisms appear to rotate LP, but in opposite directions in the two eyes. Thus in addition to producing a horizontal or vertical misalignment of the gaze line, prisms induce an unexpected position dependent torsional disparity.

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574.6

A LONG-TERM STUDY OF EYE BLINK RESPONSES AFTER HYPOGLOSSAL-FACIAL ANASTOMOSIS. A. Gruart*, A. Gunkel, W.F. Neiss, E. Stennert and J.M. Delgado-García. Lab. of Neuroscience, Faculty of Biology, Univ. of Seville, E-41012. Sevilla, SPAIN and E.N.T. Department and Institute I of Anatomy, University of Cologne, D-50931, Cologne, GERMANY.

Hypoglossal-facial anastomosis is used in man to restore the activity of facial muscles following irrecoverable facial nerve lesions and other pathological conditions. As the kinematic of reflexively-induced eye blinks is very well known, the nictitating membrane/eyelid response can be used as a good experimental model to study the adaptability of hypoglossal motoneurons to a new motor role and to follow the (possible) recovery of lost motor functions, as the corneal reflex. Four cats were prepared for the recording of eyelid and tongue movements with the magnetic search-coil technique. The electromyography (EMG) of both orbicularis oculi (OO) muscles was also recorded. After control recordings, a left hypoglossal-facial anastomosis was carried out in the four animals. Two of them were also prepared for the chronic recording of anastomosed hypoglossal motoneurons. Recorded units were identified by their antidromic activation from the reinnervated upper lid. The first signs of OO muscle reinnervation were observed 6 weeks after surgery. The innervated lid moved in complete synchronicity with tongue displacements. Reflex blinks in response to corneal air puff or flashes of light were not recovered up to three months after reinnervation, as noticed in EMG recordings of the OO muscle. The discharge rate of anastomosed hypoglossal motoneurons was always related to tongue movements. However, a significant increase in the gain of the eye retraction system, mediated through accessory abducens motoneurons, was noticed even before reinnervation. The present results indicate that hypoglossal motoneurons are unable to adapt their discharge properties to a new motor task, but some plastic changes seem to be induced in the retractor bulbi system, which is non-directly affected by the anastomosis.

574.7

ACTIVITY OF PONTINE OMNIPAUSE NEURONS DURING EYE BLINKS. L. E. Mays* and D. W. Morrisse. Dept. of Physiological Optics and Vision Science Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Pontine Omnipause Neurons (OPNs) are known to play a crucial role in the generation of saccades and quick phase eye movements. These neurons are located in the *nucleus raphe interpositus* of the monkey, near the midline and just ventral and rostral to the abducens nucleus. OPNs are tonically active in the awake monkey and cat and cease firing just before and during saccades and quick phases of nystagmus. OPNs have been shown to inhibit saccadic excitatory and inhibitory burst cells, and electrical microstimulation of the OPN region suppresses saccades and quick phases but not slow eye movements.

Several investigators have briefly noted that OPNs pause for blinks as well as saccades. Since blinks are often associated with saccades, it can be difficult to determine whether a cessation of OPN activity is associated with a blink or the eye movements which accompany a blink. In experiments using rhesus monkeys trained to maintain fixation on a visual target, we have also observed that OPNs pause during blinks. Since the animals are actively fixating the targets, the eye movements which accompany the blinks are often quite small (<2°) and brief. We have observed that during blinks the duration of the pause is often greater than 100 ms, a value which is much larger than that seen for small saccades. This suggests that cessation of OPN activity may be related to blinks *per se* and not simply to the eye movements which accompany the blinks. Furthermore, the duration of the pause is positively correlated with the duration of the blink. Analysis of data obtained using lid coils shows that OPNs pause ≈ 10 ms before the blink onset and that the pause lasts considerably longer than the downward phase of the movement. (Supported by EY03463 and EY03039).

574.8

THE ULTRASTRUCTURE OF EYELID MOTONEURONS IN THE MACAQUE. P.J. May* Departments of Anatomy, Neurology and Ophthalmology, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

Eyelid movements are primarily controlled by two muscles: the orbicularis oculi, which produces blink down-phases, and the levator palpebrae, which produces blink up-phases and moves the lid in conjunction with vertical gaze changes. The motoneurons which control these two muscles have been identified using retrogradely transported WGA-HRP/HRP. The ultrastructure of these identified lid motoneurons and the axon terminals synaptically contacting them have been characterized to provide a basis for determining the pattern of inputs that produce blink and gaze-related lid movements. Levator motoneurons have a relatively low density of synaptic contacts upon the plasma membrane of their somata and proximal dendrites. In addition they ocassionally display short somatic and dendritic spines. A variety of different terminal classes contact the surface of levator motoneurons. Type 1 profiles have large numbers of clear, spherical synaptic vesicles (40-50 nm) and make asymmetric synaptic contacts. Some examples of these extend along the postsynaptic membrane and make multiple synaptic contacts. Type 2 profiles contain clear pleomorphic vesicles and a very small number of dense-cored vesicles. They make symmetric synaptic contacts. Type 3 profiles are similar to Type 1, but also contain larger (90 nm) scattered, dense-cored vesicles. Their synaptic densities are variable in extent. Orbicularis motoneurons have a higher density of synaptic contacts on their somatic and dendritic plasma membranes, with terminals often forming a continuous lining along some membrane segments. An unusual feature of these motoneurons is the ocassional presence of a subsurface cystern immediately beneath a synaptic contact. The same three classes of profile were observed in contact with orbicularis oculi motoneurons, but there appeared to be two subclasses of Type 1 ending. One [Type 1A] was densely packed with vesicles, while the other [Type 1B] had fewer, scattered vesicles. The greater synaptic density present along the membranes of orbicularis oculi motoneurons may corelate with the very high firing rates these cells must attain during the phasic action of blinking. Supported by NIH grant EY09762.

574.9

EFFECTS OF SUPERIOR COLLICULUS STIMULATION ON THE BLINK REFLEX IN MONKEYS. S-M Lu*, B Breznen, MA Basso, C Evinger and JW Gnadt, Departments of Neurobiology & Behavior and Psychology, SUNY Stony Brook, NY 11794.

In alert Rhesus monkeys, the effects of SC stimulation on reflex blinks were studied by delivering 100-300 ms trains of electrical pulses beginning 30-50 ms before a 30-50 ms air puff to one eye. Eye and lid position were monitored using the search coil technique. Location within the SC motor map was determined with 10-25 μA biphasic pulses at 250-300 Hz. The threshold current for blink suppression was always higher than for saccades. The most effective sites for blink suppression was located in the deep layers of SC near the rostral pole, where the ratio of thresholds was roughly 1.5. This ratio increased to 3 or greater at superficial and caudal sites.

The gradient of relative threshold within the SC correlates well with the gradient of excitatory projections of the SC to brainstem omnipause neurons (OPN). Moreover, stimulation of OPN suppresses blinks. Nevertheless, the difference in the threshold for producing blink suppression and saccades suggests that the underlying neuronal circuitry may differ for the two processes.

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574.10

BRAINSTEM LINKAGE BETWEEN THE SUPERIOR COLLICULUS AND BLINKING. M.A. Basso*, and C. Evinger, Depts. of Psychology, Neurobiology & Behavior and Ophthalmology. SUNY Stony Brook, NY 11794.

Both clinical and experimental data show that the basal ganglia can dramatically modulate the brainstem blink reflex circuit. Previously, we have demonstrated that the superior colliculus (SC) is an important mediator of these effects, such that substantia nigra inhibition of the SC increases blink magnitude. The goal of the current study was to identify the brainstem neurons that mediate the SC modulation of blinking. The inhibitory omnipause neurons (OPN) are one candidate because electrical stimulation of the OPN region suppresses blinks (Mays, 1994) and stimulation of the rostral SC, a region which provides a large input to the OPN, is the most effective for suppressing blinks with electrical stimulation. However, since blink suppression with SC stimulation has a higher threshold than that for evoking saccades or producing fixation (Lu *et al.*, 1994), it is also possible that a nonsaccadic group of brainstem neurons mediate SC blink suppression.

By presenting a single, electrical pulse to the SC in urethane anesthetized rats, we determined the latency of SC suppression of orbicularis oculi EMG (OOemg) activity to be 7 ms. Single unit recordings in the brainstem revealed a group of tonically active neurons caudal to the OPN that paused with reflex blinks. Stimulation at the site of these neurons produced a decrease in OOemg activity within 5 ms. Stimulation at other brainstem sites, however, produced blink suppression at much longer latencies.

Consistent with SC stimulation in monkeys and behavioral studies in humans, the SC appears to modulate reflex blinking through brainstem neurons other than those involved in saccadic eye movements.

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574.11

GRAVITY-DEPENDENCE OF TORSIONAL OPTOKINETIC GAIN IN RHESUS MONKEYS. D Straumann*, M Dieterich, V Henn, BJM Hess. Neurology Department, University Hospital, CH-8091 Zürich, Switzerland.

In three rhesus monkeys we have measured torsional optokinetic nystagmus using the three-dimensional search coil technique. The animals were exposed to 30 seconds of stimulation by an optokinetic sphere, which rotated about the naso-occipital axis at different velocities. Gain (eye-stimulus-velocity coefficient) during the last 5 seconds of stimulation was determined for upright and supine body position. Compared to stimulations in the supine position, we found that torsional gain of optokinetic nystagmus was strongly attenuated in the upright position with angular eye velocity saturating at 5-10 deg/s (see also Schiff et al. (1986) Soc Neurosci Abstr 12: 774). Gain values in supine position varied considerably among the three monkeys, and we found a correlation with the time constant of the corresponding torsional vestibulo-ocular reflex. The data can be interpreted that the velocity-storage mechanism actually enhances torsional optokinetic nystagmus is supine position. In upright position, however, the rotation axis of torsional nystagmus does not coincide with the gravity vector. Hence, velocity storage is inhibited and torsional optokinetic gain decreases.

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574.12

LEAKY NEURAL INTEGRATION OF TORSIONAL VESTIBULAR SIGNALS IN HUMANS. S.H. Seidman*, R.J. Leigh, R.L.Tomsak, M.P.Grant, and L.F.Dell'Osso, University of Rochester Med Ctr, Dept of Neurol., Rochester, NY, Case Western Res. Univ, and the Veterans Admin. Med. Ctr., Cleveland, OH.

We investigated the dynamic properties of the human torsional vestibulo-ocular reflex (VOR) during roll head rotations, and compared them with those of the horizontal VOR. Differences were related to the contributions of the extraocular plant and the neural integrator.

Three subjects underwent position-step stimuli in the roll plane, and the resulting torsional eye movements were recorded using the magnetic search-coil technique. Using optimal parameter estimation techniques, the data were fit to a pulse-step model of the torsional VOR, which incorporated plant parameters obtained through previous forced-duction experiments. Following the position step, the eye drifted back to is resting position in the torsional plane, demonstrating a time constant of neural integration typically <2.5 sec. Despite previous evidence of an asymmetry in the ocular-motor plant between intorsion and extorsion, torsional eye movements appear to be well yoked during roll stimulation.

When subjects underwent similar VOR stimulation in the yaw plane, the eyes held their new horizontal position for a much longer period of time, always yielding a time constant of neural integration of much greater than 20 sec.

We hypothesize that the incomplete integration of vestibular signals for torsional eye movements reflects different visual demands placed on this reflex. In addition, the yoking of binocular torsional eye movements despite possible asymmetries on the plant may demonstrate a larger separation of control of individual muscles than previously thought to hold true.

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574.13

THREE-DIMENSIONAL CONSTRAINTS ON COORDINATED EYE-HEAD GAZE SHIFTS IN THE MONKEY. D.Guitton* and J.D.Crawford. Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, Montreal, Canada, H3A 2B4.

We have studied the normal orienting behaviour of one head-free *Macaca Fascicularis*. Dual search coils were implanted in one eye, and temporarily screwed onto the skull cap. Eye and head position quaternions were recorded while the animal looked at visual targets presented randomly throughout its gaze range. With the head fixed, the eye was constrained to a Listing's plane with a torsional standard deviation of ~1°. Listing's plane was no longer maintained with the head free, because VOR slow phases produced torsional changes in eye position. However, opposite torsional components in the rapid eye movement components of the gaze shift prevented accumulated torsion. Both the head and eye-in-space loosely followed a strategy resembling a set of Fick gimbals (Glenn & Vilis, *J.Neurophysiol*, 1992): horizontal rotations occurred about a relatively fixed vertical axis, but vertical rotations occured about a horizontal axis that tended to rotate with the head. These axis tilts were not as stereotyped as those observed with the head fixed, and thus the ranges of head and eye position-in-space were not so tightly constrained (torsional standard deviations were ~4° and ~4.5° respectively). However, these ranges showed the curvature characteristic of the Fick gimbals strategy: Up-left & down-right gaze directions were accompanied by a clockwise torsional tilt of the eyes & head, and down-left & up-right gaze had counterclockwise tilts (in earth-fixed coordinates). These results agree with human studies (Glenn & Vilis, 1992), suggesting that the monkey provides an appropriate experimental model for invasive studies of this behaviour.

CONTROL OF POSTURE AND MOVEMENT VIII

575.1

A NEURAL MODEL OF VOLUNTARY MOVEMENT AND PROPRIOCEPTION. D. Bullock*, P. Cisek, S. Grossberg. Boston University, Dept. of Cognitive and Neural Systems, Boston, MA 02215.

A neural model of voluntary movement and motor proprioception is developed within constraints from neurophysiology and motor psychophysics. The model generates trajectories to a desired endpoint in spatial coordinates, while using motor command corollary discharges and muscle spindle error feedback to construct an accurate perception of position. Responses of model elements resemble observed cortical and subcortical physiological data while functional characteristics suggest explanations of proprioceptive illusions and load effects. Simulations show the system's ability to execute voluntary movements while reducing oscillations caused by limb dynamics and compensating for external forces such as those caused by loads or obstacles. Further simulations demonstrate how the network can self-organize its sensorimotor transformations through spontaneous co-contractions of synergistic muscles.

Supported in part by the National Science Foundation (IRI 90-24877, IRI 90-00530) and Office of Naval Research (ONR N00014-92-J-1309, ONR N00014-93-1-1364).

575.2

DYNAMIC NEURAL FIELD THEORY OF MOTOR PROGRAMMING. W. Erlhagen, G. Schöner*, Inst. Neuroinformatik, Ruhr-Universität Bochum, 44780 Bochum, Germany

Recent evidence suggests that motor programs are specified gradually (Hening W., Favilla M., Ghez C.: Exp. Brain Res. 71, 116-128 (1988)), can be continuously updated, and are part of a closed feedback loop. The implied theoretical challenge is to address the dynamic properties of motor programs. We present a dynamic field theory of motor programs that is based on a neural representation of movement parameters such as movement direction and amplitude. The topology of such parameters is expressed by structuring the neural representations as fields. Motor programs are stable localized solutions in such neural fields. They evolve under two types of external influences: (1) localized input representing the perceptual specification of movement parameters and (2) input representing the pre-structuring of motor plans and thus reflecting the nature of the task space defined by the experimental paradigm. The distance of this pre-shape from the specified motor program in a dynamic sense determines the time need to generate the correct motor program and therefor affects reaction time in the theory. We compare our model to experimental results of Ghez and colleagues on the gradual build-up of motor programs. We account for the Hick-Hyman law, the dependence of reaction time on the metrics of tasks, on pre-cued information, and discuss effects such as range or bias effects and deprogramming. The longer term goal of integrating such a dynamic description with a dynamic account of coordination (Schöner G., Kelso J.A.S.:Science 239, 1513-1520 (1988)) is sketched.

575.3

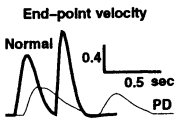
A DYNAMIC THEORY OF THE WALK-RUN TRANSITION: THE EFFECTS OF GRADE. F. J. Diedrich* and W. H. Warren, Jr. Department of Cognitive and Linguistic Sciences, Brown University, Providence, RI 02912.

Why do humans shift from walking to running when speed increases? We have proposed a dynamic theory of human locomotion in which preferred gaits are attractors that exhibit stable phase relationships, and gait transitions are bifurcations characterized by a loss of stability (Diedrich & Warren, in press). According to this theory, manipulation of the location of the attractors should have an effect on the speed of the transition. As suggested by energy expenditure data, one way to manipulate the attractors is to vary the inclination of the treadmill. Six participants walked and ran on a treadmill at grades of 0% and 10% while speed was varied. As expected, the speed at which fluctuations of the ankle-hip phase relationship were at a minimum shifted downward from 1.63 m/s on a 0% grade to 1.54 m/s on a 10% grade, consistent with movement of the attractor to a lower speed. Similarly, the mean transition speed, averaged over ascending and descending trials, shifted downward from 2.19 m/s at 0% to 2.00 m/s at 10%. In sum, changing treadmill inclination led to movement of the attractors and corresponding changes at the walk-run transition. These directional shifts were generally consistent with those expected on the basis of previous energy expenditure data.

575.4

A NEURAL NETWORK MODEL OF BASAL GANGLIA THALAMOCORTICAL RELATIONS IN NORMAL AND PARKINSONIAN MOVEMENT. J.L. Contreras-Vidal* and G.E. Stelmach. Arizona State University, Laboratory of Motor Control, Tempe, AZ 85287.

Anatomical, neurophysiological, and neurochemical evidence support the notion of segregated parallel basal ganglia-thalamocortical motor channels. We developed a neural network model for the function of this system in simple and complex (sequential and simultaneous) movements in normal and Parkinsonian (PD) behavior. Non-linear differential equations are used to model the membrane potential for each cell type (short-term activation), its physiological action (inhibitory or excitatory), as well as neurotransmitter/neuromodulators (medium-term dynamics: e.g. GABA, enkephalin, substantia P). The model reproduces motor impairments in PD due to dopamine depletion such as akinesia, bradykinesia, and pauses between movement components (the figure shows a normal and PD simulation of a two-stroke sequential movement). Specific observations of the effect of subthalamotomy and pallidotomy on PD improvement are made. The model also suggests how the motor load is distributed across several brain structures during multijoint movements.



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575.5

ACUTE EFFECTS OF LEVODOPA ON WRIST MOVEMENT IN PARKINSON'S DISEASE: KINEMATICS, VOLITIONAL EMG MODULATION, AND REFLEX AMPLITUDE MODULATION MTV Johnson*, A Mendez, AN Kipnis, P Silverstein, F Zwiebel, & TJ Ebner Departments of Neurology, Neurosurgery, and Physiology, University of Minnesota, and Minneapolis Clinic of Neurology, Minneapolis, Minnesota, USA

Acute changes in motor performance due to levodopa were evaluated by a series of four motor tests unified by their focus on wrist flexion-extension movements. Subjects with idiopathic Parkinson's disease were evaluated with a test battery consisting of a repetitive self-paced movement in which velocity was to be maximized, visually guided tracking of a sinusoid and a square wave, and an assay of stretch reflex modulation during volitional sinusoidal tracking. The maximal wrist joint velocity of self-paced reciprocating flexion and extension movements increased after levodopa (ON), without significant changes in the movement period or amplitude. In the two tracking tasks, the RMS error, peak velocity, or peak movement amplitude did not change after levodopa. Significant and consistent changes did occur in an assay of reflex modulation during error-constrained tracking after levodopa. The amplitude of volitional EMG increased after levodopa, concurrently with a reduction in reflex EMG. These changes are consistent with the noted increase in movement velocity. These results show that the effects of levodopa on movement velocity were not translated into increased accuracy. Also, the changes in the long latency reflex gain argue for a central control of this reflex, mediated by structures sensitive to levodopa. Supported by NIH grants R44 NS28633 and T32 NS07361

575.6

THE EFFECTS OF GRAVITATIONAL CUES ON THE TRANSFORMATION BETWEEN KINESTHETIC AND VISUAL INFORMATION TO REACHES TO REMEMBERED TARGET LOCATIONS A.J. Butler*, W.G. Darling, M.A. Pizzimenti. Department of Exercise Science, University of Iowa, Iowa City, IA 52242.

The abilities of human subjects to point to kinesthetically presented targets which are no longer present during a reaching movement was studied under 2 gravitational and 3 reaching conditions: (1) VR/NG, VR/G - visual reach, no gravity, gravity: (2) BR/NG, BR/G - reach with vision occluded, no gravity, gravity: (3) VRPT/NG VRPT/G - visually reach with pointer, no gravity, gravity. The ability to specify target locations on the basis of kinesthetic information alone has been found to be accurate (Darling and Miller, *Exp Brain Res*, 1993, Vol. 93 pp 534). Reaching with a pointer requires transformation of kinesthetically derived limb coordinates into a visual coordinate system. Availability of gravitational information about target locations was not manipulated in previous studies and is thought to be used in the kinesthetic representation of upper limb orientation. Thus, the main purpose of this experiment was to study the abilities of subject to reach to a remembered kinesthetic target location with or without gravitational information on target location. We presented 10 targets in two planes using a programmable robot arm. Positions of the arm, hand, fingertip and target object in three-dimensional space were recorded using optoelectronic techniques (WATSMART system, Northern Digital). Manipulating gravitational information on target location had little influence on directional and distance errors during visually guided reaching with the hand. When using the pointer, there was a substantial increase in distance errors, but not in direction errors. Availability of gravitational information had little influence on performance when reaching with the pointer. Consistent with the findings of previous work, subjects' tended to overshoot close targets and undershoot far targets during visually guided reaching to kinesthetic targets. These results suggest that removing gravitational information about kinesthetic target location does not interfere with reproducing a kinesthetically remembered hand location or with transformation of kinesthetic information into a visual coordinate system.

575.7

VISUAL PERCEPTIONS OF EARTH- AND BODY- FIXED AXES. W.G. Darling*, T. Gebhardt, S. Liu. Department of Exercise Science, University of Iowa, Iowa City, IA 52242.

Target location encoded by the visual system must be transformed into kinesthetic and motor coordinate frames for the upper limb to perform reaching movements. Recent studies in this laboratory have shown that the perceptually preferred anterior/posterior (a/p) axis of kinesthetic coordinate system is trunk-fixed (Butler et al. 1993 - Soc. Neurosci. Abstr. 19, 550) and for the visual system is also likely trunk-fixed (Darling and Williams 1993 - ASB Proceedings of 17th Ann. Mtg., pp. 115). The earth-fixed gravitational axis is preferred by the kinesthetic system at the perceptual level (Darling, manuscript in preparation). The purpose of this investigation was to determine if the visual system prefers the earth-fixed gravitational axis to body-fixed axes at the perceptual level.

Ten normal healthy individuals positioned a hand-held lighted wand under four different experimental conditions that were each run under light and dark conditions: (1) earth-fixed -subjects stood in anatomical position and moved the wand parallel to the gravitational axis, (2) earth - same as (1) except that subjects head and trunk orientations were varied, (3) head - same as (2) except that subjects positioned the wand parallel to their head-fixed longitudinal axis and (4) same as (2) except that subjects positioned the wand parallel to the trunk-fixed longitudinal axis. Orientations of the head, trunk and wand were recorded optoelectronically. The results clearly showed that subjects were most accurate at positioning the wand parallel to the gravitational axis even when head and trunk orientations were varied ($p < .001$). Control experiments showed that kinesthesia was not used for precise positioning of the hand-held wand. Thus, the visual system prefers the earth-fixed gravitational axis to body-fixed longitudinal axes at the perceptual level and coordinate transformations at this level are simplified because of the parallel axes for the visual and kinesthetic coordinate systems.

575.8

ATTRACTION OF VOLUNTARY MOVEMENT TO INTRINSIC OSCILLATORS: A SYSTEM INHERENT PHENOMENON ? G. Staude[1], W. Wolf[1]* and J. Elek[2]. [1]Universität der Bundeswehr München, D-85577 Neubiberg. [2]Medizinische Hochschule Hannover, D-30625 Hannover, Germany

Attraction of motor coordination to oscillatory components is one of the fundamental features of biological systems. Recently, we reported a systematic phase relationship between tremor and the onset of fast voluntary motor responses in patients with Parkinson's disease (PD) (Staude, G et al. Soc.Neurosci.Abstr. 1992;18:862). Experiments with control subjects mimicking tremor showed similar results. The aim of this study was to establish a functional model for the attractive effect of the tremor oscillator in the framework of the equilibrium point (EP) hypothesis. According to the EP hypothesis, there is only one central variable that controls the behavior of a muscle, the threshold λ of its tonic stretch reflex (Feldman, AG Biophysics. 1966;11:565-578). Single joint movements are induced by a shift (virtual trajectory) in control variables λ_A,λ_N of at least one pair of antagonist muscles from an initial value (that depends on the actual phase of the tremor cycle) to a final state. With the virtual trajectory known, both the movement trajectories and the electromyographic patterns of the antagonist muscles can be predicted. Movement patterns of PD tremor patients and control subjects mimicking tremor were recorded under both isometric and kinetic reaction time paradigms and compared to computer simulations. The EP hypothesis proved to reliably predict the observed movement patterns under various conditions. We conclude that attraction of voluntary motor response to intrinsic oscillations is a system inherent phenomenon of motor control and does not depend on the origin of the oscillatory component.

575.9

FOCAL INTRASPINAL NMDA IONTOPHORESIS PRODUCES DISTINCT ACTIVATION PATTERNS IN THE FROG. P. Saltiel and E. Bizzi. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

In the spinalized frog, microstimulation of the lumbar grey produces only a few possible orientations for the convergent force fields recorded in the horizontal plane from the isometrically contracting hindlimb: flexor towards body, rostral flexor, outward extensor and medial extensor. To determine if this represents activation of fibres of passage or of neurones, and as a preliminary step to anatomical studies aimed at identifying such neurones, NMDA (4mM,-100nA x 30 sec.) was iontophoresed at cord sites where microstimulation through the same pipette had been effective. Forces and 11 EMG's were recorded.

38% of sites (32 sites in 7 frogs) did not respond to NMDA. Of the NMDA-responsive sites (average latency 16 sec., range 4-29 sec.), the forces evoked by chemical stimulation had the same four types of orientation than seen with microstimulation, actually matching them at 68% of sites. For the EMG's, a close match was seen in only 38% of cases.

However the NMDA-evoked EMG's fell in a few distinct patterns which could be related to the forces produced. One pattern (8/17 sites) consisted of a nearly simultaneous onset of a tonic or rhythmic coactivation of all muscles except for RA (rectus anterior), VI (vastus internus) and GA (gastrocnemius) which were only weakly activated. With one exception, this pattern produced flexion towards body forces. In a second pattern (6/17 sites), muscle recruitment occurred progressively over a succession of cycles, with the production at first of rostral flexion and then flexion to body forces. RA, VI and GA were among the most active muscles. Cord sites eliciting the first and second patterns were generally located caudally and rostrally to the 8th root respectively. A third pattern (2/17 sites) produced outward extensor forces.

In conclusion, focal intraspinal NMDA iontophoresis may be helpful to localize groups of neurones responsible for the generation of distinct EMG patterns which produce the same set of force orientations previously identified with electrical microstimulation. (Supported by ONR:N00014/K/0372).

575.10

MOTOR TEMPLATES IN TYPING. §$P. Viviani* and §G. Laissard. §Faculty of Psychology and Educational Sciences, Univ. of Geneva, Geneva, Switzerland and $Dept. of Cognitive Science, Istituto S. Raffaele, Milan, Italy.

We investigated the performance of professional typists transcribing texts presented visually. The timing of the keystrokes was analyzed as a function of the following factors: 1) the combination of fingers used for typing digrams; 2) the relative frequency of the digrams in the language; 3) the word-context in which trigrams are embedded; 4) the total duration of the typing sequence for the word. Biomechanical and linguistic factors were found to influence the interstroke intervals. However, over and above these factors, we demonstrated that words (i.e. well-formed lexical items) are typed with sequences of keystrokes whose temporal structure depends idiosyncratically on the word and on the typist. Within the range of variability of the rhythm that is normally observed in transcription, this structure - defined as the set of all ratios between interstroke intervals - remained unchanged (homothetic invariance). The results suggest that these highly complex motor sequences are controlled by global motor plans that include an abstract representation of the timing of the keystrokes. A simple counting mechanism was postulated to explain the findings. We contrasted four variations of the mechanism on the basis of the pattern of temporal variability.

575.11

HINDLIMB MUSCLES OF THE RAT: FORCE FIELDS AND REDUNDANT SUMMATION M.C. Tresch*, E. Loeb, and E. Bizzi. Dept. Brain & Cog. Sci., M.I.T., Cambridge, MA 02139.

Previous work from this laboratory has described a set of movement primitives encoded in the frog spinal cord. In order to examine the generality of these findings, we are currently exploring the movements encoded in the spinal cord of the rat. As a first step in this project, we describe here the pattern of position dependent forces, i.e. the force fields, produced by rat hindlimb muscles and the result of combined stimulation of two muscles. In the frog, simultaneous stimulation of two muscles produces a force field which is the vector summation of the force fields of the two muscles stimulated separately. This property of summation holds for the non-redundant case, with the force transducer attached to the ankle, as well as for the redundant case, with the force transducer attached to the foot. We found a similar pattern of results in the rat: the force field predicted by summation of the two separate muscle fields was highly correlated with the field produced by the costimulation of the muscles for both the redundant and non-redundant case. This result implies that, at least for the degrees of redundancy in these experiments, the combination of muscles can be approximated as a linear vector sum, potentially simplifying the control of the hindlimb. Supported by NIH NS09343 and AR26710 and ONRN00014/90/J/1946.

575.12

THE ROLE OF DOPAMINE TERMINALS IN THE NUCLEUS ACCUMBENS IN LOCOMOTION INDUCED BY ACTIVATING THE HIPPOCAMPAL → ACCUMBENS GLUTAMATE PROJECTION.

M. Wu and S. M. Brudzynski*. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Earlier electrophysiological evidence has shown that the stimulation of the hippocampal input to the nucleus accumbens evokes short-latency, short-duration, glutamate-mediated excitatory responses in the nucleus accumbens neurons (Yang & Mogenson, 1984). It has also been shown in our recent behavioral studies that injecting glutamate receptor agonists, NMDA or AMPA, into the nucleus accumbens activates the glutamate receptors located on mesolimbic dopamine terminals (Wu et al., 1993). This evidence suggests that glutamate in the nucleus accumbens may act on glutamate receptors, both on mesolimbic dopamine terminals and accumbens output neurons. The objective of this study was to investigate the role of the mesolimbic dopamine terminals in the nucleus accumbens in the initiation of locomotion by activating the hippocampal excitatory input to the nucleus accumbens.

Locomotion (measured in an Opto-Varimex-3 activity cage) was significantly increased by unilateral injections of NMDA into the ventral subiculum of the hippocampus. This NMDA-induced locomotion from the hippocampus was abolished after the destruction of the mesolimbic dopamine terminals in the accumbens, by injecting 6-OHDA into the ventral tegmental area (VTA). The results suggest that the mesolimbic dopamine terminals are essential in transmitting hippocampal signals to the output neurons within the nucleus accumbens.
(Supported by MRC of Canada)

575.13

A PHA-L STUDY ON DESCENDING PATHWAY FOR VOCALIZATION FROM THE PERIAQUEDUCTAL GRAY TO THE LOWER BRAINSTEM IN CATS. Y. Kobayashi, A. Katada, H. Myoga and T. Sakamoto*, Dept. of Physiol., Asahikawa Med. Col., Asahikawa 078 JAPAN.

Tonic electrical stimulation to the midbrain periaqueductal gray (PAG) induced naturally sounded vocalization. The stimulation to the pontine call site (PCS) within the ventrolateral pons also induced vocalization. To know whether the PCS is the descending pathway for vocalization from the PAG to the lower brainstem, an anterograde tracer, Phaseolus vulgaris leucoagglutinin, was applied at sites located in the lateral PAG where the electrical stimulation induced vocalization.

At the level of the caudal superior colliculus, labelled fibers sparsely distributed and passed laterally through the dorsal part of the midbrain tegmentum where the stimulus threshold for vocalization was higher than the PAG. At the level of the inferior colliculus, they could be traced ipsilaterally to the ventral area along the medial lemniscus and consisted of a narrower bundle around the PCS where the stimulus threshold was the lowest.

At the level of the upper pons, they occupied a broad but thin region in the dorsal portion of the trapezoid body ventral to the medial lemniscus. Further caudally, they appeared to be concentrated in a discrete area ventral to the inferior olive and dorsal to the pyramidal tract, and could be continuously traced to the upper cervical spinal cord.

It was known that the PAG stimulation induced vocalization associated with rage, while the PCS stimulation induced vocalization without rage. The present results suggested that the PCS is the descending pathway specific for vocalization from the PAG to the lower brainstem.

575.14

ACTIVITY IN VENTROLATERAL THALAMIC AREAS DURING CONDITIONED VOCALIZATIONS IN CAT. G.R. Farley*, Research Division, Boys Town National Research Hospital, Omaha, NE 68131.

Although ventrolateral thalamus has been implicated in other motor control tasks, its role during vocal behavior might be questioned. Jürgens and colleagues, based on electrical stimulation and lesion data, indicated that it was not part of a core system of brain structures capable of producing vocalizations in the absence of concomitant reinforcing potential. In the current study, we recorded single- and multi-unit activity from sites in the ventrolateral thalamic area in awake, behaving cats. A substantial proportion of recording sites showed strong temporal correlations in neural activity with various aspects of the instrumentally conditioned vocalization paradigm, including both vocal production and feeding behavior. Activity related to feeding was generally strongest and most prevalent, typically involving bursts of firing during ingestion of the food reward. However, there were also numerous examples of increased and decreased firing during vocalizations, as well as changes in firing rate immediately following vocalization. There were no obvious changes in firing related to background respiratory activity. However, a number of loci showed bursts of activity apparently related to laryngeal EMG activity not associated with vocalizations. Finally, we also noted apparent long-term fluctuations in background firing rate associated with periods of intense behavioral activity versus periods of behavioral quiescence. These data are consistent with a possible role of this structure in motor control during vocal production. (Supported by NIH grant 5-RO1-DC01535-01.)

575.15

MOTION SICKNESS HELPS PRESERVE SENSORIMOTOR COORDINATION IN THE PRESENT AND FUTURE. D.W. Jensen*. Department of Biology, Tomball College, Tomball, TX 77375.

A new hypothesis is proposed for the function of the mechanisms of motion sickness. The hypothesis accounts for data from plasticity of postural control, development of sensorimotor coordination, motion sickness, vomiting, pharmacology, and neurotoxicology.

The mechanisms giving rise to the early symptoms of motion sickness impel an individual to remove the sensory conflicts of an offending motion. The early symptoms are a variety of ill feelings and actions that do not interfere greatly with motor control. The sufferer can still remove sensory conflicts, so the brain is not stimulated to undergo long-term adaptive changes that alter sensorimotor coordination. Thus, the early symptoms and their mechanisms serve to resist redefining normal motion while concordantly preserving the existing sensorimotor coordination. Later developing symptoms of vomiting and severe nausea, however, impede motor control profoundly: in such a state one cannot control much of anything. This extremely ill feeling is the same as what is produced by certain neurotoxins. The state of being poisoned by provocative motion is strongly associated with the motion. When the same kind of sensorimotor-coordination-altering motion occurs again that association is recalled. Acting on this recall and avoiding that motion preserves the existing sensorimotor coordination in the future.

575.16

ESTIMATION OF IDENTIFIED INTERNEURONS FORMING A FUNCTIONAL GROUP CONTROLLING ABDOMINAL POSITIONING IN THE CRAYFISH. L.D. Brewer, and J.L. Larimer* Dept of Zoology, Univ. of Texas at Austin., Austin, TX 78712.

Several studies have indicated that the command elements controlling abdominal positioning (flexion and extension) in the crayfish *Procambarus clarkii* are organized into groups through extensive synaptic connections (Larimer 1988, Trends in Neurosci.: 11). We have designed a method to estimate the size of these functional groups. Fictive abdominal positioning was observed in isolated nerve cords by recording from motor roots while stimulating single command elements intracellularly. Extracellular recordings of interneurons were made from rostral and caudal connectives or hemiconnectives* (1-2 and 5-6). When one of these command elements is stimulated it activates a number of previously silent command elements and increases or inhibits the spontaneous activity of still other interneurons. We then compared spontaneous with evoked activity. The largest number of recruited interneurons was 64, the smallest was 4, and the average was 23 (n=15). Some of these command elements are identified cells based on morphology and physiology. Data was gathered from one identified cell on three separate occasions. A comparison of these data revealed that although the number of recruited cells and their firing frequencies were not identical they were surprisingly similar. These data support the functional group hypothesis, and suggest that on average about 23 interneurons controls each abdominal positioning behavior. Furthermore, these data and previous studies indicate that the interneurons within a group may or may not be tightly coupled to each other, and their recruitment may be based upon such factors as stimulus intensity and synaptic efficacy. *Counts from only hemiconnectives were doubled since motor output and nerve cord activity are usually symmetrical. (Supported by NIH Grant NS 05423, to J.L.L.)

575.17

SEPARATE PERIPHERAL NERVES INNERVATE THE HEADS OF THE HUMAN LATERAL PTERYGOID MUSCLE, M. A. Aziz and R. J. Cowie* Anat. Dept, Col. of Med., Howard Univ., Washington, D.C., 20059.

Anatomical, developmental and biomechanical studies of the human lateral pterygoid (LPt) muscle indicate that it is divisible, at least partially, into two heads. Electromyographic (EMG) studies supporting the independent action of the superior (SLPt) and inferior (ILPt) heads during the masticatory cycle have been questioned. The precise peripheral nerve path(s) to the two heads and the full structural complexity of the masticatory space remain uninvestigated. Therefore, we have grossly dissected the LPt muscle and nerves, and measured the muscle fiber orientations of 7 dissection room specimens. Photographs, drawings, and whole sections through the masticatory space were analyzed. Our results show that: i) the two heads are independently innervated, such that the SLPt receives a single branch from the root of the masseteric nerve, while the ILPt receives two or more branches from the root of the long buccal n., as well as loops bridging the anterior and posterior mandibular (V3) divisions; ii) the origins of the heads are easily recognizable, but in two cases each head shows further horizontal segmentation; iii) the mean insertion orientations of SLPt and ILPt fibers are 27° ($\pm 6^{o}$) and 338° ($\pm 4^{o}$) from the Frankfort plane, respectively.

These findings support: 1) a claim that the two LPt heads are enclosed in separate fascial compartments; 2) EMG studies indicating their independent functions; 3) the likely segregation of their central control mechanisms. These data are prerequisite to a planned histochemical demonstration of the latter in primates.

575.18

NOREPINEPHRINE MODIFIES THE BACKGROUND FIRING RATE OF RED NUCLEUS NEURONS OF THE RAT. G. LI VOLSI*, F. LICATA, G. MAUGERI, F. SANTANGELO, Istituto di Fisiologia umana Viale Andrea Doria, 6 - 95125 CATANIA - Italia

A significant concentration of norepinephrine (NE) has been detected in mesencephalic red nucleus (RN). Although the role of this amine on the RN function remains unknown, it has been observed that NE amount in the RN appears strongly modified in various syndromes.

The aim of this work was to establish whether this amine influences the rubral neuronal activity. In anaesthetized rats, we analized the firing rate of single rubral neurons during microiontophoretic injections of NE and its agonists or antagonists.

More than 90% of the tested RN neurons modified their background firing rate in response to microiontophoretic NE. Decreases of the firing rate were recorded in 50%, enhancements in 30% and biphasic effects (decreases followed by enhancements) in 20% of the cases. Phentolamine and yohimbine antagonized the inhibitory and unaffected, or eventually enhanced, the excitatory responses, that were reduced by timolol. Clonidine mimicked the NE-evoked decreases of the firing rate.

It is concluded that norepinephrine can influence the RN neuronal activitby using both α- (mostly α_2) and β-receptors. A modulatory action of noradrenergic pathways on the rubral function, and therefore on the motor systems, is proposed.

575.19

MODULATIONS OF THE NORMAL LOCOMOTOR PATTERN DURING VISUALLY EVOKED YAW TURNS IN THE LAMPREY. P.Wallén*, F.Ullén, T.G.Deliagina, G.N. Orlovsky, and S.Grillner Dpt. of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, SWEDEN

Negative phototaxis in the river lamprey (*Lampetra fluviatilis*) was characterized behaviourally, and the strategies for performing yaw turns away from light were investigated with video recordings, combined with EMG-recordings from the myotomal musculature, in freely behaving animals. In swimming lampreys, illumination from one side evoked a yaw turn away from the light. An increase in light intensity resulted in a significant increase in mean turning angle. Strong lateral illumination frequently evoked an escape response, characterized by a decrease of swimming speed, an approximately 180° yaw turn either away from or towards the light, and subsequent locomotion in a direction opposite to the initial one. During yaw turns the locomotor rhythm became slower and asymmetrical, so that the lateral displacement of the undulatory wave was larger in the direction of the turn, and smaller on the other side. In the EMG-records, the intensity and duration of individual locomotor bursts were highly variable also during straight swimming, which presumably reflects variations in the activity of individual motor units between cycles. Stronger turns (> 60°) were correlated with an asymmetrical increase in burst intensity and duration on the ipsilateral side, and a slowing down of the rhythm. During weak turns no consistent changes in the EMG-pattern were seen. Eye illumination of quiescent lampreys, attached with their suckers to the bottom, evoked a strong yaw turn away from light, and locomotion. The initial turn was reflected in the EMG-recording, as a unilateral burst of high intensity and long duration. Burst intensity was positively correlated with the turning angle.

575.20

AN ASYMMETRICAL VISUAL INPUT CAN ABOLISH POSTURAL DISTURBANCES EVOKED BY UNILATERAL LABYRINTHECTOMY IN THE LAMPREY. T.G.Deliagina*. Nobel Institute for Neurophysiology, Department. of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden.

In all classes of vertebrates, removal of the vestibular organ (unilateral labyrinthectomy, UL) results in severe motor disorders. In the lamprey (a lower vertebrate) one of the most pronounced symptoms induced by UL is continuous rotation (rolling) around the longitudinal axis during swimming. The aim of the present study was to abolish this symptom by means of an artificially organized, asymmetrical visual input. This idea emerged from our finding that an asymmetrical visual input exerts a strong influence upon the vestibular-driven roll control system (Deliagina et al., Exp. Brain Res. 95, 421-428,1993). Swimming of the lampreys subjected to UL (control), and the effects produced by different types of asymmetrical visual inputs combined with UL were investigated. The animals of the first control group (UL only, n=11) rotated continuously during swimming, and this symptom lasted up to 15 weeks after surgery. In the animals of the second group (UL plus bilateral blinding, n=14) rotation during swimming was observed even longer, for 6-10 weeks. However, if a removal of the labyrinth was accompanied by a removal of the eye on the same side, the animals (n=17) swam normally, without rotation, immediately after surgery. In animals subjected to UL and bilateral blinding, which were rotating continuously, the rotation terminated immediately with onset of electrical stimulation (10 Hz) of the stump of the optic nerve ipsilateral to the intact labyrinth (n=5). In the lamprey deprived of the labyrinth and the eye on the same side, input from the remaining eye was able not only to functionally substitute the lost vestibular input, but also to induce adaptive changes in the roll control system: a removal of the remaining eye in 10-30 days after the first surgery did not destabilize the postural control, and the animal driven by only one labyrinth swam normally, without rotation.

575.21

MASTICATORY FUNCTION IN PATIENTS WITH CORTICAL BRAIN INFARCTION AND HEMIPLEGIA. [1]P. Kemppainen*, [1]A. Waltimo, [2]M. Kaste, [2]H. Palomäki and [3]O. Salonen. [1]Dept. of Prosthetic Dentistry, Univ. of Helsinki, [2]Dept. of Neurology and [3]Dept. of Radiology, Helsinki Univ. Central Hosp., Finland.

The masticatory function of sixteen patients (11 men and 5 women) with severe hemiparesis caused by brain infarction (A. cerebri media) was studied by means of interview, clinical examination and bite force measurements. The location of the infarction was assured with computer tomograghy and and magnetic resonance imaging to be localized in the facial primary motor cortex. The Rankin Disability Score and the Scandinavian Stroke Study Group Index showed that patients had an infarction causing marked invalidity, eg. facial paralysis and a marked loss of hand grip force on the contralateral side of the cortical lesion. However, the patients reported no change in their chewing ability, although four of them reported hoarning of food in the buccal sulcus area of the paralysed side. The clinical examination revealed no major signs of craniomandibular disorders, and masticatory muscles contracted symmetrically when examined by palpation. Bite force measurements also indicated symmetrical contraction, since we could not detect any difference in the maximal bite force between the healthy and paralysed side. These results are in agreement with recent laboratory fingings in monkeys supporting the importance of bilateral representation and cortical projections in the production and and coordination of orofacial functions.

576.1

MULTI-JOINT COORDINATION DURING THE WIPING REFLEX IN FROGS. M.G. Sirota, R. Dubuc and A.G. Feldman* CRSN, Université de Montréal H3C 3J7 and Dép. de kinanthropologie, Université du Québec à Montréal, Québec, Canada H3C 3P8.

Intact and spinal frogs produce non-rhythmical target-directed hindlimb movements in response to stimulation of the skin (the wiping reflex or WR). Analysis of the WR allows us to study the problems of redundancy in terms of degrees of freedom . We studied the redundancy problem by analyzing interjoint coordinations with kinematic techniques for the WR in response to rostral body surface stimulation in grass frogs (n = 9). These frogs were spinalized between the 1st and 2nd vertebrae under tricaine anesthesia. WRs were elicited by a series of 3-5 light pricks with a needle. Hindlimb movements were recorded in two dimensions using a video system (60 Hz) and light-reflecting markers (8 points on the hindlimb joints and body). Movement duration was effectively increased by cooling the preparation. Trajectories of the limb's endpoint and angle-angle diagrams were analyzed. Results show that there are phases of the WR in which a significant excursion in a single ("leading") joint is associated with relatively small excursions in the other joints. The leading joint can be different for different phases of the WR. However, there are phases in which joint angles change simultaneously. In the latter case, the coordination between joints was not fixed and might be changed in repeated movements. It is concluded that target-directed reaching can be accomplished by relatively independent movements in the joints despite motor task constraints.
Funded by a Group Grant from MRC Canada and FRSQ Québec.

576.2

ORGANIZATION OF CENTRAL CONTROL SIGNALS ASSOCIATED WITH MULTIPLE DEGREE OF FREEDOM ARM MOVEMENTS. L.E. Sergio* and D.J. Ostry. McGill University, Montreal, PQ, Canada H3A 1B1

To gain insight into the organization of central control signals associated with motion in one or more degrees of freedom, we examined the behaviour of bi-articular muscles during movements in which they acted as agonist in one degree of freedom and antagonist in another. As an example, the activity of biceps brachii, which acts to flex and supinate the forearm, was studied during a series of flexing pronations, where it acted as an agonist to the flexion movement and an antagonist to the pronation movement. We addressed the following question: Will bi-articular muscles display purely agonist or antagonist activity depending on factors such as movement amplitude or required torque, or will the muscle display both agonist and antagonist activity. Subjects performed flexions or extensions of a fixed amplitude while simultaneously performing supinations or pronations of increasingly larger amplitude. The activity of eight muscles about the elbow was recorded using surface electrodes while arm position was monitored using OPTOTRAK*. Results show that bi-articular muscles display an activity pattern which has both agonist and antagonist components. For example, during a small flexion combined with a large supination, pronator teres, which acts to flex and pronate the forearm, displays phasic activity concurrent with brachialis, a pure agonist. It also displays a burst of activity concurrent with pronator quadratus, a pure antagonist. This suggests that central commands for motion in individual degrees of freedom may be specified independently and superimposed.

576.3

EXAMINATIONS OF POSSIBLE EXPLANATIONS FOR TRAJECTORY CURVATURE IN MULTI-JOINT ARM MOVEMENTS. Osu, R.*[1,2], Uno, Y.[2], Kojke, Y.[2], & Kawato, M.[2] [1]Dept. of Psychol., Fac. of Letters, Kyoto Univ. [2]ATR Human Inf. Processing Res. Labs., Kyoto, Japan.

We discuss the coordinate frame in which visually guided human multi-joint arm movements are planned. In point-to-point planar reaching movements, hand paths tend to be gently curved. Models that rely on intrinsic-dynamic coordinates such as the minimum-torque-change model predict curved paths whereas models that rely on extrinsic-kinematic coordinates such as the minimum-jerk model predict straight paths. To decide between these two coordinate frames, we tested following three possible explanations for observed curvature in extrinsic-kinematic models (Wolpert, et al., 1993): (1) The planned trajectories are straight but imperfection of control causes the curvature. (2) Visual misperception causes the curvature. (3) Arm trajectories are controlled using straight virtual trajectories and the biomechanics of the arm causes the curvature. In Exp. 1 we examined the movements from the side of the body to the front of the body. We found that if subjects were explicitly instructed to generate straight paths for such movements, these movements were straighter than those generated spontaneously. This result argues against (1). In Exp. 2, subjects generated spontaneously curved trajectories even in the fronto-parallel plane where visual misperception was not expected to be a factor. This result argues against (2). In Exp. 3, EMG signals of 6 related muscles of the same movements as Exp. 1 suggest that subjects can make straighter paths without raising stiffness. This poses a problem for the portion of (3), namely, the virtual trajectory hypothesis that accounts for the performance of straighter trajectories using straight virtual trajectories by raising stiffness. Thus our results support the hypothesis that the CNS plans trajectories using intrinsic-dynamic coordinates.
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576.4

LEARNING AND TRAJECTORY PLANNING IN KINEMATIC ALTERATION OF JOINT ANGLES. H. Imamizu*, Y. Uno and M. Kawato ATR Human Information Processing Research Labs, Kyoto, Japan

We investigated whether trajectories of the human arm are planned solely in an extrinsic (kinematic) space or in both an intrinsic (dynamic) space and an extrinsic space. We virtually minified (1/2) the elbow angle and magnified (5/4) the shoulder angle of normal human subjects while they were aiming at targets. A position marker was attached to the subject's hand and its current altered position was displayed as a cursor on a CRT screen. This linear transformation in joint angles corresponds to nonlinear transformation between the hand plane and the screen, and makes a straight trajectory in the hand plane a curved one on the screen. If trajectories are planned in an intrinsic space then they are invariant in the intrinsic space (the hand plane) and distorted in the extrinsic space (the screen) under the kinematic alteration. On the other hand, if they are planned in the extrinsic space, they are invariant in the extrinsic space and distorted in the intrinsic space. The task for the subjects was to move the cursor to a target within a short time period (900 ms). The aiming error decreased with practice. We compared the trajectories after 320 trials of training to those under no alteration on the screen. There was significant difference between them. This suggests that the trajectories were distorted in the extrinsic space. The result supports the hypothesis that trajectories are planned in the intrinsic space. We also investigated intermanual transfer of the learning effect to obtain evidence of internal representation of intrinsic coordinates (joint angles) in the central nervous system.

576.5

The Three-dimensional Curvature of Unrestrained Straight-ahead Movements. F. E. Pollick*, G. Ishimura. ATR Human Inf. Processing Res. Labs, Kyoto, Japan.

We examined the three-dimensional curvature of unrestrained point-to-point hand movements in the forward direction. Subjects moved their hand from a position above the start point to a forward position above targets of different size and distance. Paths showed curvature resulting from an initial lateral and primarily downward movement that was compensated for in the second half of the movement. The downward component of motion was temporally coupled to the forward motion, with the maximum drop occurring at the time of peak forward velocity. The curvature was greatest for movements to the near target and decreased as target distance increased. Analysis of the relationship between velocity and radius of curvature showed that velocity was related to radius of curvature by a power law with an exponent of 0.59, different from the value of 1/3 typically obtained in curvilinear drawing motions. Aspects of the downward motion suggest that it was purposeful in speeding up the movement and offer explanation to the deviation from the typically reported exponent.

576.6

LEARNING AND GENERALIZATION OF LIMB DYNAMICS.
R.L. SAINBURG* & C. GHEZ Ctr. for Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have recently shown that impaired control over interaction torques in proprioceptive deafferentation can be dramatically reduced when, prior to movements performed without vision, patients are allowed to view their limb in motion. We therefore proposed that vision updated an internal model of limb dynamics used to program movement. We now ask whether intact subjects also use feedforward mechanisms to control interjoint dynamics and examine the learning and use of internal limb models.

Subjects were to trace linear hand-path templates presented on a computer screen with rapid, overlapping out-and-back movements. Their arms were supported in the horizontal plane by a low-inertia friction-free system. An outrigger to which weights could be attached enabled the distribution of mass of the forearm-hand segment to be varied. Subjects were trained in a given direction, and with a given inertial configuration, by providing them with visual feedback of the screen cursor. Accuracy was then tested during movements in other directions and with different inertial configurations.

After training, subjects made errors in initial direction, movement linearity and interjoint coordination. Simulations showed that these errors resulted from the production of torques appropriate to the inertial conditions during training. Such after-effects also occurred for movement directions that differed from the training direction, however, their magnitude was greatest in the trained direction. We conclude that the normal control of interjoint dynamics is dependent on feedforward mechanisms that employ internal models of limb mechanics that can be rapidly recalibrated. However, recalibration through training in a single direction allows only incomplete generalization to untrained directions.
(NS22715).

576.7

SPATIAL ORGANIZATION OF KINEMATIC AND DYNAMIC CHANGES DURING PREHENSION IN THE CAT. J.H.Martin*, S.Cooper, A.Hacking, C. Ghez. Ctr. Neurobiol & Behav, Columbia Univ. and NYS Psychiatric Institute, New York, NY 10032

The present study examines the spatial control of distal joint angles when interaction torques produced by the motion of proximal joints vary. Cats reached to grasp a piece of beef from the end of a narrow food-well at different inclinations and heights. The coordinates of markers at the shoulder, elbow, wrist, MCP and 4th digit were acquired at 100Hz for kinematic and dynamic analysis. Joint torques were partitioned into components reflecting inertia of segments distal to the joint, interjoint interactions, gravity and a residual term that includes active muscle contraction and passive visco-elastic resistance.

Paw paths comprised two linear segments in which the paw was aimed first to a via point in front of the food-well (lift), and then, within the well, to the food (thrust). During lift, kinematic changes, including wrist speed and angular motions of wrist and elbow, were scaled with reach height. During thrust, distal kinematics were independent of target height but varied with target inclination.

Initially during lift, passive interaction torques flexed the wrist and digits; later, as the via point was approached, they extended them. Although the late extensor torque scaled with height, and thereby could have extended the wrist and digits more at higher targets, the animals compensated by varying residual (muscle) torque and muscle activation so distal angles stayed independent of reach height.

Our findings indicate that torques and muscle patterns at distal joints are adapted to both the interactions produced by motions of proximal joints and to the spatial demands of the task. Cats apparently do not adjust proximal kinematics to vary interaction torques acting on distal joints, for example, to reduce the contraction required of distal muscles. (NS31391)

576.8

ADAPTATION TO DISPLAY ROTATIONS AND ALTERED GAINS IN PLANAR REACHING MOVEMENTS. Z.M. Pine*, J. Gordon, and C. Ghez. Dept. Rehab. Med., Prog. Phys. Ther., Ctr. Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have previously shown that direction and extent of hand movement represent independently planned features of reaching movements. The purpose of this study was to compare the time courses of adaptation to experimentally imposed rotations and gain alterations in the virtual workspace. Human subjects made aimed movements on a horizontal digitizing tablet. However, vision of the hand and arm was blocked, and virtual targets and hand positions were displayed on a computer screen. We separately applied either a rotation or a change in gain to the screen display of hand path while leaving the virtual targets unchanged. The hand display was blanked during movement, but the path taken by the virtual hand was displayed following each movement as knowledge of results.

Adaptation to both types of distortion showed evidence of two distinct processes: a relatively rapid reduction in mean error over trials, and a more gradual reduction in variability of movement error. Adaptation to rotational distortions, and re-adaptation to control conditions after rotations, was generally slower than for gain changes. Increased variability was also more pronounced and prolonged during adaptation to rotational distortions.

Differences between directional and extent adaptation support previous findings of independent specification of direction and extent. The learned visuomotor transformation between target and hand direction is generally resistant to short-term adaptation, perhaps because this involves altered specification of which muscles to contract. The target-extent relation is more rapidly recalibrated, perhaps because it involves shifting a scaling factor. (Supported by HD 01018, NS 22715)

576.9

DIRECTIONAL BIASES IN ARM MOVEMENTS REFLECT BIASED ESTIMATES OF INITIAL HAND POSITION. M.F. GHILARDI*, J.E. GORDON, C.P. GHEZ INB, CNR, H.S.Raffaele, Milano; Ctr. Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have previously reported that when subjects reach to visual targets without seeing their limb, movements show directional biases that vary with the initial position of the hand. The spatial organization of these biases suggest that in planning movement direction the nervous system underestimates the distance of the hand from a bias-free position between midline and shoulder. Since this is the habitual workspace of the hand, we hypothesized that these errors reflect this prior experience. We now asked: (1) Can directional bias in a specific location be eliminated by training with visual feedback? (2) Does such training alter the biases in other untrained areas of the workspace?

Subjects moved their hand on a digitizing tablet from different central points to 12 radial targets displayed on a computer screen. Subjects were trained to perform accurate movements from initial positions normally associated with clockwise or counterclockwise biases by providing visual feedback of cursor and hand during movement. They were then tested without feedback for movements initiated from the same and other points. Training significantly reduced or abolished directional biases for movements initiated in the region of training. Movements initiated from other locations, including the previous error-free region, showed new biases that again represented underestimates of the distance of the initial hand position, but from the new trained location. The present findings are compatible with the hypothesis that training in a particular region of workspace resets subjects' default estimate of their initial hand position. Kinematic planning is highly dependent on learned representations of the hand in the workspace. (NS 22715)

576.10

PRACTICE AND MOTOR LEARNING. R. Barth-Neander, J. Hynes & A.M. Gentile*. Teachers College, Columbia Univ, NY 10027.

Learning a novel movement pattern requires developing a new dynamic model of limbs and objects ("getting into the ball park" Greene, *Prog Theoret Biol,* 1972). It is proposed that initial learning is enhanced under consistent practice conditions which permit stabilization of the dynamic model. Practice under variable conditions of high similarity (requiring scaling) precludes stabilizing the dynamic model and disrupts learning. These hypotheses were tested with similar tasks (SIM): using chopsticks to pick up and transport small, medium or large buttons to a container; or dissimilar tasks (DIS): chopsticks/medium button, card sorting, mirror tracing. Adult subjects (N=36), naive in chopstick use, were assigned to one of 4 practice conditions: Blocked/SIM (15 successive trials on one task before switching to another), Random/SIM (15 trials on each of 3 tasks randomly ordered), Blocked/DIS, Random/DIS. Movement time was assessed on 15 acquisition trials (medium button), immediate and delayed retention tests and 2 transfer tests (new button sizes). Findings confirmed predictions: Blocked/SIM yielded better learning than Random/SIM practice. DIS conditions did not differ in retention but tended to be better than Random/SIM. These results contradict reports (Magill & Hall, *QJEP,* 1990) favoring random practice over blocked ("contextual interference effect").

576.11

THE RELATIVE CONTRIBUTIONS OF GEOMETRIC VERSUS BEHAVIORAL CONSTRAINTS IN RESOLVING JOINT-LEVEL MOTOR EQUIVALENCE. Rebecca A. States* & Charles E. Wright. Psychology Dept., Columbia Univ., 406 Schermerhorn Hall, New York, NY 10027.

Motor equivalence exists when a task may be performed using various movement patterns. For example, in a pointing task, all arm configurations that suffice to position the hand at a desired location constitute a "motor equivalence set." Motor equivalence provides flexibility, but also complicates motor planning. Whenever a movement is performed, subjects must select a single arm configuration from those available in the motor equivalence set.

Previous work has shown that, at the end-point of aimed, arm movements, much of the indeterminacy associated with joint-level motor equivalence can be accounted for (Soechting and Flanders, 1989; States & Wright, 1993). Two factors may contribute to the predictability of final arm configurations: geometric constraints imposed by a fixed linkage system, and behavioral strategies. We evaluate their relative contributions, by investigating aimed, arm movements performed with two excess degrees of freedom.

Arm configurations are measured while five subjects make repeated pointing movements from 30 starting points to 1 target. Five targets are tested on separate days. Motion is permitted in a horizontal plane, using four degrees of rotational freedom: one each at the right wrist, elbow and shoulder, and one at the torso.

We compare the variability in arm configurations sampled at the end-point of movements to a single target with the variability that would exist if all configurations within the motor equivalence set were sampled. Results show that behavioral strategies make a substantial contribution for all subjects, even though individuals' strategies differ dramatically. Effects of particular strategies are explored through simulation modelling.

Results suggest new techniques for evaluating models of joint-level motor equivalence (Rosenbaum et al., in press, Bullock & Grossberg, 1992; Bruwer & Cruse, 1990) by testing the extent to which they replicate the partitioning of variance between geometric and behavioral strategies observed here.

576.12

EFFECTS OF SOME CONSTRAINTS FOR SINGLE-JOINT MOVEMENTS. Di-An Hong°, G. L. Almeida°+, D. M. Corcos++, G. L. Gottlieb*°.
*°Rush Medical Center, Chicago, IL 60612, ++Universidade Estadual de Campinas, São Paulo, Brazil, and University of Illinois at Chicago, Chicago, IL, 60608 .

Four healthy subjects performed mechanically constrained single-joint elbow-flexion movements, in a transverse plane, and unconstrained single-joint elbow and shoulder flexion movements, in a sagittal plane.

In the unconstrained paradigm, the movements of the elbow were accompanied by varying degrees of movement at the shoulder and movements of the shoulder were accompanied by varying degrees of movement at the elbow. The pattern of muscle activity was the same for mechanically unconstrained single-joint elbow movements. The duration of the agonist EMG bursts scaled with distance while their rate of rise remained constant. The antagonist muscles were activated earlier for shorter distances This uniform initial rising phase was also observed for torque.

At the shoulder-task, the biceps long head and the anterior deltoid displayed strong coupling in terms of the muscle activity burst. At the elbow-task, for the unconstrained movement, the anterior deltoid also scaled with distance but it was less coupled with the biceps long head than in the shoulder-task. These findings are interpreted in terms of rules that control the activation of different muscles to suggest the forces sufficient and necessary to perform the movement tasks.

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576.13

UNCONSTRAINED SINGLE-JOINT MOVEMENTS IN INDIVIDUALS WITH DOWN SYNDROME. G. L. Almeida+,++, Di-An Hong+, D. M. Corcos*, G. L. Gottlieb+. +Rush Medical Center, Chicago, IL 60612, *University of Illinois at Chicago, Chicago, IL, 60608 and ++Universidade Estadual de Campinas, SP, Brazil.

Three individuals with Down syndrome performed unconstrained single-joint elbow and shoulder flexion movements, in a sagittal plane. First, the subjects were instructed to move one joint (the "focal joint") "as fast as possible", while keeping the "non-focal" joint stationary. These movements were performed over three different distances. Second, the subjects were instructed to move a "focal joint" at three different speeds, over one distance.

The movements of the focal joint were accompanied by varying degrees of movements at the non-focal joint. On average, the movements at the non-focal joint were twice as large as those observed in neurologically normal individuals, and they displayed slower movement speed at the focal joint. For the movements performed over three distance, the pattern of muscle activity was typical for "speed insensitive strategy". The duration of the agonist EMG bursts scaled with distance while their rate of rise remained constant. Nevertheless, there was more variability related to the antagonist latency. One subject presented a pattern of co-contraction, whereas for the others, the antagonist latency did not scale with distance.

For the movements performed at three different speeds, the pattern of muscle activity was typical of "speed sensitive strategy". The rate of rise of the agonist EMG burst scaled with distance with the antagonist latency being activated early for fast movements.

These findings suggest that, in comparison with neurologically normal individuals, individuals with Down syndrome use similar rules to control the muscle activation. Nevertheless, they seem to have a coordination problem which is reflected in their poor coupling of the muscle activity burst and in the large range of the movements in the "non-focal joint".

This work was supported by NIH grants NS 01508, AR 33189.

576.14

ADAPTIVE BEHAVIOR OF THE MONKEY MOTOR SYSTEM TO VIRTUAL ENVIRONMENTS. F. Gandolfo, R. Shadmehr, B. Benda and E. Bizzi*. Dept. Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

It has been shown (Shadmehr and Mussa-Ivaldi, J. Neurosci., in press) that humans can adapt to virtual dynamic environments. We are interested in finding the neural mechanisms underlying this phenomenon. As a first step, before beginning the recording of single cells from cortical areas, it is necessary to assess how well the data observed in human subjects apply to macaque monkeys. Psychophysical tests were performed in an awake behaving monkey using the following paradigm: The monkey was trained to move a manipulandum to a set of targets sequentially appearing on a computer screen and hold the cursor in the target for at least one second in order to get a reward. In the baseline condition, the manipulandum behaves like a damper in order to help the monkey move smoothly. In the perturbation condition, non-homogeneous viscous field is superimposed on the baseline field. In the latter condition, a dramatic alteration of the trajectory was observed initially, followed by a gradual return to the baseline trajectory. This is consistent with the hypothesis of an underlying kinematic planner cascaded with an execution module cancelling out the dynamics of the environment by learning a model of the disturbing field. We ruled out the possibility of co-contraction, which would be an alternative explanation of the data, by looking at the after effects obtained by restoring the baseline condition. The after-effect appeared as a disturtion in the path opposite to that induced by the field. Such after-effects decay repidly when the monkey is no longer exposed to the field. Acknowledgements: NIH grants NS09343 and AR26710, and ONR grant N00014/K/0372. FG was supported by a fellowship by SISSA.

576.15

ANALYSIS OF KINEMATIC AND DYNAMIC PARAMETERS OF HUMAN MOTOR LEARNING. S.J. Shanbhag* and T.J. Ebner. Departments of Neurosurgery and Physiology, and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

In order to more fully understand the characteristics of human motor learning, human subjects were evaluated using a step-movement task. Subjects were seated in front of a video display and controlled the movement of a cursor on the display with the right hand using a planar 2-joint manipulandum. A sling supporting the elbow constrained movement to the horizontal plane to permit explicit determination of shoulder and elbow joint angles and torques. The task consisted of maintaining the cursor in a central "start box" until one of eight equidistant "target" boxes appeared; the cursor then had to be moved to and held in the target box within a movement time window. After practicing on a normal hand-cursor movement relationship, the gain was changed, forcing the subjects to learn a new relationship between hand and cursor movement. Once the subjects' performance had returned to an acceptable level, the gain was restored to the initial conditions. Initially, subjects' hand paths were nominally straight with characteristic bell-shaped hand velocity profiles and biphasic accelerations. Biphasic ("accelerating" and "decelerating") torque profiles varied predictably with direction during the normal gain condition. Upon initial presentation with the new gain, movement errors increased, with larger errors in directions involving larger shoulder torques, and smaller errors in directions involving larger elbow torques. Compared to other targets, learning in these directions appeared to be more difficult. The "accelerating" phases of the joint torques followed a stereotyped profile, while the "braking" phases of the joint torques were prolonged in the early stages of learning, regaining their characteristic form as net performance improved. The correlation of the shoulder/elbow torques with the spatial dependence of the errors suggests that the control scheme for reorganization of two-joint movements is more complex than that for single-joint movements.

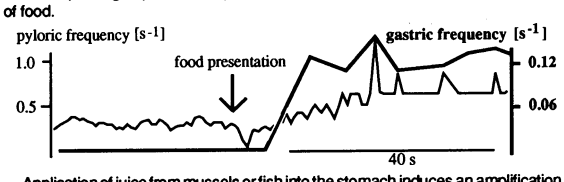
Supported by NIH Grants NS 18338 and NS 31530.

CIRCUITRY AND PATTERN GENERATION III

577.1

FUNCTION OF STOMATOGASTRIC NETWORKS IN INTACT CRAYFISH. H.-G. Heinzel*, H. Böhm, P. Eitner, D. Hellbusch and E. Messaï. Inst. of Zool., Uni. Bonn, Poppelsdorfer Schloß, D-53115 Bonn, Germany.

Rhythmic-motor patterns of the dorsal ventricular nerve of the stomatogastric ganglion were monitored with chronically implanted electrodes in intact crayfish, *Orconectes limosus*, while the activity of the gastric mill and the cardio-pyloric valve was monitored by endoscopic imaging. Feeding experiments of free-moving crayfish demonstrate a decrease in the pyloric activity during presentation and grabbing of food which is followed by a strong increase in the pyloric frequency. After food intake the gastric activity starts vigorously and stays on a high frequency level for long duration (see Figure). The activity level increases further depending on the amount of food.



Application of juice from mussels or fish into the stomach induces an amplification of movements of the gastric mill as well as a modification in the opening and closing of the cardio-pyloric valve. Gastric movements often show pyloric modulations which reflect complex gastro-pyloric patterns probably due to changes in the consistency of food. The investigations on the function of the stomatogastric system and neuroanatomical studies of the networks indicate the importance of senso-motoric mechanisms of integration which are involved in initiation and feedback-control of the stomach's rhythmic motor-patterns. Supported by HFSP.

577.2

INITIATION AND MODULATION OF GASTRIC MILL ACTIVITY BY MECHANOSENSORY ORGANS IN THE LOBSTER.

M. E. T. Boyle*, A. M. Schweins, and A. I. Selverston Dept. of Biology, University of California at San Diego, La Jolla, CA 92093-0357.

The lobster gastric mill (GM) consists of three teeth in the stomach, two lateral and one medial, which masticate food and are under the control of the stomatogastric ganglion (STG). The posterior stomach receptor organs (PSRs) are mechanoreceptors which monitor medial tooth activity. There are two clusters of 15–20 PSRs located in the ventral branch of the posterior stomach nerve (v-psn). By injecting neurobiotin intracellularly into the PSRS we show that the PSRs are dye-coupled to a subset of neurons in the STG. We have been able to trace PSR processes leading into the main STG output nerve, the lateral ventricular nerve (lvn). Our aim is to find out how sensory input from the PSRs affects the gastric motor output pattern produced by the STG. In both the intact and semi-intact animal, we record from STG neurons and the v-psn while initiating gastric mill activity in response to pumping sea water into the stomach. We use an endoscope to observe, quantify, and compare GM movement patterns produced by sensory stimulation in the intact and deafferented animal. In five preparations the d-psns were bilaterally cut while monitoring the sensory induced GM activity. Minutes after the d-psns were cut, GM activity was substantially lower. These preliminary results suggest that the PSRs may mediate the sensory induced chewing.

Supported by ONR N00014-91J-1720 and NIH RO1-09322.

577.3

NOVEL DEPOLARIZING GLUTAMATERGIC CONDUCTANCE EXPRESSED IN CRUSTACEAN STOMATOGASTRIC NEURONS IN PRIMARY CULTURE.
T.A. Cleland* and A.I. Selverston, Dept. of Biology 0357, Univ. Calif. San Diego, La Jolla, CA 92093-0357.

The stomatogastric ganglion (STG) of the Pacific spiny lobster, *Panulirus interruptus*, is one of the most well-defined central pattern generators studied at the intercellular and circuit level. In this study, STG neurons were individually isolated in primary culture in order to investigate cellular membrane properties under improved space-clamp conditions and without the complicating effects of other networked cells. Glutamate-induced currents were studied using two-electrode voltage clamp in these cultured neurons. The dominant glutamatergic response in most cells examined was a moderately calcium-dependent depolarizing current mediated by a conductance increase and reversing at -30 ± 15 mV. This stands in sharp contrast to all previously described glutamate responses within the intact ganglion, which are all chloride or potassium-mediated ipsps. Biophysical and pharmacological characterizations of this glutamatergic conductance are presented.

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577.4

MODES OF OSCILLATION AND SETTING RELATIVE PHASE IN SMALL MOTOR PATTERN GENERATING NETWORKS.
P.F.Rowat* and A.I.Selverston Biology Department, U.C. San Diego, La Jolla, CA 92093–0357

We have constructed a simplifying, biologically constrained, network model of the gastric mill CPG which shows that only a few basic mechanisms are sufficient to produce the relatively complex patterns characteristic of the cycling gastric mill. These mechanisms include a cell model having a fast current with an N-shaped I-V curve and slow inward and outward currents with linear steady-state I-V curves; graded synaptic transmission; and "slow" synapses. The cell model captures important characteristic behaviors of gastric mill cells such as plateau potentials, post-inhibitory rebound, and endogenous osillations. The reciprocal inhibitory pair is an essential sub-network of the gastric mill network, which enables the model network to produce a pattern whether or not the individual cells are endogenous oscillators.

An isolated cell has six distinct behaviors: endogenous oscillations; silence; almost an oscillator; plateau potentials; tonically firing; hyperpolarized. Using pairs of two-dimensional phase-portraits, we have studied in detail the modes of oscillation of two cells connected with reciprocal inhibitory synapses, in which the center of the synaptic transfer function, or "threshold", was allowed to be different for the two synapses. Every combination of behaviors in the individual cells can give rise to oscillations, provided the thresholds and weights are correctly adjusted. In the symmetric cases, the cells are exactly out of phase, but when assymetries are allowed many other phase relationships are obtained. In addition, one can get 1:N (N=1,2,3) or even M:N phase locking, and chaos from the reciprocal inhibitory pair.

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577.5

PHYSIOLOGICAL AND MOLECULAR HETEROGENEITY OF PY MOTONEURONS OF THE PYLORIC NETWORK.
R.M. Levini, D.I. Baro, S.A. Hughes and R.M. Harris-Warrick*. Dept. of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The 8 PY motoneurons in the stomatogastric ganglion of the spiny lobster *Panulirus interruptus* are integral members of the pyloric central pattern generator and innervate 13 intrinsic constrictor muscles on the pylorus. Electrophysiological differences between cells of this class have been acknowledged for many years (Hartline, et al., 1987). Consistent with previous studies we found two distinct physiological phenotypes which can be quickly identified. PY1 cells do not respond to stimulation of the hepatopancreatic duct nerve *(hdn)* at 20 Hz, respond strongly to puffer (10^{-3}M) or bath (10^{-4}M) applied dopamine (DA), are strongly electrically coupled to LP cells (-5 nA into PY cell), are strongly active during pyloric cycling, and can overlap the LP firing phase in a combined preparation. PY2 cells do respond to *hdn* stimulation, respond weakly or not at all to DA application, are not detectibly electrically coupled to LP (-5 nA into PY), are often silent during the ongoing pyloric rhythm and never overlap LP activity in a combined preparation. Four superficial muscles (p2,8,10,12) were used to test for differences in innervation patterns between the PY subtypes. The results showed no segregation of innervation on the muscles by either subtype: each muscle is innervated by at least one neuron of each type. We are currently looking at PY heterogeneity at the level of expression of mRNA for the *shaker* K^+ channel family using *in situ* hybridization. Supported by NIH NS17323 and 5PO1 NS25915.

577.6

EFFECT OF DOPAMINE ON THE TRANSIENT POTASSIUM CURRENT AND THE HYPERPOLARIZATION-ACTIVATED CURRENT IN PY AND LP NEURONS OF THE LOBSTER STOMATOGASTRIC GANGLION.
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The stomatogastric ganglion of the lobster, contains a network of neurons, called the pyloric network, which controls rhythmic movements of the pylorus, part of the lobster foregut. The pyloric motor pattern is altered by the endogenous neuromodulator dopamine. The effect of dopamine on the motor pattern is due to both effects on synaptic connections between neurons of the pyloric network and direct effects on the neurons themselves. We show that in two pyloric cell types, PY cells and LP cells, dopamine specifically affects the transient potassium current, Ia. In the PY cell type, dopamine decreases Ia by reducing its maximum conductance, shifting its voltage of half-activation in the depolarized direction, and increasing its rate of inactivation. In the LP cell, dopamine also decreases Ia by reducing its maximum conductance and shifting its voltage of half-activation in the depolarized direction. In the LP cell, however, dopamine has no effect in the rate of inactivation of Ia. In addition, in the LP cell dopamine affects a second current, the hyperpolarization activated current Ih. Dopamine effectively increases Ih by shifting its voltage-dependence of activation in the depolarizing direction and increasing its rate of activation. Understanding the specific effects of dopamine on these neurons is important in understanding how alterations in particular neuronal properties can underlie the alteration of a motor pattern.
Supported by NIH grant NS17323.

577.7

DISTRIBUTED AMINE MODULATION OF GRADED CHEMICAL SYNAPTIC INTERACTIONS BETWEEN NEURONS OF THE PYLORIC MOTOR NETWORK.
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Dopamine (DA), serotonin (5HT) and octopamine (Oct) are endogenous neuromodulators in Crustacea, and each evokes a distinct motor pattern from the quiescent pyloric network in the stomatogastric ganglion of the spiny lobster. We examined amine effects on graded chemical interactions between synaptically isolated pairs of neurons in the pyloric network to determine if these could contribute to the amine-generated motor patterns. Each amine had a unique and distributed spectrum of effects on graded chemical synaptic strength across the pyloric synapses. DA (10^{-4}M) enhanced some pairs of synaptic interactions and weakened others. 5HT (10^{-5}M) also enhanced and weakened different pairs of synaptic interactions but to a lesser degree than DA. Oct (10^{-5}M) strengthened some synaptic interactions and had no effect on others. At some previously described pyloric chemical synapses (AB-IC and PY-IC, for example), graded chemical transmission could not be detected in the absence of modulatory inputs in the control TTX-saline (20°C). In these cases, amine application (especially DA) created functional synaptic interactions. This suggests that neuromodulators are required for the functional expression of some chemical synapses within the pyloric network. This completes our survey of amine effects on all 18 of the graded chemical and electrical synaptic interactions within the pyloric network. Supported by NIH grant NS 17323 and the Human Frontier Science Program.

577.8

FUNCTIONAL ROLE OF ELECTRICAL COUPLING IN CONTROLLING THE LOCAL ACTIVITY OF A MODULATORY PROJECTION NEURON.
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Intra-axonal recordings at the entrance to the stomatogastric ganglion (STG) of the crab *Cancer borealis* show that the STG terminals of modulatory commissural neuron 1 (MCN1) excites the pyloric and gastric mill rhythms in the STG, receives IPSPs from the lateral gastric (LG) neuron and is electrically-coupled to LG (Nusbaum et al., J Neurosci 12:2706). The LG inhibition of MCN1 controls the gastric mill rhythm-timed activity of MCN1 in the STG (Coleman & Nusbaum, J Neurosci, in press).

Despite the synaptic inhibition from LG to MCN1, LG can also influence MCN1 via the electrical coupling between these two neurons. For example, depolarizing LG to levels subthreshold for transmitter release activates MCN1. Slightly stronger LG depolarization first activates and then inhibits MCN1. When the LG-mediated inhibition is eliminated by bath-applying picrotoxin (10^{-5} M), LG stimulation activates MCN1 enough to enable MCN1 to excite its STG targets.

During MCN1-elicited gastric mill rhythms, the MCN1 hyperpolarization is largest at the start of each LG burst. This response in MCN1 then exhibits a depolarizing sag, despite little decrease in the LG firing frequency or its underlying membrane potential. This enables MCN1 to escape from inhibition before the end of the LG burst. We are studying whether the electrical coupling between LG and MCN1 contributes to this escape from inhibition by MCN1.

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577.9

MUSCARINIC MODULATION OF EXCITABILITY AND SYNAPTIC OUTPUT IN A GASTRIC PATTERN-GENERATING NEURON OF THE LOBSTER STOMATOGASTRIC GANGLION. R.C.Elson* and A.I.Selverston
Department of Biology, UCSD, La Jolla CA 92093.

In the isolated stomatogastric ganglion of spiny lobsters, the muscarinic agonist, pilocarpine, activates rhythm generation in the gastric mill CPG. The lateral gastric neuron, LG, an important pattern-generating cell, goes from quiescence to spontaneous spiking and then to firing strong bursts underlain by plateau potentials.

Even before the onset of spontaneous activity, however, one can detect a large increase in the strength of LG's inhibitory outputs to other gastric neurons. IPSPs evoked by ortho- and antidromic spikes grow larger, the input-output function for graded synaptic transmission gets steeper, and the threshold for transmitter release is seemingly reduced. We used electrotonic coupling potentials to monitor neuropilar input resistance during synaptic potentiation, and found a large increase in synaptic conductance. The resting input resistance of LG could also increase, but is not clear whether this can account for all the observed potentiation.

Concurrently, pilocarpine decreases LG's spike threshold and increases the slope of the spike frequency - voltage plot. Together with potentiated synaptic output, these changes enhance the role of spike-mediated transmission at low presynaptic depolarizations. Also induced is a slow, seemingly active, depolarization, which may be the plateau current, and which is a good driver of graded transmission.

A possible interpretation of these results is that muscarinic modulation leads to enhanced activation of calcium currents in LG's distal neurites, allowing greater transmitter release. Present experiments seek to identify which conductances are modulated in LG and to clarify how these changes relate to modifications of excitability and synaptic efficacy.

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577.10

RECIPROCALLY INHIBITORY NEURAL NETWORKS: EFFECTS OF VARIATIONS IN INTRINSIC PARAMETERS. A.A. Sharp*, F.K. Skinner, and E. Marder. Ctr. for Complex Systems and Dept. of Biology, Brandeis University, Waltham MA 02254-9110.

We characterize how modulation of an intrinsic conductance (I_H) alters the responses of a half-center oscillator formed by reciprocally inhibitory connections. We use a combined theoretical/experimental approach employing conventional model neurons and experiments using the dynamic clamp (Sharp et al., J.Neurophys., 69:992,1993). A plot of network period vs. synaptic threshold produces an inverted U-shaped relationship (see accompanying abstract). As the maximal I_H conductance is increased or the I_H activation curve is moved in a depolarizing direction, the period vs. synaptic threshold relationship is shifted in the depolarizing direction. This indicates that these changes cause a frequency increase if a synaptic escape mechanism is being used, and a frequency decrease if a synaptic release mechanism is in operation. For a small range of synaptic threshold values, it is also possible for the system to switch between synaptic escape and release modes for these changes. If the maximal conductance is made too small or the activation curve is shifted too far in the hyperpolarizing direction, oscillations cease. This indicates that there is a critical relationship between synaptic and intrinsic properties in order to generate a robust half-center oscillator. This interactive approach allows for a detailed understanding of these relationships. Supported by NIMH MH46742 and NSF BNS9009251.

577.11

RECIPROCALLY INHIBITORY NEURAL NETWORKS: EFFECTS OF VARIATIONS IN SYNAPTIC PARAMETERS. F.K. Skinner*, A.A. Sharp, and E. Marder. Ctr. for Complex Systems and Dept. of Biology, Brandeis University, Waltham MA 02254-9110.

Reciprocally inhibitory circuits are ubiquitous components of oscillatory neural systems. We characterize how modulation of synaptic properties alter the responses of such a two cell system (half-center oscillator) using a combined theoretical/experimental approach. We employ two computational models: (i) a simple model consisting of I_H, leak, and synaptic currents and (ii) a compartmental model which includes HH-like spikes. Experimentally, we take advantage of the dynamic clamp technique (Sharp et al., J.Neurophys., 69:992,1993) to couple pairs of gastric mill neurons in the stomatogastric ganglion, and to introduce artificial I_H. As the synaptic threshold is increased, the frequency first decreases and then increases. This frequency transition occurs as the system switches from a synaptic escape mechanism to a synaptic release mechanism (Skinner et al., J.Computational Neurosc., in press). As the synaptic time constant is increased, the network frequency and oscillator amplitude both decrease. Increasing the maximal synaptic conductance causes a decrease in frequency and an increase in oscillator amplitude. For large synaptic conductances, oscillations can not be initiated for values of the synaptic threshold which fall in the transition between synaptic escape and release mechanisms. This interactive approach allows for a detailed understanding of how interactions between synaptic and intrinsic parameters generate network behaviour. Supported by NIMH MH46742 and NSF BNS9009251.

577.12

EFFECTS OF INFERIOR VENTRICULAR NERVE STIMULATION ON GASTRIC MILL AND PYLORIC CENTRAL PATTERN GENERATORS IN SPINY LOBSTER. M.S. Beauchamp* and A.I. Selverston. UCSD, La Jolla, CA 92093-0357.

The inferior ventricular nerve (IVN) links the brain to the stomatogastric ganglion (STG) in the spiny lobster, *Panulirus interrupus*. The STG is a network of neurons comprising two central pattern generators (CPGs) which control the lobster's digestive behaviors, and a role for the IVN in coordinating swallowing movements has been proposed (Sigvardt & Mulloney *J. Exp. Biol.* 97:153-168.) The effects of IVN stimulation were studied under a muscarinic agonist, pilocarpine, which generates stable, reproducible rhythms in the gastric mill and pyloric CPGs. Spontaneous IVN bursts have a long period and consist of three sequential phases: low frequency spike discharge, high frequency, intermediate frequency. Identified STG neurons were recorded intracellularly during stimulation with artificially generated bursts, whose temporal pattern closely matched that of spontaneous bursts. To better understand the mechanisms of IVN action, post-synaptic potentials (PSPs) and rhythm-altering effects of simple spike trains of various lengths and frequencies were also examined.

IVN stimulation has a powerful influence on the CPGs, interrupting ongoing gastric and pyloric activity. In the gastric mill network, LPG neurons are active during the first phase of the burst, then become inhibited by high frequency IVN firing, then fire a rebound burst. This corresponds to the observed excitatory response in LPG to a long low frequency spike train and the observed inhibitory response to a short high frequency spike train. Other identified gastric and pyloric neurons also have complex responses to IVN bursts that are well predicted by their responses to simple spike trains. Consistent with these results, low frequency IVN stimulation gives small EPSPs in LPG, small IPSPs in GM and large EPSPs in VD, PD and CD2. Both short and long-lasting alterations in rhythmic activity occur following stimulation. Gastric rhythm phase shifting, in which GM and AM fire synchronously, occurs in the first few cycles following stimulation. Pronounced pyloric rhythm alterations, consisting of several-fold increases in period and burst length, take tens of seconds to return to pre-stimulation values, suggesting that the IVN may also release a neuromodulator. Supported by HHMI fellowship to MSB and NIH 09322 to AIS.

577.13

GROSS MORPHOLOGY OF LOBSTER PYLORIC NEURON TYPES. J.B. Thuma* and S.L. Hooper. Dept. Bio. Sci., Ohio U., Athens, OH 45701

The pyloric network neuron types are distinctly different on the basis of muscle innervation, electrophysiological activity, and synaptic connectivity in the network. We examined whether the individual neuron types also have different morphologies by injecting neurons with the fluorescent dyes Lucifer yellow or rhodamine dextran and reconstructing the neurons with a Eutectic three dimensional neuron tracing system.

In agreement with the results of King (1976) and Selverston and Mulloney (1973), the main neurite of the Ventricular Dilator (VD) (n=4) neuron splits in the neuropil into two processes, each of which continues to form one of the neuron's axons. In addition, we have observed that the VD neuron consistently also has a process that leaves the main neurite at or well before the axon bifurcation and that branches extensively within the neuropil. Detailed analysis of the neuropilar arrays of the two axonal processes reveals they occupy non-overlapping regions of the neuropil.

The pyloric network has two Pyloric Dilator (PD) neurons that seem to be identical electrophysiologically. However, these neurons appear to have two distinct morphologies. In one class (n=4), the main process loops over itself as it courses through the neuropil. The first process of these looped PD neurons is always relatively close to the soma (av. distance ± s.d., 36±26μm). The other class of PD neurons (n=7) do not loop and have more distant initial branches (av. distance from soma, 133±41μm). In four double PD neuron fills, one of each class was present in the ganglion. In a fifth very poor double fill, it appeared that two unlooped PD neurons were present.

These results suggest that at least some the pyloric neuron types have distinct morphologies. Perhaps more interestingly, the distinct volumes occupied by the VD neuron neuropilar arrays could provide a basis for anatomical segregation of the stomatogastric neuropil.

577.14

DORSAL DILATOR MUSCLES IN *PANULIRUS* MAY EXPRESS BOTH PYLORIC AND GASTRIC MILL MOTOR PATTERNS. L.G. Morris* and S.L. Hooper. Dept. of Biological Sci., Ohio Univ., Athens, OH 45701

The two pairs of dorsal dilator muscles (cpv1a and b) are innervated by the Pyloric Dilator (PD) neurons and have been assumed to express a pyloric motor pattern. We examined the isotonic contractions of these muscles in *in vitro* preparations maintained in oxygenated saline with glucose. In 3 of 5 preparations, we observed no or only very small (<0.1mm) pyloric timed contractions. In the other preparations, the muscles produced large (~1mm), long duration (~4s) contractions every 6 to 8 sec., very different from the PD neuron burst length (~250ms) and cycle period (~1s) present in these preparations. In 10^{-6}M dopamine (DA), all preparations showed large (up to 2mm) contractions, with long durations and periods. Large (>0.1mm) pyloric timed activity was never observed in either saline or DA.

In two experiments, we observed that the large contractions were phase locked with Gastric Mill (GM) neuron activity, and that contraction duration covaried with GM neuron burst length. In one experiment we stimulated the anterior lateral (aln) nerve, which contains GM neuron axons; this elicited, at constant short latency, contractions whose duration covaried with the stimulation parameters. Strikingly, the relaxation time of the large contractions was much longer than the pyloric period, suggesting that even if pyloric activity could induce large contractions, the muscles would not fully relax within a pyloric period. This idea was tested by applying DA to augment contraction amplitude and stimulating the lateral ventricular (lvn) nerve or aln using parameters (40Hz for 250ms every 1s) mimicking pyloric neuron bursts. Under these conditions the contractions summated, resulting in a sustained contraction.

These results suggest that the dorsal dilator muscles, at least under some conditions, 1) may respond to pyloric input with sustained contraction and 2) can express gastric mill timed and duration motor output.

577.15

CONTROL OF FOLLOWER NEURON PHASE IN THE PYLORIC NETWORK. S.L. Hooper*. Dept. of Bio. Sci., Ohio U., Athens, OH 45701

The rhythmic pattern produced by the lobster pyloric network maintains relatively constant phasing as cycle frequency is altered by current injection into the pyloric pacemaker. Perfect phase maintenance requires appropriate changes in both pacemaker burst duration and firing delay of follower neurons after inhibition. Previously I showed that when trains of hyperpolarizing current pulses are injected into isolated pyloric neurons, firing delay of Inferior Cardiac (IC) and Lateral Pyloric (LP) neurons primarily depends on pulse amplitude, whereas Pyloric (PY) neuron firing delay primarily depends on the temporal characteristics of the input.

Intracellular recordings from LP and IC neurons in the intact network show that inhibition amplitude in these neurons changes as pyloric frequency is altered. However, this change is small and opposite to the change expected if the cellular properties of these neurons play a role in phase control. Re-analysis of prior experiments with respect to delay after the pacemaker burst, rather than phase, shows these neurons fire with a constant lag after pacemaker activity.

In the intact network, PY neurons do show appropriate changes in firing delay, and thus the firing delay properties described previously likely are relevant to their phasing. This work did not directly address whether PY neuron firing delay depends on pulse length or interpulse interval. Experiments in which these lengths were varied independently show that interpulse interval, not pulse length, mainly determines PY neuron firing delay.

Maintenance of LP and IC neuron phase as pyloric frequency changes thus is largely due to the associated changes in pacemaker burst length. PY neuron phase maintenance, alternatively, is likely associated with PY neuron cellular properties that alter neuron firing delay as pyloric frequency is altered. However, this delay may be primarily determined by the length of the PY neuron burst in the preceding pyloric cycle, not the changing duration of pacemaker inhibition received by the PY neuron.

577.16

TWO DISTINCT PATHWAYS FOR ACTIVATING cGMP IN THE CRAB STOMATOGASTRIC NERVOUS SYSTEM. N. L. Scholz†, M. F. Goy*‡, J. W. Truman†, and K. Graubard†. †Univ. of Washington, Dept. of Zoology, Seattle, WA 98195 and ‡Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

We are investigating pathways of cGMP activation in the crab stomatogastric nervous system (STNS), a collection of motor circuits known to be extensively modulated by both synaptically-delivered transmitters and circulating neurohormones. Using antisera selective for cGMP, we have conducted parallel radioimmunoassay (RIA) and immunocytochemical (ICC) studies to screen for stimuli that activate cGMP synthesis in the STNS. We have found two classes of activators that are effective: nitric oxide (NO) donors and peptide-containing extracts of crab sinus gland.

In RIA studies, three NO donors (SNP, SNAP, and SIN-1) were all found to produce large increases in cGMP levels when applied with the phosphodiesterase inhibitor IBMX. A similar result was obtained with ICC studies: treatments with NO donors and IBMX consistently caused the appearance of cGMP immunoreactivity in a subset of neurons. We used an arginine-to-citrulline conversion assay to screen tissues in the vicinity of the STNS for a nitric oxide synthase (NOS), and found a candidate in crab heart. This tissue contains an arginine-metabolizing enzyme with properties that resemble a constitutive NOS, including calcium-dependence, NADPH-dependence and sensitivity to arginine analogs. No such activity was observed in the STNS, the supraoesophageal ganglion, or muscle (GM1).

The sinus gland is a crustacean neurohemal organ that is known to contain peptides (members of the CHH/MIH family) that stimulate cGMP synthesis in a variety of target tissues. In RIA studies, extracts of crab sinus gland strongly elevated the cGMP content of the STNS when applied with IBMX. We are currently using cGMP ICC to identify which cells are targeted by the active component of the extract; preliminary results indicate that the peptide extract has a different target specificity than do the NO donors. Based on these results, we suggest that NO and a sinus gland peptide represent two non-overlapping pathways for activating cGMP in the STNS. Supported by an NRSA traineeship to N.S., NIH grants (NS15697 to K.G. and NS 25915 to M.G.) and NSF grant IBN-9242993 to J.T.

577.17

MORPHOLOGY OF STOMATOGASTRIC NEURONS OF *CANCER BOREALIS*. K. Graubard*, A. E. Wilensky. Department of Zoology NJ-15, University of Washington, Seattle, WA 98195.

The stomatogastric ganglion (STG) is a small pattern generator, comprised of 30 neurons. For most neurons, including DG, LP, and PD, the primary neurite exits the ganglion to become a single large axon that does not divide until it nears its target muscles. However, some neurons, including IC, GM and VD, have axon divisions that occur close to or within the ganglion. The primary neurite of the IC neuron first branches to produce smaller neurites that terminate within the neuropil and then bifurcates near the edge of the neuropil into two equal-diameter axons which exit the neuropil without further branching. GM neurons have a similar pattern; the primary neurite branches repeatedly to produce 3-5 axons of unequal diameters, with no further neuropil branching. In contrast, the VD neuron's primary neurite splits early in its course into two equal-diameter neurites; they then branch extensively to produce smaller neuropil-contained neurites, after which the two large-diameter axons exit the neuropil. The regions of neuropil innervated by the VD's fine neurites are distinct, with little overlap. Since the VD has two sites of spike generation, it is interesting to speculate that this unique neuropil organization subserves some function. Supported by NIH grant NS15697.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

578.1

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF LAYER I NEURONS IN RAT NEOCORTEX. F.M. Zhou* and J.J. Hablitz, Neurobiology Research Center, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The basic morphological properties of layer I neurons were described by Ramon y Cajal about 100 years ago. Yet, the electrophysiological properties of these neurons has remained an enigma and their neuronal nature has been questioned. Using an in vitro slice preparation, we have examined the morphological and electrophysiological properties of visually identified layer I neurons. Whole cell patch clamp techniques were used in slices from 2-3 week-old rats. Biocytin labeled cells were multipolar with somas 10-20 μm in diameter. Most cells processes were smooth and spread horizontally for up to 1.5 mm within layer I. Some presumed axonal processes going to layers II-IV were observed. All the layer I cells (N=30) tested had electrophysiological properties typical of interneurons. Action potentials evoked by depolarizing current pulses showed minimal or no frequency adaptation. Action potential durations, measured at the base, were short compared to pyramidal neurons recorded under similar conditions (2.5 vs 5 ms at 22°C). In the presence of 20 μM APV and 10 μM CNQX, bicuculline-sensitive spontaneous IPSCs were recorded in the majority of cells. The amplitude of sIPSCs ranges from less than 10 pA to more than 200 pA, at a holding potential -60 mV in symmetrical Cl^-, and reversed at 0 mV. Adding 0.5μM TTX reduced sIPSC frequency and blocked large amplitude sIPSCs. These results suggest that layer I neurons have firing properties characteristic of interneurons and receive inhibitory inputs. They appear to have extensive processes which may serve in controlling cortical excitability. (Supported by NS18145 and NS22373)

578.2

PROJECTIONS FROM THE PARATENIAL NUCLEUS OF THE THALAMUS. T. van Groen* and J.M. Wyss. Dept. of Cell Biology, University of Alabama, Birmingham, AL 35294-0019

Previous studies indicate that the paratenial (PT) nucleus of the thalamus projects primarily to anterior cingulate cortex (area infraradiata; IR), and ventral striatum. However, our anterograde tracing studies suggest that many PT axons extend beyond these areas to synapse in more caudal limbic cortical areas. The present study used anterogradely and retrogradely transported tracers to characterize the projections of PT. Injections into PT reliably label a terminal field in the anterior olfactory nucleus, in the medial orbital and rostroventral IR (IRbα) cortices, in the lateral and basolateral nuclei of the amygdala, in the ventral subiculum, and in the caudal entorhinal and perirhinal cortices. In addition to the cortical projections, PT injections label a dense terminal field in the ventral striatum and a less dense terminal field in the olfactory tubercle and in the ventrolateral part of the lateral septal nucleus. PT projections display a general topographic organization, i.e., rostral parts of PT project to more rostral parts of IRbα, whereas caudal parts of PT project to more caudal parts of IRbα. Further, caudal parts of PT project predominantly to rostral areas of limbic cortex (i.e., IRbα and orbital cortices), but rostral parts of PT project to both rostral and caudal areas of limbic cortex (i.e., subicular, entorhinal, and perirhinal cortices). Axons of PT terminate primarily in layers I and III/IV in most cortical areas, but the PT projections to the subiculum end in layer I, and the PT projections to the caudal entorhinal cortex terminate in the deep layers (i.e., layers V-VI). These results demonstrate that PT projects to functionally distinct limbic areas, e.g., regions implicated in learning and memory versus regions involved in cardiovascular regulation and control of emotions.

578.3

EFFERENT PROJECTIONS FROM THE ROSTRAL THALAMIC RETICULAR NUCLEUS TO THE IPSILATERAL AND CONTRALATERAL THALAMUS : AN ANTEROGRADE TRACING STUDY IN THE RAT. G. Battaglia* and C. Lizier. Dept. of Neurophysiology, Neurological Institute "C. Besta", 20133 Milano, Italy.

The conventional view that the interthalamic connections are sparse or absent has been recently challenged by anatomical studies from different groups (Bentivoglio et al.,'93; Paré and Steriade,'93; Battaglia et al.,'94). These studies, mainly based on retrograde tracing techniques, pointed out that in different mammalian species the thalamic reticular nucleus (R), and particularly its rostral part, projects to contralateral as well as to ipsilateral dorsal thalamic nuclei. The anterograde transport of biotin-dextran amine (BDA) was employed in the present study to reinvestigate the pathways connecting different regions of the rostral pole of R to the ipsilateral and contralateral dorsal thalamic nuclei. Small iontophoretic injections of BDA were delivered into the medial, ventral, and lateral regions of the rostral pole of R. After a survival time of 7 to 10 days, the rats were perfused with 1% paraformaldehyde followed by 4% paraformaldehyde, the brains coronally cut with a vibratome, and the sections processed with an antibiotin antibody to reveal the anterogradely transported BDA.

After BDA injections in the medial region of the rostral R anterogradely labeled fibers were almost symmetrically found in the anterior thalamic nuclei of the two sides, and in the corresponding medial region of the contralateral R. The midline nuclei of both sides were also symmetrically labeled, particularly after the most rostrally placed injections. After ventral injections, anterograde labeling was particularly evident in the rostral intralaminar and mediodorsal nuclei of the two sides, but the ipsilateral labeling was predominant. After lateral injections, anterograde labeling was evident in the medial part of the ventrolateral nucleus, in the ventromedial nucleus, and in the caudal regions of the intralaminar nuclei. The labeling was bilateral, though clearly more evident on the ipsilateral side. No labeling in the contralateral thalamus was ever observed after injections not involving the rostral pole of R.

The present data demonstrate that the interthalamic connections in the rat thalamus are more dense than previously thought. The data confirm moreover a functional heterogeneity between different sectors of the reticular nucleus, and that its rostral part plays a pivotal role in the crosstalk between the two thalamic sides.

578.4

SYNAPTIC PHYSIOLOGY OF THE AMYGDALA-PERIRHINAL PATHWAY STUDIED *IN VITRO* J.M.Beggs* and E.W.Kairiss. Department of Psychology and Center for Theoretical and Applied Neuroscience, Yale University, New Haven, CT 06511.

Although cortical and limbic areas have been suggested to play complementary roles in mammalian memory, little work has been done to study synaptic interactions between these two regions.To address this issue, we have been engaged in the development of an *in vitro* slice preparation which maintains connectivity between perirhinal cortex (PRh) and the lateral nucleus of the amygdala (LA). Here we describe our procedures for reliable preparation of these slices and present the results of studies of plasticity in this system.

Coronal slices 400μ thick were taken from rat brain in the range of -2.2 to -3.8 from bregma. Stimulation in LA using 100μA produced field potentials in PRh in 33% of these slices. Current-source density analysis reveals a current sink restricted to layers II-III of PRh. Injection of the lipophilic tracer Di-I also confirms that a fiber pathway from LA to the superficial layers of PRh is contained in the slice.

To identify a monosynaptic component in PRh which responds to stimulation of LA, high-frequency following tests and intensity-latency measurements were performed. Recordings from PRh indicate that there is a component that can follow at 100Hz with a constant latency of 5ms over a range of stimulation intensities (40-220μA).

To examine use-dependent synaptic plasticity, field potentials were recorded in superficial layers of PRh while applying high-frequency stimulation in LA. Preliminary results indicate that this amygdalar pathway can be potentiated. Stimulation applied to superficial layers of adjacent cortical areas can either potentiate or depress responses recorded in superficial PRh. We are investigating whether there exists cooperativity or associativity between the LA and intracortical inputs to PRh. Future studies will examine whether this plasticity is NMDA-dependent, and if the input from LA can serve as a "reinforcement signal" to consolidate intracortical plasticity in PRh.(Supported by NIH and Yale University)

578.5

A PHA-L STUDY OF THE PERIRHINAL PROJECTION TO THE THALAMUS IN THE RAT R.D. Burwell*[1], M. Caballero[2], M.P. Witter[2] and D.G. Amaral[1]. [1]The Center for Behavioral Neuroscience, SUNY at Stony Brook, Stony Brook, NY 11794-2575. [2]Anatomy and Embryology, Vrije University, Amsterdam, The Netherlands.

The precise relationship of certain thalamic nuclei with distinct regions of the neocortex has provided one mechanism for defining the cytoarchitectonic boundaries of the cortical mantle. This study was designed to examine whether projections from various locations of the perirhinal cortex might differentiate subdivisions of this region. Using adult male Sprague-Dawley rats as subjects, the anterograde tracer, *Phaseolus-vulgaris* leucoaglutinin (PHA-L), was iontophoretically injected at twenty-one sites along the rostrocaudal extent of perirhinal cortex, in areas 35 and 36 according to borders adapted from Swanson (1992). The thalamic projections arise from deep layers of perirhinal cortex, and those from area 36 are more robust than those from area 35. All portions of perirhinal cortex project lightly to midline thalamic nuclei, terminating in the reuniens nucleus and the rhomboid nucleus. Importantly, subregions of areas 35 and 36 can be distinguished by the patterns of their efferent connectivity with other thalamic regions. For example, rostral area 36 projects primarily to the posterior nuclear group, while mid-rostrocaudal levels project primarily to the central medial nucleus; and caudal levels project to the anterior nuclear group and the lateral nuclear complex. Rostral area 35 is distinguished by a moderately dense projection to the central division of the mediodorsal thalamus. These data about the definition of thalamic nuclei in receipt of perirhinal regions will be used to define the functional roles for perirhinal subdivisions.

578.6

INTRINSIC CIRCUITS OF FUNCTIONALLY SPECIALIZED SUBDIVISIONS OF HUMAN CEREBRAL CORTEX. M.F. Kritzer*. Dept. Neurobiology and Behavior, SUNY @ Stony Brook, Stony Brook, N.Y. 11794-5230.

Analyses of intrinsic cortical circuits in non-human primates have shown that the organization of these connections is region-specific (e.g., Lund et al., Cerebral Cortex 3:'93; Harel and Malach, J. Comp. Neurol. 334:'93). This study indicates that local circuitry, and particularly that of upper layers, likewise provides anatomical signatures to functionally specialized cortices in man.

Intrinsic circuits were labeled with dil in post-mortem human primary motor, somatosensory and visual cortex, and orbitofrontal, inferotemporal and posterior parietal cortices of 7 adult males. Comparison of circuits among corresponding layers of these areas revealed i) region-specific differences in the local circuits of supragranular layers and ii) cortical-wide similarity in the wiring of infragranular laminae. In area 17, for example, supragranular sites yielded the expected pattern of strong bilaminar lateral labeling of comparable density and breadth (3-5mm) in layers II/III and V (Burkhalter et al., J. Neurosci. 13, '93). Similar injections in somatosensory cortex, however, were distinguished by lateral labeling in layers II/III which was not only more dense but extended almost twice as far (~6mm) as labeling in layer V. Layer II/III sites in areas 40 and IT also produced dense lateral labeling (3-4mm) in layers II/III; however, a sparse complement of fibers in layer V which consistently spread further laterally (5-7mm) than supragranular axons was characteristic of these injections. Finally, layer II/II sites in motor cortex were unique in yielding laterally restricted labeling (1-3mm) in all layers. These areally-specific supragranular patterns contrasted with labeling produced by crystals placed in layers V or VI which was laterally extensive (3-7mm) in layers III and V, or layer VI (5-7mm), respectively, in every region. The local circuits of supragranular layers may thus be of particular importance to the generation and/or elaboration of the computations/physiology which distinguish functionally specialized domains of the human cerebral cortex.

578.7

EVOLUTION OF THE FRONTAL LOBES: AN MRI STUDY ON APES AND HUMANS. K. Semendeferi[1], H. Damasio[2*] and G.W. Van Hoesen[2,3]. Depts of Anthropology[1], Neurology[2,3] and Anatomy[3], Univ. of Iowa, Iowa City, IA 52242.

How much did the frontal lobes (FL) enlarge during hominoid evolution and which of the subdivisions (dorsal, mesial, orbital) changed the most? We studied all 5 living hominoids (human, chimpanzee, gorilla, orangutan, gibbon) and one monkey (macaque). Thin cut MRs were obtained from each non-human brain specimen and from a living human subject. All were reconstucted in 3-D. Major landmarks were identified and used to separate the frontal lobe from the rest of the hemisphere. We subdivided the cortex and the immediate subjacent white matter in 3 sectors (orbital, dorsal and mesial). The remaining tissue was considered another sector and contained white matter and subcortical grey structures.

Relative to the rest of the hemisphere, the size of the FL is largest in the chimpanzee and human, where it is nearly identical (36.3% and 36.9% respectively). The macaque and gibbon have the smallest (31.4% and 31.5%). The FL show a trend towards increased relative size in hominoid evolution, but the 3 sectors are more conservative. Across the species the dorsal, mesial and orbital sectors are close in relative size and, as expected, the dorsal is largest followed by the mesial and orbital. A noteworthy difference was found in the fourth sector, which was largest in the human followed by the chimpanzee and gorilla. The most marked difference was seen in the anterior third, which contains the white matter, underlying prefrontal cortices.

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578.8

Ultrastructural analysis of the A-subtype of the α_2-adrenergic receptor in the monkey prefrontal cortex reveals enrichment of post-synaptic and axonal labelling in lamina 5. C. Venkatesan[1*], H. Kurose[2] and C. Aoki[1]. [1]Center for Neural Science, New York University, NY, NY 10003 & [2]Univ. of Tokyo, Dept. of Toxicology and Pharmacology, Hongo 7-3-1, Bunkyo-ku, Tokyo 113.

Activation of α_2-adrenergic receptors (α_2AR) in monkey prefrontal cortex has been shown to enhance memory and learning. Recently, antibodies were produced against the A, B and C subtypes of the α_2AR. Using an antibody against the A subtype, we show that the distribution of perikarya labelled for the α_{2A}AR resembles the pattern obtained by Nissl staining. Puncta, ca. 1 - 2 μm, can be visualized over cells bodies. Electron microscopy reveals these puncta to reflect labelled endoplasmic reticulum and Golgi apparatus within perikarya and intensely labelled axons which course along the perikaryal plasma membrane. The immunoperoxidase product is also present within glial, presynaptic, postsynaptic and nonsynaptic profiles. Immunoreactive presynaptic and postsynaptic profiles form both symmetric and asymmetric junctions. Semi-quantitative analysis reveals that the relative proportions of labelled profiles are distinct within and across cortical laminae. The majority of labelled profiles in layers II, IV, V and VI are axonal. Layer V, which contains the highest noradrenergic fiber density, also contains the highest areal density of profiles labelled for the α_{2A}AR. Moreover, this layer exhibits a relatively higher proportion of ir-axons and ir-postsynaptic densities than the other laminae. These results demonstrate that the functionally and anatomically distinct laminae also contain unique subcellular distribution patterns of the receptor. CA is supported by NIH EY08055 (FIRST), NS30944, NSF RCD9253750 (Presidential Faculty Fellowship) & HFSP RG-16/93.

578.9

COMPARISON OF INTRA- AND INTER-AREAL PATTERNS OF CONNECTIVITY IN MONKEY PREFRONTAL CORTEX. M.L. Pucak*[1], J.B. Levitt[3], J.D. Classey[3], J.S. Lund[3], and D.A. Lewis[1,2]. Depts. of Psychiatry[1] and Neurosci.[2], U. of Pittsburgh, Pittsburgh, PA 15260, and Dept. of Visual Science[3], U. of London, England.

The excitatory axon terminals that furnish intra- and inter-areal connections in monkey prefrontal cortex (PFC) are both arranged in discrete clusters. However, little is known about the relations between these two types of terminal fields, or about the distribution of their neurons of origin. Single injections (350 µm diam.) of biotinylated dextran amine in superficial layers of areas 9 or 46 of macaque monkey PFC produced two distinct types (intra- and inter-areal) of clusters of anterogradely-labeled axon terminals. Within areas 9 or 46, labeled terminals formed multiple stripes (.45 × 1.4 mm) in layers 1-3 that were similar to the intrinsic lattice structure formed by the intra-areal axon collaterals of layer 2/3 pyramids as revealed by the tracer biocytin (Levitt et al., 1993). Terminals from the same injection also formed bands (.49 × 1.2 mm) at substantially greater distances from the injection site. These axon terminals, which were distributed across all cortical layers, arose from labeled axons in the underlying white matter. Similar injections of cholera toxin B (CtB) revealed two analogous types of clusters of retrogradely-labeled pyramidal neurons. Within areas 9 and 46, labeled neurons formed stripes (.42 × 1.2 mm) primarily in layers 2/3, whereas in other PFC regions, clusters of labeled neurons were present in both superficial and deep layers. Anterogradely-labeled CtB axon terminals overlapped both the intra- and inter-areal cell clusters, revealing the reciprocity of these connections. These findings confirm the discontinuous distribution of both intra- and inter-areal connections in monkey PFC. They also demonstrate that each point in the intrinsic lattice furnishes divergent output to multiple zones within the lattice as well as to other PFC regions, and receives convergent input from these same zones. These patterns of reciprocal intra- and inter-areal connections provide an anatomical substrate for the simultaneous and recurrent activation of specific distributed networks of neurons in monkey PFC.

578.10

MORPHOLOGY AND EXTRINSIC TARGETS OF PYRAMIDAL NEURONS FURNISHING INTRA-AREAL CONNECTIONS IN MONKEY PREFRONTAL CORTEX. D.S. Melchitzky*[1,2], M.L. Pucak[1], R.S. Dammerman[2], and D.A. Lewis[1,2]. Depts. of Psychiatry[1] and Neurosci.[2], University of Pittsburgh, Pittsburgh, PA 15260.

Pyramidal neurons in the superficial layers of monkey prefrontal cortex (PFC) furnish horizontally-spreading axon collaterals which form a lattice-like structure comprised of multiple stripes (Levitt et al., 1993). Because the function of this intra-areal circuitry depends on the input and output of the cells which furnish it, we investigated the dendritic morphology and extrinsic projections of those neurons. Small iontophoretic injections of biotinylated dextran amine (BDA) were made into areas 9 or 46 of cynomolgus monkeys. We analyzed those retrogradely-labeled neurons 1) which were labelled via their axon collateral projections and 2) for which BDA provided complete filling of dendrites. Typical features of these pyramidal neurons included: location in layers 2/3, an axon which emitted collaterals on its course toward the white matter, apical dendrites extending into layer 1, and basilar dendritic trees which were confined to layers 2/3. The horizontal extent of the dendritic arbor (250-450 µm) was similar to the width of intrinsic lattice stripes (Levitt et al., 1993), suggesting that these neurons sample input from a single stripe. In order to determine the destination of the extrinsic projection of these neurons, dual injections were made: 1) cholera toxin B (CtB) in areas 9 or 46, to label neurons with intrinsic axon collaterals within these areas, and 2) Fast Blue (FB) in another area of PFC, to label associationally-projecting neurons. In locations where the tracers overlapped, 46.5% (144/310) of FB-labelled neurons were also labelled with CtB. Thus, neurons which furnish axon collaterals of the intrinsic lattice also provide associational projections. The laminar location of the dendritic arbor of these neurons indicates that they may receive intra- and/or inter-areal input. These features suggest that the intrinsic lattice plays an important role in cortical information processing by permitting the simultaneous activation of discrete, spatially segregated groups of neurons, resulting in a specific pattern of output from the PFC to other cortical areas.

578.11

DENDRITIC MORPHOLOGY OF NEURONS WITH DIFFERENT AXONAL PROJECTIONS IN MONKEY PREFRONTAL CORTEX. A.S. Soloway*[1], M.L. Pucak[2], and D.A. Lewis[1,2]. Depts. of Neuroscience[1] and Psychiatry[2], Univ. of Pittsburgh, Pittsburgh, PA 15260.

The dendritic morphology of pyramidal neurons, the major source of excitatory intra- and inter-areal cortical connections, has been reported to differ depending upon the target of their efferent axons. In addition, separate populations of neurons have been shown to furnish associational and callosal projections from a given region of monkey prefrontal cortex (PFC). In this study, injections of the fluorescent tracer Fast Blue (FB) into areas 9 or 46 of cynomolgus monkey PFC were used to identify associational and callosal neurons in these areas. Retrogradely-labeled neurons were then intracellularly injected with Lucifer Yellow in fixed slices (300 µm), converted to an immunoperoxidase label, and reconstructed using the Eutectic Neuron Tracing System. Neurons providing associational projections were pyramidal or modified pyramidal cells located predominantly in layers 2-3, with a smaller number in layers 5-6. Associational neurons in the superficial layers shared the following characteristics: an apical dendrite which extended into layer 1, a basilar dendritic tree which was confined to layers 2-3, and a dendritic arbor with a horizontal extent of 200-400 µm. The laminar location, vertical and horizontal spread of the dendritic tree, and the pattern of dendritic arborization of associational neurons is quite similar to that of neurons whose axon collaterals form the lattice-like structure of intra-areal connections. These similarities are consistent with other evidence indicating that a subpopulation of PFC neurons furnishes both inter-areal and intra-areal connections (Melchitzky et al '94). Callosal neurons were also similar to associational neurons in their laminar distribution and somal morphology; quantitative assessments of dendritic arborization and complexity, as well as measures of excitatory input, will be used to directly compare these two non-overlapping cell populations.

578.12

CALRETININ-IMMUNOREACTIVE NEURONS IN MONKEY PREFRONTAL CORTEX: ULTRASTRUCTURE AND ASSOCIATIONS WITH DOPAMINE AFFERENTS. D.A. Lewis*, C.L. Snyder, and S.R. Sesack, Depts. Psychiatry and Neuroscience, Univ. Pittsburgh, Pittsburgh, PA, 15213.

The calcium-binding protein, calretinin (CR), is present in a morphologically distinct population of local circuit neurons in superficial layers of monkey prefrontal cortex (PFC). This morphological cell class has previously been shown to target both pyramidal and non-pyramidal neurons. Furthermore, the morphological features and laminar position of CR-containing cells suggest that they may be synaptic targets of dopamine (DA) afferents. We examined the ultrastructural features of these neurons in area 9 of macaque monkey using a pre-embedding immunogold marker and investigated their association with DA terminals by combining immunogold staining with peroxidase immunoreactivity for tyrosine hydroxylase (TH). CR-positive axon terminals made synaptic contacts with cell soma, as well as large and small diameter dendrites. These contacts were primarily of the symmetric type. Many of the small dendritic targets had the morphological characteristics of non-pyramidal neurons, but were not immunoreactive for CR. CR immunoreactivity was also observed in small non-pyramidal neurons whose dendrites were distinctly varicose and received abundant synaptic input. In double labeled sections, immunomarkers for CR and TH were frequently observed in adjacent areas of the neuropil. However, appositions between labeled processes were rare, and specific instances of synaptic input were not observed. Although our previous findings demonstrated that DA terminals directly innervate some GABAergic interneurons in the PFC, the present findings suggest that CR-containing interneurons are not included in this target population. However, a more indirect modulatory interaction between CR and TH-containing processes is suggested by their common distribution.

578.13

REGIONAL NONHOMOGENEITIES IN CORTICAL ASTROGLIA IN ADULT MONKEYS. PERSISTENCE OF TRANSITIONAL FORMS. J.A.Colombo* and V.Puissant. Programa Unidad de Neurobiología Aplicada (PRUNA)(CEMIC-CONICET), Av.Galván 4102, 1431 Buenos Aires, Argentina.

Cerebral cortex organization during development depends on radial glia. Both, radial- and transforming (intermediate) glia reportedly do not persist into late postnatal life. We report findings in three adult, Cebus apella monkey brains processed for vimentin and GFAP, utilizing the peroxidase-antiperoxidase procedure. Regional non-homogeneities in the distribution of cortical, vimentin + and GFAP + astrocytes were characterized by dense patches of astroglial processes with no obvious relationship to vascular elements, followed by others with scatttered immunoreactive material. Additionally, relatively abundant, long astrocytic processes were observed stemming from layer I through III/IV in several areas of the temporal lobe, medial- and dorsolateral frontal cortices, as well as cingulate cortex. Persistent presence of "transforming" astrocytes in adult life of the New World monkey Cebus apella are of unknown physiological role. These observations reinforce the notion that astroglia plays ubiquitous roles in cortical function during postnatal life.- Acknowledgments: CONICET, Fundación Conectar, Petrolera Argentina S.Jorge, Universidad de Belgrano.

578.14

AGE-DEPENDENT $GABA_A$ RECEPTOR REGULATION IN RAT SENSORY AND ASSOCIATION CORTEX. J.C. McEachern, J. Church*, C.A. Shaw. Depts. of Physiology, Anatomy & Opthalmology, Vancouver, BC V6T 1Z3.

Sensory cortex exhibits age-dependent differences in magnitude and direction of ionotropic receptor regulation in response to agonist and depolarizing stimulation (Shaw & Scarth, *Mol. Brain Res.*, 14 (1992) 207). These developmental changes in receptor regulation correspond temporally with the critical period for long-term potentiation (LTP) (Perkins & Teyler, *Brain Res.*,439 (1988) 222) and are postulated to be an important mechanism underlying this form of neuroplasticity. Neuroplasticity in association cortex, however, may not be limited to a critical period. If this is the case, its developmental profile of receptor regulation may differ from that characteristic of sensory cortex. A profile of $GABA_A$ receptor regulation at various ages was prepared for frontal association areas Fr 1, 2 and 3 (Kolb & Tees, *The Cerebral Cortex of the Rat*, 1990) versus sensory cortex areas. Cortical slices from these areas in rats aged 10d, 16d or >70d were treated with veratridine or muscimol, labeled using [^{3}H]-SR 95531 and compared to controls. In both sensory and association cortex veratridine treatment resulted in a decrease in receptor number at 10d, but an increase at 16d and older. At all ages muscimol treatment resulted in a decrease in receptor number in both cortical areas. These comparable profiles suggest either: 1) similar age-dependent characteristics of receptor regulation in the two areas, and hence a) association cortex critical period for neuroplasticity may be identical to that of sensory cortex , or b) age-dependent receptor regulation is not a key determinant of critical periods; or 2) these frontal areas in the rat are not true association cortex .

578.15

CORTICOCORTICAL CONNECTIONS MEDIATE DIRECTED ATTENTION IN RATS: BEHAVIORAL AND ANATOMICAL EVIDENCE. R.L. Reep*, J. Vandevelde, E. Duckworth, M. Stoll and J.V. Corwin, Department of Physiological Sciences, University of Florida, Gainesville, FL 32610; and Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

We investigated the role of corticocortical connections in directed attention by selectively disconnecting areas AGm and PPC, then testing animals for signs of hemispatial neglect. In rats, these two cortical areas are involved in spatial orientation and are highly interconnected via rostrocaudally directed axons traveling both directions in the deep gray matter. Therefore, coronally oriented knife cuts which extend into layer VI but do not encroach upon the white matter should selectively transect these axons without interrupting those directed subcortically.

Knife cuts extending into layer VI (N=6) produced neglect, whereas control cuts extending only as deep as layer V (N=4) did not ($p<.02$). The deeper knife cuts resulted in appreciably less axonal labeling than normal and a very sparsely labeled region rostral to the cut, whereas control subjects with shallower cuts exhibited labeling which was comparable to normals.

These results suggest that the corticocortical axons linking AGm and PPC play a pivotal role in directed attention.

578.16

APOMORPHINE ADMINISTRATION PRODUCES ACUTE DOSE-DEPENDENT RECOVERY FROM NEGLECT FOLLOWING UNILATERAL POSTERIOR PARIETAL CORTEX LESIONS IN RATS. J.V. Corwin*, G.I. Hix, K.J. Burcham, and C. Gordy. Psychology Dept., Northern Illinois Univ., DeKalb, IL 60115.

Previous research from our laboratory (Corwin et al., 1986; King and Corwin, 1990) has indicated that apomorphine (apo) has an acute dose-dependent therapeutic effect on neglect induced by unilateral destruction of the medial agranular prefrontal cortex (AGm), suggesting that neglect may result from a disruption of dopaminergic mechanisms. To extend the potential applicability of these findings to humans, in which the vast majority of cases of neglect are produced by unilateral destruction of the posterior parietal cortex (PPC), the present study examined the effects of apo administration on neglect induced by unilateral destruction of the PPC.

Subjects were 20 Long-Evans hooded male rats. After extensive handling, the subjects received an ablation of either the left or right PPC via aspiration. At 48-96 hrs post-surgery, the subjects were tested for the presence of multimodal neglect, determined by the degree of head orientation in response to presentation of visual, tactile, or auditory stimuli. Subjects which met the criterion for severe neglect (defined by a ratio of responsiveness of the neglect side:non-neglect side of $\leq$1:3) were randomly assigned to one of four apo dosage groups: vehicle, 0.1, 0.3, or 0.5 mg/kg. Apo or the vehicle was administered i.p. 45-60 min after behavioral testing. Eighteen minutes post-injection, the subjects were retested. All behavioral testing was done with the experimenter "blind" with respect to the dosage administered.

There was a significant reduction in the severity of overall neglect in the 0.3 and 0.5 mg/kg groups ($p<.05$), but not in the vehicle or 0.1 mg/kg groups. The results indicated that apo produces an acute dose-dependent therapeutic effect on multimodal neglect produced by PPC lesions. As found for the AGm, neglect produced by unilateral PPC removal may be a result of disruption of dopaminergic mechanisms.

578.17

FUNCTIONAL NEURAL IMAGING OF NAUSEA IN HUMANS. T.Roberts, A.Miller+, H.Rowley and J.Kucharczyk*, Neuroradiology Section, UCSF, San Francisco, CA 94143 and + Lab.Neurophysiol., Rockefeller Univ., New York, NY 10021

Considerable progress has been made in recent years in determining the CNS mechanisms which mediate vomiting. However, the neuronal substrates that subserve nausea are completely unknown. The present study used functional neuroimaging techniques to determine if a localized cortical area is activated during nausea. Functional data were obtained using a 37-channel SQUID-based magnetic source imaging (MSI) device (Magnes, Biomagnetic Technologies Inc., San Diego, CA) and were overlaid on neuroanatomical information from high-resolution 3D SPGR MRI. Data were analyzed for the occurrence of transient bursts of magnetic activity (exceeding 400fT when data were filtered in the range 8-100Hz). These were used to derive the location of the responsible neural source using the single equivalent dipole model. Data have been obtained from one volunteer in which nausea was produced on two separate days by either oral ingestion of syrup of ipecac or by vestibular stimulation (induced by head movements during yaw-axis rotation, while wearing vision-reversing goggles). Current dipoles indicative of neuronal activation were localized to a 2-3cm diameter region of cortex in the inferior frontal gyrus, when nausea was produced by either stimulus. Such activity was not observed during baseline controls or during sequences involving swallowing or exaggerated respiratory movements. While preliminary, the data suggest that MSI may provide a quantitative means of measuring the effects of various pharmaceutical interventions on nausea and suggest a new CNS target for such intervention.

Supported by grants from NIH (NS20585 to AM).

COMPARATIVE NEUROANATOMY: SENSORY SYSTEMS

579.1

SOME MORPHOLOGICAL FEATURES OF A VISUAL THALAMIC NUCLEUS IN A REPTILE. M.B.Pritz*. Sect. of Neurol. Surg., Indiana U. Sch. of Med., Indianapolis, IN 46202-5124.

Nucleus rotundus is a prominent nucleus in the dorsal thalamus of all non-mammalian vertebrates. In one group of reptiles, Caiman crocodilus, this neuronal aggregate contains relay cells only and lacks local circuit neurons. The present study investigated some aspects of rotundal cytoarchitecture based on examination of Nissl morphology. Neurons were not uniformly distributed throughout the nucleus but were grouped in clusters commonly ranging from 2 to 5 cells. Somas were round, oval or triangular in shape. The following neuronal morphological features were investigated: area, perimeter, and roundness or eccentricity expressed as the ratio of greatest width to greatest length. Preliminary observations, mean ± standard deviation, examined neurons in each of 3 planes: horizontal (N=50), sagittal (N=50), and transverse (N=50). Measurements were as follows: (1.) area (μm^2) = 196.88 ± 46.13 - horizontal; 205.58 ± 50.69 - sagittal; and 202.93 ± 39.12 - transverse; (2.) perimeter (μm) = 55.32 ± 7.64 - horizontal; 56.46 ± 7.35 - sagittal; and 54.96 ± 5.70 - transverse; (3.) roundness: 0.67±.13 - horizontal; 0.62 ± .10 - sagittal; and 0.71 ± .11 - transverse. Additional observations will be made to determine whether these morphological features identify subpopulations of relay cells in nucleus rotundus.

579.2

A COMPARISON OF THE EIMER'S ORGANS OF THE STAR-NOSED MOLE (*CONDYLURA CRISTATA*), THE HAIRY TAILED MOLE, (*PARASCALOPS BREWERI*), AND THE EASTERN MOLE (*SCALOPUS AQUATICUS*). K. C. Catania* Neurobiology Unit, Scripps Institution of Oceanography and Dept. of Neurosciences, School of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093-0201.

Eimer's organ is a tactile sensory structure found on the snouts of moles and some shrews. It consists of a raised dome of epidermis containing a column of cells associated with sensory receptors. Scanning electron microscopy and light microscopy were used to compare the structure and distribution of these organs in three North American moles.

Eimer's organs are visible on the skin surface of the hairy tailed mole and the star-nosed mole, but not the eastern mole. The external appearance, distribution, and internal structure of the Eimer's organs of the hairy tailed mole are similar to those most commonly found in other species. The structure of the Eimer's organs of the star-nosed mole and the eastern mole diverge from this basic form in seemingly opposite directions. The Eimer's organs of the star-nosed mole are more numerous, smaller, highly organized units with little variability and a consistent pattern of terminal swellings within a cell column, just below a thin keratinized epidermis. By contrast, the Eimer's organs of the eastern mole lie below a thick keratinized epidermis, are larger and more variable in structure, and have no central cell column. These differences may be influenced by the soil type through which each species burrows; saturated mud allowing a more elaborate and delicate sensory apparatus in the case of the star-nosed mole, and dry abrasive soil requiring a thick keratinized epidermis over the receptor complex in the case of the eastern mole. Supported in part by NIH grants NS24869 to R. Glenn Northcutt, and 5t32-GM 08107 (Biology Training Grant).

579.3

THE ANURAN DORSAL COLUMN NUCLEI: ORGANIZATION, IMMUNOHISTOCHEMICAL CHARACTERIZATION AND FIBER CONNECTIONS. A. Muñoz, M. Muñoz, A. González and H.J. ten Donkelaar* Dept. of Cell Biology, Fac. Biol. Complutense University of Madrid, 28040 Madrid, Spain, and *Dept. of Anatomy and Embryology, Fac. Medical Sciences, University of Nijmegen, P.O. Box 9101 6500 HB Nijmegen, The Netherlands.

The organization and fiber connections of the dorsal column nuclei (DCN) of the anurans Rana ridibunda and Xenopus laevis are studied with immunohistochemistry and tract tracing. Primary afferents from cervical, thoracic and lumbar spinal dorsal roots reach the DCN somatotopically arranged. Primary afferents from the cranial nerves V, VII, IX and X innervate the DCN via the descending trigeminal tract. Nonprimary afferents arise bilaterally in cells of the rhombencephalic reticular formation, area octavolateralis, cerebellar nucleus, raphe and throughout the spinal cord. The main efferent projection of the DCN, i.e. the medial lemniscus is formed by DCN fibers that cross the midline at the level of the nuclei and innervate contralaterally the rhombencephalic reticular formation, the octavolateral area, cerebellum, the principal, magnocellular and mainly the laminar nucleus of the torus semicircularis, the anterodorsal and anteroventral (including the red nucleus) mesencephalic reticular areas and in X. laevis the intermediate and deep layers of the tectum. At diencephalic levels the medial lemniscal fibers innervate the posterior tubercle, the ventral thalamus (ventromedial and ventrolateral nuclei), and sparsely the posterior and central nuclei of the dorsal thalamus. Ipsilateral, extralemniscal, DCN efferents reach the granular layer of the cerebellum and descending efferent fibers innervate the spinal cord. Histochemical and immunohistochemical observations of the DCN area revealed the presence of GABAergic, glycinergic, as well as parvalbumine (in X. laevis also calbindin), and NADPH diaphorase positive neurons in the DCN. Terminal structures positive for 5-HT, CGRP, SP, NPY and NADPH diaphorase are present in the DCN.

579.4

A NOVEL CUTANEOUS SENSORY SYSTEM IN HAGFISH. C.B. Braun* Neurobiology Unit, Scripps Institution of Oceanography and Dept. of Neurosciences, School of Medicine, University of Calif., San Diego, La Jolla, CA 92093-0201.

Ultrastructural and neuroanatomical investigations of the skin of the Pacific Hagfish, *Eptatretus stouti,* have revealed sensory buds, similar to those previously described on the barbels (Georgieva et al. 1979, Zoo. Scr. 8:61-67), in large numbers in the skin of the prenasal duct and on the head, trunk, and tail. Densities are highest near the snout and mouth (mean densitiy 24 buds per mm^2) and decrease caudally to about 11 buds per mm^2 on the trunk and tail. Interestingly, sensory buds are uncommon or absent from most of the oral epithelium and pharynx. These organs resemble taste buds, and are likely chemosensory, but differ from taste buds in their distribution and innervation. Unlike vertebrate taste buds, hagfish sensory buds are innervated by both cranial and spinal nerves. All internal sensory buds, and those on the snout and rostral head are innervated by branches of the trigeminal complex. All other sensory buds are innervated by spinal nerves. Such a pattern of innervation is unique among vertebrate special sensory organs and may explain some aberrant features of the myxinoid hindbrain. A large portion of the sensory trigeminal nucleus is argued to actually represent the rostral segment of the special visceral sensory (gustatory) column.

Supported in part by NIH grant DC01081 to R.G. Northcutt

579.5

PROJECTIONS OF THE NERVUS TERMINALIS IN THE THORNBACK RAY, (*PLATYRHINOIDIS TRISERIATA*)
M.H. Hofmann, R.G. Northcutt and T.H. Bullock*. Dept. Neurosciences, U.C. San Diego, La Jolla, CA 92093-0201.

The nervus terminalis (NT) was discovered more than a century ago in elasmobranchs. Presumed homologs of this cranial nerve have been investigated in many vertebrate classes. However, only a few data are available on the connectivity of the NT in elasmobranchs.

We investigated the projections of the NT by means of biotinylated dextran amine (BDA) injections into the olfactory bulb (OB) and rostral telencephalon at the entrance of the NT in the thornback guitarfish, *Platyrhinoidis triseriata.*

Injections of BDA into the OB revealed labeled fibers entering the forebrain in the NT, proceeding caudally in a medial position adjacent to the recessus neuroporicus and penetrating septal and preoptic areas. Labeled cell bodies were observed only in the medial part of nucleus preopticus, above the optic chiasm. Injections into the area where the NT enters the forebrain revealed labeled cells in all of the ganglia along the course of the NT towards the lateral part of the OB. Previous work has shown that the NT carries tonic efferent activity [Bullock and Northcutt Neurosci Lett 44(1984)155-160]. The labeled cells we found in the nucleus preopticus are possibly the source of these efferents.

Supported by a DFG grant to MHH and NIH grants to RGN and THB.

579.6

MIDBRAIN ROOF-DORSAL THALAMIC CONNECTIONS IN RANID FROGS. T.J. Neary* and W. Wilczynski. Anatomy Div., Creighton Univ., Omaha, NE 68178 and Psychology Dept., Univ. of Texas, Austin, TX 78712.

Iontophoretic injections of WGA-HRP were made in the anterior nucleus and middle thalamic zone (MTZ) of the dorsal thalamus in bullfrogs (R. catesbeiana) and midbrain roof populations examined for labelled cells. Injections involving the anterior nucleus consistently labelled cells bilaterally in the pretectal grey and ipsilaterally in the principal toral nucleus (TSp), particularly in its lateral, somatosensory part. Medial applications in the MTZ labelled cells in the ipsilateral pretectal grey and in the TSp, where most labelled cells were present medially and centrally. Injections in the intermediate MTZ resulted in greater labelling in the lateral part of the TSp and less in its medial part. They also labelled cells in the pretectal grey and a few cells in laminae 6 and 8 of the optic tectum. Lateral injections of the MTZ labelled more cells in laminae 6 and 8 of the optic tectum and also labelled cells in the pretectal grey and the lateral part of the TSp. In general, dorsal thalamic injections of WGA-HRP only rarely labelled cells in the laminar or magnocellular toral nuclei. Our findings suggest that the anterior nucleus may be more concerned with somatosensory than auditory information and that there is a crude topographic map between the tectum/torus and the middle thalamic zone.

579.7

AFFERENT AND EFFERENT CONNECTIONS OF THE VESTIBULOLATERAL CEREBELLUM IN THE CHANNEL CATFISH R.C. Bayborn and J.G. New*, Dept of Biology and Parmly Hearing Institute, Loyola University Chicago, Chicago, IL 60626.

The vestibulolateral cerebellum of the catfish is comprised of two discrete granule cell nuclei; a lateral (EGl) and medial (EGm) eminentia granularis. Previous studies have demonstrated that these two areas receive afferent nerve fiber input from lateral line (mechanosensory and electrosensory) and octaval nerves respectively. Axons of granule cells in the eminentiae form a portion of the cerebellar crest that projects back over the medullary octavolateralis nuclei and modulates the activity of ascending medullary sensory neurons. The purpose of this study is to determine other sources of afferent input to the eminentia granularis

Channel catfish were anesthetized with MS-222 a nd the divisions of the eminentia granularis were labelled with either horseradish peroxidase or DiI. After suitable transport times the brains were sectioned and viewed via light or fluorescence microscopy.

The bilateral eminentiae granularii are heavily interconnected by commissural fibers that decussate through a deep cerebellar commissure dorsal to the cerebral acqueduct. Additional afference arises bilaterally from the inferior olivary nuclei and two basal medullary nuclei. One of these nuclei is located in the posterior brainstem dorsal to the inferior olive, the other is positioned more rostrally and laterally. In the metencephalon both the nucleus praeeminentialis and the nucleus of the medial longitudinal fasciculus provide bilateral afference to the EG.

The eminentia granularis is part of a descending central control system that provides gain control and adaptive filtering to the octavolateralis sensory systems. Our results indicate several new sources of afference that presumably play a role in modulating the flow of ascending information. These sources may provide proprioceptive and somatosensory reafferent input to the brain which is then filtered to increase the signal to noise ratio in the sensory lemniscal system.

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579.8

MULTIPOLAR INTRAZONAL NEURONS IN THE MORMYRID ELECTROSENSORY LATERAL LINE LOBE: MYELINATED DENDRITES AND RECIPROCAL SYNAPTIC CONNECTIONS INVOLVED IN CENTER SURROUND INHIBITION. J. Meek[1], K. Grant[2]* and T.H.M. Hafmans[1] [1]Department of Anatomy and Embryology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands, [2]Institut Alfred Fessard, CNRS, 91190 Gif sur Yvette, France

The mormyrid electrosensory lateral line lobe (ELL) compares primary sensory electroreceptive input with electromotor command feedback to localize objects in the environment of the fish. To investigate the neuronal and synaptic basis of the underlying sensorimotor integrative mechanisms, we injected neuroanatomical tracers into the ELL (phaseolus-leucoagglutinin and Biotinylated Dextran Amine), analyzed the labeled neurons and terminals light- and electronmicroscopically, and extended the results with GABA immunohistochemistry. Tracer injections in the deep layers of the ELL resulted in the labeling of large and smaller neurons in the ipsi- and contralateral ELL, corresponding to the intrazonal neurons described previously (Bell CC, Finger TE and Russell CJ, 1981, Exp Brain Res 42:9-22). The large neurons exclusively have myelinated processes: a single one with clear axonal features and a number of others probably representing myelinated dendrites. They are GABAergic, have only a few synaptic terminals on their cell bodies, and make contacts with gap-junction-like specializations on granule- and intermediate layer cells. These contacts might well allow for reciprocal signal transport, i.e. excitation of the intrazonal neurons by primary afferents via the granule- and intermediate layer cells, and inhibition of the latter neurons by intrazonal neuron activity. Thus, they probably subserve the center-surround inhibition described by Bell on the basis of intracellular unit recordings (1990, J Neurophysiol 63:304-318).

579.9

THE DEVELOPMENT OF THE ELECTROSENSORY LATERAL LINE LOBE IN THE CATFISH *AMEIURUS NEBULOSUS* (SILURIFORMES: SILUROIDEA). M.J. Lannoo* and S.J. Lannoo. Muncie Center for Med. Educ., Indiana Univ. Sch. of Med., Muncie, IN 47306

Within electrosensory siluriform teleosts, generalized, passively electroreceptive species (siluroids) form an electrosensory lateral line lobe (ELL) consisting of one somatotopic map. Derived species (gymnotoids) are actively electroreceptive with an ELL composed of four somatotopic maps. This system is an experimentally accessible model for the study of multiple map formation. The development of the gymnotoid four map nucleus has been described previously. Here, we begin to describe the development of the one map siluroid nucleus for comparison with the gymnotoid four map system. *Ameiurus* embryos and larvae were staged according to Armstrong and Child, in which hatching occurs at stage 43. Using nissl-stained histological sections of *Ameiurus* larvae, juveniles, and adults cut at 6 μm in transverse and sagittal planes we report that: 1) the ELL can be distinguished as a subpial, laterally positioned cellular region located dorsal to the central projection of the unfused anterior ganglion by stage 35, the youngest stage in our sample; 2) the ELL cellular layers develop from the extreme dorsolateral periventricular region; 3) the ELL molecular layer begins to form by stage 45; 4) the ELL can be distinguished from the (mechanosensory recipient) nucleus medialis by stage 50; and 5) lamination of ELL cellular layers is visible by stage 53. Supported by NIH grant NS 30702, to M.J.L.

COMPARATIVE NEUROANATOMY: CHEMICAL ANATOMY

580.1

COMPARATIVE LOCALIZATION OF NITRIC OXIDE SYNTHASE IN THE VERTEBRATE SPINAL CORD. G. Brüning* Department of Anatomy, Free University of Berlin, D-14195 Berlin, Germany.

The localization of nitric oxide synthase was investigated in the spinal cord of mouse, chicken, turtle, frog and goldfish by NADPH diaphorase histochemistry. Specificity of the histochemical detection method was verified by colocalization with an antiserum against nitric oxide synthase purified from porcine cerebellum. In all vertebrate species, the motoneurons were, as a rule, unstained. Strongly stained neurons were concentrated in the dorsal horn. Widespread areas of the dorsal horn were filled by a dense terminal plexus which appeared to be formed chiefly by intrinsic neurons as dorsal root fibers were mostly negative. Intensely stained neurons were also scattered in the intermediate grey and the ventral horn. In the mouse, intensely positive neurons were clustered in the preganglionic sympathetic and parasympathetic nuclei. In the corresponding cell columns of nonmammalian species, stained neurons were detected only occasionally. In summary, these results indicate that, despite certain species differences, there is a considerable conservation in the expression of nitric oxide synthase in the spinal cord, suggesting that nitric oxide may influence sensory information processing throughout vertebrate species.

580.2

DISTRIBUTION OF THE SUPEROXIDE DISMUTASE 1 (SOD1) IN THE MAMMALIAN NERVOUS SYSTEM. CA Pardo*[1], Z-S Xu[2], DW Cleveland[2], and DL Price[1,3,4]. Departments of [1]Pathology, [2]Biological Chemistry, [3]Neurology, and [4]Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

SOD1, an enzyme participating in the free radical scavenger system, protects against oxidative stress. Recent evidence indicates that some cases of familial amyotrophic lateral sclerosis are linked to mutations in SOD1, but it is not clear how these mutations cause degeneration of motor neurons. A first step in understanding these processes is to define the cellular distributions of SOD1 in the nervous system. To this end, we have produced new specific antibodies to be used in Western blot and immunocytochemical studies. In human, monkey, baboon, rat, and mouse, SOD1 immunoreactivity (SOD1-IR) was identified primarily in neurons in the CNS and PNS. In spinal cord, SOD-IR was present in motor neurons, interneurons, and diffusely in the substantia gelatinosa. Immunoelectron microscopic analysis disclosed that SOD1 was localized predominantly in the perikaryon, axonal and dendritic compartments, and euchromatic areas of the nucleus, and there was evidence of different levels of SOD1-IR among cell groups. In the hippocampal formation, neurons of the CA3-CA4 sectors were intensively SOD1 immunoreactive, whereas CA1 neurons showed less immunoreactivity. SOD1-IR was also demonstrated in large- and medium-sized pyramidal neurons of neocortex, subsets of neurons in pyriform cortex, amygdala, striatum, and thalamus. These findings indicate that SOD1 is present in many neuronal populations throughout the nervous system and that certain subsets of nerve cells, including motor neurons in spinal cord and brainstem, are enriched in this enzyme.

580.3

NITRIC OXIDE SYNTHASE IN THE LOCUS COERULEUS OF THE CHICK: DEVELOPMENT, DISTRIBUTION AND LACK OF CORRELATION WITH TRANSMITTER PHENOTYPES. A. Schober[1,2], M. Bothwell[1], and C.S. von Bartheld[1]*. [1]Department of Physiology & Biophysics (SJ-40), University of Washington, Seattle, WA 98195; [2]Department of Anatomy & Cell Biology, University of Heidelberg, D-69120 Heidelberg, FRG.

The distribution and morphology of nitric oxide synthase (NOS)-containing neurons was investigated in the locus coeruleus complex (LC) of chick embryos. The NADPH-diaphorase technique was used to detect the presence of NOS at the incubation days E8, E12, E18 and 14 days posthatch (P14). The first NOS expression in LC was found at age E12. The number of NOS-positive cells in the locus coeruleus proper (LoC) increased from E12 to E18 (450 to 850) and decreased slightly after hatching (P14). In the locus subcoeruleus (SC), NOS-containing neurons could be identified first at age E18. The number of NOS-expressing neurons increased from E18 to P14 (70 to 270). To determine which classical transmitter phenotype may be coexpressed with NOS, adjacent sections were immunolabeled for choline acetyltransferase (ChAT), for somatostatin (SOM) and for the low-affinity neurotrophin receptor (P75) as a marker for noradrenergic LC neurons (von Bartheld and Bothwell, 1992, J.Comp.Neurol. 320:479-500). In addition, NADPH-diaphorase histochemistry was combined with immunocytochemistry of P75, ChAT and SOM in the same sections. NOS is not colocalized with the P75 receptor (noradrenergic cells) or SOM, and only few cholinergic cells (<10%) were double-labeled with NOS. In birds, NOS is an excellent marker for a major subpopulation of LC neurons. None of the classical neurotransmitters examined is colocalized in NOS-positive LC neurons. These results indicate that major differences exist between species, as the rat LC contains only few NOS-positive cells (Xu et al.,1994, Exp. Brain Res. 98:75-83). The expression of NOS may indicate a role of nitric oxide in developmental and physiological aspects of the LC in the chick brain.

Supported by DFG grant Scho 489/1-1 and NIH grants NS 30305 and HD 29177.

580.4

DISTRIBUTION OF CATECHOLAMINE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE LIZARD *ANOLIS SAGREI*. A Rehn, P Ekström and T Östholm*, Dep. of Zoology, Univ. of Lund, Sweden.

Adults of the "brown anole", *Anolis sagrei* (fam. Iguanidae, order Squamata) caught in Miami, Florida, USA and juveniles obtained from our own breeding colony were used in this study. Immunohistochemical methods with antibodies against the transmitter dopamine (DA) and the synthetic enzymes, tyrosine hydroxylase (TH), dopamine β–hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) were used to locate the distribution of catecholamine neurons in the anole CNS. Catecholaminergic neurons were observed in all parts of the brain. In the olfactory bulb, TH and DA immunoreactive (ir) neurons were observed in the glomerular layer and the external plexiform layer. In the telencephalon, THir neurons were seen in the nucleus septalis medialis. In the diencephalon, THir and DAir neurons were present in the periventricular preoptic area, the periventricular hypothalamic nucleus and the lateral hypothalamic area. CSF-contacting neurons in the periventricular hypothalamic organ were only DAir. THir neurons were present in the posterodorsal nucleus. Large groups of catecholaminergic neurons were found in the mesencephalon. THir and DAir neurons were located in the ventral tegmental area, the substantia nigra and the presumed reptilian equivalent of the mammalian A8 group. THir neurons were seen on the both sides of the cerebral aqueduct. In the rhombencephalon, THir, DAir and DBHir neurons were found to exist in the isthmic region, the nucleus of the solitary tract and in the area postrema. THir, DAir and PNMTir neurons were observed in the lateral part of the reticular formation. In the spinal cord, THir and DAir CSF-contacting neurons were present in the ventral wall of the central canal. The distribution of catecholaminergic neurons in *A. sagrei* is generally similar to that in previously studied reptiles (*Anolis carolinensis, Gekko gekko, Varanus exanthematicus, Chameleon ssp, Pseudemys scripta elegans and Python regius*). However, a number of interspecific differences were observed.

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580.5

DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE BRAIN OF A GYMNOPHIONAN AMPHIBIAN.
A. Gonzalez*, M. Munoz, A. Munoz, O. Marin and W.J.A.J. Smeets. Dept. Cell Biology, Fac. Biology, Univ. Complutense of Madrid, Spain and Research Inst. Neurosci. Vrije Univ. Amsterdam, The Netherlands.

The aim of the present study was to extend our knowledge of catecholamine systems (CA) in amphibians to the limbless amphibians, i.e. the gymnophionans. For that purpose, the distribution of CA neuronal structures in the permanent aquatic gymnophionan *Typhlonectes compressicauda* was studied by means of tyrosine hydroxylase (TH)-immunohistochemistry. A total of 14 TH-immunoreactive (THi) cell groups were found. They are located in the olfatory bulb, preoptic area, hypothalamus, pretectum, mesencephalic-isthmic- and rhombencephalic tegmental areas, the nucleus of the solitary tract, the spinal cord and the retina. In addition, elaborated plexusses of THi fibers occur throughout the brain. Compared to the distribution patterns observed in anuran and urodele species, major differences were observed in the innervation of the pallial regions, the septum and the mesencephalic tegmentum. Nevertheless, when the distributional patterns of TH immunoreactivity of representatives of the three amphibian orders (Anura, Urodela and Gymnophiona) are compared with each other, it is obvious that there exists a basic pattern. Moreover, the organization of the CA in *Typhlonectes compressicauda* shares many features not only with other anamniotes, but also with amniotes.

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580.6

THE DISTRIBUTION OF GABA-LIKE IMMUNOREACTIVITY IN THE MIDBRAIN OF THE ATLANTIC STINGRAY.
R.B. Leonard*, R.L. Puzdrowski and G.A. Kevetter, Depts of Anat. & Neurosci, Physiology & Biophysics, Otolaryngology and Marine Biomedical Institute, Univ. TX Med Branch, Galveston, TX. 77555

As part of a study of projections to and from the midbrain in elasmobranch fishes, we have begun to examine the distribution of neurotransmitters within the brainstem of the Atlantic stingray (*Dasyatis sabina*). Because there are some theories about the distribution of inhibitory neurotransmitters within the brain, we have begun our study by examining the distribution of GABA. Our most consistent results to date have been obtained using a polyclonal antiserum against GABA conjugated to BSA (Sigma) combined with the ABC (Vector) method. Although we have not undertaken specificity controls in this species, specificity is suggested by the staining observed in other areas (e.g., cerebellum, and cerebellar crest). A large number of stained cells are observed in the optic tectum. The density of stained cells is greatest superficially, dropping to essentially zero in the deepest layers. Stained cells extend forward into portions of the pretectal area. There is a high density of stained cells in the medial mesencephalic complex, particularly in the ventral portion. In contrast, few stained cells are observed in the lateral mesencephalic nucleus. The exception is a population that extends laterally from the medial mesencephalic complex that appears to overlap the ventral portion of the lateral mesencephalic complex. Labeled cells are scarce in other areas of the midbrain. Labeled axons and terminals are numerous in the optic tectum, particularly in the superficial layers. Labeled terminals are observed around motoneurons in both the dorsal and ventral subdivisions of the oculomotor nucleus. Terminal label is also present in the Edinger-Westphal nucleus, is sparse in the periaquaductal grey and may be present in the red nucleus. Supported by grants from the NIH, DRF and Sealy Foundation.

580.7

LHRH AND FMRF-AMIDE IMMUNOREACTIVITY IN THE GREEN TREEFROG.
W. Wilczynski* and R. G. Northcutt. Dept. of Psychol., Univ. of Texas, Austin, TX 78712 and Dept. of Neurosci., UCSD, La Jolla, CA 92093.

Telencephalic gonadotropin releasing hormone cells are thought to be the principal regulators of gonadotropin, and hence sex steroid, secretion in vertebrates (Muske, BBE, *42*:215-230, 1993). In some vertebrates, these cells are immunoreactive for both mammalian LHRH and FMRF-amide. We used standard immunocytochemistry methods to visualize these peptides in male *Hyla cinerea*. LHRH-ir cells were seen only in the medial septum and in a cluster rostral and dorsal to the anterior preoptic area (POA). LHRH-ir fibers appeared outside these areas in the medial and dorsal pallia, lateral septal nucleus, and nucleus accumbens. A few fibers extended to the olfactory bulbs and dorsal lateral pallium. Caudally, LHRH-ir fibers traveled periventricularly through the POA and in the medial forebrain bundle (MFB). Some fibers left the MFB to travel dorsally to the habenula. Most MFB fibers continued caudally to pretectal levels, where LHRH-ir fibers were also seen in the posterior tuberculum and dorsal hypothalamus. This system eventually formed a dense subpial plexus beneath the ventral hypothalamus. Some fibers continued caudally in the lateral and ventral midbrain fiber tracts. The periventricular system diminshed caudal to the POA, but remained medial to the posterior tuberculum and dorsal hypothalamus and continued through the midbrain around the cerebral aqueduct and in the laminar toral nucleus. Caudal to the isthmis, only a few fibers remained beneath the 4th ventricle above the raphe nuclei. FMRF-amide immunoreactivity was much more extensive. Cells were present in the medial septum and POA where LHRH-ir cells were seen, as well as in the medial pallium, suprachiasmatic nucleus, posterior tuberculum, and dorsal hypothalamus. FMRF-amide-fibers were seen in all areas expressing LHRH-ir fibers, plus in a dense periventricular bundle adjacent to the ventral hypothalamus, in the deep layers of the optic tectum, and scattered in the diencephalon, midbrain, and reticular areas of the medulla as far caudal as the nucleus solitarius. (Supported by NIMH MH45350 and NIH NF24869.)

580.8

DISTRIBUTION OF IMMUNOREACTIVE GLUCOCORTICOID TYPE II RECEPTOR STAINING IN THE BRAIN OF *Anolis carolinensis*.
Cliff H. Summers*, Amy-Lacy Adams, Jennifer Anderson, and Gary Ten Eyck. Department of Biology, University of South Dakota, 414 E. Clark, Vermillion, SD 57069-2390.

Brains of the lizard *Anolis carolinensis* were either frozen in tissue freezing medium or dehydrated, cleared, and embedded in wax. Following sectioning, 5 - 12 µ, brains were incubated at 4°C for 48 h with a polyclonal rabbit-anti-human glucocorticoid type II receptor antibody (Affinity Bioreagents). This antibody was raised against amino acids 346 - 367 from the amino terminus of the human glucocorticoid receptor. Receptor staining was accomplished by diaminobenzidine reaction to a biotinylated secondary antibody (goat-anti-rabbit). Most immunoreactive staining was localized in cell nuclei.

The area with the greatest concentration of glucocorticoid immunoreactive staining was the anterior dorsal ventricular ridge. This area has also been referred to as the hypopallium and hyperstriatum anterius in lizards. Staining is heavy at and adjacent to the ventral margins of the lateral ventricles at the level of the optic chiasm and epiphysis. More anteriorly, dense staining was seen in the margin of the anterior dorsal ventricular ridge between the lateral and olfactory ventricles. Staining was also prevalent in cells of the medial cortex, also referred to as the hippocampal cortex or hippocampus pars dorsomedialis.

580.9

DISTRIBUTION OF LAMPREY GONADOTROPIN- RELEASING HORMONE-III IN LAMPREY BRAINS.
S. A. Sower*[1], M. Nozaki, A. Gorbman, J.H. Youson and S.A. Tobet[2]. [1]Dept. of Biochem.& Molec. Biol.,Univ. of N.H., Durham, NH, 03824 and [2]Prog. Neuroscience, Shriver Center, Waltham, MA 02254 and Harvard Medical School, Boston MA 02115.

Two immunoreactive forms of gonadotropin-releasing hormone (GnRH), lamprey GnRH-I and lamprey GnRH-III, were found in the neurons in larval and adult sea lamprey, *Petromyzon marinus*. Using antisera preferentially directed against either lamprey GnRH-I or -III, dense reaction product was seen in cell bodies in the rostral hypothalamus and preoptic area. Reaction product was also dense in fibers to and within the neurohypophysis, in addition to numerous fibers which projected caudally, beyond the neurohypophysis through the mesencephalon. The majority of immunoreactive GnRH in larvae was lamprey GnRH-III, and when lamprey GnRH-I was seen, it was in cells that appeared to contain both forms of GnRH. A small number of cells were found in the caudal hypothalamus of larvae but not adults. These cells contained only immunoreactive lamprey GnRH-III and may constitute a functional subgroup within the population of GnRH neurons. In animals undergoing metamorphosis there was a large increase in reaction product in all GnRH containing cells and fibers. The results are consistent with the hypothesis that lamprey GnRH-III is an important form of GnRH during the maturation of GnRH cells and fibers, and further indicates that these cells have attained their normal positions in the preoptic area and hypothalamus before metamorphosis. (Supported by Great Lakes Fishery Commission, and NSF DCB-9004332 and -8904919).

580.10

MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES IN THE PIGEON TELENCEPHALON: DISTRIBUTION OF PIRENZEPINE-SENSITIVE AND AF-DX 116-SENSITIVE SITES.
E.C.Kohler*, #W.S.Messer, Jr. and V.P.Bingman. Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403 and #Dept. of Medicinal & Biological Chemistry, College of Pharmacy, University of Toledo, Toledo, OH 43606.

There is evidence suggesting the existence of at least five subtypes of muscarinic acetylcholine receptors (mAChRs). Furthermore, autoradiographic studies have demonstrated a heterogeneous distribution of mAChR subtypes in the mammalian brain. Data from studies on birds (Kohler et al., Nsci Abstr. 19:1131, '93; Tietje et al., J. Biol. Chem. 266:17382, '91 & 265:2828, '90) suggest that there may also be more than one type of mAChR in the avian brain. Here we examine the possibility of multiple mAChRs by investigating the binding of [^{3}H]-(R)-quinuclidinyl benzilate ([^{3}H]-(R)-QNB) and the ability of the M_1 receptor antagonist pirenzepine (PZ) or the M2 receptor antagonist AF-DX 116 (AF) to reduce the binding of [^{3}H]-(R)-QNB in the pigeon telencephalon. Autoradiograms indicate high densities of mAChRs in dorsal archistriatum, combined hyperstriatum dorsale and ventrale, parolfactory lobe, and paleostriatum augmentatum (490-620 fmoles/mg protein) and low densities of mAChRs in nucleus basalis (Bas), ectostriatum (Ecto), and paleostriatum primitivum (275-380 fmoles/mg protein). An ANOVA indicated that sites with significantly higher affinity for PZ (vs. AF; suggesting relatively higher M1 density) were found in most telencephalic regions. These analyses also indicated that sites in Ecto and septum showed no significant difference in affinity for PZ or AF while sites with significantly higher affinity for AF (vs. PZ; suggesting relatively higher M2 density) were found in Bas. These data will be useful in determining some of the specific roles of the cholinergic system in studies of avian behavior. Current investigations include the role(s) of putative mAChR subtypes in avian learning and memory. This work was supported in part by NS 01493.

580.11

DISTRIBUTION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE FOREBRAIN OF THE TURTLE CHRYSEMYS PICTA. A.S. Powers* and M. Rodriguez. Dept. of Psychology, St. John's Univ., Jamaica, NY 11439.

Neuropeptide Y (NPY), a 36-amino-acid peptide, has been shown to be localized in neurons of the cerebral cortex, striatum, and amygdala of mammals. The present study examined its distribution in the forebrain of the painted turtle (Chrysemys picta). We used fluorescence immunohistochemistry to visualize the NPY cells and fibers. Sections were incubated in a polyclonal primary antibody against synthetic NPY made in rabbit (Accurate) and then in a biotinylated anti-rabbit secondary, followed by avidin conjugated to the fluorophore FITC. In the telencephalon, NPY-positive cells were found in the tuberculum olfactorium, in the medial, dorsomedial, dorsal, and lateral cortices, and in the dorsal ventricular ridge. NPY-positive cells were also found in the paleostriatum augmentatum (equivalent to the striatum), in area d (the reptilian equivalent of nucleus accumbens), and in the amygdala. Few cells were seen in globus pallidus or in the septal area. NPY-positive fibers were widely distributed throughout the telencephalon, but were especially prominent in the molecular layer of the medial and dorsomedial cortices. In the diencephalon, NPY-positive cells were seen only in the dorsal nucleus of the supraoptic decussation, which is equivalent to the thalamic reticular nucleus of mammals. Fibers were present in the hypothalamus, including a dense plexus in the nucleus ventromedialis hypothalami. These results are similar to those seen in mammals, especially in the telencephalon, and together with results of studies of other nonmammals, show continuity in forebrain systems during the course of evolution. Supported by NS31785 to ASP.

BRAIN METABOLISM AND BLOOD FLOW: BASIC MECHANISMS II

581.1

CEREBRAL BLOOD FLOW MEASUREMENT USING RADIOACTIVE Xe^{133} UNDER PENTOTHAL AND FENTANYL ANESTHESIA. A.Elrifai*, J.Bailes, S.Govindan, E.Teeple, M.Taylor, S.Shih, M.Adatepe and J.Maroon. Allegheny General Hospital, Allegheny-Singer Research Institute and The Medical College of Pennsylvania, Pittsburgh, PA 15212.

rCBF was measured twice in six dogs within one week. The first measurement was done while animals were sedated using Pentothal, a short acting barbiturate, 20 mg/kg and in the second Fentanyl was titrated as 50 μg/dose for a maximum of 10 doses. The rCBF was measured using a modified NOVO 10a Cerebrograph using two detectors on each side; the remaining detectors were shielded. Animals were sedated, endotrachealy intubated & connected to the cerebrograph. ECG, heart rate, respiration and tidal volume were monitored. The end tidal PCO_2 was kept at 35-40 mmHg. Radioactive Xe^{133} (Dupont Pharmaceuticals) was mixed with low dissolved oxygen saline (LDO). Following registration of background activity for 0.5 min, a bolus of Xe^{133} in saline was injected in a cephalic vein. Xe^{133} clearance was recorded throughout 11 minutes and expired air was monitored to supply the air curve. CBF results were expressed in ml/100gm/min as the initial slope index between 30 and 90 sec, as CBF15 and as the fast compartment flow F1. Corrections for hemoglobin and CO_2 were not instituted. Hemispheric CBF was treated as the average value of the 2 detectors on each side & an average for each animal was calculated. ANOVA showed a difference in rCBF between the 2 measurements as described in ISI, CBF15, and F1. There were no differences in PCO_2, hemoglobin levels or the injected Xe^{133}.

581.2

RELATION BETWEEN CYCLIC GMP GENERATION AND CEREBROVASCULAR REACTIVITY: MODULATION BY NPY AND α-TRINOSITOL J. P. You, W. H. Zhang, I. Jansen, D. Erlinge* and L. Edvinsson Dept. of Exp. Res, Malmö General Hospital, S-214 01 Malmö, Sweden

The present study examines the possible correlation between cerebrovascular vasodilator agents and generation of cGMP in guinea pig cerebral vessels. Acetylcholine (ACh), substance P (SP), nitroglycerine (NTG) and sodium nitroprusside (SNP) not only elicited concentration-dependent relaxation of basilar artery segments; but also significantly increased the generation of cGMP of cerebral vessels. Neuropeptide Y (NPY) increased the generation of cGMP by 2%-46% of control levels (at 10^{-7}-10^{-6}M of NPY; * $p<0.05$). In addition, NPY (10^{-6}M) induced a transient relaxation of the precontracted guinea pig basilar arteries with endothelium. This transient relaxation was blocked by nitro-L-arginine (NOLAG 10^{-4}M). α-trinositol did not alter the formation of cGMP. In the presence of α-trinositol NPY (10^{-7}-10^{-6}M) did not significantly elevate the production of cGMP. The rise in cGMP induced by ACh, SP and NTG was slightly increased by the addition of NPY ($3x10^{-7}$M). ACh and SP induced an endothelium-dependent relaxation of the precontracted guinea pig basilar arteries, while SNP and NTG induced an endothelium-independent relaxation. ACh, SP and NTG induced concentration-dependent relaxations of basilar artery, respectively. This relaxation elicited by ACh or SP, but not NTG, was markedly attenuated by NPY ($3x10^{-7}$M). This inhibitory effect of NPY on vasomotor responses was completely reversed by α-trinositol (10^{-6}M). In conclusion, there is a close correlation between relaxation and cGMP formation induced by ACh, SP, NPY, SNP and NTG. The relaxant response to ACh, SP and NPY are mediated via the release of endothelium-derived relaxing factor/nitric oxide (EDRF/NO), while SNP and NTG act directly on the guanylyl cyclase. The inhibitory effect of NPY on endothelium mediated relaxation by ACh and SP are not mediated via cGMP, but inhibited by α-trinositol.

581.3

SODIUM CYANIDE MICROINJECTED INTO THE ROSTRAL VENTROLATERAL MEDULLA (RVL) ELEVATES CEREBRAL BLOOD FLOW (rCBF) AND EXCITES CEREBRAL CORTICAL "VASODILATOR" NEURONS. E.V. Golanov* and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. College, New York, NY 10021.

Sympathoexcitatory reticulospinal neurons of RVL are central oxygen detectors rapidly and reversibly excited by ischemia, hypoxia, or NaCN (Sun and Reis, *J. Physiol.* 476:101, 1994). As a consequence, they increase sympathetic nerve activity and arterial pressure (AP) (Sun and Reis, *Am. J. Physiol.* 266:R245, 1994). The facts that systemic hypoxia increases rCBF without metabolism, stimulation of RVL (Underwood et al., *J. Cereb. Blood Flow Metab.* 12:844, 1992) replicates this response, and bilateral lesions of RVL reduce, by over 50%, hypoxic vasodilation (Underwood et al., *Brain Res.* 635:217, 1994) suggests that hypoxic stimulation of RVL neurons may increase rCBF. To test the hypothesis, NaCN was microinjected (150 pM, 5 nl) into RVL of anesthetized rats. CN rapidly (<2 sec), site-specifically, dose-dependently and reversibly increased rCBF (measured by laser-Doppler flowmetry) to 21±6% ($p<0.01$, n=8) and AP to 138±13 mmHg ($p<0.05$). After spinalization, NaCN only increased rCBF. CN also increased the numbers of cortical burst-rCBF wave complexes (Golanov and Reis, *Am. J. Physiol.* 266:R204, 1994) and excited neurons in lamina V which discharge during the negative potential of spontaneous or RVL-evoked burst-wave complexes ("vasodilator neurons") (Golanov and Reis, *Am. J. Physiol.*, in press). The cerebrovascular and neuronal responses were blocked by intracortical tetrodotoxin. We conclude that hypoxic excitation of O_2-sensitive RVL neurons in RVL increases cortical rCBF and activates "vasodilator" neurons of cerebral cortex. The results add support to the hypothesis that hypoxic cortical vasodilation is, in part, neurogenic resulting from reflexogenic activation of a pool of cortical neurons by oxygen-sensitive neurons in RVL.

581.4

RESPONSES OF CEREBRAL COLLATERAL VESSELS TO INHIBITION OF ATP-SENSITIVE POTASSIUM CHANNELS. C.A. Mazzochi, J.S. Gerdes, M.G. Muhonen, S.C. Robertson* and C.M. Loftus. Div. of Neurosurgery, Univ. of Iowa College of Medicine, Iowa City, IA 52242 and Veterans Affairs Medical Center, Iowa City, IA 52246.

The mechanism of collateral vasodilatation following cerebral arterial occlusion is not completely understood. The canine cerebral vasculature was investigated for the presence of ATP-sensitive potassium channels, and effects of an inhibitor to these channels as they relate to collateral blood flow.

In eight mongrel dogs a left sided craniotomy was performed with temperature controlled artificial cerebrospinal fluid (aCSF) superfused over the brain. Normal cerebral artery pressure was measured with a glass micropipette. A branch of the middle cerebral artery (MCA) was cannulated and back pressure measured. Collateral-dependent tissue was identified using the "shadow flow" technique. Glibenclamide (10^{-5}M) was added to the aCSF. Regional cerebral blood flow (rCBF) to subcortical cerebrum was measured with radioactive microspheres.

Baseline blood flow to ipsilateral normal cerebrum was 98 ± 16 ml/100g·min^{-1} and did not change during glibenclamide. Flow to collateral-dependent tissue was 93 ± 13 ml/100g·min^{-1}, and 68 ± 7 ml/100g·min^{-1} following glibenclamide ($p<0.05$). The attenuation of collateral blood flow was associated with a profound decrease in cannulated MCA branch back pressure. Pressure in the normal MCA branch was unchanged.

When ATP-sensitive potassium channels, which probably participate in compensatory vasodilatation were inhibited, a reduction in rCBF occurred in collateral-dependent tissue, but not in normal cerebrum. This would imply that ATP-sensitive potassium channels are involved in collateral vasodilatation following MCA occlusion, but may not be involved in maintenance of resting basal tone of cerebral arteries.

581.5

STIMULUS PARAMETERS INFLUENCE MAGNITUDE AND TIME COURSE OF OPTICAL INTRINSIC RESPONSES IN RODENT BARREL CORTEX A.J. Blood*,S.M. Narayan, A.W. Toga , Laboratory of Neuro Imaging, UCLA School of Medicine, Los Angeles, CA 90024.

Optical imaging of intrinsic signals was performed in primary somatosensory cortex of the rat during single whisker deflections of varying frequencies and durations, as well as during competitive stimulation of forelimb and whisker. A dose-response relationship was shown between whisker stimulus parameters and the intensity and spatial/temporal characteristics of the optically recorded intrinsic signal. When frequency was constant, longer durations of stimulation resulted in larger magnitude responses, as determined by pixel intensity in a statistically defined region of interest. Similarly, when duration was constant, higher frequencies of stimulation resulted in larger magnitude responses. In addition, greater numbers of deflections per trial resulted in higher magnitude responses than relatively smaller numbers of stimuli. Conversely, there were no differences in response magnitude between different combinations of frequency and duration which had in common the same number of stimuli. Temporally, there is evidence that the time between stimulus onset and peak response is influenced by frequency, with higher frequency stimulation resulting in a faster time-to-peak. Registration with certain aspects of vascular anatomy and stereotaxic coordinates demonstrated that intrinsic responses were well spatially localized with both types of fiducial points. Electrophysiological recordings demonstrated that optical response regions were coincident with areas of neuronal activity, although the time course of optical responses was slower than that of neuronal firing. Finally, competitive forelimb stimulation caused decreases in whisker response magnitude, and the intensity of forelimb stimulation determined the degree to which the whisker response decreased. Thus, both whisker stimulus parameters and introduction of competitive stimuli are critical variables in determining the magnitude and/or timing of optical intrinsic responses in rat barrel cortex.

581.6

FUNCTIONAL NEURAL-VASCULAR CONGRUENCE OVER SOMATOSENSORY CORTEX

S. M. Narayan*, A.J.Blood and A.W.Toga
Laboratory of Neuro Imaging, Dept of Neurology, UCLA, Los Angeles CA.

We examined the relationship between cerebral blood volume (CBV) and activation of primary somatosensory cortex (S-I) in the rat, using plasma-fluorescence, optical intrinsic signals and single unit recordings. Cortices of urethane-anesthetized male Sprague-Dawley rats were exposed, and fluorescent Texas Red Dextran dye (MW 70,000) given i.v. Vibrissal deflection or foredigit electroshock were associated with dye-related fluorescence increases over contralateral posteromedial barrel subfield or forelimb S-I. Fluorescence change foci overlay areas of increased cortical layer III cell firing on single unit recordings. Surface boundaries of even the smallest foci overspilled the electrophysiologic center receptive field. Larger stimuli caused fluorescence foci to enlarge considerably while the receptive field did so only slightly, increasing this spatial discrepancy. Fluorescence change was delayed and prolonged, suggesting that CBV increases at 1-1.5 s, and peaks 2-2.5 s after the onset of 1-2 s stimulation in both regions. Interleaved optical intrinsic studies revealed reflectance decreases between 550-850 nm, with similar timing and location to fluorescence increases after identical stimuli.

Thus, we observed delayed increases in vascular dye-fluorescence (related to CBV) over activated cortex. The striking similarity between optical reflectance and fluorescence suggest that changes in intrinsic cortical reflectance are strongly related to changes in CBV.

581.7

BLOOD FLOW RESPONSES IN THE RAT BARREL CORTEX DURING WHISKER STIMULATION. D. Liu, J. Dowling, M.E. Spence, C. Royainen, and T. Woolsey*, Dept. of Neurol. and Neurol. Surg. and of Cell Biol. and Physiol., Washington Univ. School of Medicine, St. Louis, MO 63110.

Arteriovenous transit times (AVTTs) of 0.001% FITC-dextrans were measured by videomicroscopy through a closed cranial window to examine blood flow responses during whisker row and single whisker stimulation. Areas of cortical activity during stimulation were targeted with optical imaging of "intrinsic" responses. Whiskers were stimulated at 4-5 Hz for 1 min. Whisker row stimulation produced a mean AVTT decrease of 24.0% (n=24, SD±11.61, $p<0.0001$), whereas single whisker stimulation resulted in a mean decrease of 16.5% (n=68, SD±9.0, $p<0.0001$). Single whisker stimulation produced a significantly smaller decrease AVTT ($p<0.001$) in vessel pairs studied. Decreases in AVTTs could be detected within 5 seconds of the onset of whisker stimulation. Recovery to baseline values occurred within 5-10 seconds after stimulation ceased. These studies show that targeted measurement of AVTTs can be used for measurements of quantitative localized changes in blood flow in response to natural stimuli.

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581.8

WHISKER STIMULATION INCREASES BLOOD FLOW AND GLUCOSE UTILIZATION IN THE TRIGEMINAL PATHWAY OF AWAKE RAT. C. Royainen*, L. Wei, J. Fenstermacher, T.Woolsey. Dept. of Neurol. Neurol. Surg., & Dept. Cell Biol. Physiol., Washington Univ., Sch. Med., St. Louis, MO 63110, and Dept. Anesthesiology, Henry Ford Hospital, Detroit, MI 48202

Local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU) in response to natural stimulation of the trigeminal (whisker) pathway were investigated in conscious rats. LCBF and LCGU in the trigeminal (V) pathway were measured autoradiographically by ^{14}C-iodoantipyrine and by 2-deoxyglucose respectively. Structures measured in the trigeminal pathway were: V nerve and tract; V motor root and nucleus; V spinal nuclei and principal nucleus; ventrobasal thalamus and barrel cortex. LCBF and LCGU were compared bilaterally after mechanical stimulation of the right whiskers. Whisker stimulation increased LCBF 1.29-1.95 X and LCGU 1.27-1.76X in all sites of gray matter along the trigeminal pathway. The greatest increases of LCBF and LCGU occurred in left barrel cortex. LCGU values of various structures during whisker stimulation were highly correlated ($r = 0.95$) with their corresponding LCBF values. The unstimulated and stimulated sides had identical slopes indicating close coupling between LCBF and LCGU during whisker stimulation.

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581.9

COMPARISON OF NEURAL AND VASCULAR UNITS IN MOUSE BARREL CORTEX. J. Sui, C.M. Royainen, T.A. Woolsey and A.J. Cohen*. Depts. Neurol. & Neurosurg., Cell Biol. & Physiol., and Ophthal. & Vis. Sci., Washington Univ. Sch. Med., St. Louis, MO 63110.

Blood flow increases locally during stimulation of single whiskers in rodent barrel cortex. The control and precision of the vascular response depends in part on the structure of cerebral vascular units: a terminal arteriole, the capillaries it feeds and venules draining those capillaries. Vascular units were demonstrated in barrel cortex by dye injection into single arterioles. Adult mice were anesthetized and perfused with 4% paraformaldehyde in HEPES-saline from the abdominal aorta. The aim of fixation was to prevent further constriction, dilation or changes of relative resistance in the cerebrovascular network. A craniotomy was opened over the left somatosensory cortex. Pial vessels were visualized by steady perfusion at 60cm H_2O with 1% Procion Red in 150 mM NaCl. Single penetrating 7-23 µm arterioles were impaled with glass micropipettes and injected with 0.4% Patent Blue or 2% FITC-albumin. Injected markers emerged in 1-7 venules <0.5 mm from the arteriole. An arteriolar domain was defined as the polygon of the injected arteriole and its emerging venules at the pial surface. Arteriolar domains were approximately the same size as projected layer IV barrels stained for cytochrome oxidase but formed independent maps. Portions of arteriolar domains overlapped, indicating shifts in perfusion of internal capillaries. Injections of internal arteriolar branches may clarify the structure of finer capillary modules in somatosensory cortex.

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581.10

INTRINSIC CORTICAL RESPONSES IN THE RAT BARREL CORTEX WITH PROLONGED WHISKER STIMULATION. M. Henegar, J. Dowling, D. Liu, C. Royainen, T. Woolsey and R. Grubb*. Dept. of Neurosurgery and Dept. of Cell Biology and Physiology, Washington Univ. Sch. of Med., St. Louis, MO 63110

Intrinsic cortical responses were examined over the rat barrel cortex during continuous whisker stimulation. Videomicroscopy through a closed cranial window was recorded before and during stimulation of C-row and single whiskers at 4-5 Hz for 10-30 min. Computer analysis of these recordings and of "live" images revealed "intrinsic" responses localized to the appropriate areas of barrel cortex by later histology. Responses remained localized to these areas for up to 10 min., but subsequently appeared to spread. The quality of images constructed from video recordings was comparable, though slightly inferior, to live images. These studies demonstrate that "intrinsic" optical signals persist and remain localized for at least several minutes of continuous whisker stimulation. Video tape recording facilitates subsequent analysis of "intrinsic" optical responses.

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581.11

RED CELL FLUXES CHANGE WITH STIMULATION OF RAT WHISKER BARREL CORTEX. G. E. Liang, B. Gilmore, M.E. Spence, D. Liu, C.M. Royainen, T.A. Woolsey and R.E. Schmidt*. Depts. of Neurosurgery, of Cell Biology and Physiology and of Pathology, Washington University School of Medicine, St. Louis MO 63110.

Fluorescent erythrocytes (fRBCs) were used to measure blood flow changes in cerebral cortex with whisker stimulation. fRBCs (PKH2-GL, fluorescein isothiocyanate, and fluorescent beads) are long lived intravascular markers ($t_{1/2} \approx 2$ hours) permitting changes to be evaluated prior to, during and after controlled whisker stimulation. fRBCs were recorded continuously by video microscopy through a cranial window over rat whisker-barrel cortex and were counted as they emerged from cortical venules. "Optical intrinsic" signals were used to target activated barrel cortex; after the experiments vessels and barrels were mapped by histology. Stimulation of a row of whiskers promptly and significantly increased flux draining optically active cortex; flux recovered quickly to baseline after stimulus termination. Venules draining non-optically active barrel cortex have reduced or unchanged fluxes. Venule diameter did not change. Localized changes in RBC flux were consistent with previous measurements by other methods. In contrast to plasma markers, fRBCs permitted continuous evaluations over long periods and allowed selection and detailed sampling of multiple venules from images $\approx 1mm^2$.

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COGNITION IV

582.1

CORPUS CALLOSUM DEVELOPMENT IN CHILDREN. L.A.Rowe[a], J. Kranzler[a], L.J. Lombardino[b], L. Mercado[b], S.R. Browd[c], G.W. Hynd[d], O.F. Agee[e], W.G. Luttge[c] & C.M. Leonard[c]. Depts. of Education[a], Communication Processes[b], Neuroscience[c], Radiology[e], Univ. of FL, Gainesville, FL, 32610 & School of Professional Studies[d], Univ. of GA, Athens, GA 30602.

The corpus callosum (CC) can be easily visualized and measured on midsagittal magnetic resonance imaging (MRI) sections. Variations in the size of anatomically defined subregions, such as the genu, body, isthmus and splenium, have been associated with verbal fluency, language lateralization, handedness, sex, and age. In this study these subregions of the CC were measured using Witelson's method. Intra-rater and inter-rater reliability estimates ranged from 0.87 to 0.94. We investigated the development of these regions in a group of 32 control children between the ages of 5.7 and 12.5 (18 boys and 14 girls) who had received the Test of Nonverbal Intelligence (TONI, mean score = 106). The area of each region was divided by the area of the total intracranial volume to correct for overall differences in brain size. After this correction, the body of the corpus callosum was the only region that was significantly smaller in girls ($F_{1,30} = 6.42$, $p < 0.02$). The body and the isthmus were the only regions to show a significant increase in size with age (isthmus: $r = 0.42$, $p < 0.02$; body: $r = 0.39$, $p < 0.03$). In girls, the size of the genu showed a trend toward correlation with the TONI score ($r = 0.48$, $p < 0.10$). In boys, none of the subregion correlations with the TONI were greater than 0.15. We are currently studying the relation between subregion size and asymmetries in association cortex. (Supported in part by Social and Behavioral Sciences Research Grant No 12 FY93-0551 from the March of Dimes Birth Defects Foundation.)

582.2

SYLVIAN FISSURE ANOMALIES IN CHILDREN WITH LEARNING DISABILITIES. L.R. Mercado[a], S.R. Browd[b], C.M. Leonard*[b], L.J. Lombardino[a], K.K.S Voeller[c], J.J. Ross[d], K. Noffsinger[d], O.F. Agee[e]. Depts. of Communication Processes[a], Neuroscience[b], Psychiatry[c], Pediatrics[d] and Radiology[e], University of Florida, Gainesville, FL 32610.

Previous research has indicated that individuals with dyslexia have an increased incidence of multiple Heschl's gyri (MH) and anomalies in the region of the planum temporale (P) and the supramarginal gyrus (S). We analyzed these regions in 32 control children [mean age: 9.0 yrs; 19 males (M), 13 females (F)] and 31 children with language and learning disabilities [LD, mean age: 10.4 yrs; 19 M, 12 F] using thin sagittal magnetic resonance images. Three raters trained to reliability in classification schemes developed by Steinmetz (extra or missing supramarginal gyri) and Witelson [missing horizontal (H) or vertical (V) banks of the planum] identified sylvian fissure morphology. Additionally, Heschl's gyrus was categorized as being single or multiple. The distributions of anomaly types in the two groups were significantly different ($x^2 = 8.69$, $p = 0.013$). In the controls, anomalies in P or S occurred more often than MH (15 P/S, 5 MH), while in LD, MH anomalies occurred more often (8 P/S, 15 MH). In both groups, P/S anomalies occurred on both left and right (left: 35%, right: 28%) while MH occurred more frequently on the right (left: 8%, right: 24%). Multiple Heschl's gyri may arise as a result of errors in cell migration or axonal pathfinding during fetal development. Such errors may lead to scrambled auditory connections that put children at risk for auditory processing problems such as those reported by Tallal and Elliott.

(Supported in part by Social and Behavioral Sciences Research Grant No 12 FY93-0551 from the March of Dimes Birth Defects Foundation.)

582.3

INCREASED ASYMMETRY OF THE PLANUM TEMPORALE PARALLELS IMPROVED PHONOLOGICAL CONCEPTUALIZATION IN CHILDHOOD. C.M. Leonard[a], L.J. Lombardino[b], L. Mercado[b], J.I. Breier[a], A.L. Foundas[c], S.R. Browd[a], O.F. Agee[d], K.M. Heilman*[e]. Depts. of Neuroscience[a], Communication Processes[b], Radiology[d], Neurology[e], Univ FL, Gainesville, FL, and Dept. Psychiatry & Neurology[c], Tulane Univ, New Orleans, LA.

Asymmetry of the planum temporale may be the most robust anatomical correlate of functional localization for language. The fetal planum demonstrates asymmetry but developmental changes have not been investigated. We used a 3d volumetric MRI sequence to study 32 normal children aged 5.7 to 12.5 (19 male, 13 female, 17 strongly righthanded, 15 nonrighthanded). The area of the planum temporale (defined as the lower bank of the sylvian fissure between Heschl's gyrus and the posterior ascending ramus) was measured between 46 and 54 Talairach units. The children received the Lindamood test of auditory conceptualization (LAC). Between ages 5 and 10, planar asymmetry increased [$r(n = 25) = 0.51$, $p < 0.01$]. Over the entire age range, a multiple regression analysis indicated that LAC scores increased with age ($F_{3,28} = 5.8$, $p < 0.0001$) as well as with planar asymmetry, independent of age ($F_{3,28} = 2.2$, $p < 0.05$). Consistent with Galaburda's work, increased asymmetry was accompanied by decreased size of the right planum. Studies of brain damaged children suggest a developmental reduction in the right hemisphere's ability to mediate language. This reduction may be due to the loss of right planar tissue. It is not known whether planar asymmetry changes in individual children. The possibility of structural changes in perisylvian regions during childhood should be verified in a developmental study. (Supported by Social and Behavioral Sciences Research Grant No 12 FY93-0551 from the March of Dimes Birth Defects Foundation.)

582.4

ABNORMAL CORTICAL ACTIVATION GENERATED BY SIMPLE AUDITORY STIMULATION AND MUSIC IN CHILDHOOD AUTISM. M. Zilbovicius*, B. Garreau, C. Barthélemy, Ph. Remy, A. Syrota, G. Lelord and Y. Samson. SHFJ, CEA, Orsay and INSERM U 316, CHU Bretonneau, Tours, France.

Abnormal behavioral and electrophysiological responses to auditory stimuli are typical of childhood autism, and may reflect an abnormal cortical processing of auditory inputs. We investigated a putative abnormal pattern of cortical activation in autistic children using an auditory stimulation rCBF paradigm. Eleven autistic children (mean age ± sd : 7 ± 2.4 years) were compared to five non-autistic children (mean age ± sd : 8.5 ± 3.5 years). rCBF was measured under premedication using SPECT and ^{133}Xenon at 1) rest, 2) during simple binaural auditory stimulus (750 Hz tones, duration of 200 msec, intensity of 80 dB, frequency of one tone per second), and 3) while listening to music (Schubert piano sonata # 9). No rCBF abnormality was observed at rest. The *simple auditory stimulus* induced a rCBF increase in the **left** temporo-occipital cortex in controls (+5% ± 3%; $p<0.005$). Autistics showed a rCBF increase in the **right** temporo-occipital cortex (+3% ± 4%; $p<0.005$). Comparison between both groups revealed a significant difference in the **left** temporo-occipital region ($p<0.05$). *Listening to music* induced in autistic children a rCBF increase in the **right** anterior temporal (+5% ± 4%; $p<0.001$) and in the temporo-occipital regions (left: +6% ± 8%; $p<0.02$; right: +6% ± 12%; $p<0.05$). Controls showed a trend toward activation in the **left** temporal anterior region (+3% ± 5%; $p=0.09$). Comparison between both groups revealed a difference in the **right** anterior temporal region ($p<0.05$). Thus, autistic children seem to have an abnormal lateralization pattern of cortical activation induced by auditory stimulations, with deficient **left** cortical processing of short simple auditory stimuli, and increased **right** cortical processing of music. *Supported by France-Telecom and INSERM network # 489001.*

582.5

VISUAL RECOGNITION MEMORY IN HUMAN INFANTS. O. Pascalis* and S. de Schonen. Developmental Unit, Lab. of Cognitive Neuroscience, C.N.R.S., Marseille, France.

Visual recognition memory was assessed in 3-day-, 2-month-, and 3-month-old infants with a paired-comparison task. Infants were first habituated to a picture of a face using an infant-controlled procedure, and, after a 2-min retention interval, they were presented with the familiar face and a novel face for two retention periods of 16 sec for the youngest group and 20 sec for the oldest groups. Time spent looking at either stimuli during the two retention periods was measured and percent fixation to the novel face was calculated. At the age of 3 days and 3 months, infants looked significantly longer at the novel face ($p < .001$), whereas at the age of 2 months, infants looked longer at the familiar face ($p < .01$). These data demonstrate the presence of visual recognition memory for delays of 2 min as early as 3 days of age in human infants. In addition, the change in preferential viewing in 2-month-old infants (preference for familiar instead of novel face) suggests a discontinuity in the development of this memory system. Together with the lack of novelty preference found in amnesic patients (McKee & Squire, 1992, J. Exp. Psychol.) and in infant monkeys with early medial temporal lobe lesions (Bachevalier et al., 1993, Neuroreport), the findings indicate that, in human neonates, the medial temporal lobe makes a critical contribution to recognition memory. These results are discussed in relation with neuroanatomical data on the maturation of the visual system and medial temporal lobe structures in human and non-human primates.

582.6

RELATIONSHIP OF PLASMA MHPG TO COGNITIVE FUNCTIONING IN CHILDREN WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER. L.H. Pick, J.M. Halperin*, J.H. Newcorn and S.T. Schwartz. Dept. of Psychology, Queens College, Flushing, NY 11367 and Dept. of Psychiatry, Mt. Sinai Sch. of Med., New York, NY 10029.

Data suggest that central noradrenergic (NA) mechanisms are involved in the mediation of attention and information-processing. Psychopharmacologic evidence also indicate a role for NA mechanisms in the pathophysiology of children with attention-deficit hyperactivity disorder (ADHD). Studies comparing ADHD children to controls on urinary measures of the NA metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), have yielded inconsistent results. The inconsistent findings may be due in part to the heterogeneity of ADHD children with regard to both MHPG levels and a variety of cognitive functions. This study examined measures of cognitive and academic function in 44 boys with ADHD who were divided into three groups based upon plasma MHPG level (low, medium and high). Plasma, rather than urinary, levels of the metabolite was assessed because it has been found to be more highly correlated with central MHPG in both animals and humans. Academic achievement test scores in reading, spelling and arithmetic were all linearly related to MHPG level. The low MHPG group scored significantly higher than the high MHPG group, and those with medium MHPG levels scored between the other two. The groups did not differ in IQ, as assessed by the WISC-R, however the high MHPG group scored significantly lower on the academically-related Information and Arithmetic subtests. On a computerized continuous performance test, the high MHPG group made significantly more inattention errors, but not more impulsivity errors. Finally, the three MHPG groups did not differ on parent or teacher ratings of hyperactivity or aggression. These data suggest that central NA function may be related to a dimension of inattention/learning problems in children with ADHD.

582.7

AUDITORY EVOKED POTENTIALS AND VERBAL AND SPATIAL ABILITIES IN PREADOLESCENT CHILDREN. J.L. Shucard*, D.W. Shucard, G.L. Ciupak, C.P. Schaeffer, R.L. Clopper, T. Quattrin, M.L. Voorhess. Departments of Neurology and Pediatrics, SUNY @ Buffalo School of Medicine, 100 High Street (D6), Buffalo, NY 14203.

Evidence from both behavioral and electrophysiological studies has supported the notion that there are differences between the sexes in both anatomical/functional brain organization as well as in cognitive abilities, such as visual spatial information processing. Our laboratory has been exploring these purported cognitive sex differences using both neuropsychological and electrophysiological techniques. In the present study, we examined the pattern of cerebral activation across a well characterized sample of pre-adolescent males and females in order to further explore possible relationships between cognitive abilities and electrophysiological indices of cortical organization.

Boys and girls (20 each) between 8 and 11 years of age were studied. Children were determined to be prepubertal by the Tanner Scale of Pubertal Development. Subjects participated in (a) neuropsychological assessment and (b) electrophysiological testing to obtain auditory evoked potentials (AEPs) to probe stimuli from Frontal, Temporal, Central and Parietal scalp sites during the performance of spatial and verbal tasks.

Subjects were categorized into high, medium, and low spatial abilities groups based on a linear combination of three behavioral measures of visual-spatial skills. Differences in AEP amplitude occurred between the high and medium versus low spatial abilities groups obtained during the performance of spatial tasks. High and medium spatial subjects showed greater amplitude AEPs as compared to the low spatial subjects. These differences were most prevalent in the parietal leads and were not seen during the verbal task. High spatial subjects, regardless of sex, showed this AEP pattern.

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582.8

COGNITIVE DECISION-MAKING STRATEGIES AS A FUNCTION OF GENDER AND HANDEDNESS. S. R. Ginn* and D. A. Whetstone. Dept of Psychology, East Carolina Univ, Greenville, NC 27858-4353.

This abstract reports the results of a cognitive bias task (CBT) using both right- and left-handed subjects of both sexes. During the CBT, the subject is presented with a circle or square target stimulus followed by two choice circle or square stimuli. Target and choice stimuli are constructed along 3 dimensions: size (large or small), color (red or blue), and contour (filled or outlined). Subjects are instructed to indicate a preference for either of the choice stimuli after target presentation. In this study, subjects' response biases were tested by varying target-choice similarity; reaction time before choice was also recorded. However, in contrast to Goldberg, et al., we found no significant differences for either gender or handedness on response choice. Results indicate that both men and women, right- and left-handed, make choices in this task in a context-dependent manner.

582.9

THE RELATIONSHIP BETWEEN DERMATOGLYPHIC ASYMMETRY, SEX, AND COGNITIVE STYLE: DISSOCIATION IN THE PERFORMANCE OF SPECIFIC SPATIAL TASKS. C.M. Fernandez-Carol*, B.D. Fantie, D. Rabois, and C.A. Patterson. Human Neuropsychology Laboratory, Psychology Department, The American University, Washington, DC 20016-8062.

Previously, Kimura and Carson (1993) reported a correlation between cognitive pattern and finger ridge asymmetry on tasks traditionally associated with sex differences in performance. Specifically, both male and female subjects who had a greater number of loops in the right thumbprint compared to the left thumbprint performed better on cognitive tests usually associated with enhanced performance by males (e.g., spatial processing, math). In contrast, subjects with higher left-hand counts did better on tests frequently associated with superior performance by female (e.g., fluency, perceptual speed). In this preliminary attempt to replicate Kimura and Carson's findings, young adult male and female subjects performed a variety of paper-and-pencil cognitive tasks and their thumbprints were examined. As expected, based on this earlier work, the males, overall, had significantly more thumbprint loops than did the females. We also found that all subjects performed equivalently on a putative gender-neutral task (i.e., vocabulary). On three tests of spatial ability, however, there was a dissociation between dermal ridge asymmetry and sex. The Hidden Patterns test showed a greater relationship between dermatoglyphic asymmetry than it did a sex difference. Surface Development and Card Rotation test performances, on the other hand, were more greatly related to sex differences. Spatial ability is not a unitary function. Dermatoglyphic asymmetry may be related directly to the amount of material in the sex chromosomes. We speculate, however, that there may be different events or mechanisms, other than those associated with gender (e.g., the presence of hormones in the developing brain), that contribute to the development of spatial performance.

582.10

SEX DIFFERENCES, SPATIAL WORKING MEMORY AND EVENT-RELATED POTENTIALS. J. M. Hoesing,* J. D. Lewine, R. Yeo, & C. Edgar. Depts. of Psychol., Univ. of New Mexico, Albuquerque, NM 87131 and Radiology, V.A. Medical Center, Albuquerque, NM.

Research on individual differences in cognitive functioning is beginning to reveal important sex differences in problem-solving strategies. It has been found that males outperform females on certain tasks that require imaginary manipulation of objects, navigating through a route, guiding or intercepting projectiles, and mathematical reasoning tasks. Females do better on tasks in which perceptual speed is important (eg., identifying matching items). They have greater verbal fluency, are superior in arithmetic calculation, recalling landmarks along a route, and manual tasks that require fine-motor coordination.

Here we report a novel sex difference. The task, based upon a procedure described by Sternberg (1969), is presented in two phases. Phase 1 requires the subject to memorize a) an array of letters, or b) the locations occupied by letters in an array. In phase 2 the subject is shown single letters and is asked to identify whether the letter a) was a member of the memorized array (letter identification), or b) was in a location occupied by any letter in the memorized array (location identification). Females take the same amount of time for letter identification as for location identification. Males respond significantly faster in the location verses letter identification conditions. This suggests that males use different processing mechanisms to solve location versus item-recognition memory tasks. Females appear to use a common processing mechanism for the two tasks.

582.11

GENDER DIFFERENCES IN SENSORY INTERHEMIPSHERIC TRANSMISSION TIMES. S. Caillé and M. Lassonde*. Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal, Qué., Canada.

The fibers of the corpus callosum (CC) connect the hemispheres following a rostro-caudal distribution: visual fibers course through the splenium, auditory fibers through the posterior body (isthmus) and somesthetic fibers through the anterior body. The aim of this study was to estimate the interhemispheric transmission time (ITT) in the visual, auditory and somesthetic modalities in men and women, given that previous data suggest a gender dimorphism favoring women in the posterior part of the CC (splenium and isthmus). Sensory ITTs consisting of lateralized flashes of light, puffs of air and pure tones were presented to 50 normal dextral subjects (25 males and 25 females) to evaluate visual, somesthetic and auditory ITT respectively. Results indicated that while somesthetic (13.61 ms) and visual (3.75 ms) ITTs did not differ according to gender, auditory ITTs of men (2.52 ms) and women (0.09 ms) differed significantly. This finding is cautiously interpreted as the consequence of women having a larger isthmus than men. Moreover, it offers an anatomical substrate for the reported weaker lateralization of function in women.

582.12

SEX DIFFERENCES IN NUMERICAL DENSITY OF NEURONS IN HUMAN AUDITORY ASSOCIATION CORTEX. S. F. Witelson*[1], I. I. Glezer[2] and D. L. Kigar[1]. [1]Dept. of Psychiatry, McMaster Univ., Hamilton, ON, L8N 3Z5; [2]Dept. Cell Biol. Anat. Sci., CUNY Med. School, New York, N.Y.

Brain size is approximately 10% larger in men than women. This sex difference could be reflected in some microscopic difference: e.g., in the total number, size or numerical density of neurons. Sex differences have been documented in the gross anatomy of and in the cognitive functions mediated by parietotemporal regions (Witelson & Kigar, JCN, 1992). The aim of the present study was to assess microscopic features of cytoarchitectonic area TA_1 (von Economo), auditory association cortex in the planum temporale within the Sylvian fossa. The brains of 5 women and 4 men were studied (mean age = 54 and 49 yr, respectively), all selected to be of documented normal neurologic and cognitive status and consistently-right-handed. Cortical depth (D), the number of neurons in a column under 1 mm^2 surface area through the depth of the cortex (N_c), and the number of neurons per unit volume (N_v) were obtained using direct counting under the light microscope. Counts were made from 25μm Nissl-stained sections from right and left hemispheres. These three measures were also obtained for each of the 6 cortical layers. Results indicated that regardless of hemisphere, D did not differ between sexes, but N_v did by 11% ($df = 7$, $p < .008$, two-tailed t-test). There was no overlap between the sexes. This sex difference was observed for layers II and IV only. These findings suggest that sex differences in architectonics and, as a consequence, possibly in synaptology, are expressed mostly in non-pyramidal, granular layers associated with reception and distribution of information to the cortex. Such a sex difference could have consequences for neural function and cognition.

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582.13

SEXUAL ORIENTATION AND ANATOMY OF THE CORPUS CALLOSUM. A. Scamvougeras[1], S. F. Witelson[1], M. Bronskill[2], P. Stanchev[2], S. Black[3], G. Cheung[4], M. Steiner[1]* and B. Buck[1,3]. [1]Dept. of Psychiatry, McMaster Univ., Hamilton, ON, L8N 3Z5; Depts. of [2]Medical Imaging, [3]Medicine (Neurol.) and [4]Radiol., Sunnybrook Health Science Centre, Univ. of Toronto.

An increased prevalence of left handedness (defined as not exclusive right-hand-preference) was found in gay men compared to the general population (McCormick & Witelson, Psychoneuroendoc., 1991). Left handedness and, by inference, hemispheric functional asymmetry, was found also to be correlated with increased cross-sectional area of the isthmus of the corpus callosum in men (Witelson & Goldsmith, Brain Res., 1991). Based on these and other findings it was hypothesized that the isthmus is larger in gay than heterosexual men even when hand preference is controlled. Eleven gay men and 10 heterosexual men matched for age and educational level, all selected to be in good health and consistently-right-handed, underwent a research MR scanning procedure specifically designed for this study. Callosal measurements were obtained from 4 mm midsagittal slices. Area measures of the total corpus callosum, the isthmus and other subregions were obtained. The isthmus was larger in gay men by 13% ($p = .05$, one-tailed t-test). Similar results were reported for the anterior commissure (Allen & Gorski, PNAS, 1992). The isthmus interconnects right and left parietotemporal regions involved in linguistic and spatial cognitive functions, tasks on which gay and heterosexual men differ (e.g., McCormick & Witelson, 1991). Our findings add to the evidence of a neurobiological factor in the origin of male sexual orientation. Moreover, our results indicate that sexual orientation is linked to functional lateralization of higher cognitive functions and may be part of a larger constellation of cortical and cognitive characteristics.

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582.14

SEMANTIC INTERHEMISPHERIC INTEGRATION IN THE SPLIT-BRAIN: MORE ILLUSORY THAN REAL

Alan Kingstone & Michael S. Gazzaniga*
Center for Neuroscience, University of California, Davis, 95616

Subcortical semantic transfer is strongly suggested when a callosotomy patient draws pictures that *integrates* the meaning of words lateralized to the separated hemispheres. In our study patient J.W. drew pictures with the left hand (right hemisphere) or the right hand (left hemisphere), with or without visual feedback of the drawing. Sometimes the presented words were conceptually ambiguous (e.g., words "toad" and "stool" may be drawn as a toad sitting on a stool, or as a single emergent object -- a mushroom). Results indicated that word integration (e.g., a toad sitting on a stool) occurred mainly when visual feedback was provided; hemispheric control of the *ipsilateral* as well as the contralateral response hand was observed; and emergent objects were never drawn when words were lateralized. We conclude that what might first appear as evidence of subcortical semantic integration is in fact each hemisphere drawing its own separate picture with the same response hand on the same piece of paper. Our results support the view that the evidence for interhemispheric semantic integration in the split-brain patient is not compelling. (Supported by NIH/NINDS P01 NS17778).

582.15

THE RELATIVE VALUE OF SPEECH ARREST, READING AND REPETION ERRORS TO DETERMINE CEREBRAL DOMINANCE FOR LANGUAGE DURING THE INTRACAROTID SODIUM AMYTAL TEST. J.Robidoux* & I.Rouleau. Université du Québec à Montréal & Hôpital Notre-Dame, Montréal, Québec, Canada.

The intracarotid Sodium Amytal (ISA) test is commonly used to establish cerebral dominance for language (CDL) prior to surgery for intractable epilepsy. CDL determined clinically is based on a general analysis of deviant productions during the ISA test but the respective lateralizing value of speech arrest, reading and repetition errors has not been fully examined. To address this issue, the ISA sessions of 30 (clinical CDL: 25 left (L), 2 right (R) and 3 mixed) right-handed epileptic patients, with a unilateral temporal focus, were examined retrospectively. For each patient, presence of speech arrest and dysphasic errors during reading and repetition of words and phrases were compared for L and R injections. Speech arrest was observed in 28 patients (L only: 21; R only: 3; L & R: 4). The presence of suppression corresponded for all patients but 2 (with mixed dominance) with the established clinical CDL. The analysis of 114 reading errors produced by the patients with a L CDL revealed the expected dissociation between neglect error (R inj.) and phonemic paralexia (L inj.). However, morphological errors were found after both injections. In contrast, repetition errors were observed only following the dominant injection and were mostly phonemic transformations. Taken together, these results suggest that speech arrest and repetition errors are specific to the injection of the dominant hemisphere while reading errors are not. Wether these findings would apply to all epileptics (e.g., left-handers, frontal focus etc.) will also be dicussed.

582.16

NEUROSOMATIC ASYMMETRY AND HANDEDNESS. T.J. Andrews, L.E. White*, M.F. Groelle, D. Purves. Dept. of Neurobiology, Duke University Medical Center, Durham NC 27710.

We examined hand size in 50 adult volunteers who described themselves as right-handers, and 50 who considered themselves left-handers. Volumetric measurements of the two hands, assessed for each subject by a simple water displacement technique, showed that right-handed individuals have larger right hands than left (mean difference = $3.5 \pm 0.4\%$; $p<0.0000001$). In contrast, the hands of left-handers are much more nearly symmetrical (mean difference = $0.3 \pm 0.5\%$; $p<0.5$). Based on what is known about trophic interactions between neurons and their targets, these findings predict a corresponding asymmetry of the relevant parts of the sensorimotor system in right handers. This implication accords with preliminary studies of the human sensorimotor cortex, which show a leftward hemispheric asymmetry in the region where hand and upper limb are represented (White et al. *Nature* 368: 197-198, 1994). Corresponding studies of the spinal cord show a rightward asymmetry at the level of the cervical enlargement (mean difference of the cross-sectional area of the hemicord = $6.3 \pm 2.7\%$, $n = 5$; see also Nathan et al. *Brain* 113: 303-324, 1990). Measurements of hand size and related neural structures in infants could address whether these asymmetries arise from differential use or intrinsic factors.

582.17

FUNCTIONAL HEMISPHERIC ASYMMETRY IN HUMANS: METABOLIC EVIDENCES OF DIFFERENT CONNECTIVITY IN FOCAL HIPPOCAMPAL LESION. F. Parker and M.F. Lévesque*. Epilepsy and Brain Mapping Program. Los Angeles, CA 90048

To investigate different patterns of neuronal connectivity in human brain, interhemispheric differences in metabolic activity were analyzed in subjects with unilateral hippocampal lesion causing focal hippocampal epilepsy. 16 right-handed patients with proven unilateral Right (R : n=8) or Left (L : n=8) focal hippocampal onset by stereoelectroencephalograhic (SEEG) criteria were selected. Interictal metabolic changes with fluoro-deoxyglucose positron emission tomography (18F-FDG PET) were quantified using an accurate stereotactic methodology and correlated with the side of seizure onset by SEEG. For each patient, 46 individual anatomical regions (ROI) were defined on stereotactic MRI and superimposed to stereotactic PET. For each ROI the number of count per pixel was computed. An asymmetry index (onset side - opposite side / onset side + opposite side) was defined and analyzed with T-tests and Wilcoxon tests. The Left subgroup showed lateralized dysfunction for the hippocampus* (p=.001), the lateral temporal neocortex* (p=.002), thalamus* (p=.003),and medio-lateral frontal cortex(p=.001)* while the Right subgroup showed preferential desactivation of the pallidum* (p.0001), cingulum* (.003) and supplementary motor area (p=.03). These results suggest intrinsic differences in connectivity between right and left hemisphere in response to an identical functional lesion. . * significant at .05 level after Bonferroni correction

582.18

Regional Variation of Parvalbumin-Immunoreactive Neurons in Human Auditory and Language Cortices Jeffrey J. Hutsler* and Michael S. Gazzaniga, Center for Neuroscience, University of California, Davis, CA 95616

Regional variation in the architecture (Brodmann '09) and distribution of chemically identified cell groups within the cerebral cortex likely contributes to functional specialization. Homologous regions of the right and left parietal-temporal junction vary in their contribution to language, but little attention has been focused upon the presence or absence of asymmetric neuronal microcircuitry within these areas.

Parvalbumin-immunoreactivity (PV-IR) has been shown to label a subgroup of GABAergic interneurons in the cerebral cortex (Van Brederode et al '90). This cell group possesses distinct physiological properties (Kawaguchi & Kubota '93) and thus contributes uniquely to cortical processing. An examination of the distribution of PV-IR neurons within auditory, auditory association, and putative language regions of two whole postmortem human brains revealed regional differences at various auditory processing levels, as well as differences between hemispheres. Specifically, the density of PV-IR neurons is lowest within anterior portions of the superior temporal gyrus (STG) and is greatest within cortical regions of the planum temporale, posterior STG, and supramarginal gyrus. An analysis of this density variation within individual laminae reveals a three-fold increase in the number of PV-IR cells within infragranular layers of posterior regions. An examination of homologous regions from each hemisphere shows density differences within individual lamina of the posterior locations. These results suggest that inhibitory interneurons make a unique contribution to cortical processing at different locations within the auditory cortex as well as within homologous regions of the hemispheres that are functionally unique. (This research was supported by the McDonnell Pew Foundation)

582.19

IMMUNOCYTOCHEMISTRY OF CALCIUM-BINDING PROTEINS REVEALS FUNCTIONAL LATERALIZATION IN HUMAN BRAIN. I.I. Glezer[1]*, S.F. Witelson[2], D.L. Kigar[2]. [1]Cell Biol. Anat. Sci. CUNY Med. School, New York, NY 10031,USA, [2]Dept. Psychiatry, McMaster University, Hamilton, Ont., CA.

Light microscopic immunocytochemical analysis of three calcium-binding proteins: calretinin (CR), calbindin (CB) and parvalbumin (PV) was applied to 24 adult, 14 male, 10 female human brains (left and right hemispheres) obtained postmortem from cognitively normal individuals with tested psychological characteristics including hand preference. The 50 μm vibratome sections from the posterior part of the superior temporal gyrus (area TA_1, von Economo) were incubated with primary polyclonal antibodies against CR, CB and PV. It was found that in human neocortex all three calcium-binding proteins were well expressed and represented in three different populations of non-pyramidal neurons. The degree of the immunostaining, especially for the PV-positive neuronal population was associated with hand preference. In the group of consistent right-handers (CRH), all 13 cases showed high degree of immunostaining (grades 3 to 4 on a scale from 0 to 4). In contrast 7 of the 11 nonCRH people showed a very low degree of immunostaining (grades 0-2) in either both or in one of the hemispheres. The difference between the CRH and nonCRH groups was significant ($\chi^2 = 11.7$, df=1, $p<0.001$). These results are the first demonstration of an association between biochemical characteristics of cortical non-pyramidal neurons and hemispheric lateralization of human cognition. Supported by grants:NS18954, MRC(CA) MA-10610, EJLB Fdn(CA), CUNY, RF-776615.

582.20

GENETIC AND ENVIRONMENTAL CONTRIBUTIONS TO CRANIAL SIZE IN BLACK AND WHITE ADOLESCENTS. J. P. Rushton* and R.T. Osborne. Department of Psychology, University of Western Ontario, London, Ontario, N6A 5C2, Canada.

Data from 236 pairs of twins (472 individuals) aged 12-18 years were used to examine genetic and environmental factors influencing cranial size, an indirect estimate of brain volume. Measures were taken of zygosity, head length, head breadth, age, sex, race, height, and weight for 187 males and 285 females, 222 whites and 250 blacks. Cranial size was calculated from head length and head breadth using standard equations. Group differences were found. Cranial size increased from ages 13 to 17 from 1,223 to 1,279 cm^3. After adjusting for the effects of age and body size, boys averaged 1,290 and girls 1,229 cm^3, whites averaged 1,269 and blacks 1,251 cm^3. Intraclass correlations were calculated and models fitted of proportionate environmental and genetic contributions to variance. The heritability for the sample as a whole was 51 percent with 6 percent due to common environment and 43 percent due to unique environmental factors including error variance.

NEURAL PLASTICITY I

583.1

RECOVERY OF A VIBRISSAE-DEPENDENT BEHAVIORAL RESPONSE FOLLOWING PHOTOTHROMBOTIC INFARCTION OF SOMATO-SENSORY CORTEX IN RATS A.J. Pazos*, M.D. Mayberry, A. Font, P.M. McCabe, W.D. Dietrich, E.J. Green. Dept. of Psychology, University of Miami, Coral Gables, FL 33124

Previous work indicates that rats trained to perform an appetitively motivated vibrissal sensory discrimination task exhibit substantial behavioral deficits following an infarction of the primary somatosensory cortex (SmI). These deficits recover gradually over the course of post-infarct testing. The present study sought to determine whether this behavioral recovery requires the involvement of either ipsilateral secondary somatosensory cortical tissue (SmII) or the homologous SmI area in the contralateral hemisphere.

Sixteen adult male Wistar rats were trained to turn ipsilaterally in a T-maze following unilateral vibrissal stimulation, and contralaterally in response to control stimulation. Rats received 50 randomly alternating daily trials until they achieved criterion (>80% correct). Rats then received either a unilateral infarction of SmI, a unilateral infarction involving both SmI and SmII, bilateral SmI infarcts, or sham surgical procedures. The cortical infarcts were produced through the interaction of a photosensitive dye (rose bengal) injected into the rat's tail vein, and the direct irradiation of the cranium with a light beam (Watson et. al., 1985). Shams underwent the same procedure, except saline was injected in place of rose bengal.

Post-infarction behavioral testing revealed that animals in each of the infarct groups required a significantly greater number of days to reattain criterion than did sham rats. Nevertheless, each animal eventually recovered to pre-infarct levels of responding. These results indicate that none of the somatosensory areas evaluated are *essential* for behavioral recovery from SmI infarcts in this task. The data thus suggest that either a) recovery from these deficits results from the dissolution of remote effects of the infarcts on some undetermined neural region, or b) behavioral recovery requires neural plasticity in that region. Supported by AHA 93GIA/960

583.2

MK-801 FAILS TO INDUCE RECOVERY FROM CHRONIC TACTILE PLACING DEFICITS AFTER UNILATERAL LESIONS INCLUDING THE ROSTRAL AND CAUDAL FORELIMB AREAS OF THE RAT SOMATIC SENSORIMOTOR CORTEX. S. Barbay* and T.M. Barth. Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

Previous studies have shown that unilateral lesions of the caudal forelimb area (CFL) of the rat somatic sensorimotor cortex (SMC) produce a transient ipsilateral somatosensory asymmetry on a bilateral-tactile stimulation test and transient contralateral impairments in tactile-forelimb placing (lasting 14-28 days). Recovery from these deficits is facilitated by a regimen of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 beginning 16 hrs after the lesion. The present study investigated whether a similar regimen of MK-801 would attenuate chronic deficits produced by a unilateral lesion including the CFL and the rostral forelimb area (RFL) of the rat SMC. Rats received large unilateral lesions of the SMC followed by a regimen of MK-801 (1 mg/kg) beginning at 16 hrs after the brain injury. In untreated control rats (i.e. SMC lesions + saline) severe contralateral tactile-forelimb placing deficits were observed for the duration of the postoperative test period (i.e. 6 months) and no recovery was apparent. Control rats also showed an ipsilateral asymmetry on the bilateral-tactile stimulation test that partially recovered in the 6 month postoperative observation period. Treatment with MK-801 failed to induce recovery of the tactile-placing deficits but facilitated the rate of recovery on the bilateral-tactile stimulation test. These data suggest that while MK-801 may be effective in facilitating the rate of recovery from transient deficits, there is no evidence to suggest this agent has the potential to induce recovery when no restoration of function is expected. Moreover, the present results indicate that an intact rostral forelimb area may be involved in the process of recovery from unilateral CFL lesions because removing this structure (along with the CFL) prevents the occurrence of recovery.

583.3

UNILATERAL DAMAGE TO THE FORELIMB SENSORIMOTOR CORTEX: LONG-TERM EFFECTS OF TWO-WEEK LIMB IMMOBILIZATION ON DENDRITIC GROWTH AND FORELIMB FUNCTION. D.A. Kozlowski* & T. Schallert. Institute for Neuroscience & Department of Psychology, University of Texas at Austin, Austin, TX 78712.

Unilateral damage to the forelimb-representation area of the sensorimotor cortex in adult rats results in time-dependent enhancement of dendritic processes in layer V pyramidal neurons of the homotopic contralateral cortex and compensatory over-reliance on the ipsilateral/unimpaired forelimb (Jones & Schallert, 1992). Immobilization of the ipsilateral forelimb, by a one-sleeved cast, prevents this anatomical event (Jones & Schallert, 1994). In addition, behavioral function, when examined two days following cast removal, is more impaired by immobilization of the ipsilateral limb than of the contralateral limb. Thus, dendritic enhancement and functional recovery may be driven by compensatory forelimb use.

At this time, however, the long term effects of limb immobilization are unknown. In the present study, the unimpaired forelimb in groups of unilaterally lesioned rats was immobilized with a one-sleeved cast during the arborization period (Days 1-15 post-lesion). Analysis of dendritic arborization using Golgi-Cox procedures and tests of limb function were performed at various times following cast removal, for up to 60 days post lesion. Ipsilateral immobilization of the forelimb caused a chronic disruption of function, and substantially reduced the rate, and maximal extent, of dendritic arborization enhancement. Supported by NS 23964 & AA 07471.

583.4

EFFECTS OF SCOPOLAMINE AND MK-801 ON RECOVERY OF FUNCTION FOLLOWING SENSORIMOTOR CORTEX LESIONS IN THE RAT. R.M. Saponjic*, M.R. Hoane, S. Barbay, and T.M. Barth. Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

Following brain injury there is an excessive release of glutamate and acetylcholine which may lead to secondary cell death proximal and distal to the site of the trauma. Previous research has suggested that agents which antagonize glutamatergic and cholinergic receptors may serve as neuroprotective agents and facilitate behavioral recovery. Although research has demonstrated the efficacy of treatment with MK-801 (a noncompetitive antagonist at the N-methyl-D-aspartate [NMDA] receptor) and scopolamine (an antagonist at the muscarinic acetylcholine receptor) following brain injury, very few studies have systematically examined the "window of opportunity" for these drugs. Moreover, the effects of treatments with a combination of neuroprotective drugs are largely undetermined. The present study examined the "window of opportunity" for MK-801 and scopolamine by themselves and in combination. Rats received unilateral lesions of the forelimb representation in the somatic sensorimotor cortex (SMC) and a single injection of either scopolamine (1 mg/kg), MK-801 (1mg/kg) , scopolamine + MK-801, or saline. In addition to the type of treatment, the timing of the drug injections was varied among groups with some animals receiving their treatments at 15 minutes, 2 hrs, or 48 hrs after the brain lesion. Behavioral tests included measures of forelimb tactile placing and locomotor placing. The results were that although scopolamine and MK-801 by themselves facilitated the recovery of function on some of these tests when given at 15 minutes or 2 hrs, the combination treatment provided the most consistent beneficial effects regardless of the time of injection or the behavioral test. These data support the idea that activation of both muscarinic and NMDA receptors are involved in the production of secondary brain damage and that combination treatments with antagonists may provide the most consistent facilitative effects on behavioral recovery.

583.5

AFFERENT INPUT INTEGRATION AND SEGREGATION IN LEARNING ARE INPUT TIMING DEPENDENT X. Wang*, M. Merzenich, K. Sameshima, W. Jenkins. Keck Center, Coleman Laboratory, UCSF, San Francisco CA 94143-0732.

Earlier studies suggest that a) changes in coincident input-based, Hebb-like synaptic effectiveness underlie the afferent input co-selection that marks the emergence of behaviorally-important stimulus-specific neuronal responses in the cerebral cortex, and b) representational segregation results from consistently non-correlated inputs. Here, we tested both hypotheses by documenting the cortical representations of the hands in area 3b of adult owl monkeys trained to discriminate multiple-site tactile stimuli. Unrestrained monkeys received sequences of tactile stimuli delivered to their fingers via two narrow bars. One bar simultaneously struck limited surfaces of the distal segments of fingers 2, 3 &. 4; the second struck surfaces of the proximal segments of the same digits. Background stimuli were step indentations (50μ, 50ms) delivered alternatively by the two bars (ISI>200ms). The monkey's task was to detect two consecutive step indentations delivered to the same skin site by either the distal or proximal bar. After weeks of training with monkeys performing >300 daily trials, cortical neuronal responses to these and other general tactile stimuli were defined with 30-50 samples/mm^2 derived all across the area 3b zone engaged by these behaviorally important stimuli. Many sampled neurons had cutaneous receptive fields that were comprised of the discontinuous skin patches on two or three digits that were co-stimulated by either the proximal or distal bar stimuli. At the same time, non-simultaneously excited distal and proximal skin surfaces were largely or completely segregated from one another representationally, and in some cases were further separated by a band of cortex representing inputs from the hand dorsum. No comparable multiple-digit receptive fields were recorded in the hand zone of ventroposterolateral nucleus of the thalamus that was also mapped in detail. These findings support the conclusion that coincident input co-selection operates to create neuronal response specificity in learning. They provide a further demonstration that discontinuities in cortical maps are functional and not anatomical entities, and that temporally non-simultaneous inputs are sorted by cortical networks into largely-segregated zones. (Supported by the Coleman Fund, HRI and NIH Grant NS-10414)

583.6

EVIDENCE FOR EXTENSIVE REORGANIZATION OF THE SOMATOSENSORY CORTEX IN ADULT HUMANS AFTER NERVOUS SYSTEM INJURY. T. Elbert*, H. Flor, N. Birbaumer, S. Knecht, S. Hampson, E. Taub. Institute of Experimental Audiology, University of Münster, 48149 Münster, Germany.

In five patients with unilateral upper extremity amputation, we demonstrated alteration of the topographic representation of the cortical face area and the cortical upper arm (stump) areas which lie adjacent to the former receptive field of the amputated limb. Magnetic source imaging revealed that the focus of cortical activation elicited by facial stimulation was shifted 1-2 cm toward the receptive field which would normally receive input from the now amputated hand and fingers. A similar tendency was observed for the receptive field for the upper arm (stump). Additional responsivity of these reorganized cortical areas was evidenced by an enhanced evoked electroencephalographic potential and magnetic field, compared to stimulations on the intact side. Observed alterations provide evidence for extensive plastic reorganization in the adult sensory cortex of humans following nervous system injury. This may constitute the neurophysiological basis of such phantom limb phenomena like the persistent sensory mislocation; however, they are not a sufficient cause of the phantom phenomenon termed "facial remapping" (i.e. of the phantom hand on the skin of the face; Ramachandran, Science, 1992).

583.7

FUNCTIONAL MAPPING OF THE REORGANIZATION OF HUMAN HAND SENSORIMOTOR CORTEX AFTER PERINATAL INJURY USING MRI TECHNIQUES. Y. Cao, P.R. Huttenlocher*, E.M. Vikingstad, V.L. Towle and D.N. Levin. Departments of Radiology, Neurology and Pediatrics, The University of Chicago Hospitals, Chicago, IL 60637

Functional magnetic resonance imaging was used to map the hand sensorimotor cortex of hemiparetic patients who had suffered unilateral brain damage in the perinatal period. Six patients (mean age: 15 years) who had porencephaly or diffuse cortical atrophy performed a finger opposition task during functional MRI scanning. The control group consisted of eight right-handed normal subjects. T_2*-weighted images were obtained with a modified gradient-echo pulse sequence during a baseline scan period followed by 4-8 alternating task and rest periods. The results indicated that the intact hemispheres of the patients had approximately equal cortical volumes activated during contralateral (normal hand) and ipsilateral (paretic hand) finger movements. In contrast, the left or right hemispheres of right-handed normal subjects were primarily activated by contralateral finger movements, and had little ipsilateral sensorimotor activation. Our study of patients with unilateral perinatal brain damage indicates that their intact hemispheres are much more strongly linked to the ipsilateral hand than is the case in the normal subjects. In two patients, cortical regions distant from the central sulcus were activated by paretic hand movements. Our findings are consistent with previous clinical observations and animal experiments which suggest that the immature brain is able to reorganize in response to focal injury.

583.8

PERIPHERAL NERVE CROSS INDUCES PLASTICITY IN THE SOMATOSENSORY CORTEX OF ADULT MACAQUES. J. Hahm*, R.W. Luethke, J. Danielsson & T.P. Pons. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892, and Johns Hopkins University, Baltimore, MD 21205.

The contributions of peripheral versus central factors to reorganization of cortical representational maps after peripheral nerve injury are not well understood. To explore contributions mediated by peripheral regeneration, we surgically altered the pattern of sensory innervation in the hand, then mapped the cortex. In two adult macaques, the sensory components of the distal median and ulnar nerves were transposed at the level of the palm. This procedure induces regeneration of the median nerve into skin normally subserved by the ulnar nerve, and ulnar nerve regeneration into median nerve territory. The radial nerve was severed and sutured into muscle in the forearm. Motor portions of the nerves were not altered. After a 6 or 9 month recovery period the hand representation in cortical areas 3b and 1 was mapped using multiunit electrode recording techniques. Our data indicate that cortex became reorganized according to the altered input. Instead of the normal mediolateral topographic progression from D5 to D1, we found a disordered progression from D1 to D5 across this dimension; the D4 and D5 representations were located where D2 and D3 representations are normally found, and the reverse. Two D1 representations were found, one located next to the forearm representation, and one next to face representation, presumably due to a remaining intact branch of the median nerve. 10% of the recording sites had discontinuous receptive fields of a type not seen in normal monkeys. The representation of the palmar pads were disproportionately large relative to the representation of distal digits. Since regeneration of the nerves in the proximal portion of the hand occurs prior to regeneration in the distal finger segments, the palmar pads may have already "claimed" cortical territory before the distal finger segments could compete for cortical space. These results suggest that the pattern of central reorganization that occurs after nerve damage is mediated by both the spatial and temporal regeneration pattern of peripheral nerves.

583.9

COORDINATED SYNAPTIC AND ASTROCYTIC STRUCTURAL CHANGES IN RATS REARED IN COMPLEX ENVIRONMENTS.
T.A. Jones*, N. Hawrylak, and W.T. Greenough. Dept. Psychology and Neuroscience Program, Beckman Institute, Univ. of Illinois, Urbana, IL, 61801.

Rats in a complex environment (EC) have larger neuronal dendritic fields and an increased number of synapses per neuron in the visual cortex in comparison to animals housed individually (IC) or socially (SC) in standard laboratory cages. These neuronal effects are accompanied by hypertrophy and proliferation of astrocytes expressing glial fibrillary acidic protein (Sirevaag & Greenough, 1991). To more closely assess the relationship between synaptic and astrocytic changes, astrocytes were examined at the electron microscopic level in layers I, II-III and IV of the visual cortex in rats following 30 days of EC, IC, or SC housing. Stereological measures of the volume fraction and surface density of glial processes showed little change in these size variables following exposure to EC. However, there was a specific increase in the region of contact between glial processes and pre- and post-synaptic elements in EC rats, measured as the surface density of glial membrane in direct apposition to these synaptic elements. That is, glial processes become more ensheathing of synapses, an effect which may be indicative of greater involvement in synaptic activity and/or a response to elevated synaptic activity. The increased glia-synapse apposition bears a resemblance to the glial response previously reported in relation to increases in synapse efficacy (Wenzel et al., 1991). Ongoing work is assessing the possibility that structural and positional changes in perisynaptic astrocytic processes occur in close temporal coordination with synaptic events. Supported by MH35321 and MH10422.

583.10

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVITY IS ALTERED IN THE RAT VISUAL CORTEX FOLLOWING MONOCULAR DEPRIVATION DURING DEVELOPMENT
N. Hawrylak*, W.T. Greenough Neurosci. Prog., Dept. of Psych. and Cell & Struct. Bio., Beckman Inst. Univ. of Illinois, Urbana 61801

Astrocytes demonstrate dynamic experience-dependent growth and possibly proliferative responses in the visual cortex of rats reared in a complex environment compared to those raised in individual cages (Sirevaag and Greenough, 1991 Brain Res. 540;273-278). To further delineate the effect of experience on the growth and differentiation of astrocytes in the visual cortex, monocularly deprived rats were examined with GFAP.

Eye lids were sutured at P12. Sutured rats were assigned to one of two groups; those with sutures that remained in place until P80 (MD, n=6); those that had the sutures removed at P75 and were allowed 5 days of light exposure (MD+L, n=6). A nonsutured control group P80 (CL, n=6) was used for comparison. Optical disectors and random systematic sampling were used to estimate the numerical densities (Nv) of astrocytes, neurons and total glia. A stereological cycloid intersection method was used to estimate the surface density (Sv) of GFAP immunoreactive processes. The numerical densities and (Sv) were estimated in layers 2/3, 4, 5, 6.

The Nv of astrocytes was not significantly different between the three groups. Group MD had a significant (35%) decrease in the Sv in layer 4 compared to control. The ratio of Sv to Nv of neurons (Sv/Nv), an estimate of the amount of GFAP-immunoreactive astrocyte surface area per neuron, was significantly decreased in layer 4 in both monocularly deprived groups compared to control. These results suggest that early experience has a profound effect on the morphological characteristics of astrocytes in the mature brain. Supported by MH35321.

583.11

TIME-DEPENDENT EFFECTS OF OPTIC NERVE CRUSH ON SUPERIOR COLLICULUS LOCAL CEREBRAL GLUCOSE USE.
U. Schmitt°, B.A. Sabel°, R. Cross†, F.E. Samson† and T.L. Pazdernik†*. °Inst. Med. Psychol., Univ. Magdeburg, Germany. †R.L. Smith Res. Cent., Univ. Kansas Med. Cent., Kansas City, KS 66160.

Rats given an optic nerve crush regain visual function despite a 90% loss of retinal ganglion cells. Thus, there is either restoration of information transfer from retina to brain or there is an adaptation in that visual function occurs with less information transfer. The 2-[^{14}C]-deoxyglucose technique was used to determine local cerebral glucose use (LCGU) as an assessment of information transfer from retina to brain visual centers. Male adult Long Evans rats (6/group) were given a mild crush to the right optic nerve and LCGU was assessed 2, 9, or 22 days later. LCGU procedure was determined during stimulation with a flashing strobe-light and rotating bar pattern with or without physostigmine (300 μg/kg; i.m.), a cholinesterase inhibitor known to activate retinocollicular pathways. Qualitative inspection of LCGU autoradiograms from rats 2 days after crush revealed a marked reduction in LCGU in the left superior colliculus, especially the superficial layer. There was only minimal restoration of LCGU at 9 or 22 days after crush, restoration was greatest in the portion of the superior colliculus adjacent to the brain midline. Quantitation of LCGU according to the Sokoloff equation is in progress. These results provide evidence that visual function is restored after optic nerve crush in spite of a marked dysfunction in retinocollicular information transfer. Supported by BMFT 07 NBI 04/3.

583.12

A NITRIC OXIDE SYNTHASE (NOS) INHIBITOR REDUCES THE OCULAR DOMINANCE SHIFT AFTER MONOCULAR DEPRIVATION.
N.W. Daw*, S.N.M. Reid, D. Czepita and H. Flavin. Dept. of Ophthalmology, Yale Univ. Sch. of Med., New Haven, CT 06520.

Nitric oxide (NO) is an intercellular messenger. This intercellular messenger has been shown to modify activity-dependent synaptic efficacy in hippocampal slices (Schuman & Madison, *Science* 245:1503-1506, 1991; O'Dell et al., *Pro. Natl. Acad. Sci.* USA 88:11285-11289, 1991). We are interested if NO is also involved in activity-dependent modification of eye-specific connections occurred in the visual cortex during the critical period for monocular deprivation. Eight kittens were monocularly deprived for 10 days starting at 44-46 days of age. Four of the kittens also received infusion of Nω-nitro-L-arginine methyl ester (NAME) into one visual cortex by an osmotic minipump while deprivation. At the end of the monocular deprivation, the ocular dominance of visual cortical cells was evaluated electrophysiologically. Our data showed that the ocular dominance histogram in NAME treated cortex shifted less than un-treated cortex. However, NAME did not completely abolish the effect of monocular deprivation. Preliminary results also showed that the effect of this NOS inhibitor on the ocular dominance shift was found in layers I-V but not in layer VI. Our results suggest that nitric oxide may play a role in modification of eye-specific connections in the visual cortex. However, the activity of nitric oxide synthase is probably not essential for the modification of the connections. Supported by EY 06474 and NS 29343.

583.13

INHIBITION OF NITRIC OXIDE SYNTHASE BLOCKS TOUCH AND VISUAL LEARNING AND INHIBITION OF HEME OXYGENASE-2 INHIBITS TOUCH LEARNING IN *OCTOPUS VULGARIS* .
J. David Robertson*, J. Bonaventura, Adam Kohm, Amy Lane, Nicolas Limthong and Kenneth Harbaugh. Duke University Marine Laboratory, Pivers Island, Beaufort, NC. 28516.

We have recently demonstrated that inhibition of nitric oxide synthase by intramuscular injections of 75 mg/kg of N-w-nitro-L-arginine methyl ester (L-NAME) one hour before each training session blocks touch learning in *Octopus vulgaris* (*Proc. Roy. Soc., London, B* -in press). We used discrimination between ~2 cm. black plastic balls, one smooth and one rough as the learning paradigm. The balls could not be discriminated by vision alone but were readily distinguished by touch. We have now extended these studies to visual learning using discrimination between vertical and horizontal white rectangles as the learning paradigm. We have now begun a study of the effects on touch learning of intramuscular injections of Zn-protoporphyrin-9 at 10 mg/kg. After a few days this resulted in significant inhibition of touch learning. Since this inhibits heme oxygenase-2, the source of CO in brain tissue, it seems that CO, as well as NO, is necessary for touch learning. Wayne, Bonaventura and Sheetz have recently shown that NO, at low concentrations, causes active extension of filopodia in neuronal cultures (*Proc. Soc. Neurosci.* 1993). We have shown that cytochalasin D, which also blocks filopodial extension, blocks touch learning in Octopus, so our findings suggest that filopodial extension may be essential to touch learning in Octopus. There is always some doubt when using drugs to inhibit learning as to whether the primary effect is on sensory or motor output rather than on the learning process itself. To address this question, we have shown that animals treated with L-NAME can recall a previously learned touch paradigm but cannot learn a reversal of the paradigm. This suggests that the drug acts directly on the learning process.

583.14

ARC, AN ACTIVITY-REGULATED CYTOSKELETAL PROTEIN, IS A BRAIN SPECIFIC IMMEDIATE EARLY GENE THAT ASSOCIATES WITH THE CYTOSKELETON.
G.L. Lyford, K. Yamagata, W. Kaufmann, A. Lanahan, and C.A. Barnes# and P.F. Worley* Department of Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205 and #ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

To define the molecular mechanisms of neuronal plasticity, we have identified novel proteins that are rapidly regulated by neuronal activity. In particular we have isolated a novel gene, *Arc*, which demonstrates co-localization and interaction with the neuronal cytoskelecton. *Arc* was isolated by differential cloning techniques from electrically stimulated hippocampus. The message encodes for a 55kD protein that is homologous to loops 21 and 22 of the structural protein, α-spectrin. *Arc* mRNA and protein are rapidly and transiently induced by seizure and LTP stimuli and mRNA induction is not blocked by pretreatment with cyclohexamide. Basal *arc* mRNA expression shows developmental regulation with levels rising in post-natal brain and peaking at P16. Immunohistochemistry demonstrates Arc expression is restricted to neurons with protein detected in both cell soma and dendrites. Confocal images show that Arc co-localizes with the actin cytoskeleton in the sub-membranous cell cortex. *In situ* hybridization after seizure shows signal in both the cellular and molecular layers of the dentate gyrus, suggesting that *arc* mRNA is induced not only in the cell somas but also in the dendritic tree of the granule cells. Biochemical data corroborate the evidence from sequence homology and protein localization that *arc* codes for a cytoskeletal-related protein. We have demonstrated in a cellular fractionation from brain that greater than 90% of Arc immunoreactivity resides in the cytoskeletal pellet which is resistant to 2M KCl and 1% triton washes. We have further demonstrated that Arc copurifies with actin up to the last steps of an actin preparation. Finally we have demonstrated that an *in vitro* Arc translation product cosediments with F-actin. Taken together these evidence suggest that *arc* is a cytoskeletal-associated protein which may play a role in dendritic structural changes underlying neuronal plasticity. This work is supported by EY09374 and AG09219.

583.15

HEBBIAN PAIRING OF TACTILE STIMULATION. I. CORTICAL PHYSIOLOGY: RAPID TOPOGRAPHIC REORGANIZATION OF SOMATOSENSORY CORTEX OF ADULT RATS. B. Godde, F. Spengler, H.R. Dinse* Institut für Neuroinformatik, Theoretische Biologie, Ruhr-Universität Bochum RUB, D-44780 Bochum, Germany

We address the problem of fast reorganizational plasticity using a protocol of simultaneous, paired peripheral tactile stimulation (PPTS), motivated by the postulate of Hebb that temporal coincidence of external events are required to evoke plastic changes. In studies of cortical reorganization in the hindpaw representation of somatosensory cortex of adult rats, a few hours of PPTS induced a dramatic reorganization that included: 1) enlargement of receptive fields (RFs) that always comprised the RFs of the stimulated sites. Quantitative analysis of response planes confirmed these findings. 2) RF overlap increased significantly between RFs representing stimulated skin fields 3) Cortical representational areas of the stimulated skin fields were severalfold enlarged and were paralleled by a shift of the hindpaw representational borders. 4) Response durations were elongated due to enhacement of late response components. 5) Computer-based reconstruction of somatosensory maps revealed distortions that were selectively related to the stimulated skin sites, but the overall topography was mostly preserved. 6) All effects were fully reversible after 6 to 8 hours after termination of PPTS. 7) Response amplitudes and latencies were not effected. The implications of residual plasticity for short term plasticity in representational maps of adults are now investigated in computer simulations based on self-organizing maps, which include modifications of synaptic coupling without involving anatomical changes.

583.16

HEBBIAN PAIRING OF TACTILE STIMULATION. II: HUMAN PSYCHOPHYSICS: CHANGES OF TACTILE SPATIAL AND FREQUENCY DISCRIMINATION PERFORMANCE. H.R. Dinse, B. Godde, F. Spengler, B. Stauffenberg, R. Kraft (sponsor ENA) Institut für Neuroinformatik, Theoretische Biologie, Ruhr-Universität Bochum RUB, D-44780 Bochum, Germany

We address the problem of fast reorganizational plasticity using a protocol of simultaneous, paired peripheral tactile stimulation (PPTS). We demonstrated that a few hours of PPTS induced dramatic reorganization in the hindpaw representation of somatosensory cortex of adult rats (Godde et al. 1994, this meeting).

In order to study the functional implications of this short-term representational plasticity at a behavioral level, we initiated human psychophysical experiments using spatial (two-point discrimination) and frequency discrimination performance (in the flutter range). The index finger tip of the right hand was stimulated with a protocol analogous to the electrophysiological experiments (Godde et al. 1994, this meeting). We found a significant increase of the spatial discrimination performance after 6 or 2 hrs of passive stimulation (PPTS), but not after 30 min. Also, we found full reversibility of this improvement 8 hrs after termination of PPTS. The modifications of the frequency discrimination task were more complex including a transient detoriation of the performance.

The results are discussed in relation to the observed changes of RF size and overlap. We suggest that an increased number of neural elements involved in this task may be causally related to the improvement of the global processing.

NEURAL PLASTICITY II

584.1

GENERATION OF A SUBTRACTED cDNA LIBRARY FROM THE CEREBRAL CORTICIES OF AN ENVIRONMENTALLY ENRICHED RAT AND A STANDARD ENVIRONMENT RAT. S.A.Brooks*, D.T. Rivera†, M.C.Diamond, and J.L.Martinez,Jr†, Department of Integrative Biology and Department of Psychology†, University of California, Berkeley, CA 94720

Environmental enrichment in rats, when compared to rats housed in a standard environment, induces a number of anatomical and structural changes in the cerebral cortex including: an increase in dendritic branching (Holloway 1966, *Brain Res.*, 2:393-396), an increase in dendritic spine counts (Globus et al. 1973, *J. of Comp. Physiol. Psych.*, 82:175-181), an increase in glial cell counts (Altman et al., 1964, *Nature*, 204:1161-1163), and an increase in the hybridization of rat cerebral cortical RNA to unique rat DNA sequences (Uphouse et al., 1975, *Dev. Psychobiology* 8(2): 171-178). Together these results suggest that changes in gene expression underlie the enrichment-induced changes in the cerebral cortex. In an effort to identify some of the up-regulated genes, we performed a cDNA library subtraction, using a ten-fold excess of standard cortex cDNA as the negative, and generated an environmentally enriched subtracted cDNA library. We have begun characterization of the subtracted cDNA library and to date have isolated two genes. Preliminary analysis indicates that these genes are differentially expressed. Further analysis will include sequencing the genes and *In Situ* hybridization with the genes in the brains of both enriched and standard environment animals. Supported by Rennie Fund to Joe L. Martinez, Jr.

584.2

SYNERGISTIC SENSORY INNERVATION OF THE DORSAL AND VENTRAL HAND SURFACES IN PRIMATES. S. Seto*, J.P. Noonan, M. Steinschneider, J.C. Arezzo, P.E. Garraghty and C.E. Schroeder, Depts. Neurosci. and Neurol., Albert Einstein Coll. Med., Bronx, NY 10461 and Dept. Psych., Indiana Univ., Bloomington, IN 47405.

Previous studies have established the presence of nondominant radial nerve inputs within the median nerve representation of monkey Area 3b. Such co-representation of inputs may underlie the preferential patterns of hand map reorganization seen after peripheral nerve section.The present study assessed the degree of similar co-representation in Areas 4, 5, 7 (IP) and S2. Macaques were surgically prepared for chronic awake electrophysiologic recording. Linear array, multicontact electrodes were used to record laminar current source density and concomitant multiunit activity (MUA) profiles generated from electrical stimulation of the median, radial and ulnar nerves in the forearm. Within glabrous surface representations in S1, median or ulnar nerve stimulation produced a "dominant" laminar response profile characterized by robust short latency activation in Lamina 4, and later activation of supra- and infragranular laminae. Radial nerve stimulation produced a "nondominant" profile- small initial PSPs centered on Lamina 4, without clear MUA correlates, followed by a longer excitatory phase consisting of large PSPs and increased MUA. A qualitatively similar dominant/nondominant co-representation was found in all areas studied. Co-localization of inputs in these areas may, as in S1, influence the pattern of reorganization following peripheral injury. However, its role in normal function may be a counterpart to that of synergistic muscle innervation. That is, integration of temporally correlated inputs from the dorsal and volar hand surfaces may be important to somatosensory perception and routine hand use. (Supported by MH06723, MH47939, & by a J.S. McDonnell Foundation Grant).

584.3

PREOPERATIVE AND POSTOPERATIVE PRACTICE AFFECT THE RECOVERY OF LOCOMOTOR PLACING FOLLOWING UNILATERAL CORTICAL DAMAGE IN THE RAT. T.M. Barth*, S.L. Irish, and S. Barbay, Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

Behavioral compensation is one mechanism of recovery of function after brain injury. In this case, the brain-damaged organism makes learned adjustments in behavior to compensate for the lost function. Although this mechanism of recovery is well noted, it has not been systematically studied. Experimental methods used to determine if behavioral compensation is the mechanism of recovery involve manipulating preoperative and postoperative experience with the task. If recovery is related to learning new behaviors then it is reasonable to predict that task-specific experience should facilitate this process. The present experiments were designed to investigate whether pre- or postoperative practice can affect the recovery of locomotor placing following unilateral lesions of the rat somatic sensorimotor cortex (SMC). Locomotor placing was measured with the foot-fault test. Animals were placed on an elevated grid floor and allowed to freely locomote for 2 minutes. Rats without lesions use the rungs for footholds but occassionally a limb slips through one of the openings in the grid (i.e. a foot-fault). Rats with unilateral SMC lesions make a significant number of foot-faults with the forelimb contralateral to the lesion. Previous studies have suggested that recovery on the task is related to learning to inhibit the continuation of inaccurate forelimb placing movements based on somatosensory and proprioceptive feedback. In Experiment 1, rats received unilateral SMC lesions and either 7 days of preoperative practice or no practice. In Experiment 2, postoperative experience was manipulated by beginning to test the animals either 2 (practice group) or 14 (no practice group) days after the SMC lesion. The results of these experiments were that both pre- and postoperative task-specific practice facilitated the rate of recovery when compared to the no practice groups. These data support the idea that recovery of locomotor placing is related to behavioral compensation.

584.4

PREFRONTAL CORTEX INJURY TIME-DEPENDENTLY ALTERS STRIATAL *ZIF268* AND *JUN B* EXPRESSION. J.F. Marshall* and J.M. Vargo. Dept. of Psychobiology, University of California, Irvine, CA 92717-4550.

The nigrostriatal dopaminergic system has been implicated in the behavioral neglect and recovery seen following unilateral ablation of the of the medial agranular region of prefrontal cortex (AGm). In this study, immunohistochemistry for immediate early gene (IEG) protein products was used to examine alterations in striatal neuronal response at 5 days (neglect group) or 21+ days (recovered group) after unilateral AGm ablation. Basal and dopamine agonist-induced (5 mg/kg d-amphetamine [AMPH]) levels of IEG expression were examined. Adjacent brain sections were reacted with antisera to Zif268 or Jun B, and the amount of immunoreactivity in the CPu was determined through image analysis. At 5 days postlesion, basal and AMPH-induced Zif-immunoreactive (IR) nuclei in the ipsilateral CPu were fewer in number (25-30%, $p<.003$) and smaller in size (6%, $p<.003$) compared to those in contralateral CPu. For Jun-IR nuclei at 5 days postlesion, no hemispheric asymmetries in basal levels were evident, while AMPH-induced levels were reduced by 25% in the ipsilateral CPu ($p<.03$). This reduction was due to decreases in both number (20%) and size (10%) of Jun-IR nuclei. For both Zif and Jun, hemispheric asymmetries were primarily restricted to dorsolateral CPu, the region receiving dense afferents from AGm. In contrast to the 5-day findings, all hemispheric asymmetries were significantly diminished or not evident in recovered rats (21+ days).

584.5

PREFRONTAL CORTEX INJURY TIME-DEPENDENTLY ALTERS STRIATAL [^{3}H]GLUTAMATE BINDING. J.M. Vargo*, S.J. O'Dell, J.D. Blakey, and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717-4550.

Unilateral removal of the medial agranular region of prefrontal cortex (AGm) results in behavioral neglect of contralateral stimuli followed by recovery in about 3 weeks. The present study used autoradiography to examine regional alterations in striatal NMDA receptors at 5 days (neglect group) and 21+ days (recovered group) after unilateral AGm ablation. Time-dependent asymmetries in [^{3}H]glutamate (GLU) binding were seen. [^{3}H]GLU binding in the ipsilateral caudate-putamen (CPu) was decreased by 13% at 5 days postsurgery and increased by 9% at 21+ days postsurgery (p's<.05). These effects were restricted to the dorsolateral CPu, the region receiving dense afferents from AGm. Controls demonstrated symmetrical binding. These results are unlikely to be due to changes in CPu neuronal density following partial deafferentation since no lesion effects on [^{3}H]SCH-23390 (D_1 receptor) binding were evident in adjacent brain sections. These findings indicate that a partial loss of corticostriatal afferents leads to an initial reduction followed by an upregulation of striatal NMDA receptor binding, perhaps as a compensatory response to the loss of GLU input. These NMDA receptor changes may be partly responsible for the behavioral neglect and recovery, and for the time-dependent alterations of striatal immediate early gene expression seen following unilateral AGm ablation (see Marshall and Vargo, this volume).

584.6

NONCOMPETITIVE NMDA ANTAGONIST AND ANTIOXIDANT DRUGS FACILITATE BEHAVIORAL RECOVERY FOLLOWING ELECTROLYTIC LESIONS OF THE RAT CORTEX. M.R. Hoane* and T.M. Barth. Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

Following brain injury due to ischemia, hypoxia or fluid percussion trauma there is an excessive release of glutamate that appears to trigger a sequence of events leading to secondary cell death proximal and distal to the initial site of damage (i.e. excitotoxicity). Models of traumatic brain injury suggest that excitotoxicity may be attenuated by neuroprotective drugs that either prevent or limit the activation of the N-methyl-D-aspartate (NMDA) receptor by glutamate or prevent the breakdown of the cell membrane by free radicals (i.e. free radical scavengers). These neuroprotective drugs may facilitate behavioral recovery by limiting the extent of secondary brain damage. The present experiment investigated the possibility that neuroprotective drugs facilitate behavioral recovery following electrolytic lesions of the cortex. Three drugs were studied: MK-801 (a noncompetitive NMDA antagonist working at the PCP binding site); magnesium chloride ($MgCl_2$; a voltage dependent noncompetitive NMDA antagonist); and N-tert-butyl-α-phenylnitrone (PBN; an antioxidant and free radical scavenger). Rats received unilateral electrolytic lesions of the somatic sensorimotor cortex (SMC) and a regimen of MK-801 (1 mg/kg), $MgCl_2$ (1 mmol/kg), PBN (100 mg/kg) or saline beginning 15 min after the lesion. The rats were tested on a locomotor placing task. Saline treated animals showed the expected severe impairment in placing the contralateral forelimb during locomotion on the grid floor. In contrast, rats receiving MK-801, $MgCl_2$ or PBN showed a reduction in the magnitude of the initial deficit as well as an acceleration of recovery. These results suggest that neuroprotective drugs identified in models of ischemia and traumatic brain injury have the expected behavioral effects in an electrolytic lesion model. Moreover, they suggest a process similar to that of excitotoxicity may occur following electrolytic brain lesions causing transneuronal degeneration in areas distant from the lesion site.

584.7

WHAT ARE THE NECESSARY AND SUFFICIENT CONDITIONS FOR DENDRITIC REORGANIZATION AFTER CORTICAL INJURY? M.L. Forgie*, R. Gibb, S. Rowntree, and B. Kolb. Department of Psychology, The University of Lethbridge, Lethbridge, Canada, T1K 3M4.

There are several reports of dendritic reorganization after neocortical injury in adult rats. We have found that prefrontal, cingulate, and motor cortex lesions all lead to a chronic increase in dendritic arbor in parietal cortex ipsilateral to the lesion. Jones & Schallert (1992) also found increased dendritic growth in the normal hemisphere of rats with unilateral motor cortex lesions, and they correlated this with behavioral asymmetry. In contrast, we found that large unilateral strokes result in chronic dendritic atrophy ipsilateral to the lesion and no change in the normal hemisphere, in spite of a large behavioral asymmetry. The presence or absence of dendritic growth following these lesions may be due to differential astrocyte reactivity, lesion size, or be secondary to behavioral change. We therefore made unilateral motor cortex lesions of varying sizes and at different ages throughout the lifespan and measured behavioral asymmetry. Animals were sacrificed at different postoperative intervals for GFAP, bFGF, vimentin, synaptophysin, and OX-42 immunohistochemistry, or Golgi-Cox analysis. Dendritic change was related to age, the magnitude of the astrocytic reaction, and lesion size, but we found no relationship between behavioral change and dendritic growth in the normal hemisphere.

584.8

TACTILE STIMULATION ENHANCES RECOVERY AND DENDRITIC GROWTH IN RATS WITH NEONATAL FRONTAL LESIONS. Bryan Kolb*, Grazyna Gorny and Robbin Gibb. Dept of Psychology, University of Lethbridge Lethbridge, Canada, T1K 3M4.

Rats were given medial frontal lesions or sham operations on postnatal day 4. Beginning on day 5 half of the animals were given 15 min of tactile stimulation with a small paint brush three times per day until weaning at day 21. At 60 days the animals were trained in the Morris water task and the Whishaw reaching task. The behavioral results showed large behavioral deficits in lesion animals relative to normal control rats. These deficits were significantly attenuated by the stroking. At the end of testing the brains were processed for a modified Golgi-Cox stain or immunohistochemistry. Frontal lesions reduced brain weight and decreased dendritic branching. The tactile stimulation significantly reversed these effects, with the effects being larger in female than male rats. Spine density was decreased by tactile stroking in both normal and operated rats. GFAP, OX-42, and synaptophysin immunochemistry is in progress. Overall, the results suggest that tactile stimulation during infancy may modulate the effects of perinatal cortical injury and may affect synaptic development in the normal brain.

584.9

NEUROCHEMICAL AND BEHAVIORAL RECOVERY AFTER COLCHICINE LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN RATS. L. W. Shaughnessy*[1], S. Barone Jr.[2], W. R. Mundy[3] & H. A. Tilson[3]. [1]Curriculum in Neurobiology, UNC, Chapel Hill [2] METI & [3] Neurotoxicology Division, U.S. EPA, RTP, NC 27711.

It has been proposed that the cholinergic system plays a critical role in learning and memory (Deutsch, 1971, *Science* 174:788-794; Bartus *et al.*, 1985, *Ann NY Acad Sci* 444:332-358). In support of this hypothesis are data demonstrating that cortical cholinergic hypofunction correlates with cognitive deficits in humans (Perry *et al.*, 1978 *Br Med J* 2:1457-1459; Wilcock *et al.*, 1982 *J Neurol Sci* 57:407-417). In the present study, infusions of colchicine into the basal forebrain have been used to further explore the relationship between cholinergic and behavioral function. Bilateral infusions of either colchicine (3.0μg/0.5μl/site) or vehicle (0.5μl/site) were made in the nucleus basalis magnocellularis (NBM) of male Long-Evans rats (n=12/group). Four weeks post-lesion, behavioral assessments were made for one-half of the rats in each group. Five weeks post-lesion, these rats were sacrificed and ChAT activity was measured in several brain regions. The second half of the rats were tested behaviorally 11 weeks post-lesion and sacrificed twelve weeks post-lesion. Five weeks after colchicine infusion, there was a significant decrease in cortical ChAT activity and passive avoidance cross-over latency and a deficit in the acquisition rate of a water maze task. Twelve weeks after colchicine infusion, ChAT activity in the parietal cortex and acquisition of the water maze task were not significantly different than vehicle-infused rats, although a significant decrease was seen in the passive avoidance cross-over latency. These data suggest an association between time-dependent recovery of a cholinergic deficit and recovery of a spatial learning task. In contrast, the passive avoidance task had an equivalent deficit at both timepoints. In summary, these data show task-specific behavioral recovery associated with time-dependent recovery in a specific regional cholinergic marker.

584.10

BEHAVIORAL EFFECTS OF BILATERAL CORTICAL LESION ON REACTION-TIME PERFORMANCE IN THE RAT C Baunez.,P Salin, A. Nieoullon and M. Amalric* Cellular and Functional Neurobiology Laboratory, CNRS, 13402 Marseille cx 9 (France).

There is substantial evidence that suppression of cortical projections enhance striatal dopaminergic activity. Cortical ablation however does not result in obvious motor deficits on spontaneous behaviors. The effect of a bilateral cortical lesion were thus studied in a conditioned motor task known to be sensitive to striatal dopamine activity. Rats were trained to depress and hold a lever until the presentation of a visual conditioned stimulus (CS) and then to release it with a reaction time (RT) of less than 500 ms for food reinforcement. Cortical lesions were performed by thermocoagulation of fronto-parietal areas. Such lesions are known to induce a progressive degeneration of cortical layers. The animals were tested daily from day seven to thirty-five post-lesion. A dramatic short-lasting increase in the number of delayed responses (over 500 ms), recovering after 20 days, could be observed in the reaction-time task. This effect was classically considered as a motor impairment. In contrast, a slight and long-lasting increase in the number of anticipated responses (premature release of the lever before the CS) was observed throughout the 35 days of testing. These results show that an extensive lesion of the fronto-parietal cortical areas induced a mixed effect with different time recovery. The long-lasting effect on anticipated responses could be related to the effects produced by a dopaminergic hyperactivity in the striatum. The short-lasting effect on delayed responses is suggested to result from damage of the direct motor pathways following extensive cortical lesions that are rapidly compensated by other mechanisms.

584.11

THE CONTRIBUTION OF TASK-SPECIFIC EXPERIENCE TO THE AMPHETAMINE-INDUCED FACILITATION OF RECOVERY IN RATS WITH CORTICAL DAMAGE. T.D. Schmanke* and T.M. Barth. Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

In an early study it was shown that amphetamine facilitates the recovery of beam-walking ability in rats with unilateral sensorimotor cortical damage (SMC) only if the animals receive task-specific practice while under the influence of the drug. More recently it has been suggested that amphetamine and practice have independent effects on the recovery of beam-walking. The present study evaluated the effects of amphetamine and practice on the recovery of tactile forelimb placing following unilateral SMC lesions. This behavior was chosen because amphetamine facilitates recovery from placing deficits even though the animals do not show placing reactions during the time of drug intoxication. If task-specific experience has a role in the facilitation of recovery it does not require the execution of the motor response while under the influence of the drug. Experiment 1 tested whether amphetamine (2 mg/kg) and practice interact to affect recovery. Four groups were used: drug + practice (dp); drug + no practice (dnp); saline + practice (sp); saline + no practice (snp). The dp group showed the fastest rate of recovery. Statistical analyses showed that there were main effects for drug and practice but no significant drug x practice interaction. These data suggest amphetamine and practice have independent effects on recovery of tactile placing. Experiment 2 attempted to isolate the type of experience necessary to facilitate recovery of forelimb placing. Unilateral SMC damaged rats were given amphetamine and assigned to one of four different groups: practice on the contralateral side only; practice on the ipsilateral side only; handling only; no practice. The results were that rats receiving either ipsilateral or contralateral experience during the period of drug action recovered significantly faster than animals receiving no practice. These data suggest that either task-specific somatosensory stimulation contralateral to the lesion or ipsilateral sensory and motor experience on the task facilitate recovery of tactile placing.

584.12

FAST AND REVERSIBLE REORGANIZATION OF THE THALAMO-CORTICAL PATHWAY OF ADULT RATS INDUCED BY INTRACORTICAL AND INTRATHALAMIC MICROSTIMULATION. R.F. Zepka, F. Spengler and H.R. Dinse. (sponsor EBBS) Inst. Neuroinformatik, Theoret. Biol. Ruhr-Univ. Bochum RUB, D-44780 Bochum, Germany

Recent experiments revealed rapid reorganization of the cortical representations of the primary somatosensory cortex (SI) of adult rats following intracortical microstimulation (ICMS) [Recanzone et al. (1992) Cerebr Cor 2: 181; Dinse et al. (1993) Neuroreport 5: 173; Spengler & Dinse (1994) Neuroreport 5:]. While there is a substantial body of information about the reorganization at a cortical level, little is known about the nature of subcortical plasticity.

The present study investigated the contribution of the thalamic ventral posterior lateral nucleus (VPL) to the cortical reorganization induced by ICMS and the degree and nature of reorganization of the somatosensory representation in this thalamic nucleus induced by direct intrathalamic microstimulation (ITMS). The experiments were performed in adult rats anaesthetised with Urethane. Neurons within the hindpaw representation in SI and in VPL were recorded with glass microelectrodes, microstimulation was applied at a selected cortical (ICMS) or thalamic (ITMS) site for 2 to 4 hours. We studied the effect of 1) ICMS on VPL; 2) ITMS on VPL; and 3) ITMS on SI. In contrast to the extensive cortical reorganization following ICMS, small effects in the reorganization of the somatosensory map at the VPL were found. ITMS elicited significant changes of the RFs in VPL. However, unlike the cortical ICMS effects, no spatial expansions of the skin representations surrounding the RF of the stimulated site were observed neither in VPL nor in SI after ITMS.

The present results indicate that a few hours of ITMS leads to plastic reorganization at the VPL. However, the changes are small compared to those describe for SI, suggesting that this type of fast plasticity is a predominantly cortical phenomenon.

Supported by Coordenadoria de Aperfeicoamento de Pessoal (CAPES - Brazil).

584.13

QUANTITATIVE DENDRITIC AND SPINE ANALYSES OF HUMAN PREFRONTAL AND OCCIPITAL CORTICES. L. Larsen, R.L. Swanson, M.L. Wainwright, and B. Jacobs.* Laboratory of Quantitative Neuromorphology, Dept. of Psychology, The Colorado College, Colorado Springs, CO 80903.

A fundamental issue in quantitative neuromorphology is the extent to which processing demands affect the complexity of dendritic systems. To this end, the basilar dendritic systems of supragranular pyramidal cells in human supramodal association cortex (prefrontal, area 10) were compared with those in unimodal association cortex (occipital, area 18). Tissue obtained from the left hemisphere of 5 neurologically normal subjects was processed with a modified rapid Golgi technique. Quantification of 100 neurons (10 neurons per tissue block) was performed on a Neurolucida system (Microbrightfield, Inc.) according to accepted criteria (Jacobs & Scheibel, 1993, *J. Comp. Neurol., 327,* 83-96). Dependent measures were total dendritic length (TDL), mean dendritic length (MDL), dendritic segment count (DSC), and dendritic spine number (DSN). A distinction was also made between proximal (1st, 2nd, and 3rd order) and the ontogenetically later developing distal (4th order and above) dendritic branches.

Despite interindividual variation, neurons in area 10 consistently exhibited higher TDL (19.8%), DSC (19.9%), and DSN (31.6%) values than those in area 18. Proximal-distal distribution was similar between both cortical areas for TDL, DSC, and DSN, but distal segment MDL was an average of 79.6% greater than proximal segment MDL (which is consistent with Jacobs et al., 1993, *J. Comp. Neurol., 327,* 97-111). An order by order analysis of the dendritic envelope revealed that differences between the two cortical areas were most pronounced in 4th order dendritic segments. Significant age-related decreases in TDL, MDL, and DSN were also noted. This finding of greater dendritic neuropil in area 10 over area 18 provides tentative support in humans for the notion that dendritic system complexity roughly reflects the computational demands placed on those systems. (Tissue generously provided by Dr. D. Bowerman, El Paso County Coroner, and Dr. R. Sherwin, Penrose Hospital)

584.14

LEARNING INDUCED PLASTICITY OF CORTICAL MAPS - LACK OF CHANGES IN REPRESENTATIONS OF ADJACENT, UNTRAINED SENSORY RECEPTORS. M. Kossut* and E.Siucińska. Dept. of Neurophysiology, Nencki Institute, 3 Pasteur st. 02-093 Warsaw, POLAND.

Sensory training is known to produce an enlargement of cortical representations of receptors activated during the training (Jenkins et al.1990, Recanzone et al., 1992, Weinberger et al., 1993). Increase of cortical representations of trained receptors was accompanied by a decrease in extent of adjacent representations of receptors not involved in training. We have previously demonstrated that short lasting classical conditioning involving stimulation of vibrissae results in enlargement of vibrissal cortical representation. In the present experiment we examined if the enlargement of the trained row B of whiskers representation takes place at the expense of neighboring, untrained rows.

During training stimulation of row B was paired with electrical irritation of the tail. Four pairings per minute were repeated for 10 min. a day for 3 days (altogether 120 pairings). A day after completion of training 2-deoxyglucose mapping was performed, in which cortical representation of the trained row B and control row B on the other side of the snout were visualized. As we have shown previously, representation of the trained row B was broader by 45 %, expanding into territories of row C and A. In another group od animals we examined if cortical representation of rows A and C bordering the trained row B, are narrower than usually. Analysis of 2DG autoradiograms revealed that labeling induced by stimulation of rows A and C is not decreased either in area or in intensity compared to control. The cortex corresponding to the trained row B (unstimulated during 2DG mapping) showed higher labeling than on the control side. The results indicate that training-induced expansion of row B representation is a dynamic phenomenon, where regions of cortex are activated by a new input without loosing normal reactivity to the old one.

NEURAL PLASTICITY III

585.1

DIFFERENTIAL EXPRESSION OF THE TRANSCRIPTION FACTOR ZENK IN RESPONSE TO ENVIRONMENTAL COMPLEXITY AND HANDLING. C.S. Wallace*, D.F. Clayton & W.T. Greenough Neuronal Pattern Analysis, Beckman Inst., Univ. Illinois, Urbana, IL 61801.

Exposure to environmental complexity (EC) rapidly induces morphological plasticity in the visual cortex of weanling rats (Wallace et al., 1992). Previously we reported (Withers et al., SN Abstr.1991) that a transcription factor, ZENK, associated with LTP (Cole et al., 1989, Wisden et al., 1990) is elevated in the visual cortex of EC animals following short term exposure as well. ZENK is also known as Zif/268, Egr-1, NGFI-A and Krox 24. To assess the behavioral regulation of ZENK, we used quantitative *in situ* hybridization to measure the expression of ZENK mRNA after 2-4 days of either: 1) group housing in a complex environment (EC); 2) individual housing with once-a-day handling (HIC) and 3) housing in complete isolation (IC). Active exploration (EC) resulted in significant increases of ZENK in visual cortex (OC2) of HIC and IC, suggesting behavioral regulation of ZENK in a region that supports robust plasticity. HIC alone significantly elevated ZENK above IC values. No significant differences were observed in frontal region FR2. ZENK levels were significantly increased in hippocampal subfield CA1 following EC. Significant elevations of ZENK in the striatum were only observed in the EC animals, suggesting plasticity in this region may be induced by EC. Regardless of condition, ZENK was distributed in a modular pattern in many cortical regions. The sensitivity of ZENK to behavioral context indicates that experience can dynamically regulate neuronal gene expression. Supported by MH35321 & Kiwanis Foundation to WTG and NS25742 to DFC.

585.2

LONG-TERM POTENTIATION AND KINDLING-INDUCED POTENTIATION OF FIELD POTENTIALS IN ENTORHINAL CORTEX EVOKED BY PYRIFORM CORTEX STIMULATION. C.A.Chapman* and R.J.Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S-4K1.

Long-lasting potentiation phenomena in the rat entorhinal cortex have not been completely described in the chronic preparation. Monopolar field potentials (EPs) were recorded in the entorhinal cortex in response to pyriform cortex test-pulses (.1ms, 16-1259μA). The major component was a negative-going potential, ≈0.75mV in amplitude with a ≈16ms latency to peak. Responses were tested 4 times over a 7 day baseline period. Five animals then served as controls, 5 received high-frequency trains to the pyriform cortex (40-60 400Hz trains of 16 .1ms 1259μA pulses), and 5 were begun on a 6 day pyriform cortex kindling procedure (1sec 60Hz trains of 1ms 600μA pulses 3 times/day). EP amplitudes remained stable in the control group. Kindling-induced potentiation of EP amplitudes (mean=201% of baseline) was observed during the kindling protocol, and declined over the next two weeks to 137% of baseline. Peak LTP at test-pulse intensities of 501 or 794μA was 156% of baseline levels on average. However, the amount of potentiation observed roughly tripled to 240% when each high-frequency train was subsequently embedded in a frequency potentiating pyriform cortex train (11 pulses, 15Hz). The responses showed little decay over a 7 wk testing period except at the highest test-pulse intensities. These results indicate large, reliable, and long-lasting potentiation effects in entorhinal cortex EPs in the chronic preparation.

585.3

SPATIAL MEMORY FOLLOWING PERIPHERAL NERVE GRAFTS AND FORNIX-FIMBRIA TRANSECTION. A.D. Davis,[1]* L.F. Kromer[2], and L.A. Rothblat[1] [1]Dept. of Psychology, George Washington Univ., Washington, DC 20052 and [2]Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

The purpose of this study was to determine whether peripheral nerve grafts in the CNS can promote axonal regeneration resulting in behavioral recovery. Fourteen rats received bilateral knife-cut lesions of the fimbria-fornix. Eight of the animals also received a sciatic nerve transplant placed between the septum and hippocampus. Four animals served as normal controls. Beginning 2 weeks after the transplant procedure, all animals were tested on a discrete-trial rewarded alternation task and the Morris water maze. Testing continued for 2 months. Both experimental groups were signficantly impaired on the spatial tests compared to normal controls throughout testing. Moreover, preliminary analysis of the results indicate that the sciatic nerve transplants had no facilitating effects on spatial performance. Correlations between cholinergic innervation and behavior are currently being conducted.

Sponsored Society of Neuroscience/NIMH and The George Washington University Facilitating Fund.

585.4

Null mutation of *c-fos* Impairs Functional and Structural Plasticities in the Mammalian Nervous System. Yoshinori Watanabe[1,4], Randall S. Johnson[2], Linda S. Butler[1], Bruce M. Spiegelman[2], Virginia E. Papaioannou[3], and James O. McNamara[1]* [1]-Duke Univ. Med. Center, [2]-Harvard Med. School , [3]-Colombia University, [4]-Fukushima Medical College.

Fleeting changes of neuronal activity produce a lasting reorganization of synaptic connections in development of the mammalian nervous system. Activity-dependent interactions also contribute to a formation of abnormal synaptic connections in the mature nervous system. Expression of the immediate early gene, *c-fos*, has been postulated to link fleeting changes of neuronal activity to lasting modifications of neuronal function and structure in the mammalian nervous system. To test this idea, we examined behavioral and electrophysiologic indices of kindling development and kindling-induced sprouting of hippocampal granule cell axons in wild type (+/+), heterozygous (+/-), and homozygous (-/-) mice carrying a null mutation of *c-fos*. Defects of both electrophysiologic and behavioral features of kindling development were evident in null mutants. Kindling-induction of granule cell axon sprouting measured by Timm staining was significantly attenuated in null mutants compared to +/+ mice with intermediate values evident in +/- mice. We conclude that *c-fos* is necessary for both normal development of kindling and complete expression of kindling-induced synaptic reorganization of granule cell axons. We suggest that the null mutation of *c-fos* produces this phenotype by altering seizure-induced transcriptional activation of target genes.

585.5

RHEB, A RAS HOMOLOG ENRICHED IN BRAIN, INTERACTS WITH RAF1 SERINE/THREONINE KINASE IN AN ACTIVITY-REGULATED MANNER. W. M. Yee*, A. Lanahan, and P.F. Worley. Department of Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205

It has been postulated that production of specific gene products in response to neuronal stimulation is required for the establishment and maintenance of neuronal plasticity. In our efforts to characterize activity-regulated gene products associated with neuronal plasticity and long-term potentiation (LTP), we have isolated and characterized Rheb, a novel Ras homolog enriched in brain that is induced by LTP and seizure stimuli and is regulated as an immediate early gene. Rheb is also induced by NGF, EGF, and FGF in neuronal cell lines and is enriched in the granule cell layer of the hippocampus following stimulation. Rheb has significant homology with human H-Ras and yeast RAS1 in conserved domains required for GTP-binding and hydrolysis, and appears to be a functionally close relative of human H-Ras as determined by Rheb's ability to transform mammalian cells. In order to characterize Rheb's physiological role in neurons following neuronal stimulation, we have utilized the yeast two-hybrid system of identifying protein-protein interactions to isolate upstream and downstream interactors of Rheb. We report here that Rheb interacts with Raf1, a serine/threonine kinase required for the oncogenic activity of human H-Ras. Using an *in vitro* co-precipitation assay, we have confirmed that Rheb interacts with Raf1 kinase isolated from the hippocampus and cortex of postnatal day 21 rats. In addition, we have experimental evidence suggesting that the interaction between Rheb and Raf1 may be regulated by neuronal activity. This line of evidence suggests that Rheb may be acting as an activity-regulated "molecular switch" that activates Raf1 serine/threonine kinase following depolarizing stimuli or neurotransmitter binding.

585.6

A NOVEL PROTEIN REGULATED BY SYNAPTIC ACTIVITY IN VISUAL CORTEX AND HIPPOCAMPUS. P. R. Brakeman*, A. Lanahan and P. F. Worley. Depts. of Neuroscience and Neurology, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

A novel activity-regulated developmental immediate-early gene (Ard62) was cloned by differential screening of a rat hippocampal cDNA library. Ard62 encodes a brain-specific 6.5 kB mRNA with a 186 amino acid open reading frame that is not homologous to known proteins. Sequence analysis predicts that Ard62 has multiple phosphorylation sites, but no glycosylation sites. Antisera raised against a bacterial fusion protein containing the 186 amino acid open reading frame recognizes a single 28 kD band in HEK 293 cells transiently transfected with an expression construct containing the open reading frame and a 28 kD doublet in rat hippocampus. This 28 kD doublet is rapidly induced in hippocampus following seizure with a maximal induction at 4 hours. Fractionation of hippocampal tissue shows that Ard62 is primarily cytosolic, but not nuclear, and also is associated with the membrane fraction. Northern blot analysis shows that Ard62 is induced in hippocampus within 30 minutes following seizure and is super-induced by pre-treating with cycloheximide. In situ hybridization studies show that Ard62 is strikingly inducible in visual cortex. Exposing dark-reared rat pups to light dramatically increases Ard62 mRNA in visual cortex, and unilateral intraocular injection of TTX reduces basal expression of Ard62 in the contralateral visual cortex. A role for Ard62 in synaptogenesis is suggested by the redistribution of Ard62 message in cortex during a critical period of excitatory synaptogenesis in the second post-natal week in rats. The distribution of Ard62 changes from a diffuse cortical distribution in day 10 rats to a specific localization in cortical layers II and V/VI in day 14 rats. In addition, ARD62 is strongly induced in dentate gyrus in an NMDA-dependent manner following a high frequency LTP stimulus. Ard62 is a novel protein regulated by synaptic activity in cortex and may be involved in cortical synaptogenesis. Supported by EY09347.

585.7

VARIABILITY OF POPULATION SPIKE AMPLITUDE IN LONG-TERM POTENTIATION IN BEHAVING RATS. S.J. Jones, D. Moore, and M.E. Corcoran*. Dept. of Psychology, University of Victoria, POB 3050, Victoria, B.C., Canada, V8W 3P5.

Although long-term potentiation (LTP) has been postulated to be a mechanism of learning and memory, relatively few investigations of LTP have been performed in awake rats carrying chronic electrodes, and, of those, large variation in the amount of LTP has been observed across laboratories. To study factors influencing LTP, we measured the amplitude of the population spike (PS) evoked in the dentate hilus by stimulation of the perforant path in awake rats. In Experiment 1, LTP was induced in 1 group with hilar chemitrodes receiving infusions of aCSF, in another group with chemitrodes but no infusions, and in a third group with electrodes. The electrode group exhibited greater LTP than either chemitrode group. Experiment 2 tested the effects of behavioural state on LTP. Data from 1 group were collected only when rats were immobile; data from group 2 were collected irrespective of ongoing behaviour. All rats in Experiment 2 received i.p. injections of saline 30 min before tetanization, to mimic conditions of drug administration. No LTP was observed in either group at 60 min post-tetanization, whereas significant LTP was observed 24 hr later. Experiment 3 tested the hypothesis that the saline injection may have suppressed LTP at 60 min. Group 1 received an i.p. injection of saline prior to tetanization, and group 2 received no injection. At 60 min, only group 2 exhibited significant LTP, whereas both groups showed LTP at 24 hr. In Experiment 4, one series of tetanic stimulations was applied each day for 9 days. Significant LTP was observed only after session 2 and asymptoted after session 6.

We conclude that the magnitude of LTP is affected by the presence of a chemitrode and by prior injections and that induction of LTP is more reliable with multiple sessions of tetanization. (Supported by NSERC)

585.8

DEHYDROEPIANDROSTERONE SULFATE INCREASES PS IN DENTATE GYRUS LTP IN INTACT RATS. A.Yoo, J.Harris* and B.Dubrovsky. Dept. of Physiology, McGill University Montreal, Quebec, CANADA H3A1A1

Dehydroepiandrosterone Sulfate (DHEAS), a neurosteroid secreted by both the adrenal cortex and the glia, has been shown to behave as a non-competitive antagonist of the $GABA_A$ receptor which exerts inhibitory effects on LTP development when activated. Thus, it could be hypothesized that DHEAS would enhance LTP formation. Experiments were performed in intact anaesthetized rats (urethane,1.5mg/Kg). Electrodes were stereotaxically positioned in the perforant path (bifocal for stimulation) and in the dentate gyrus (monofocal for recording). DHEAS (20 mg/Kg) was injected in the femoral vein, dissolved in Nutralipid 10% which served as control injection. Our data showed that after hormonal treatment, 10 minutes before priming tetanic stimulation (8 trains of impulses, total duration of 1 sec, train rate of 0.03 Hz, frequency of 350 Hz and pulse duration of 500 μsec each half wave), the amplitudes of the population spike (PS) increased approximately 2 fold over the ones from control experiments. In contrast, slopes of EPSPs did not change. Both latencies of EPSPs and PSs were shifted towards shorter durations with the hormone. The results,consistent with the work of Meyer et al.(1993) in CA1 area of rat hippocampal slices, further suggest that hormonal compounds could differentially affect selected neuronal loci.

585.9

REWARD-SCHEDULE EFFECTS AND GRANULE CELL NEUROGENESIS IN THE DENTATE GYRUS OF THE RAT

G.Y. Espinoza & A. Amsel*. Dept. of Psychology & Inst. for Neuroscience, University of Texas, Austin, TX 78712

Granule cell neurogenesis in the rat dentate gyrus has been shown to continue well into adulthood, although peak neurogenesis occurs at the end of the first postnatal week. We recently examined the effect of a number of kinds of learning on granule cell neurogenesis in 11-12-day-old pups (Espinoza & Amsel, Soc. Neurosci. Abs., 1993). No significant training effect was found, but there was a significant effect due to handling. The experiment has now been replicated with 17-18-day-old pups. They were trained in 6 sessions over 2 days (3 sessions per day), 30 trials per session. There were 3 groups defined by reinforcement schedule: continuous reinforcement, partial reinforce-ment, or reinforced in a pattern of single alternation (PSA). A fourth group, a handled control, got no runway training, but was put through all other specific aspects of handling in the training situation. A fifth group was an unhandled control: no handling and no training. At the end of each of the 2 training days, pups from all groups were injected intraperitoneally with Methyl-^{3}H-Thymidine (activity 5Ci/mmol, 5mCi/gm wt) in order to label newly forming cells. Coronal brain sections were coated with Kodak NTB-2 emulsion, exposed, developed and then counterstained with cresyl violet. Computer-aided counts revealed no significant differences among all five groups including no handling effect. At 17 days of age, rat pups can learn PSA with intertrial intervals (ITIs) up to 60 seconds, whereas 11-day-olds can learn PSA only at an 8-second ITI. To test the possibility that neurogenesis of granule cells in the dentate gyrus is influenced only at longer ITIs, the first four 17-day-old experimental groups are being replicated with longer ITIs. Data are also being collected on the effects of postnatal alcohol administered by artificial rearing from days 4 - 9.

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585.10

LESION-INDUCED COLLATERAL SPROUTING AND THE BRAIN'S RESPONSE TO INJURY. J. Knapp*, J.J. Norden. Dept. of Cell Biology, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232

Growth-associated protein-43 (GAP-43) is a nervous system-specific phosphoprotein whose expression is correlated with axon growth during regeneration and development. We are interested in the involvement of GAP-43 in axon elongation and employ lesion-induced collateral sprouting to study the correlation between axon growth and GAP-43 in the adult rat CNS. One of the most widely used paradigms to study collateral sprouting is unilateral removal of the entorhinal cortex (EC). This results in a partial denervation of the dentate gyrus (DG) of the hippocampus, and subsequent growth and reinnervation of the denervated areas by other afferents projecting to the DG. Preliminary data suggest that the sprouting response induced by electrolytic and aspiration methods may be qualitatively and quantitatively different. While under nembutal anesthesia (50 mg/kg), the right EC is lesioned (aspiration or electrolytic) in male Sprague Dawley rats. The animals survive for 2, 6, 15, or 30 days before sacrificing. The brain is removed, embedded, sectioned, and then immunoreacted with GAP-43 or GFAP antibodies. Following electrolytic lesions of the EC, the inner molecular layer of the DG expands to a greater width than following aspiration lesions as evidenced by GAP-43 immunoreactivity of brain sections. In addition, astrocytes are more immunoreactive with GFAP antibodies indicating a more robust gliosis following electrolytic lesions than after aspiration lesions. We are currently investigating other differences between the two types of lesions in generating a sprouting response in the DG. We hypothesize that seizures generated by electrolytic lesions influence the sprouting and astrocyte response following electrolytic lesions.

585.11

SYSTEMIC ADMINISTRATION OF NALOXONE AFFECTS EPSP-SPIKE COUPLING IN LONG-TERM POTENTIATION OF THE LATERAL PERFORANT PATH. R.D. Kirkby*, C.R. Bramham, and J.M. Sarvey, Department of Pharmacology, Uniformed Services University, Bethesda, MD 20814.

Previous research suggests that activation of opioid receptors is necessary for the induction of long-term potentiation of field responses evoked in the dentate gyrus by electrical stimulation of the lateral perforant path, the terminals of which may release both glutamate and opioid peptides. To further assess the role of opioid receptors in long-term potentiation in this pathway, we systemically administered naloxone (10 mg/kg; i.p.) 60 min prior to tetanic stimulation of the lateral perforant path in urethane-anesthetized adult rats. Tetanic stimulation consisted of 10 trains (60-s intertrain interval) of 8 pulses (200-μs duration; 400 Hz).

Contrary to expectation, naloxone- and saline-treated rats demonstrated similar increases in the initial slope of the excitatory postsynaptic potential (EPSP) recorded in the hilus. On the other hand, the magnitude of the population spike as a function of the slope of the EPSP after tetanus was somewhat smaller in rats treated with naloxone. The findings may shed light on the participation of opioid receptors in synaptic plasticity in the lateral perforant path.

Supported by NIH NS23865.

585.12

A MODEL FOR ASSOCIATIVE LTP: ROLES OF RETROGRADE MESSENGERS. F. Alicata, G.F. Ayala*, M. Migliore, Oasi Institute for Research and Treatment of Mental Retardation and Brain Aging, Troina (EN), Italy, and I.A.I.F.-Natl. Res. Council, Palermo, Italy.

We present a kinetic model of associative Long-Term Potentiation (LTP) in the hippocampus. We used an extension of a previous work on simulation of LTP (M.Migliore and G.F.Ayala, *Neur. Comp.* 5, 103 (1993)) to implement the two population of synapses activated by a weak (W) and/or a strong (S) afferent pathways to the same hippocampal dendritic region. The model is in good agreement with experimental data on the associative properties of LTP and its modifications when using different protocols of stimulation. The model suggests a possible interpretation of the experiments in terms of molecular processes and a possible key role of retrograde messengers in the induction of associative LTP. In particular, the model suggests that the associative properties of LTP could be explained in terms of the modulating effects by the retrograde messengers on the coupling mechanisms between the S and W pathways. Two possible modes of coupling the two pathways have been tested with this model, one pre- and one post-synaptic, and both are in good qualitative agreement with experimental data.

585.13

ROLES OF RETROGRADE SIGNALS IN LTP AND LTD: A COMPUTATIONAL MODEL. M. Migliore*, F. Alicata, G.F. Aya la, I.A.I.F. - Natl. Res. Council, Palermo, Italy, and Oasi Institute for Research and Treatment of Mental Retardation and Brain Aging, Troina (EN), Italy.

We propose a kinetic model that suggests an interpretation of experiments in terms of the molecular processes that control the induction and maintenance of Long-Term Potentiation (LTP) and Long-Term Depression (LTD). The model suggests that LTP and LTD could be maintained by two similar but distinct autocatalytic processes activated by the same class of retrograde messengers. In particular, we propose that these two processes could interfere with the normal synaptic transmission mechanisms, increasing (LTP) or decreasing (LTD) the amount of neurotransmitter released at each stimulus. We present simulations of the effects of application of inhibitors of retrograde signal production such as L-NAME and ZnPP, and application of Nitric Oxide scavengers, such as Hemoglobin. Simulations' results suggest an explanation of the experimental findings that retrograde messengers are ininfluent to maintain LTP or LTD, whereas an appropriate level of retrograde signal is needed to induce or maintain LTP and/or LTD during tetanic stimulation.

585.14

HIPPOCAMPALLY-DEPENDENT TRACE EYEBLINK CONDITIONING INDUCES CHANGES IN THE IMMUNOREACTIVITY FOR MUSCARINIC ACETYLCHOLINE RECEPTORS AND PKCγ IN THE RABBIT HIPPOCAMPUS. E.A. Van der Zee*, M.A. Kronforst, and J.F. Disterhoft CMS Biology, Northwestern University Med. Sch., Chicago, IL 60611.

Cholinergic neurotransmission, mediated through muscarinic acetylcholine receptors (mAChRs) and the subsequently activated Protein Kinase C (PKC) modulates neuronal activity in the hippocampus in relation to learning and memory. Previously, we demonstrated changes in the hippocampal immunoreactivity (ir) for mAChRs and PKCγ after spatial learning (Van der Zee et al., J. Neurosci. 12:4808-4815; 1992). In the present study we determined whether 500 msec. trace eyeblink conditioning induces changes in mAChR- and PKC-ir in the hippocampus. Young adult (3 months) female NZW rabbits were trace conditioned (n=8). Pseudoconditioned (n=8) and behaviorally naive animals (n=8) served as controls. 24 Hrs after a rabbit reached the 80% criterion or after the 15th day of training, the animals were transcardially perfused with 2.5% paraformaldehyde + 0.05% glutaraldehyde. Free-floating sections were immunolabeled for mAChRs (M35) or PKC-isoenzymes (PKCα, βI, βII, and γ [both for the regulatory domain (36G9) and the catalytic domain]). Naive animals showed low levels of mAChR-ir throughout the hippocampus, and only moderate levels for all PKC-isoenzymes. Conditioned rabbits showed increased ir for mAChRs and PKCγ (both the catalytic and regulatory domain) in granule cells and pyramidal cell soma and their associated apical dendrites. In contrast, no changes were observed for PKCα, βI, or βII. The level of mAChR- and PKCγ-ir in pseudoconditioned animals was intermediate between naive and traceconditioned animals. One animal which only reached 40% CR's showed similar changes for mAChRs and PKCγ as the pseudoconditioned rabbits. These results demonstrate protein-selective changes in the hippocampal principal cells in trace eyeblink conditioned rabbits which may underlie the accompanying behavioral and electrophysiological changes. (Supported by the Netherlands Organization for Scientific Research (NWO) to E.A.V.d.Z. and by NIH RO1 MH47340 and RO1 AG08796)

585.15

HIPPOCAMPALLY-DEPENDENT TRACE EYEBLINK CONDITIONING INDUCES CHANGES IN THE IMMUNOREACTIVITY FOR THE LOW SUBUNIT OF NEUROFILAMENTS IN THE RABBIT HIPPOCAMPUS. M.A. Kronforst*, E.A. Van der Zee, and J.F. Disterhoft CMS Biology, Northwestern University Med. Sch., Chicago, IL 60611.

Neurofilaments (NFs) are cytoskeletal proteins which play a fundamental role in neuronal morphology and axonal transport. Enhanced neuronal connections are formed during learning. However, changes in NFs have not been previously reported in relation to learning and memory in the brain. In the present study we determined whether the immunoreactivity (ir) for the low subunit NF changes in the hippocampus during acquisition of trace eyeblink conditioning, a hippocampally-dependent task. Young adult (3 months) female NZW rabbits were trace conditioned (n=8). Pseudoconditioned (n=8) and behaviorally naive animals (n=8) served as controls. 24 Hrs after the rabbits reached the 80% criterion, or was trained for 15 days, the animals were transcardially perfused with 2.5% paraformaldehyde + 0.05% glutaraldehyde. Free-floating cryostat sections were immunolabeled with a phosphorylation-independent (68kD, Sigma, NR4) or a phosphorylation-dependent (70kD, Chemicon, MAB1615) anti-NF-L antibody. No gross changes in staining-intensity were found for either antibody in the principal cells or interneurons between the different groups. However, trace conditioned rabbits showed a twofold increase in fiber-density for NR4 in the stratum oriens, and approximately a 50% decrease in the number of fiber crossings for MAB1615 was found in the stratum radiatum as compared to pseudoconditioned and naive animals. The increase for NR4-ir most likely results from an increase in NF-content in CA1 output fibers. The decrease in the MAB1615-positive fibers most likely indicates an increase in dephosphorylation allowing the assembly of NFs formed from the low subunit, and enabling the neurons to reshape their axons. These results indicate region-selective changes in the hippocampus of trace eyeblink conditioned rabbits which may underlie learning and memory processes. (Supported by the Netherlands Organization for Scientific Research (NWO) to E.A.V.d.Z. and by NIH RO1 MH47340 and RO1 AG08796)

585.16

TIME-FREQUENCY ANALYSIS OF EEG FROM ENTORHINAL CORTEX AND DENTATE GYRUS. Y.Xu[1,2], S.Haykin[1], R.J.Racine*[2] and C.A.Chapman[2], Communications Research Lab.[1], Dept. of Psychology[2], McMaster University, Hamilton, Ontario, Canada, L8S 4K1

Neurobiological signals, such as the EEG, are usually nonstationary over time. One way to account for the temporal nonstationarity of a signal is to use time-frequency analysis to reveal its time-frequency relations. In this report, the short-time Fourier Transform (STFT), an extension of the Fourier Transform, has been used to analyze the EEG recorded from entorhinal cortex (EC) and dentate gyrus (DG). The resulting 2-dimensional time-frequency spectrum image is obtained via a 2s sliding window with a 1.9s overlap. Thirty second samples of EEG (sampled at 256 Hz) were recorded simultaneously from the EC and the DG of behaving rats. During the middle 10s of each 30s epoch, a low intensity train of electrical pulses at one of 5 frequencies (2,6,10,14,18Hz) was applied to the pyriform cortex (PC), which projects to the EC. The EC projects, in turn, to the DG. Although there was no or little visibly apparent change in the EEG produced by the stimulation, the time-frequency spectrum image showed clear peaks at the stimulation frequency and its harmonics. The time-frequency form of the coherence function was calculated based on the STFT (15 EEG epochs) to study the connectivity between the EC and the DG. Coherence was high for the theta frequency, which fluctuated around 7-9 Hz during the 30s sampling period, and was also enhanced during stimulation at the stimulation frequency and its harmonics. The STFT was also used in signal synthesis (or extraction). The portion of the time-frequency image at the stimulation frequency and its harmonics (±1-2Hz) were extracted and an inverse Fourier transform was then performed on the extracted signal. The effect of the stimulation train, originally embedded in the raw EEG, showed up clearly in the reconstructed signal. Our results indicated that the STFT is an effective technique for signal processing of the nonstationary EEG.

585.17

BRIEF TETANIC STIMULATION CAUSES SELECTIVE TRANSIENT INHIBITORY SUPPRESSION IN THE DENTATE GYRUS N. W. Milgram* and J. Ferbinteanu University of Toronto, Scarborough Campus,1265 Military Trail, Ontario, Canada, M1C-1A4

Several studies have reported that repeated activation of hippocampal afferents leads to a loss of recurrent inhibition. These findings suggest that inhibition is susceptible to use dependent breakdown. To further understand the conditions necessary for such breakdown, we have investigated the effect of brief stimulus trains to the perforant path on both paired pulse inhibition and facilitation in the dentate gyrus with urethane anesthetized male hooded rats. A three pulse procedure with a reference pulse, a variable intensity conditioning pulse (C-pulse) and a test pulse (T-pulse) was used. The interval between the C and T pulse was set at 25 msec and the intensity of the T pulse was held constant. Under the control condition, there was paired pulse facilitation with a low intensity C pulse, while a high intensity C pulse caused paired pulse inhibition. Trains with 5 pulses were applied at frequencies of 2 or 200 Hz, 15 seconds before delivery of the C-T pulse pair, caused a loss of both paired pulse facilitation and inhibition. Increasing the number of pulses to 17 at a 2 Hz frequency had no incremental effect on paired pulse suppression, but attenuated the loss of paired pulse facilitation. There was evidence that the inhibitory loss was transient, since it was reduced when the delay between the trains and the C-T pulse pair was set at 1 minute. Work currently in progress is examining whether this use dependent inhibitory loss is enhanced by treatments which induce epileptic seizures.

585.18

A NOVEL NEURAL ACTIVITY-REGULATED PENTRAXIN EXPRESSED IN THE BRAIN. C. C. Tsui* and P. F. Worley. Department of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent studies indicate that activity is tightly correlated with the expression of immediate early genes which may regulate long-term neuroplastic changes. To examine cellular mechanisms involved in activity-dependent plasticity, we have used a differential screening strategy to identify genes that are rapidly induced in neurons by depolarizing stimuli. Here we describe a novel immediate-early gene that we term *narp* (neural activity-regulated pentraxin) because of its sequence homology with known pentraxins, which include serum amyloid protein (SAP) and C-reactive protein (CRP). A full length *narp* cDNA has been identified and sequenced. The open reading frame predicts a 443 a.a. protein that contains a pentraxin family Ca^{2+}- binding domain and an eight amino acid pentraxin family signature sequence. The overall level of aa sequence identity between Narp and CRP is only 20%, however within the 15 a.a putative calcium and the 17 a.a. phospholipid binding domains these proteins are 87% and 53% identical, respectively. Pentraxin family proteins are known to be secreted and possess N-terminal hydrophobic signal sequences. Narp is also predicted to include an N-terminal signal sequence. Using *in vitro* transcription and translation assays in the presence of canine microsomes, we have determined that Narp protein is expressed in the intravesicular compartment consistent with it being a secreted protein.

narp mRNA is expressed at high levels in normal cortex, hippocampus, habenula, trigeminal ganglia, and ventral medial hypothalamus. Basal expression is rapidly responsive to natural synaptic activity as the level of *narp* mRNA in the visual cortex decreases after intraocular injection with TTX. *narp* mRNA is also rapidly induced in the dentate gyrus of the hippocampus by high frequency synaptic stimulation using the LTP paradigm and is dependent on NMDA receptor activation.

Pentraxins share the property of binding to specific glycoproteins and may function as natural lectins. We propose that Narp may function as an activity-regulated lectin to modify neuronal interactions with extracellular glycomolecules. Research supported by EY09374.

585.19

AGING DOES NOT AFFECT SYNAPSE RESTRUCTURING ASSOCIATED WITH THE INDUCTION OF LONG-TERM POTENTIATION. Y. Geinisman*, L. deToledo-Morrell and F. Morrell. Dept. of CMS Biol., Northwestern Univ. Med. Sch. and Dept. of Neurol. Sci., Rush Med. Coll., Chicago, IL 60611.

Aged rats can be potentiated to the same degree as young ones by perforant path stimulation, but they lose their potentiated response in the dentate gyrus more rapidly (Barnes, *J. Comp. Physiol. Psychol.*, 1979, *93:* 74; deToledo-Morrell et al., *Neurobiol. Aging.*, 1988, *9:* 581). We showed earlier that the induction of LTP in the dentate gyrus of young rats is followed by a selective increase in the number of axospinous synapses with multiple, completely partitioned transmission zones (Geinisman et al., *Hippocampus.*, 1993, *3:* 435). The aim of this study was to determine whether structural synaptic plasticity induced by LTP in aged animals is similar to that observed in young adults. Aged (27 mo. old) F344 rats were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Potentiated rats were stimulated (with fifteen 20 ms bursts of 400 Hz delivered at 0.2 Hz) on each of 4 consecutive days and sacrificed 1 h after the fourth stimulation. Aged animals did not differ significantly from previously studied young adults in terms of the extent of potentiation. The number of synapses per neuron was differentially estimated for various synaptic subtypes in the middle (MML) and inner (IML) molecular layer of the dentate gyrus using the unbiased disector technique. Only axospinous synapses with multiple, completely partitioned transmission zones were significantly increased in numbers in the MML (but not in the IML) of potentiated aged rats relative to their stimulated (at a frequency of 0.2 Hz) or implanted controls. The magnitude of this change was practically the same in old and young rats as compared to their respective controls. This finding suggests that the observed structural synaptic modification may account for the equivalent extent of potentiation in rats of different chronological ages.

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586.1

SELECTIVE SYNAPTIC PLASTICITY IN THE CEREBELLAR CORTEX OF THE RAT FOLLOWING COMPLEX MOTOR LEARNING. J.A. Kleim, R.M.A Napper, R.A. Swain*, K.E. Armstrong, T.A. Jones and W.T. Greenough. Depts of Psych. and Cell & Struct. Bio., Neurosci. Prog., and Beckman Inst. Univ. Illinois, Urbana, IL 61801.

Following complex motor learning, rats exhibit an increase in the number of synapses/Purkinje cell in the cerebellar cortex. This experiment examined which synapse types are altered. Female rats, approximately 8 months of age, were randomly assigned to an Acrobatic motor learning Condition (AC), a Voluntary Exercise Condition(VX) or an Inactive Condition (IC). AC animals were trained for 30 consecutive days on a motor learning task which consisted of a series of obstacle laden pathways requiring a significant amount of motor coordination to complete. The VX animals were housed with running wheels attached to their home cages to which they had unlimited access and the IC animals were subjected only to a daily handling equivalent to that experienced by the other groups. The density of synapses was obtained using the physical disector on serial electron micrograph montages taken within the molecular layer of the cerebellar cortex and synapses were classified as to their pre and postsynaptic origins. Purkinje cell density was obtained using the physical disector on serial 1 µm sections of the cerebellar cortex and data were expressed as synapses/Purkinje cell. Results indicate that the AC animals have a greater number of parallel fiber to Purkinje cell synapses in comparison to controls. Detailed assessment of other synaptic subtypes is in progress. Supported by AG 10154, NSF BNS 88 21219 and NSERC.

586.2

FORMATION OF DOUBLE SYNAPSES IN THE CEREBELLAR CORTEX OF THE RAT FOLLOWING MOTOR LEARNING. K. Federmeier, J.A. Kleim, B.J. Anderson* and W.T. Greenough. Depts of Psych. and Cell Struct. Bio., Neurosci. Prog., and Beckman Inst., Univ Illinois, Urbana, IL 61801.

Following complex motor learning, rats exhibit increased numbers of synapses/neuron in the cerebellar cortex. Here we present two experiments examining the structural plasticity of the parallel fiber following motor skill acquisition. Female rats were randomly assigned to an Acrobatic motor learning Condition (AC), a Voluntary Exercise Condition (VX) or an Inactive Condition (IC). AC animals were trained for 30 consecutive days on a complex motor learning task which consisted of a series of obstacle laden pathways requiring a substantial amount of motor coordination to complete. The VX animals were housed with running wheels attached to their home cages to which they had unlimited access and the IC animals received no motor skill training or access to running wheels. The mean intervaricosity distance along Golgi impregnated parallel fibers did not significantly differ among the three groups suggesting that few new presynaptic varicosities had developed. The second experiment utilized the same behavioral paradigm and examined the number of single varicosities forming multiple synaptic contacts (double synapses) using unbiased stereological measures from serial electron micrographs. AC rats had significantly more double synapses/Purkinje cell than VX or IC rats. The increase in synapses in the cerebellar cortex following motor skill acquisition appears to involve the formation of new synapses on existing parallel fiber varicosities. Supported by AG 10154, NSF BNS 88 21219 and NSERC.

586.3

FOS EXPRESSION IN THE MOTOR CORTEX OF THE RAT DURING THE ACQUISITION OF A COMPLEX MOTOR LEARNING TASK.
E.R. Schwarz, J.A. Kleim* E. Lussnig, T.A. Comery and W.T. Greenough. Depts. Psych. Cell Struct. Bio. Neurosci. Prog. and Beckman Inst., Univ. Illinois, Urbana, IL. 61801.

Following training on a complex motor learning task, structural changes have been observed in the rat motor cortex(see adjacent poster). Previous research has also shown FOS to be expressed during learning. In this study, the expression of FOS was examined during the acquisition and maintenance phases of training on a complex motor learning task. Female rats were randomly assigned to an Acrobatic motor learning Condition (AC), a Forced Walking Condition (FW) or an Inactive Condition (IC). AC animals were trained to complete an elevated, obstacle laden course which required a substantial amount of motor skill to traverse. Each AC animal was pair matched with a FW animal which was forced to travel down a flat elevated runway equal in length to the acrobatic course. The IC animals received no motor training or activity but were handled daily. Five animals from each condition were sacrificed after 1, 2, 5, 10 and 20 days of the experimental manipulation. Four 60 µm coronal sections containing motor cortex (1.6 mm to -1.4 mm from Bregma) were taken from one hemisphere of each animal and immunohistochemically stained for FOS. Sections were Nissl counterstained and the percentage of FOS positive cells was counted through a volume of tissue within Layer II/III of the motor cortex. Results show the AC animals to have a greater percentage of FOS positive cells during the acquisition phase (days 1, 2 and 5) versus the maintenance phase (days 10 and 20) of training. FOS expression was not significantly changed as a function of time in the FW and IC animals. Supported by AG 10154, MH 40631, NSF BNS 88 21219 and NSERC.

586.4

SYNAPTOGENESIS WITHIN THE MOTOR CORTEX OF THE RAT FOLLOWING COMPLEX MOTOR LEARNING. E. Lussnig, J.A. Kleim, E.R. Schwarz, T.A. Comery and W.T. Greenough*. Depts. Psych. Cell and Struct. Bio. Neurosci. Prog. and Beckman Inst., Univ. Illinois, Urbana, IL. 61801.

Following training on a complex motor learning task, changes have been observed in synaptic number in the rat cerebellar cortex. In this study, the number of synapses per neuron was examined in Layer II/III of the motor cortex following training on a complex motor learning task. Female rats were randomly assigned to an Acrobatic motor learning Condition (AC), a Forced Walking Condition (FW) or an Inactive Condition (IC). The AC animals were trained to complete an obstacle laden course which required a substantial amount of motor skill to traverse. Each AC animal was pair matched with a FW animal which was forced to travel down a flat elevated runway equal in length to the acrobatic course. The IC animals received no motor training or activity but were handled daily. Five animals from each condition were sacrificed after 1, 2, 5, 10 and 20 days of the experimental manipulation. Ten, 200 µm, coronal sections were taken through one hemisphere (1.6 mm to -1.4 mm from Bregma) from each animal. Blocks of tissue containing the motor cortex were removed and embedded for electron microscopy. The physical disector was used to obtain neuronal density from 80 serial 1 µm sections through Layer II/III of the motor cortex. Synaptic density was measured using the physical disector on 16 serial electron micrographs taken within Layer II/III. The number of synapses/neuron in each animal was estimated from these two measures. The AC animals had a significantly greater number of synapses/neuron than the FW and IC animals. Supported by AG 10154, MH 40631 NSF BNS 88 21219 and NSERC.

586.5

VISUALLY-GUIDED PLASTICITY IN THE AUDITORY SPACE MAP OF ADULT BARN OWLS. M. S. Brainard* and E. I. Knudsen. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401.

The optic tectum (superior colliculus) contains mutually aligned neural maps of visual and auditory space. Prior experiments have demonstrated that during development the topography of the auditory map is calibrated by visual experience: in owls reared with prismatic spectacles that optically shift the visual field along the horizon, the map of auditory space in the tectum gradually shifts to become aligned with the optically displaced visual map. In this study, we examined the capacity of the auditory space map in adult prism-reared owls to recover normal topography following restoration of normal visual experience.

Owls were reared from shortly after eyelid opening (16-19 days) with prismatic spectacles that displaced the visual field by 23° to the left or the right. After owls reached adulthood, the tectal auditory space map was assessed by measuring extracellular responses to dichotically presented stimuli of varying interaural time difference. For owls in which visual experience had significantly shifted the tectal auditory space map, prisms were removed at ages ranging from 120-1208 days, and subsequent changes in the auditory map were assessed.

In all prism-reared owls, regardless of the age at which normal vision was restored, the tectal map of auditory space recovered essentially normal topography. This recovery occurred rapidly, over periods ranging from 10-30 days. The results indicate that substantial visually-guided plasticity in the tectal representation of auditory space is maintained in adult owls. This finding contrasts with prior behavioral and neurophysiological results that have suggested a loss of plasticity in the tectal map of auditory space and in sound localization behavior beyond a critical period in development.

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586.6

SOILED-BEDDING INDUCES STRUCTURAL CHANGES OF SYNAPSES IN RAT ACCESSORY OLFACTORY BULB.
M. Ichikawa[1*], *M. Matsuoka*[1,2], *and Y. Mori*[2] [1]Dept. of Anat. & Embryol., Tokyo Metropolitan Inst. for Neurosci., Tokyo 183, and [2]Lab. of Veterinary Ethology, The University of Tokyo, Tokyo 113, Japan.

The effects of soiled bedding on synaptic morphology in the accessory olfactory bulb (AOB) were examined in adult male rats. Forty-day-old male rats were isolated. One group was exposed to bedding soiled by male and female rats (EC). Another group was only to male soiled bedding (SC). The last was exposed to clean bedding (IC). After 2 months, the animals were sacrificed for electron microscopy. The size and the numerical density of synapses were measured in the glomerulus and the granule cell layer. In the glomerulus, the length of the synaptic contact zone was significantly greater in the EC than in the SC and the IC, while there was no statistically significant difference in the density of synapses among the three groups. Two types of synapses were classified in the granule cell layer: (1) perforated synapses, which are characterized by discontinuities in their postsynaptic thickenings and (2) nonperforated synapses. For perforated synapses, the length of the synaptic contact zone was significantly greater in the EC than in the SC and the IC. For nonperforated synapses, there was no statistically significant difference in the length of the synaptic contact zone among the three groups. In the density of both perforated and nonperforated synapses, there was no statistically significant difference among the three groups. These results demonstrated that exposure to bedding soiled by female rats, which contains female odour substances (pheromones) can induce structural changes of the synapses in the AOB of male adult rats.

586.7

THE AT-14 mRNA IS ENRICHED IN SEVERAL AVIAN SONG CONTROL NUCLEI AND ENCODES AN RC3/NEUROGRANIN-RELATED PROTEIN S.M. Siepka*, J.M. George, H. Jin and D.F. Clayton. Dept. of Cell & Structural Biology, University of Illinois, Urbana 61801

Differential cDNA hybridization experiments led to the identification of a brain-specific, forebrain-enriched RNA (HAT-14) that is especially abundant in song control nuclei HVC and lMAN of canaries and zebra finches. The sequence of the HAT-14 cDNA predicts a 73 amino acid protein that contains a domain found in the GAP-43 and RC3/neurogranin proteins, where it is believed to mediate protein kinase C-regulated calmodulin binding. Further analysis of the sequence reveals the first 50 amino acids are 84% identical between rat RC3 and HAT14. The identity drops to 25% in the remaining C-terminal portion, with lack of conservation of the residues identified as "collagen-like" in the rat sequence. Antibodies raised against predicted HAT-14 peptide sequences react with neurons in the songbird telencephalon. In sharp contrast to RC3/neurogranin (which is not expressed outside the telencephalon), high levels of HAT14 expression are also observed in cerebellar Purkinje cells in the songbird.

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586.8

DEVELOPMENTAL REGULATION OF GAP43 MRNA IN AVIAN SONG CONTROL NUCLEI. H. Jin, H. B. Simpson, S. Siepka, C. Mello, M. Huecas, K.L. Nastiuk, J.M. George and D.F. Clayton*. Dept. of Cell and Structural Biol., Univ. of Illinois, Urbana, IL, 61801

GAP-43 is an axonal protein whose expression increases during neuritic outgrowth, and which undergoes phosphorylation by Protein Kinase C during long-term potentiation. Thus a central role has been proposed for GAP-43 in both neural development and adult plasticity. The avian song control system stands as one of the best models of regulated neural plasticity. To determine whether GAP-43 may regulate plasticity in the song system, we cloned the canary homolog of the GAP-43 cDNA. We used this cDNA to measure (by in situ hybridization) the expression of GAP-43 RNA in the adult canary brain, and in the zebra finch brain during the critical period for song circuit development. Two observations have emerged. First, GAP-43 is notably *less* abundant (compared to surrounding telencephalon) in the principal song nuclei HVC and RA in adult songbirds, but is relatively enriched in nucleus lMAN. Second, GAP-43 RNA levels remain relatively stable throughout zebra finch development (15-110 days of age) in most song nuclei, with one exception: RNA levels are increased in nucleus RA when the bird is first learning to sing (before day 60) then fall to the low levels observed in adults. These results suggest GAP43 gene regulation does not play a significant role in the initial establishment of connections within the song control circuit, although the gene is expressed constitutively and thus the protein itself may serve a function. In RA, downregulation of the gene may be correlated with stabilization of the motor control pathways for song production.

(Supported by NIH grant NS25742)

586.9

ESTROGEN EFFECTS ON SPATIAL PERFORMANCE AND HIPPOCAMPAL PHYSIOLOGY IN FEMALE RATS. D.L. Korol*, K. Unick, K. Goosens, C. Crane, P.E. Gold, and T.C. Foster. University of Virginia, Charlottesville, VA 22903

Gonadal steroids influence performance on a variety of spatial tasks in both humans and rodents. In addition, estrogen modifies the structure and response properties of neurons in the hippocampus, an area believed to be involved in processing spatial information. The present study examined the effects of estrogen on spatial performance and various measures of hippocampal physiology. Ovariectomized rats with and without hormone replacement (estrogen-progesterone regimen structured to mimic the natural cycle) and control rats at various stages of the estrous cycle were tested in the Morris swim task. Synaptic function and plasticity in the CA1 region of the hippocampus was then assessed *in vitro* at the same stage of the estrous cycle as at behavioral testing. A gradient of effect of hormones was observed for swim task performance and potentiation following primed burst stimulation: High levels of circulating estrogen related to poorer swim task performance and to higher levels of primed burst potentiation. Thus, a significant negative correlation between spatial memory and magnitude of synaptic plasticity emerged. We are presently examining the effects of fluctuations in estrogen on sleep and other behavioral measures in female rats. Possible actions of circulating estrogen on hippocampal function include: 1) neuromodulatory effects, via hippocampal afferents 2) direct, activational effects on hippocampal function and 3) regulation of number and/or function of synapses. Supported by AG05540 (DLK), AG07648 (PEG) and NS31830 (TCF).

586.10

CELL TYPE SPECIFIC EXPRESSION FROM THE TYROSINE HYDROXYLASE PROMOTER MAY ACCOUNT FOR THE ROTATIONAL BEHAVIOR INDUCED BY ACTIVATION OF THE PROTEIN KINASE C PATHWAY IN SUBSTANTIA NIGRA PARS COMPACTA NEURONS WITH A HSV-1 VECTOR. [1]S. Song*, [1]Y. Wang, [1]D. Hartley, [1]D. Ulkrey, [1]S. Bak, [1]O. Ashe, [1]L. Bryan, [2]J Haycock, [3]M. During, [4]R. Neve, [5]K. O'Malley, [1]A. Geller. [1]Children's Hosp., Boston MA; [2]Louisana St. Med. Ctr, New Orleans LA; [3]Yale Univ. Sch. Med., New Haven CT; [4]McLean Hosp., Belmont MA; [5]Wash. Univ. Sch. Med. St. Louis MO.

Regulation of rat motor behavior by the PKC pathway in substantia nigra compacta (SNc) neurons is being analyzed by a genetic intervention strategy with a HSV-1 vector system. Expression in cultured neurons of the rat PKC βII catalytic domain (pkcΔ), which is unregulated and constitutively active, causes activation of the PKC pathway and an activity dependent increase in neurotransmitter release (Soc. Neurosci. Abs. 1991, 17, 603). We hypothesized that using the tyrosine hydroxylase (TH) promoter to restrict expression of pkcΔ to SNc neurons would potentiate striatal dopamine release, resulting in rotational behavior. This hypothesis is being tested with vectors that use a 6.8 kb TH promoter to control expression of Lac Z (pTHlac) or pkcΔ (pTHpkcΔ).

The cell type specificity of expression directed by the TH promoter was investigated in cultured cells and in the rat midbrain. The efficiency of infection of several TH expressing (TH^+) and TH lacking (TH^-) cell lines was corrected for by counting the number of cells expressing β-galactosidase following infection with pHSVlac, which contains the constitutive HSV-1 immediate early (IE) 4/5 promoter. pTHlac directed a 10 to 20-fold increase in the number of cells expressing β-galactosidase comparing infection of TH^+ peripheral (PC12) or CNS (MN9D) cells to infection of TH^- peripheral (N18TG2) or CNS (E27) neuronal cells. pTHlac was tested *in vivo* by stereotactic injection into midbrain followed by either double staining (X-gal and anti-TH or anti-β-galactosidase and anti-TH) or cell counts based on cell morphology and location, and the results showed that over 80 % of the X-gal positive cells were neurons and 40 % of the X-gal positive cells were TH^+ SNc neurons. In contrast, after injection of pHSVlac, 37 % of the cells expressing β-galactosidase were neurons and 5 % were SNc neurons. Thus, the TH promoter directed a marked increase of cell type specific expression in TH^+ neurons; however, expression in inappropriate cell types, particularly TH^- CNS neurons following injection of pTHlac into the midbrain, may reflect either limitations of the current HSV-1 vector system or regulatory elements missing from the 6.8 kb TH promoter used in this study. The cell type specific expression directed by the TH promoter may explain the rotational behavior caused by pTHpkcΔ (Soc. Neurosci. Abs. 1993, 19, 806).

586.11

NIGROSTRIATAL PROJECTION AFTER UNILATERAL 6-HYDROXYDOPAMINE INJECTION INTO THE STRIATUM IN NEONATAL RATS. Y. Ichitani*, M. Takasuna and T. Iwasaki. Inst. of Psychology, Univ. of Tsukuba, Tsukuba, Ibaraki 305, Japan.

In order to investigate the reorganization of nigrostriatal dopamine neuron system after a lesion of this system, we examined the effects of neonatal 6-hydroxydopamine (6-OHDA) injection unilaterally into the striatum on the subsequent development of contralateral projections from the intact side of substantia nigra (SN). After a treatment of desmethylimipramine (20mg/kg, s.c.), Wistar rats received unilateral injection of 6-OHDA (10μg/5μl) into the striatum on postnatal day 2 and 4. At the age of 4 weeks to 5 months, cholera toxin B subunit (CTB, 1%) was injected (0.4μl) into the striatum ipsilateral to 6-OHDA injection. After a survival time of 48h, rats were perfused and their brain sections were treated for immunocytochemistry of CTB. We could detect almost no CTB-immunoreactive cell bodies in the SN contralateral to the injection regardless of the age of animals. Results suggest that unilateral lesion of the neonatal nigrostriatal system did not have marked effects on the formation of interhemispheric connections of this system.

586.12

MORPHOLOGICAL CHANGES IN RAT RED NUCLEUS FOLLOWING COMPENSATION FOR LIMB LESIONS. P.R.Kennedy*, N.Ishihara, L.Rutherford and J.Wilson. Neuroscience Lab., Georgia Tech, Atl., GA 30332.

Morphological changes are found in finches whose song-related nuclei regress and regrow each season. This degree of plasticity has not been described in animals where changes in cortical sensory maps have been found to be use-dependent. Such plasticity has not been shown to produce changes in the number or morphological appearances of neurons in specific nuclei. We here describe use-dependent changes in rat red nucleus.

Long-Evans rats, 3 to 8 weeks of age, were subjected to transection of their Achilles tendons and walked without a limp within 3 days. Ten to 30 days later, rats were sacrificed and brain sections stained with thionin. Prior experiments in several rats showed the caudal pole contained predominantly lumbo-sacral projecting neurons. Therefore, the caudalmost 300 microns were examined under brightfield microscopy, and neuron number and type determined. Initial results suggest that neurons in caudal RN contralateral to the lesion are reduced in number and size.

These data indicate that morphological changes occurred following compensation for the Achilles tendon lesions.

586.13

DEVELOPMENTAL CHANGES IN THE VESTIBULO-OCULAR PATHWAYS DURING METAMORPHOSIS IN FLATFISH. J.K.S. Jansen* and P.S.Enger. Dept. of Physiology and Institute of Biology, University of Oslo, 0317 Oslo Norway.

During the first two months after hatching flounders go through a very unusual metamorphosis. They tilt their bodies progressively to one side while the one eye migrates in the opposite direction across the dorsal midline to settle next to the other eye, on the opposite side of the head. During this process the vestibulo-ocular pathways are presumeably reorganized to compensate for the 90 degrees misalignment of visual and vestibular frames of reference . In the present study we have examined the vestibular nuclear complex with anterograd and retrograd neuronal tract tracing techniques (HRP and dextranamines) before and after the period of eye migration in larvae and juvenile turbots.

We find: 1. that the vestibular complex consists of spatially segregated groups of neurons selecting distinct pathways to reach their targets in rostral eye motor nuclei and in the spinal cord. 2. All groups can be recognized in premetamorphic larvae as well as in juveniles. 3. The number of projection neurons increase comparably in the vestibulo-ocular and the vestibulo-spinal components of the vestibular complex. 4. Some of the projection neurons change their terminal fields in the rostral eye motor nuclei from predominately unilateral terminals in the larvae to bilateral termination after the period of eye migration in juveniles.

We conclude that the vestibular complex is basically similar in flatfish and other teleosts. The reorganization of terminal fields in vestibulo-ocular pathway during metamorphosis may be part of the plasticity required to compensate for the eye migration.

586.14

FUNCTIONAL DESENSITIZATION OF NMDA RECEPTORS IN AN ANIMAL MODEL OF CHRONIC UP-REGULATION OF GLUTAMATE RELEASE. P. Marini, M. Di Luca, A. Caputi, L. Pastorino, M. Cimino, G. Bonanno, M. Raiteri, J. Perez* and F. Cattabeni. Institute of Pharmacological Sciences, University of Milano, 20133 Milano and Institute of Pharmacology and Pharmacognosy, University of Genova, 16148 Genova, Italy.

Rats exposed *in utero* to methylazoxymethanol (MAM) at embryonic day 15 show a profound disorganization in the CA region of the hippocampus. These animals show cognitive deficits and impairments in LTP induction (Ramakers et al., 1993). At a molecular level, in the presynaptic compartment increased phosphorylation of B-50/GAP-43 is present with a parallel and persistent increase in the membrane-associated PKC. As a consequence, increased basal glutamate release (332±23 pmol/mg of protein control; 575±50 pmol/mg of protein MAM-treated) has been observed in hippocampal synaptosomes of MAM-treated rats, whereas basal release of GABA is unaltered.

The functional state of NMDA receptors was evaluated in hippocampal plasma membranes by ^{3}H-MK801 binding under nonequilibrium conditions, with a mathematical model allowing for the study of simultaneous modulation of the receptor by two agonists, Glycine and Glutamate, according to Marvizon and Baudry, 1993. The data show that in MAM-treated rats the allosteric interaction between Glycine and Glutamate sites shows a 10 fold difference with respect to controls, without changes in the number of binding sites as measured by ^{3}H-CGP39653 binding.

It is known that phosphorylation processes can influence the function of NMDA receptor. In these animals in vivo PKC dependent phosphorylation of the post-synaptic substrate neurogranin is markedly altered, suggesting that alterations of plasticity mechanisms involve pre- and post-synaptic parameters.

Marvizon J-C. and Baudry M. Analyt.Biochem. 213:3-11, 1993.
Ramakers G.M.J. et al., Neurosci. 54:49-60, 1993.

586.15

MAP KINASE KINASE (MEK-1) IS ENRICHED IN RADIAL CELL PROCESSES IN ZEBRA FINCH BRAIN. J.M. George*, H. Jin, W.S. Woods, and D.F. Clayton. Dept. of Cell and Structural Biol., Univ. of Illinois, Urbana, IL, 61801.

A differential screening strategy previously led to the identification of cDNA clones representing RNAs enriched in parts of the songbird telencephalon (Mol. Br. Res. 12:323, 1992). The sequence of one of these clones (HAT-5) predicts a protein 95% identical to the recently cloned mammalian MEK-1 protein (Crews et al., Science 258:478, 1992), which functions as an activator of MAP Kinase/ERK-1 and is part of the signal transduction pathway for various growth factors. By Northern analysis, the HAT-5 RNA is in most and perhaps all songbird tissues (zebra finch, canary), but is noticeably enriched in the brain. Expression in brain is minimal in newly hatched finches, moderate at 7 days of age, and more abundant still in adults. An antibody raised against residues 38-50 of the MEK-1 peptide reacts with a band of ~48 kD on immunoblots of brain extracts. By immunocytochemistry, the antibody stains cellular processes that appear to arise from the ventricular lining, especially at the tips of the ventricles. In Nissl counterstained sections, the processes are occasionally associated with small elongated cells. Processes with these features have previously been identified as elements of radial cells, which serve both as neuronal precursors and as substrates for migration of young neuroblasts in the adult avian brain. The enrichment of MEK-1 in radial cell processes suggests the radial cells may respond to signals generated deep within brain tissue, far from their soma in the ventricular lining. [supported by NIH: NS 25742]

586.16

ANTIBODIES TO HAT-3, A NOVEL SONGBIRD PROTEIN, RECOGNIZE SYNAPTIC ELEMENTS IN CULTURED RAT HIPPOCAMPAL NEURONS. G.S. Withers*[1], J.M. George[2], G.A. Banker[1] & D.F. Clayton[2]. [1]Neuroscience Dept., Univ. Virginia, Charlottesville, VA 22908; [2]Cell & Structural Biology, Univ. Illinois, Urbana, IL 61801.

Previous studies of differential gene expression in songbird brain led to the identification of a brain-specific mRNA (HAT-3) which is developmentally regulated in the song control system (George et al., SN Abstr., 1993). The sequence predicts a 143 amino acid protein which contains a repeating amphipathic motif of 11 amino acids. Antibodies raised against the predicted C-terminus detected a single band (~17 kD) on immunoblots of both songbird and rat brain synaptosomal extracts, and stained zebra finch brain sections in a pattern consistent with synaptic localization.

With the ultimate goal of analyzing the function of the novel protein, we have used these antibodies to stain cultured hippocampal neurons from rat brain. Before synaptic contacts are present (e.g. 1-3 days in vitro [DIV]), HAT-3 is present in the cell body but appears diffuse and in low levels in processes. The antibodies yield a punctate staining pattern in more mature neurons (e.g. 7 DIV or older) that have established synaptic contacts. In double staining experiments, the protein is colocalized at the light microscopic level with the well-characterized synaptic vesicle protein synapsin I. Double staining with MAP2 revealed that HAT-3 puncta were concentrated around the soma and along dendrites. These results suggest that, while initially diffuse, in later stages of development HAT-3 protein becomes concentrated at regions of synaptic contact, and is localized presynaptically. Supported by NS17112 (GB), NS07199 (GW), HD07333 (JG), NS25742 (DC).

586.17

NETWORK'S AFFERENT CONNECTIVITY IS GOVERNED BY NEURAL MECHANICS. O. V. Favorov* and D. G. Kelly. Departments of Biomedical Engineering and Mathematics, The Univ. of North Carolina at Chapel Hill, N.C.27599.

Lateral interactions among neurons in a Hebbian network can modify the afferent connections to those neurons. Excitatory lateral connections cause the connected cells to acquire similar afferent connections, whereas inhibitory lateral connections cause them to acquire different sets of afferent connections. That is, excitatory lateral connections induce attracting forces, and inhibitory lateral connections induce repelling forces on the afferent connections. Thus neurons can change the positions of - i.e., move - the groups of afferent cells that project to them, via their lateral interconnections. Forces, positions, and motions are the subject of mechanics, and therefore the development of afferent connectivity to a network can be approached as a problem in *neural mechanics*. To formulate neural mechanics, with Newtonian mechanics as a paradigm, we first measured the forces in the simplest system, consisting of two neurons with a single lateral connection, each with Hebbian connections from the same afferent field; then we studied whether systems of more than two laterally-connected neurons can be analyzed vectorially in terms of their two-neuron subsystems. The force between two neurons is found to depend linearly on the strength of the lateral connection, and also nonlinearly on the distance between the afferent groups of the two neurons. Hebbian plasticity of lateral connections offers the means to alter the shape of this dependency. Forces induced by excitatory and inhibitory connections act in opposite directions. Multiple forces acting on the afferent connections of the same neuron (induced by its multiple lateral connections) add vectorially and linearly. The space in which these forces act is not identical with the physical space of the afferent network; its metric is defined by correlations in the behaviors of afferent cells, and is very dif-ferent from the familiar space of Newtonian mechanics. This formulation provides a theoretical and mathematical framework in which to analyze and predict the experi-ence-driven development of afferent connectivity of networks throughout CNS. Supported, in part, by NIMH MH48654 and NINDS NS30686.

587.1

ELECTRICAL ACTIVITY IN HEMISECTED SUPRACHIASMATIC NUCLEI (SCNs). P. Zlomanczuk, G.R. Lynch*, EPO Biology, University of Colorado, Boulder, CO 80309-0334

The phenomenon of "splitting" - separation of an overt circadian rhythm in two components - indicates the possible presence of two component oscillators in the circadian pacemaker. In mammals, a potential site for a single oscillator is each of the SCNs. A previous study (Zlomanczuk et al., *Brain Research* 559:94-99, 1991) demonstrated that the SCNs of hamsters with a split locomotor activity rhythm exhibit matching bimodal profiles of firing rate in vitro. In this study, we examine the effect of severing neuronal connections between the SCNs on firing pattern. Brain slices containing the SCNs were prepared from Djungarian hamsters which expressed a split in locomotor activity under constant light (LL, 70-80 lux, n=8) or entrainment to long day (LD, 16:8, n=7). The slice was halved into left and right hemisections, and each isolated in a recording chamber. Single units were recorded. Sampling proceeded for 18-24h.

In six LD animals the firing pattern in both nuclei was similar to that observed in an intact slice: a low firing rate (4.23±0.67) during the projected time of locomotor activity and a high firing rate (8.21±0.25) during the absence. Both SCNs had a similar firing profile. In one slice no circadian pattern was present. In six of the LL animals two peaks of electrical activity were observed in each hemisection. Peak amplitude was ipsilaterally asymmetrical (6.79±0.48 and 9.44±0.75, $p<0.01$) with the lower amplitude in one SCN coinciding with the higher amplitude in the contralateral SCN. Firing rate during both peaks was higher than during troughs (4.11±0.24). SCNs from two LL animals did not express circadian pattern. Each hemisection exhibited a bimodal firing pattern independently, although the amplitude of one peak was greater than the other. Further, the timing of maximal firing in the left hemisection is 180^0 out of phase with the right hemisection. (NIH MH52546)

587.2

DIFFUSE CLOSED HEAD INJURY INDUCES PHASE SHIFTS IN CIRCADIAN RHYTHMS AND EXPRESSION OF FOS PROTEIN IN THE SCN AND IGL IN THE RAT. K. Edelstein *, B. Robinson, and S. Amir. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada.

Diffuse closed head injury has been associated with cognitive and behavioral deficits in humans and animals. Little is known about the effects of such injury on the circadian system. Wistar rats were implanted with biotelemetry transmitters, and circadian temperature and activity rhythms were recorded under constant dark (DD) conditions for 10 days. Pentobarbital-anesthetized rats were then mounted in a stereotaxic apparatus and injured using a 250 gm weight drop device. Behavioral results indicated that such injury induced phase delays that were greater than those induced by anesthesia alone. Differences in amplitude and period were observed in injured animals as well. The effects of such injury on the circadian system was further examined using the expression of Fos protein, previously shown to be a marker of light-induced activation of cells in the suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL). Rats sacrificed up to 3 hours after head injury exhibited Fos-like immunoreactivity in the SCN and IGL, while pentobarbital-anesthetized controls did not. Therefore, the induction of Fos protein in these regions is not a specific response to photic stimulation. Taken together, these results suggest that diffuse closed head injury alters circadian rhythmicity. Some of the behavioral deficits observed in head injured animals may be secondary to the effects of such damage on the circadian system.

587.3

NICOTINE INDUCES c-*fos* IN THE FETAL BUT NOT ADULT SUPRACHIASMATIC NUCLEUS. D.A. Clegg, B.F. O'Hara*, E.S. Macdonald, V.H. Cao, H.C. Heller, J.D. Miller, T.S. Kilduff. Center for Sleep and Circadian Neurobiology, Depts. of Psychiatry and Biological Sciences, Stanford University, Stanford, CA 94305.

The suprachiasmatic nucleus (SCN) is the location of the circadian pacemaker in mammals. Circadian rhythmicity is evident in the fetal rat SCN as early as embryonic day 18 (E18), and is entrained by maternal cues. Various stimuli, the most notable being light, but also some drugs such as carbachol, can phase shift the circadian system. We examined whether nicotine was capable of causing phase shifts and inducing c-*fos* mRNA production in the SCN in a manner similar to light. Although nicotine causes phase shifts in the adult SCN *in vitro*, nicotine injection (1 mg/kg s.c.) did not induce detectable c-*fos* in the SCN *in vivo* at any circadian time (CT3, 9, 15 or 21) although c-*fos* was induced in the habenula. In contrast, nicotine administered to pregnant dams on E20 induced c-*fos* in the fetal SCN as well as in the fetal habenula. These responses were blocked by pre-administration of mecamylamine. Subsequent investigations suggested that nicotine failed to induce c-*fos* expression at E16, caused minimal expression at E18, robust expression at E20 and postnatal day 1 (P1), and no expression at P3 or thereafter.

There are several possible explanations which could account for the developmentally restricted c-*fos* induction in the SCN. 1) The composition of the nicotinic receptor (nAChR) subunits may change during these critical periods. Evidence from our laboratory and others suggests that the primary nAChRs in the adult SCN may produce inhibitory rather than excitatory responses. The fetus and neonate may express the more typical, excitatory nAChRs that would lead to c-*fos* expression. 2) Ongoing synaptogenesis may alter responses to nicotine. 3) The fetus may not yet express secondary components present in the adult SCN which generate a net inhibition. We are currently investigating these possibilities in the fetal vs. adult SCN. (Supported by NIH grants HD29732 and AG05556)

587.4

LIGHT-INDUCED EXPRESSION OF IMMEDIATE EARLY GENES IN THE SUPRACHIASMATIC NUCLEUS (SCN) AND RETINA OF C3H/HeN MICE AND THE EFFECT OF MELATONIN ADMINISTRATION. M.I. Masana*, S. Benloucif and M.L. Dubocovich. Dept. Mol. Pharmacol. and Biol. Chem., Northwestern University Medical School, Chicago, IL, 60611.

Induction of immediate early genes and behavioral responses to light ensue only with light pulses delivered during the subjective night. To investigate the possible effects of circadian fluctuations of melatonin levels on photic responses, we studied the effect of melatonin administration on light-induced phase shifts and c-*fos* mRNA expression in the SCN and retina of C3H/HeN mice. Wheel running activity was recorded from male mice (7-8 weeks old) housed in constant dark. Mice injected with melatonin (90 ug s.c.) or vehicle (10% ethanol/saline) were exposed to light (300 lux 15 min) at various CTs, with CT12 (CT = circadian time) as the onset of activity. Melatonin administration 5 minutes before the light pulse produced a phase advance at times where light produced little or no response (CT2 to CT10). At the end of behavioral measures, treatments were repeated and the brains and eyes processed for 2-[^{125}I]-iodomelatonin binding or *in situ hybridization*. Initial analysis indicated an increase in light-induced c-*fos* mRNA expression by melatonin with a dramatic gradient thoughout the SCN. In the retina, light-induced c-*fos* expression was observed in a small number of ganglion cells and specific 2-[^{125}I]-iodomelatonin binding was observed in the inner plexiform and the ganglion cell layers. These results suggest a role for melatonin receptor activation in retina and/or SCN of the C3H/HeN mice in the modulation of light responses. Supported by MH42922 and a Glaxo grant to MLD and F32-AG05608 to SB.

587.5

INDIVIDUAL DIFFERENCES IN REENTRAINMENT CORRELATE WITH SPECIFIC 2-[^{125}I]-IODOMELATONIN (2-IMEL) BINDING IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF C3H/HeN MICE. S. Benloucif,* and M. L. Dubocovich, Dept. Molec. Pharmacol. and Biol. Chem., Northwestern Univ., Chicago, IL 60611.

Melatonin entrains free running activity and facilitates adjustment to a change in the light/dark cycle. These effects appear to be mediated through activation of melatonin receptors in the "biological clock", the hypothalamic SCN. This study examined whether individual differences in rates of reentrainment and 2-IMEL binding densities in SCN of C3H/HeN mice are related. Upon establishment of stable entrained running wheel activity, the LD cycle was advanced by 6 hours. Mice were treated with either melatonin (90 µg s.c.) or vehicle (10% ethanol/saline) for 3 days at the new dark onset. Melatonin facilitated the rate of reentrainment [$F(1,29) = 6.13$, $p < 0.05$], measured by daily advances in activity onset from pre-shift baseline. Melatonin treated mice reached an average shift in activity onset of 6.07 ± 0.28 h ($n = 16$) in 5 days, in contrast to 7 days for vehicle treated mice (6.06 ± 0.23 h, $n = 14$). Mice selected for varying rates of reentrainment were sacrificed, and brain sections containing the SCN and paraventricular thalamic nucleus (PVNT) were processed for quantitative receptor autoradiography. Specific 2-IMEL binding (181 pM) defined with 1 µM melatonin was observed in both the SCN and PVNT. There was a significant correlation between 2-IMEL specific binding in the SCN and the reentrainment rate in the melatonin treated group ($r = 0.77$, $p < 0.01$, $n = 8$), but not in controls ($n = 4$). No correlation was observed with PVNT binding. These data suggest that individual differences in reentrainment in response to exogenous melatonin may be due to variations in the density and/or affinity of melatonin receptors in the SCN, and further support the SCN as the locus of melatonin's effects on circadian activity. Supported by a Glaxo grant (MLD) and NIH F32-AG05608 (SB).

587.6

The Glial Intermediate Filament Vimentin Defines the SCN in the Syrian Hamster but not the Rat. A.S. Elliott* and A.A. Nunez. Dept. of Psychology / Neuroscience Program, Michigan State University, East Lansing, MI 48224

The suprachiasmatic nucleus (SCN), the site of the biological pacemaker, has been shown to undergo circadian variations in the expression of glial fibrillary acid protein in the Syrian hamster (*NeuroReport* **4**: 1243). Vimentin, an intermediate filament that demarcates radial glia, is the dominant intermediate filament of immature astrocytes. Vimentin has also been shown to be expressed in glial cells of adult rats in the supraoptic nucleus (*J. Neuroendocrin.*, **5**:1), a nucleus in which astrocytes also have been shown to change morphology under osmotic challenges (*Progress in Neurobiology*, **34**: 437). We tested the hypothesis that vimentin may be a marker of areas that retain plasticity, and may play a role in regulation of the circadian timing by glial cells. Animals were housed in a 12:12 light:dark cycle (dim red illumination), with lights out at 12:00 noon. Animals were hooded and perfused at selected times (Hamsters 8 am, 10 am, 2 pm, 4 pm; Rats 8 am, 12 noon, 4 pm), and brains were sectioned at 40 µm. Immunocytochemistry for vimentin was performed and sections were evaluated for the presence of immunoreactivity. Vimentin was present in the SCN of the hamster, and ranged in defining only the ventrolateral portion of the SCN to being ubiquitous throughout the nucleus. Cell bodies were frequently noted along the SCN/chiasm border, with processes from these cells invading the ventral SCN. However, no circadian variation in the distribution of immunoreactivity was noted. No immunoreactivity for vimentin was seen in the SCN of the rat, but some immunoreactive fibers were seen in the retrochiasmatic area. Supported by grant IBN 9209437 from NSF.

587.7

LOCAL SYNAPTIC CIRCUITS IN THE RAT SUPRACHIASMATIC NUCLEUS. G.J. Strecker* and F.E. Dudek. Dept. Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

Previous anatomical studies have indicated that a large fraction of neurons and presynaptic terminals in the suprachiasmatic nucleus (SCN) contain GABA. Furthermore, electrical stimulation of regions outside the SCN has been found to produce GABAergic synaptic potentials in SCN neurons, but it is unclear whether such evoked potentials arise from the stimulation of GABAergic neurons within the SCN, or of fibers originating in other areas. To test whether SCN neurons form local inhibitory synapses among themselves, we applied brief pulses of glutamate (10 mM, 0.2 s) to the SCN during whole-cell voltage-clamp recording in thin (150 μm) hypothalamic slices. Such glutamate pulses would be expected to stimulate predominantly cell bodies in the SCN rather than fibers of passage.

Whole-cell voltage-clamp recordings in SCN from 12 to 18 day-old Lewis rats revealed spontaneous outward currents in all neurons (n = 33), with amplitudes ranging from 5 to 60 pA at holding potentials near 0 mV, and a frequency of occurence ranging from 0.05 to 10 Hz. Bicuculline (10 μM) blocked these events in 9 of 9 neurons, indicating that they were GABA-mediated inhibitory postsynaptic currents (IPSCs). Pressure ejection of glutamate-containing bath solution onto the SCN resulted in clear increases in the rate of IPSCs in 6 of 18 neurons. Spontaneous inward currents, which were presumably excitatory postsynaptic currents (EPSCs), were seen in fewer neurons (n = 11 of 15), possibly due to their lower rate of occurrence (about 0.1 Hz, on average). Glutamate microstimulation did not evoke EPSCs reliably in any of 12 cells. These results suggest that local synaptic cicuits within the SCN exist, and are predominantly inhibitory.

Supported by a grant from the AFOSR.

587.8

DIFFERENTIAL INNERVATION OF THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) AND INTERGENICULATE LEAFLET (IGL) BY RAPHE NUCLEI. E.L. Meyer and L.P. Morin, Dept. Psychiatry and Neurobiology, Stony Brook University, NY 11794.

The SCN and IGL, components of the circadian system, receive serotonergic (5-HT) innervation from the dorsal (DR) or median (MR) midbrain raphe. In the rat, the data are inconsistent as to which nucleus projects to SCN or IGL. Studies of DR and MR efferent pathways to the hamster circadian system have not been performed.

Retrograde (Fluoro-Gold, cholera toxin, Fast Blue) and anterograde (Phaseolus vulgaris leucoagglutinin; PHA-L) tracing techniques were used to show raphe neurons and their projections. Retrograde tracers were injected into the SCN or IGL of male golden hamsters and locations of labeled cells in DR and MR were noted. PHA-L was injected into the DR or MR and the projections to the SCN and IGL were mapped.

The anterograde data show MR, but not DR, projections to the SCN. Retrograde label placed in the SCN labels cells in the MR, but not DR. In contrast, placement of PHA-L in either the DR or MR labels fibers in the IGL. Retrograde analysis is in progress. The results are consistent with data (unpublished) showing that MR, but not DR, lesions destroy 5-HT projections to the SCN. DR, but not MR, lesions eliminate 5-HT fibers in the IGL. The anterogradely labelled MR fibers to the IGL may not contain 5-HT. This issue is being evaluated. Supported by NS22168.

587.9

LOCALIZATION OF PRETECTAL NEURONS IN THE RAT WITH EXTENSIVE PROJECTIONS TO THE SUPRACHIASMATIC NUCLEUS (SCN) AND THE INTERGENICULATE LEAFLET (IGL).
Jens D. Mikkelsen* and Niels Vrang. Inst Med Anat, Univ Copenhagen, Blegdamsvej 3, 2200 N, Denmark

A characteristic anatomical feature of the two most important components of the mammalian circadian timing system: the SCN and the IGL, is that they are bilaterally innervated from the retina. Parts of the olivary and posterior pretectal nuclei have been shown to be bilaterally innervated from the retina as well, and we therefore aimed to explore whether these two nuclei were anatomically related to the circadian system. The anterograde neuronal tract tracer, *Phaseolus vulgaris*-leucoagglutinin (PHA-L), was injected iontophoretically into different pretectal nuclei of adult male rats. Only if PHA-L was injected in the medial part of the pretectum i.e. involving the olivary and posterior pretectal nuclei, PHA-L-ir nerve fibers were observed to course laterally and rostrally into the optic tract, and, within the optic tract and chiasm, under the diencephalon to penetrate dorsally into the SCN. Along this pathway, the projection gave rise to a large number of branches, varicose nerve fibers, and nerve endings in the IGL. In the SCN, varicose PHA-L-labeled nerve fibers were found exclusively in its ventrolateral part, apparently overlapping with the retino- and geniculorecipient zones. The pretectal injections gave rise to a bilateral innervation of the SCN and IGL, but mostly ipsilaterally to the side of injection. To determine the precise location of the projecting neurons in the pretectum, the retrograde tracer *Cholera toxin*, subunit B, was iontophoretically injected either into the SCN or the IGL. The presence of labeled neurons scattered in both the posterior and olivary pretectal nuclei was observed in both experiments. Most neurons were found in the medial part of the posterior pretectal nucleus and in the dorsomedial part of the olivary pretectal nucleus ipsilateral to the injection, confirming the anterograde tracing studies. These neuroanatomical tract tracing results reveal the presence of a novel pathway linking the pretectum, the SCN and the IGL, indicating that the projecting pretectal neurons and their efferent projections are potentially involved in phase-shifting mechanisms.

587.10

THE SUPRACHIASMATIC NUCLEUS AND INTERGENICULATE LEAFLET OF ARVICANTHUS NILOTICUS, A DIURNAL MURID RODENT.
L. Smale*, Dept. Psychology, Michigan State University, East Lansing, MI, 48824

Little is currently known about how the neural substrates controlling circadian rhythms differ in diurnal compared to nocturnal species. One reason has been the absence of a suitable diurnal rodent model. Arvicanthus niloticus, a murid rodent recently imported from Kenya, exhibits a diurnal pattern of precisely timed wheel-running activity and may represent an ideal diurnal mammal for studies of the neural control of circadian rhythmicity. To provide background information for future experimental studies, we used immunohistochemical techniques to examine the distribution of peptides in the SCN and IGL of 8 adult A. niloticus (2 females and 6 males); in this initial study animals were not pre-treated with colchicine. In A. niloticus, as in other mammals, distinctly different subdivisions of the SCN contained VIP and VP immunoreactive (IR) cell bodies. Within the SCN, we did not observe cell bodies containing NPY, met-enkaphalin or substance-P immunoreactivity. NPY-IR, M-enk-IR and SP-IR fibers were present within the SCN. The IGL contained a substantial number of NYP-IR cells, as well as SP-IR and M-enk-IR terminals. Overall, the IGL and SCN of A. niloticus resembled, in many respects, those previously described in rats.

587.11

ONTOGENY OF THE MOTOR CIRCADIAN SYSTEM IN CRAYFISH.
J. Hernández-Falcón, A. de la O-Martínez and B. Fuentes-Pardo. Depto. Fisiología, Facultad de Medicina, UNAM. AP 70-250, México, 04510, D. F. MEXICO.

The ontogeny of motor circadian rhythm of the crayfish (Procambarus clarkii) was studied using specimens kept under controlled conditions of light and temperature (17°C) The motor activity of animals aged between 10 to 180 days after hatching was recorded. During 10 days the movements of unrestrained crayfish were monitored by means of a video camera coupled to a digital converter. Recordings were obtained under free-running in darkness (DD), free-running in light (LL) and LD 12:12 photoperiods. From each recording circadian parameters (amplitude, : ratio and night/day ratio) were measured; was calculated by means of periodogram technique. The mean for the youngest crayfish was 23.9 hrs in either LL or LD. In the oldest animals the mean was 22 hrs in LL, 22.9 hrs in DD, and 20 hrs in LD. The younger the crayfish the more irregular the locomotor patterns. However, the ability to be synchronized by LD photoperiods is better in the younger than in the older crayfish. The results indicate that the circadian organization appears very early in the development. They suggest also a progressive increment in the number, kind and amount of dispersion of the oscillators during the ontogenic development.

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587.12

EFFECT OF SKELETON PHOTOPERIODS UPON THE CIRCADIAN MOTOR ACTIVITY RHYTHM DURING ONTOGENY IN CRAYFISH. M.L.Fanjul-Moles*, O. Castañon-Cervantes and M. Miranda -Anaya. Lab. de Neurofisiología Comparada,Fac.de Ciencias UNAM. México D.F., 70371, México.

During ontogeny of crayfish *Procambarus clarkii* the synchronization of the circadian activity rhythm (CAR) to complete photoperiods (CP) is not clear (1) probably due to a masking effect of light. We tried to rule out this effect, studying the influence of symetrical skeleton photoperiods (SSP) upon the CAR at different stages of development. *Procambarus clarkii* juvenile instars between 10 up to 150 days after hatching were individually housed in activity recording cages under constant darkness and temperature conditions. Each crayfish was free running recorded during 15 days. Afterwards it was changed to 3 different SSP equivalent to the following CP : 12:12 , 8:16 and 20:4. After 15 days of SSP stimulation they were left free running again . The results suggest that only animals older up to 60 days are able to synchronize to SSP adjusting its maximal phase to the longest scotophase.

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588.1

THE ROLE OF CENTRAL NEURAL DIRECT CONNECTIONS ON THE CONTROL OF RAT PINEAL METABOLISM. J.Cipolla-Neto, I.Bartol, S.M.Mondoni, P.M.Seraphim, S.C.Afeche, L.G.Britto*, J.Scialfa and A.M.C.Peraçoli. Dept.of Physiology and Biophysics, Inst.of Biomed. Sci., Univ.of São Paulo, São Paulo, Brazil, 05508-900.

The aim of the present work was to study the effects of lesions on the intergeniculate leaflet (IGL) or the deep pineal / lamina intercalaris region (DP) on the diurnal profile of N-acetylserotonin (NAS) and on the nocturnal pineal inhibition process induced by acute light exposure. Male adult albino rats (n=214, 12h:12h light-dark cycle, lights on at 0600h), intact or previously lesioned, were killed along the 24 hours or during the night immediately after 1 or 15 minutes of exposure to 500 lux white lights. The pineal glands were collected and frozen (-70°C) until NAS was assayed by HPLC-ED. The 24-hour experiment shows that there is no phase shifting on the diurnal NAS curve of groups of rats with large bilateral IGL lesion compared to the controls. On the other hand there is a significant reduction on the amplitude of pineal NAS content observed in every nocturnal point of the curve ($p<0.05$).The pineal glands of IGL-lesioned rats do not respond to brief (1 min.) retinal photostimulation keeping its NAS content equal to the lesioned dark killed rats ($p=0.93$). On the other hand, 15 min. of photostimulation bring the pineal NAS content of the IGL group to nearly zero equally to the control animals. In experiments done at 2400 h, DP lesion does not modify the content of NAS in the pineal gland of rats killed in the dark. However, the pineal photo-inhibition process induced by 1 min of light exposure is impaired since the lesioned group has its NAS content reduced much less than the intact animals. These results suggest that: 1-the nocturnal photo-inhibition process of the pineal gland is partially dependent on central direct neural projections, probably from the IGL region; 2- The IGL exerts a tonic excitatory control on the nocturnal pineal NAS production, probably via its indirect projections controlling the hypothalamus-spinal cord-sympathetic pineal system. FAPESP grant # 92/1506-7 to JC-N.

588.2

INTRACELLULAR Ca^{2+} DYNAMICS IN DISSOCIATED CELLS OF THE CHICK PINEAL GLAND. T. D'Souza & S. E. Dryer*, *Program in Neuroscience, Florida State University, Tallahassee, FL 32306.*

The regulation of intracellular free Ca^{2+} concentration was examined in dissociated chick pineal cells using the fura-2 technique. Approximately 10% of cells examined exhibited spontaneous Ca^{2+} oscillations. Membrane depolarization evoked large increases in intracellular free Ca^{2+} that were dependent upon external Ca^{2+} ions. Application of thapsigargin (2 μM) evoked increases in intracellular free Ca^{2+} in the absence of external Ca^{2+} indicating the presence of internal stores. Application of 100 nM vasoactive intestinal peptide (VIP) induced a sustained increase in intracellular free Ca^{2+} and, in a small number of cells, evoked Ca^{2+} oscillations. Application of 200 μM norepinephrine had no effect on intracellular free Ca^{2+} in quiescent or oscillating pineal cells. Application of 8-Br-cyclic AMP (5 mm), papaverine (50 μM), IBMX (100 μM), or forskolin (200 nM) evoked sustained increases in intracellular Ca^{2+}. The responses to forskolin could be observed in Ca^{2+} free external salines suggesting mobilization of internal stores. These results suggest new mechanisms for the regulation of melatonin synthesis and secretion, and possible sites of action for the intrinsic circadian oscillator. Supported by AFOSR F-49620.

588.3

LOCALIZATION AND CHARACTERIZATION OF 2-[^{125}I]-IODOMELATONIN BINDING SITES IN GUINEA-PIG BRAIN I.J.M. Beresford, M.L. Dubocovich[1], J. Andrews, R. Nicholls, J.C. Coughlan, S. Iacob[1], A. G. Hayes* and R.M. Hagan Dept. Pharmacol., Glaxo Res. & Devel., Ware, Herts. UK, SG12 0DP, [1]Dept. Pharmacol., Northwestern Univ. Medical School, Chicago, IL60611.

ML-1 receptor binding sites have been localized within a number of circadian and visual areas of the mammalian and avian CNS. We have localized and characterized 2-[^{125}I]-iodomelatonin ([^{125}I]-MEL) binding sites in guinea-pig brain.

Specific [^{125}I]-MEL (90pM) binding, defined using melatonin (1μM), was identified in coronal sections of guinea-pig brain by receptor autoradiography at the level of the arcuate, suprachiasmatic and lateral geniculate nuclei, superior colliculus (SC) and nucleus accumbens. In SC and hypothalamic membranes, high-affinity, specific, saturable and reversible binding was observed. In the SC, [^{125}I]-MEL bound to a single population of high-affinity binding sites (K_D 14.1±0.4pM, B_{max} 66±6fmol/g tissue), while in hypothalamus both high and low affinity sites were identified (K_{D1} 33.3±6.5pM, B_{max1} 19.1±0.7fmol/g tissue; K_{D2} 6.6±2.8nM, B_{max2} 153±47fmol/g tissue), which appear to represent two affinity states of the ML-1 receptor. In hypothalamus, melatonin agonists inhibited binding with a rank order of potency (IC_{50} values) 2-iodomelatonin (0.16±0.02nM) ≥ N[2-(7-methoxy-1-naphthalenyl)ethyl]-acetamide (MNEA; 0.37±0.07nM) > melatonin (1.8±0.2nM) ≥ 6-chloromelatonin (3.1±1.0nM) >> N-acetylserotonin (1.4±0.3μM) >> 5-HT (>100μM). 5-methoxycarbonylamino-N-acetyltryptamine (GR135531), which has higher affinity for N-acetylserotonin-preferring (ML-2) receptors (Dubocovich et al., IUPHAR, 1994), was only weakly active (<1μM). The rank order of potencies in SC membranes was identical (correlation coefficient 0.998).

In conclusion, [^{125}I]-MEL binding sites, with the characteristics of ML-1 receptors, were localized in guinea-pig SC and hypothalamus, areas in which this receptor may regulate visual and circadian information, respectively.

588.4

NON G-PROTEIN COUPLED HIGH AFFINITY MELATONIN RECEPTORS ARE PRESENT IN THE SHEEP BRAIN. Barrett, P.*, Lawson, W., MacLean, A., Hazlerigg, D., Williams, L.M. and Morgan, P.J. Rowett Research Institute,Bucksburn, Aberdeen AB2 9SB Scotland

Melatonin receptors have been localised to both neuronal and non-neuronal sites by *in vitro* autoradiography using the radioligand 2-^{125}I-iodomelatonin. In general these receptors are characteristically of the G-protein class, as they are membrane-bound, of picomolar affinity which is modulated by guanine nucleotides. In non-neuronal ovine pars tuberalis cells, melatonin acts to inhibit forskolin-stimulated adenylate cyclase through a coupling to two independent inhibitory G-proteins. Recent evidence suggests that unique Gs-like G-protein, which is both cholera toxin-sensitive and recognised by $\alpha_S$subunit C-terminally directed antibodies mediates this response. In contrast sheep neuronal tissue has markedly different characteristics. Homogenate binding assays reveal that the hippocampal binding site has high affinity (Kd = 56 pM), yet binding is not affected by GTPγS, cholera or pertussis toxins, each of which modulate the affinity of the PT melatonin receptor. Native polyacrylamide gel electrophoresis resolves the PT receptor as a complex which migrates with a molecular mass of ~515kDa. Under the same conditions the hippocampal receptor migrates with a molecular mass of ~365kDa. This assay also provides a visual demonstration of the lack of GTPγS sensitivity of the hippocampal receptor. With this technique we also show that the melatonin receptor present in the cerebral cortex and pre-optic area have a similar mass to the hippocampal receptor and are not affected by GTPγS.This suggests that these receptors are not G-protein coupled and therefore belong to different class of receptor.

This work was funded by SOAFD

588.5

EVENING ADMINISTRATION OF MELATONIN PROMOTES SLEEP IN HUMANS. **I.V.Zhdanova, R.J.Wurtman, H.J.Lynch, J.Ives^, J.Matheson^, C.Morabito, A.B.Dollins, D.L.Schomer and C.J.Watkins.* Clin.Res.Ctr., MIT, Cambridge,MA 02139 and ^Neurology, Beth Israel Hosp., HMS, Boston,MA 02215.**

Sleep-inducing effects of low doses of melatonin were examined in young healthy volunteers. The hormone (0.3 or 1.0 mg, p.o.) or placebo was administered at 1800 or 2000 h; the lower dose elevated serum melatonin to concentrations comparable to those normally occurring nocturnally in adults (80-120 pg/ml). Effects of the treatment on performance and mood were monitored all day prior to evening treatment and until 0900 the next morning. The volunteers' subjective evaluation of the hypnotic effect showed that they could distinguish between melatonin and placebo, especially after treatment at 2000 h. Beginning 2 hours after treatment, sleep latency was measured by a switch release or by a polysomnographic system. Either dose given at either time point decreased both sleep onset latency and the most sensitive index of melatonin's effect, stage 2 sleep latency.

These observations indicate that raising blood melatonin levels to those normally occurring nocturnally promotes sleep onset in humans.

588.6

S-20098, A MELATONIN-AGONIST, PHASE SHIFTS THE CIRCADIAN CLOCK OF MICE AND SYRIAN HAMSTERS ACCORDING TO A PHASE-RESPONSE-CURVE. VAN REETH O., OLIVARES Y., ZHANG F.W., TUREK F.W., DEFRANCE R., MOCAER E.*. CERB, Université Libre de Bruxelles, Brussels ; Dept of Neurobiology and Physiology, Northwestern University, Evanston, USA ; Institut de Recherches Internationales Servier, Paris, France.

S-20098, a naphtalenic analogue of melatonin, has potent and specific agonist properties on melatonin receptors both *in vitro*(YOUS et al., 1992) and *in vivo*. Like exogenous melatonin, daily injections of S-20098 in late subjective day can entrain the activity rhythm of rats free-running in constant darkness (DD) (BONNEFOND et al., 1993). S-20098 can also accelerate the rate of re-entrainment of the activity rhythm in rats subjected to an 8-h advance shift in the light-dark cycle (REDMAN et al., 1993). The aim of this study was to see whether single administration of this new compound has any effect on the circadian system of hamsters and mice. In two separate studies, we have determined the effects of single injections of S-20098 on phase and period of the circadian rhythm of wheel running in C3H/HE mice (10 mg/kg) or (5 mg/kg). Single intraperitoneal injections (vehicle versus S-20098) were administered at one of ten different circadian times (CTs 3, 6, 9, 10, 11, 12, 15, 18, 21 and 24 ; CT 12 = onset of locomotor activity) in mice or hamsters free-running in DD(N = 7-10 animals per CT).

Phase advances in the activity rhythm were observed between CT9 and CT12 in both species. Maximal phase advances were of a larger magnitude in mice (CT11 ; mean = 37± 8 min) than in hamsters (CT 9 ; mean : 17 ± 7 min). No significant phase-delays in the activity rhythm were observed. Treatment with S-20098 had no significant effect on the period length and any of the CTs tested.

Results of these studies indicate that single injections of S-20098 can induce phase shifts in the circadian clock of rodents, and suggest a potential role of S-20098 as a chronobiotic in the treatment of disorders where biological rhythms are disrupted.

S. YOUS, J. ANDRIEUX, H. E. HOWELL, P. J. MORGAN, P. RENARD, B. PFEIFFER, D. LESIEUR, B. GUARDIOLA-LEMAÎTRE ; Journal of Medicinal chemistry 1992; 35: 1484-1485.
C. BONNEFOND, L. MARTINET, D. LESIEUR, G. ADAM, B. GUARDIOLA-LEMAÎTRE ; in Melatonin and the pineal gland. Y. Touitou, J. Arendt, P. Pevet ed. 1993, Elsevier Science Pub., 123-126.
J. REDMAN, B. GUARDIOLA-LEMAÎTRE ; in Melatonin and the pineal gland. Y. Touitou, J. Arendt, P. Pevet ed. 1993, Elsevier Science Pub., 127-130.

588.7

MELATONIN RHYTHMS IN THE JUVENILE DJUNGARIAN HAMSTER: MAGNETIC FIELD EXPOSURES AND THEIR REPRODUCTIVE CONSEQUENCES. SM Yellon*, JC Smith and H Trong Div. Perinatal Biology, Depts. Physiol. & Peds., Loma Linda Univ. Sch. of Med., Loma Linda, CA 92350

The pineal melatonin rhythm in a variety of rodents is suppressed by exposure to 60 Hz magnetic fields (MF) and may alter the clock mechanism involved in the photoperiodic control of reproduction (*J Cell Bioch 51: 394, 1993*). To determine if MF disrupts photoperiodic regulation of sexual maturation, males in long (16L) or short days (10L) were exposed each day to a 1 Gauss MF (15 min. beginning 2 h before lights out) from 18 to 25 days of age. A single exposure of juveniles or adults to this MF reduces duration and phase delays the nighttime rise in melatonin. Sham-controls were in an adjacent system but current was not applied. Hamsters were decapitated every 1 to 4 h to define the melatonin rhythm (n=6/time/group); serum and pineal gland levels were determined by RIA. In long days, testes growth in both sham-exposed and MF-treated hamsters was typical for the normal onset of puberty (group means >216 mg). In short days the testes were small, regardless of exposure (<118 mg). Therefore, daily MFs did not interfere with the photoperiodic mechanism controlling reproductive development. For the melatonin rhythm, MF exposure reduced the duration of the nighttime melatonin rise in hamsters in long days by several hours relative to that in controls. MF treatment was without effect in short days; melatonin increased in the pineal and circulation within 3 h of dark onset and remained elevated for nearly 10 h. These data support the conclusion that daily MF exposure neither alters the normal onset of puberty nor disrupts the photoperiodic mechanism that arrests sexual maturation. The findings raise the hypothesis that adaptation to prevailing MF conditions may underlie the lack of responsiveness by juvenile hamsters to repeated MF exposures, a proposal that may also apply to the adult Djungarian hamster. (Supported by NIH ES016137)

588.8

MELATONIN DURATION AND REPRODUCTIVE RESPONSES IN MALE SYRIAN HAMSTERS. J.B. Powers*, A.E. Jetton and R. A. Mangels. Departments of Psychology and Biology, Univ. of Massachusetts, Amherst, MA 01003-7710.

Syrian hamsters are long day breeders; exposure to short days causes inhibition of the reproductive system. This response requires the nightly secretion of melatonin which serves as a hormonal indicant of night length. Traditional views suggested that day lengths shorter than 12.5 hrs were equally effective in generating reproductive inhibition, independent of the actual day length used and its associated melatonin duration. In the experiments presented here we determined if variations in melatonin duration or photoperiod history would affect the rate of reproductive inhibition following melatonin treatment. Forty-five male Syrian hamsters were exposed to long days (16L:8D) for two months and then assigned to one of four groups; three remained in 16L and were pinealectomized (PINX); the fourth was shifted to a short day (7L:17D) and was sham pinealectomized (SH-PINX). The three PINX groups received nightly sc infusions for nine weeks containing either vehicle (VEH) or melatonin (MEL - 50 ng/hr) for 8.5 hrs or 12.0 hrs . The SH-PINX group was not infused. At three week intervals, testis widths were measured and blood withdrawn for assay of follicle stimulating hormone (FSH) and prolactin (PRL). Both MEL durations caused significant testicular regression but this occurred more rapidly with the longer MEL duration ($p<.05$). In a second experiment, MEL duration was held constant but groups were exposed to differing long day conditions prior to MEL treatments. Forty-eight males were divided into two groups - one exposed to 18L:6D, the other to 14L:10D for six weeks. All hamsters were then PINX and switched to 16L:8D. Within each condition, one-half the males were infused for 9.5 hrs each night for nine weeks with either MEL or VEH; their reproductive condition was assessed as in the first experiment. MEL infusions caused significant reproductive inhibition ($p<.05$) but this was only moderately influenced by photoperiod conditions prior to MEL treatment. Results derived from serum assays of FSH and PRL will be reported. Supported by HD30372, HD07673 and MH44132.

588.9

MELATONIN INDUCES OUTWARD CURRENTS IN A SUBPOPULATION OF RAT SUPRACHIASMATIC NUCLEUS (SCN) NEURONS. Z. G. JIANG* AND C.N. ALLEN Center for Research on Occupational and Environmental Toxicology and Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201.

Several lines of evidence support a role for melatonin in entrainment of circadian rhythms via direct actions on SCN neurons. However, the cellular mechanisms of melatonin's actions remain largely unknown. We used whole-cell recording techniques and brain slices to examine the membrane currents activated by melatonin. Young adult male rats were housed on a 12:12 h light-dark cycle and their locomotor activity and body temperature monitored for 2-4 weeks before the experiments. Full entrainment with the light-dark schedule was always achieved in less than a week. Animals were killed 3-5 h (CT3-5) after lights on and recordings were made during the next 1-10 h (CT5-15). Melatonin (0.1-30 μM) induced an outward current (3-40 pA, at -60 mV), in 4 of 20, 8 of 27 and 5 of 18 cells tested between CT6-CT9, CT9-CT12 and CT12-CT15, respectively. The current was associated with an increase in membrane conductance. The response was concentration-dependent with a threshold of about 100 nM and a maximum effect at 10-30 μM. The melatonin-activated currents were blocked by 1 mM Ba, partially blocked by 3 mM Cs, but not affected by TTX and/or a combination of CNQX and APV. The current amplitude was reduced and, in a few cases, reversed by a hyperpolarization over -100 mV. These data suggest that melatonin activates a potassium current thus causes inhibition in a subpopulation of SCN neurons. Study supported by grant AG10794.

588.10

Melatonin Regulates CREB Phosphorylation in Ovine *ParsTuberalis*
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Rowett Research Institute, Bucksburn, Aberdeen. AB2 9SB.
+Department of Anatomy, University of Cambridge, Cambridge. CB2 3DY.

Ovine pars tuberalis (oPT) cells containing melatonin receptors, and pars distalis (oPD) cells lacking such binding sites, were studied for the involvement of the transcription factor CREB, in the transduction of the melatonin signal. Experiments included gel shift and immunocytochemical studies to determine the presence of CRE-binding nuclear factors and to investigate the effects of forskolin and melatonin, on the level of CREB activation, measured as P-CREB immunoreactivity (P-CREBir). Gel shifts identified two specific CRE oligonucleotide-protein complexes in oPT nuclear extracts. These were displaced by excess unlabelled CRE but not by excess TRE oligonucleotides and both bands were supershifted using anti-CREB and anti-P-CREB antisera. Immunocytochemistry results were quantitated using digitised image-analysis to measure observed cell treatment differences in nuclear optical density. Increased PCREBir in response to forskolin stimulation was time and dose dependent. This change in staining was not detectable using the anti-CREB antibody, hence increased PCREBir is due to rapid phosphorylation rather than de novo CREB synthesis. The time course for forskolin stimulated P-CREBir was consistent with nuclear translocation of protein kinase A. In oPT but not oPD cells, CREB phosphorylation was inhibited by melatonin in a time and dose dependent manner, with the dose also being dependent on the forskolin concentration used. The IC50 for melatonin was around 10nM. Melatonin is therefore thought to regulate expression of cAMP-responsive gene expression through exerting an inhibitory effect, via the cAMP/PKA pathway, on the level of activation of the transcription factor CREB. *Funded by SOAFD and Wellcome trust.*

588.11

AGE RELATED DIFFERENCES IN THE STRUCTURE OF HUMAN PINEAL CALCIUM DEPOSITS: RESULTS OF TEM, SEM AND MINERALOGRAPHIC MICROANALYSIS. [1]A.W. Klein, [2]H.A. Schmid, [1]E.G. Stopa [1]J.T. Parmelee* and [1]P.McMillan. [1]Dept. of Pathology, Brown Univ. Sch. of Med./Rhode Island Island and [2]Veterans Admin. Med. Ctr., Providence, RI.

Pineal calcifications increase with advancing age in man and in many other mammals. Melatonin biosynthesis decreases with advancing age, and has several calcium dependent steps, suggests the possibility that pineal cell dystrophy related to the aging process may accelerate biomineralization in the pineal gland. Pineals from a range of aged men (14, 47, 62 and 82 years) were metallographically embedded, polished, and studied by scanning EM and transmission EM for age related differences. The data show that: 1) concentrically arranged crescent shaped lamellae increase in number and decrease in width, with advancing age. This suggests remodeling. 2) There is an increase in the calcium: phosphorus ratio (1.27-1.41 in 14 to 62 year ages), with advancing age (1.49-1.62 at 82 years) in all lamella. C/P lamellar increases may parallel cell dystrophy and dysfunction. 3) The architecture of the deposits show an age progression from a) round-smooth-stage, through a round beaded stage and into acervuli (i.e. antlered) stage. This suggests mineralization and growth by methods other than simple apposition. These results suggest a biomineralization followed by a remodeling that changes throughout the organism's life span and may relate to the decline of melatonin biosynthesis.

589.1

STRESS TRIGGERS DIFFERENT PATHOPHYSIOLOGICAL MECHANISMS IN YOUNG AND OLDER CARDIOMYOPATHIC HAMSTERS (CMHs). Q. Chang, R. Conway, J. E. Ottenweller, B. H. Natelson *. UMDNJ-GSBS, -NJMS, and -RWJMS, Newark & New Brunswick, and Neurobehavioral Unit (127A), VAMC, East Orange, NJ 07018.

Because BIO 14.6 CMHs in the lesion forming period of their disease are more susceptible to the lethal effects of stress than older CMHs[1], we hypothesized stress triggered coronary vasospasm in younger CMHs and congestive heart failure (CHF) in older CMHs. To test these hypotheses, we stressed 2.5 and 6.5 mo male CMHs (2 hr of supine cold immobilization for 5 consecutive days). Three, 5 and 7 days after stress; anesthetized CMHs were sacrificed and their hearts were mounted by the aorta for retrograde perfusion of the coronary arteries using a modified Langendorff system. Baseline developed pressure (DP), maximum dp/dt and coronary vascular resistance (CVR) were recorded at 80 mmHg perfusion pressure and following coronary infusion of arginine vasopressin (AVP) at 0.2 unit/min. In younger CMHs, stress produced a decrease in DP and maximum dp/dt on day 3, but not on days 5 or 7. Stressed older CMHs had significantly lower DP and maximum dp/dt than nonstressed on all 3 days ($p<.05$). Baseline CVR in younger CMHs was significantly higher than in older CMHs ($p<.05$). AVP infusion produced a bigger increase in CVR in younger stressed CMHs than in either younger nonstressed or older stressed CMHs ($p<.05$). The data support our hypotheses. The coronary vasculature shows increased AVP-induced vasoconstriction in stressed younger but not older CMHs. In contrast, older but not younger CMHs develop a consistent decrease in cardiac mechanics indicative of CHF. The lethal effects of stress in younger and older CMHs appear to occur because of the activation of different pathophysiologic processes. (Supported by VA Medical Research Funds)
Ref: 1. B. H. Natelson, W. N. Tapp, S. Drastal, R. Suarez, J. E. Ottenweller. Hamsters with coronary vasospasm are at increased risk from stress. Psychosom Med 1991; 53: 322-331

589.2

EFFECTS OF AEROBIC EXERCISE TRAINING ON THE RESPONSES TO CHRONIC STRESS. S.P. Bailey, A.M. York, K.W. Grasing*, S. D. Schlussman, J.P. Advis, and D. H. Overstreet. Dept. Exercise Science & Sport Studies and Dept. Animal Studies, Rutgers Univ., New Brunswick, NJ 08903. Clinical Research Center, Robert Wood Johnson Medical School, New Brunswick, NJ 08903. Skipper Bowles Center for Alcohol Studies, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27599

Aerobic exercise training (ET) modifies the behavioral responses to acute stress. The effects of ET on the responses to chronic stress are less clear. The purpose of this study was to examine the effectiveness of ET in attenuating the behavioral and endocrine responses to chronic unpredictable stress (CUS). 16 Flinders Sensitive Line (FSL) and 16 Flinders Resistant Line (FRL) rats were used in this investigation due to their respective sensitivity and resistance to CUS. Animals were subjected to 10 weeks of ET (25 $m \cdot min^{-1}$ & 7% grade, 30 min per day) or remained sedentary (SED). Animals were then subjected to 1 wk of CUS. Open field mobility (OFM) and sucrose consumption (SC) were evaluated prior to and after CUS. After CUS, blood was collected for measurement of plasma corticosterone (CORT). Following CUS the number of squares entered during OFM was reduced ($p<.05$) in sedentary animals (both FSL and FRL); however, the number of squares entered during OFM was unchanged in FSL-ET and FRL-ET. The number of rears during OFM was reduced ($p<.05$) in all animals following CUS. Following CUS, SC consumption was reduced ($p<.05$) in all groups except FRL-ET. Plasma CORT was greater ($p<.05$) in FRL-SED than FRL-ET following CUS. In comparison, plasma CORT values in FSL-SED and FSL-ET were similar to normal resting values. These results indicate that ET is effective in attenuating some, but not all, of the behavioral responses to CUS. Furthermore, FSL animals did not benefit as much from ET as FRL animals. Resistance to the behavioral effects of stress by ET was associated with lower CORT levels in FRL animals.

589.3

EFFECTS OF MILD INTERMITTENT TAIL SHOCK ON COLONIC MOTOR ACTIVITY. N.S. Morrow,[1] and T. Garrick.[1,2] Center for Ulcer Research and Education, Dept. of Veteran Affairs Medical Center,[1] Los Angeles CA 90073, Dept. of Psychiatry,[2] UCLA, Los Angeles, CA 90024.

Changes in colonic motor activity during exposure to tail shock were examined in rats chronically implanted with 2 force transducers on the proximal colon. Baseline colonic contractility was monitored in home cages for 3 days. Meal-stimulated changes in colonic activity were then examined in 2 testing periods. Following the second feeding trial, rats were randomly divided into 3 groups (n=6 each) for testing. All rats were loosely restrained in a plexiglass tube and 2 electrodes taped to the tail. One group of rats was administered unpredictable, intermittent shocks (1 ma, 5 sec, VI= 1 min) for 1 h. A second group was exposed to the tail shock chamber but was not shocked. The third group of rats received tail shocks for 1 h and then 24 h later were re-exposed to the tail shock chamber but were not shocked. Fecal output was recorded throughout the experiment and rated for consistency on a 4-point scale. Results indicated that following a meal, the duration of long duration contractions increased significantly from baseline measures. During exposure to tail shock, the amplitude of both short and long duration contractions were significantly suppressed ($p's<.05$) when compared to basal values. Tail shock increased the number of feces evacuated ($p's< .05$) and affected fecal consistency in a variable manner. Exposure to the shock chamber, alone, did not significantly alter the colonic contractility pattern or fecal output from basal values. These results indicate that alterations in colonic motor activity can be induced by exposure to environmental stressors and may be a contributing factor in stress-induced bowel dysfunction.

589.4

A NEW COMPUTER-BASED METHOD FOR ANALYZING RAT ULTRASONIC VOCALIZATIONS. J. Martinez, K. Krogh and L.D. Lytle. Department of Psychology, University of California, Santa Barbara, CA 93106.

Rat ultrasonic vocalizations have proven useful for assessing maturational changes in maternal-infant interactions and other aspects of behavior, as well as for monitoring the effects of various psychoactive drugs. In most instances, changes in the number of vocalizations are the dependent variable. Expensive equipment and time-consuming sonagram-based procedures limit more complete analyses of the vocalization response (e.g., possible changes in amplitude, duration, frequency). We now report the development of a new computer-based method that allows for a rapid, inexpensive, and relatively simple analysis of changes in the number, amplitude, and frequency of rat ultrasonic vocalizations. This new system employs a 386 SX or more advanced personal computer outfitted with an A/D converter, a timer and a counter. The computer receives its input from a zero-crossing and an envelope detector circuit which in turn receive a high frequency signal from an S-25 ultrasound detector (Ultrasound Devices, Ltd., London). A Quick Basic software routine developed at UCSB was used to analyze call frequency, amplitude, and duration in 1 msec time frames.

We used this new method to monitor maturational changes in albino rat's ultrasonic vocalizations following separation from their dams and littermates for a 30 min period (at 23±1 °C) beginning at birth, and extending at 5 day intervals until the time of weaning. The number, duration, and frequency of calls emitted changed with age in a fashion essentially replicating previously published results [Okon, E.E. *J. Zool., Lond.* (1970) *162:* 71-83] using more expensive and time consuming methodology. The number and amplitude of pup calls emitted peaked at age 10 days and declined thereafter; the average call frequency was highest between ages 5-15 days.

589.5

NEITHER PRENATAL STRESS NOR NEONATAL HANDLING ALTER RAT'S PERFORMANCE ON THE MORRIS WATER MAZE. A.C. Segarra*[1], J.L.González[2], R.Keller[2], K.Salim[2] and B.S. McEwen[2]. [1]Dept. of Physiology, Sch. of Med, Univ.of Puerto Rico, San Juan, P.R. 00936 and [2]Lab. of Neuroendocrinol., Rockefeller Univ., 1230 York Ave., NY, NY 10021.

Prenatal stress and neonatal handling are known to have long-lasting effects on rat emotionality. The hippocampus mediates, at least partially, some of these effects. In this study we investigated the effect of prenatal stress and of neonatal handling on spatial ability and learning, behaviors regulated by the hippocampus. From GD 14 to 21 pregnant Sprague-Dawley rats were restrained for 45 min 3 times/day. From PN 1-10, half of the pups were handled daily by placing them in a cage at 37°C for 3 min. At approximately 23 and 63 days rats were tested in a Morris Water Maze (MWM).

Prenatal stress had no effect on rat performance in a MWM, nor did handling. A sex difference in latency to find the platform, was observed in adult animals, males performing better than females. This sex difference was not observed in prepubertal animals. An age difference was also observed, with older animals learning to reach the platform twice as fast as prepubertal animals. Our previous results show that the prenatal stress treatment was effective since an increase in emotionality and in basal corticosterone plasma levels was observed in the same animals. These findings indicate that spatial cognitive abilities in rats are unaffected by prenatal stress and by neonatal handling.

589.6

SOCIAL AND ENVIRONMENTAL CHALLENGES PRODUCE CHANGES IN CIRCADIAN AND ULTRADIAN RHYTHMS. D.G. Harper, W. Tornatzky* and K.A. Miczek. Dept. of Psychology, Tufts University, Medford, MA 02155

If circadian and ultradian rhythms of temperature and heart rate reflect optimal homeostasis then the speed of recovery of these rhythms may reflect the time required to recover homeostasis following a challenge. In our present study, we examine the adaptation of rats to several experimental manipulations designed to disrupt or disorganize the circadian and/or ultradian rhythms of these animals. Telemetry readings of heart rate, temperature and activity were collected in 5 minute epochs in 8 adult male Long Evans rats throughout the experiment. Following implantation of a telemetry sender, animals were allowed to recover from the surgery and adapt to a 12:12 light cycle (lights off 14:00, lights on 02:00) for a period of 21 days. (1) The light cycle was then advanced 6 hours (lights off 08:00, lights on 20:00) and the animals allowed to entrain to the new rhythm. (2) The animals were given 30 ml. of a 10% sucrose solution at 10:30 (access limited to 1/2 hour) over a period of 3 weeks to test for food entrainment effects. (3) Animals were then subjected to a social defeat experience for 5 days with submissive behavior and ultrasound vocalizations monitored. Changes in the amplitudes of circadian and ultradian rhythms following social stress and light shift and entrainment effects following drinking were observed.

589.7

THE IMMEDIATE EFFECTS OF PHYSICAL EXERCISE ON PASSIVE-AVOIDANCE MEMORY AND ANXIETY IN RATS. J.E. BRYANT*, Tracey Smith, Leslie H. Hicks and Michael J. Lewis. Neurobehav Lab, Dept of Psych, Howard University, Washington, D.C. 20059

Participation in physical exercise is currently fashionable. Although many people report psychological benefits following exercise, experimental data on this issue remain sparse. The present study examined whether a brief voluntary wheel-running exercise (20 min a day for 10 days) could facilitate passive-avoidance memory while concurrently examining anxiety in young male rats. Previous exercise, when compared to a nonexercise condition, was not found to significantly facilitate passive-avoidance memory in rats. However, voluntary wheel-running did significantly increase anxiety [$t(40) = 2.43$. $p<.05$], as measured by defecation rates, in acquisition of one-trial step-through passive-avoidance. The finding that engagement in physical exercise increased anxiety is discordant with the reports of many exercisers who suggest an anxiolytic effect following exercise.

589.8

EFFECTS OF INDUCED POSITIVE AND NEGATIVE STRESSORS ON IMMUNE FUNCTIONING IN CHRONIC DEPRESSION AND CONTROLS. A.V. Ravindran*, J. Griffiths, S. Zalcman & H. Anisman. Royal Ottawa Hospital, Ottawa, Ontario, Canada K1Z 7K4.

Stressful events encountered by humans have been reported to influence immune functioning. Depending on severity, stressors may influence natural killer (NK) cytotoxicity, circulating lymphocyte subsets (e.g., NK cells), as well as cell proliferation in response to mitogens. In the present investigation a brief mild, laboratory stressor (cognitive challenge) increased NK cell numbers in controls and in dysthymic (chronic, low grade depressive) patients. The extent of the increase was directly related to plasma norepinephrine levels, and may reflect effects on cell trafficking. Likewise, a more meaningful stressor (having subjects complete the day-to-day hassles scale with subsequent discussion of the stressors) resulted in an increase of NK cell numbers in both dysthymic and control subjects. Cell proliferation in response to a mitogen was reduced in dysthymic patients who discussed hassles. These effects were less marked when patients discussed uplifts, or in control subjects who discussed their hassles. Finally, control subjects who had undergone a stressor perceived as being more severe (final academic examination) exhibited NK cell alterations, as well as variations of several other lymphocyte subsets (e.g., CD-3, CD-4 and CD-19). The data are discussed in terms of the relationship between stress and depression and the impact of stressors on immune functioning.

589.9

EXAGGERATED ACOUSTIC STARTLE IN GULF WAR VETERANS WITH PTSD. C. A. Morgan III, C. Grillon*, S. M. Southwick, and D. S. Charney. Department of Psychiatry, Yale University School of Medicine, West Haven VAMC, West Haven CT 06516.

Exaggerated startle is reputed to be one of the cardinal symptoms of Post Traumatic Stress Disorder (PTSD). Objective studies have given conflicting results as to whether or not startle is increased in PTSD. However these studies were conducted on subjects with chronic PTSD (Vietnam and Israeli combat veterans). The present study investigated the acoustic startle response of eight Gulf War veterans with acute PTSD and 15 healthy age matched controls. The eyeblink component of the startle reflex was measured in response to six blocks of pseudorandomized 40 ms white noise bursts of varying intensities (90, 96, 102, 108, 114 dB). No war related or stressful cues were presented to subjects prior to or during testing. Startle amplitude was significantly greater and habituation significantly reduced in the PTSD subjects compared to controls. Because other studies in the literature, as well as our own laboratory, which have investigated the startle response in combat related PTSD, have failed to find exaggerated startle or reduced habituation of startle at baseline (i.e. absence of stress), it is likely that the present results reflect an acute elevation of startle in this group. The higher amplitude and decreased habituation of startle in the PTSD subjects may reflect a sensitization of the fear/alarm response created by the recent stress of combat trauma consistent with preclinical studies of shock sensitization and startle.

589.10

CONTROL OVER CHRONIC STRESS REDUCES THE TERMINAL ACCURACY, BUT NOT THE ACQUISITION OF SIMPLE ALTERNATION R.A. Bauman* & G. J. Kant Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307.

The purpose of the present study was to characterize the disruptive effects of chronic stress on the acquisition and terminal accuracy of a simple alternation task in rats. All rats lived in individual cages that were exposed to a 12:12 light/dark cycle. During the preshock period, two levers were available and any leverpress resulted in the delivery of a food pellet. Subsequently, rats in group A/E were trained to (A)void/(E)scape signaled footshocks. After learning to escape, food was only available for alternating leverpresses. Alternation was also required in groups Y and C. In group Y, signals and shocks were yoked to those delivered to rats in group A/E; rats in group C were not shocked.

Although no differences were evident among groups during acquisition, terminal accuracy (percent correct alternation) was significantly higher in group C than in the A/E or Y groups. This resulted from a significantly higher mean number of correct presses (food pellets) for Group C than for either shock group. Since mean number of incorrect presses was significantly higher for group A/E than for group C or Y, the requirement to A/E footshock may have interfered with the accuracy of alternation.

589.11

DIFFERENTIAL EFFECTS OF ACUTE STRESS EXPOSURE ON RADIAL ARM MAZE PERFORMANCE. M.J. Stillman*[1], B. Shukitt-Hale[1], A. Levy[2], H.E. Modrow, and H.R. Lieberman. Military Performance and Neuroscience Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760-5007, [1]GEO-CENTERS, INC., Newton Centre, MA 02159, and [2]IIBR, Ness Ziona, ISRAEL.

This study examined radial arm maze (RAM) performance following exposure to two stress conditions and a normothermic-freely-moving control condition. Male Fischer 344 rats were trained on the win-shift RAM procedure for 7 days by which time they achieved asymptotic performance. The next day, rats were exposed to 15 min of restraint in either 37 °C water (normothermic-restraint) or in 20 °C water (cold-restraint). Rats were removed from restraint and were allowed 40 min in a dry cage prior to being tested in the RAM. Performance was measured using the following dependent variables: time per choice, the total number of choices, the percent error, and the number of correct out of the first eight choices (number correct). ANOVA indicated significant effect of stress on number correct ($p < .001$) as well as the other variables. Performance decrements were observed in both stress conditions relative to the normothermic-freely-moving condition, with the normothermic-restrained rats displaying less impairment than the cold-restrained rats (e.g., mean ± SEM values for number correct were 7.4 (± 0.22), 4.0 (± 0.52), and 1.83 (± 0.51), respectively). These findings suggest that restraint and cold stress impair performance on a memory task, with the extent of the impairment being related to stress severity.

589.12

DIFFERENTIAL PAIN TOLERANCE RESPONSE TO HIGH DOSE NALOXONE FOLLOWING EXPOSURE TO CONTROLLABLE AND UNCONTROLLABLE STRESS J. Fertig*, R. Peters, J. Leu, G. Mueller, Walter Reed Army Institute of Research, Washington, D.C. 20307

In an attempt to characterize the pain tolerance response to naloxone administration following exposure to stress, forty healthy male subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either controllable stress (CS) or uncontrollable stress (UCS) conditions. Stress induction was followed by a double blind infusion of the opiate antagonist naloxone (1.5 mg/kg) or placebo. A muscle ischemia pain tolerance test was given following drug administration. Physiologic reactivity and biochemical response were monitored throughout stress induction, drug administration and pain tolerance testing. Although naloxone administration dramatically elevated heart rate ($p<.0001$), plasma cortisol ($p<.0001$), and immunoreactive beta-endorphin ($p<.0001$), it had a differential effect on pain tolerance depending upon subject's prior exposure to CS or UCS. Pain intensity ratings were highest for CS exposed subjects and lowest for UCS subjects. Results suggest that prior exposure to UCS or CS can mediate pain tolerance following naloxone exposure.

589.13

PERSISTENT HIGH CORTISOL RESPONSES TO REPEATED PSYCHOLOGICAL STRESS IN A SUBGROUP OF HEALTHY MALES. C. Kirschbaum, J. Prüßner, A.A. Stone, I. Federenko, J. Gaab, D. Lintz, N. Schommer and D.H. Hellhammer*. Center for Psychobiology and Psychosomatics, University of Trier, 54286 Trier, Germany.

The present study tested the hypothesis that some subjects may not readily show habituation of adrenocortical stress responses to repeated psychological stress. 20 healthy males were each exposed five times to the same, brief psychosocial stressor (public speaking and mental arithmetic in front of an audience) with one stress session per day. Salivary cortisol levels were assessed as an index of adrenocortical stress responses. For the total group, cortisol levels were significantly elevated on each of the five days. The mean response decreased from day 1 to day 2, however, no further attenuation could be observed on the remaining days. Cluster analysis revealed two groups of subjects who showed completely different response kinetics. In the first group (n=13), termed "low responders", cortisol levels were elevated on day 1 only. Days 2-5 cortisol levels were unaltered. In contrast, subject in the second group ("high responders") displayed large increases to each of the five experimental treatments. This group had no significant response decrement from day 1 to days 2-4 and only a marginal response difference between day 1 and day 5. Discriminant analysis revealed that a combination of five personality scales plus the scores on a symptoms checklist significantly discriminated between high and low responders. Using this discriminant function, all 20 subjects were correctly classified to the two groups. These results are discussed with a focus on the possible impact of adrenocortical response types on health and disease.

STRESS: NEUROTRANSMITTER STUDIES

590.1

HABITUATION OF GLUTAMATE AND LACTATE RELEASE INDUCED BY STRESSFUL STIMULI IN RAT FRONTAL CORTEX. M.Takita[1] and M.OGURI[2]*. [1]Neuroinformatics Lab., Biosignalling Dep., Natl. Ins. of Bioscience and Human-Technology. Ibaraki 305, Japan. [2]Dept.Biometrics and Faculty of Sci., Toho Univ., Chiba 274, Japan.

To clarify information processing for novel stress in rat medial frontal cortex (mpFC), we developed newly system to monitor glutamate (GLU) and lactate with a high time-resolution (less than 1 min) by using *in vivo* brain microdialysis. The dialysate was mixed directly with an enzyme solution (containing GLU- or lactate-dehydrogenase and NAD^+) in a T-tube. The fluorescent of produced NADH in the mixture was measured continually by a fluorometer equipped with a flow cell. The basal level of mpFC GLU release (GR) was about 2 μM and was a one-200th lower than that of LR. The mpFC GR was clearly demonstrated to increase immediately and transiently from its steady state levels after 1min tail pinch (TP) so that the mpFC LR was increased by various stimuli (TP, 5min 100dB white noise (100dB), and 5min immobilization (IMB)). These stimuli-induced increments were suppressed partially by the 2nd trial 1 hour later in a manner of habituation (GR; TP:-32.9 $\pm$8.3%, LR; TP:-34.5$\pm$7.1%, 100dB:-35.3$\pm$9.2%, IMB:-45.5$\pm$6.2% vs. the initial responses, all was at least $p<0.05$ by paired t-test(n=4-5)), consistent with dishabituation to a novel stimulus or "below-zero" habituation by several repetitions. GR and LR both showed the decreasing response by TP in septum. The mpFC GR response was appeared under reserpinization (0.5 mg/kg, sc.) while these of LR were completely not. These results suggested that there are two different systems for stress processing specialized in rat mpFC.

590.2

EFFECT OF REPEATED FOOTSHOCK STRESS, APOMORPHINE AND THEIR COMBINATION ON THE INCIDENCE OF APOMORPHINE (APO)-ELICITED SELF-INJURIOUS BEHAVIOR (SIB) IN JUVENILE NEONATAL 6-HYDROXYDOPAMINE (6HD)-TREATED RATS. C.J. Stodgell*, S.R. Schroeder, J.M. Hyland & R.E. Tessel. Dept. of Pharmacol. & Toxicol. and The Schifelbusch Institute for Life Span Studies. University of Kansas, Lawrence, KS. 66045-2505

Male rats received bilateral intraventricular injections of 6HD (50 ug/inj.) or vehicle (0.1% ascorbic acid in saline; SAL) on postnatal day 3. At 30 days of age, half the 6HD and SAL animals were primed with 6 IP H_2O injections, or 6 APO injections (10 mg/kg; IP; every 3-4 days) unless SIB was elicited during earlier injections. If SIB was elicited, APO was replaced with H_2O injections. Half of the APO- and H_2O-primed animals were further primed with 7 1-hr footshock sessions (6 1-mA 300-V shocks/2 sec every 10 sec; every 3-4 days); the other half remained in their home cages. One week later acute APO dose-effect curves (DEC; 0-10 mg/kg IP; 30 min interinjection interval) were constructed in each rat and the behaviors manifested recorded on video tape. The following week, the animals were sacrificed for striatal catecholamine determinations. SIB was not elicited by footshock *per se* in any animal. During the DEC, 3/10 6HD animals primed only with APO displayed SIB, and 2/10 rats primed only with footshock displayed SIB. However, 7/10 6HD rats primed with the APO-footshock combination displayed SIB, (X^2; $p<0.05$ compared to both prior groups). No DEC-associated SIB occurred in any SAL rats except for one animal that had been combination-primed. 6HD decreased striatal catecholamine and metabolite concentrations by 96% in H_2O-primed, unshocked animals. However, these depletions were modestly but significantly reversed ($p< 0.01$) in combination-primed 6HD-treated animals. These and other findings from our laboratory suggest that stress can potentiate APO-induced SIB in 6HD rats by increasing dopamine release and postsynaptic dopamine receptor stimulation, and if dopamine availability is sufficiently increased, may have the capacity to elicit SIB in such animals. (Supported by grant 1 PO1 HD26927).

590.3

LEARNED HELPLESSNESS AND IN VIVO BIOGENIC AMINE RELEASE IN HYPOTHALAMUS AND CAUDATE. F. Petty*, G.L. Kramer, P. Zukas, and S. Jordan. VAMC and Dept. Psychiat., Univ. Texas Southwestern Med. Cent., Dallas TX 75216

We have developed a theoretical neuronal map of learned helplessness (LH) in the rat involving serotonin (5-HT), dopamine (DA) and GABA in medial prefrontal cortex, GABA and norepinephrine (NE) in hippocampus, and 5-HT in septum. In hypothalamus, other investigators have reported decreased 5-HT uptake into synaptosomes, and decreased basal and K^+-stimulated release from tissue slices of LH rats, as well as decreased paroxetine and unchanged β-adrenergic receptor binding. We have now applied in vivo microdialysis to measuring DA, NE and 5-HT simultaneously in hypothalamus and caudate of conscious, freely moving rats in a LH paradigm. Rats received 100 trials of inescapable tailshock stress, and after testing for LH, microdialysis probes were implanted into either caudate or lateral hypothalamus. One day later, perfusion was maintained with Ringer's solution until a stable baseline was obtained, then switched to Ringer's with high K^+. No differences were found in caudate in DA or 5-HT among any of the three experimental groups (LH, NH -- stressed, nonhelpless, or TC -- unstressed tested control). In hypothalamus, no differences were seen for DA or 5-HT. For NE in hypothalamus, significantly higher levels of basal NE were observed for LH rats, compared to NH and TC, while the NH rats had significantly higher K^+-stimulated NE release than LH or TC. For all the stressed rats, there was a significant positive correlation between shuttlebox escape latencies and basal NE release. These data suggests that the caudate may not be involved in the in vivo biogenic amine neurochemistry of LH, and that in the lateral hypothalamus, NE mechanisms predominate in this animal model of depression.

590.4

BETA-1 ADRENOCEPTOR BLOCKADE MIMICS THE BEHAVIORAL EFFECTS OF ACUTE STRESS IN MICE. E.A. Stone*, D. Quartermain, J. S. Manavalan, and Y. Zhang, Depts. Psychiatry and Neurology, New York University School of Medicine, New York, NY 10016

Reduced brain adrenergic neurotransmission has long been implicated as a factor in inducing behavioral changes after stress. The nature of the receptor at which transmission is deficient, however, has not been elucidated. The present studies were undertaken to clarify this problem by comparing the effects of acute stress with those of beta adrenoceptor blockade on behavioral function. Male Swiss Webster mice were either subjected to one of three types of stress, immobilization (IMO), tube restraint or subordination, or were given an injection of one of several types of blocking agents and then examined for swimming behavior, locomotion and grooming. Each of the stressors was found to reduce swimming and locomotion and to increase grooming. Administration of the nonselective beta blocker, propranolol, and the beta-1 selective blocker, betaxolol, reproduced all three effects of stress. The beta-2 blocker, ICI 118,551 was only partially effective and the peripherally active beta-1 antagonist, atenolol was inactive. The 5HT1A receptor blocker, WAY 100478, and the dopaminergic blocker, fluphenazine, reduced locomotion but failed to increase grooming. The findings indicate that the effects of stress can be reproduced selectively by central beta-1 receptor blockers and suggest that neurotransmission at these brain receptors is reduced after acute stress. Supported in part by grants AFOSR F49620-92-J-0084, MH45265 and MH08618.

590.5

EFFECT OF BETA-1 ADRENOCEPTOR BLOCKADE ON c-FOS RESPONSE TO STRESS IN MOUSE BRAIN. Y. Zhang* and E.A. Stone, Dept. Psychiatry, New York University School of Medicine, New York, NY 10016

We have previously shown that beta-blocker-reversible c-fos expression can be used as a marker for postsynaptic noradrenergic activity at beta receptors in the brain. The present study utilized this method to determine in which regions of the brain beta-1 receptors are activated during stress. Male Swiss Webster mice were injected with the selective beta-1 blocker, betaxolol, 20 mg/kg or not injected and 0.5 hr later were subjected to immobilization (IMO) stress or to control conditions for 1 hr. Two hrs after the end of the stress the animals were anesthetized and perfused. Brains were processed for c-fos immunohistochemistry using a fos-specific antibody by standard procedures. Noninjected-nonstressed and betaxolol injected-nonstressed mice showed little c-fos immunoreactivity in the brain. IMO stress caused widespread c-fos expression in the forebrain and brainstem. Betaxolol treatment prior to stress significantly reduced c-fos ir in most forebrain structures but not in the brainstem. These results suggest that stress activates beta-1 receptors primarily throughout the forebrain. Supported in part by grants AFOSR F49620-92-J-0084, MH45265 and MH08618.

590.6

EFFECT OF PROPRANOLOL ON STRESS-INDUCED CHANGES IN PASSIVE AVOIDANCE AND OPEN FIELD EMEMRGENCE TESTS. J.S. Manavalan*, M. Najimi, E.A. Stone and D. Quartermain, Depts. Psychiatry & Neurology, New York University School of Medicine, New York, NY 10016

The present study examined the effect of propranolol on the ability of stress to elicit behavioral inhibition in Harlan Hsd:Nd4 mice. Mice were given propranolol prior to immobilization or tube-restraint stress and then were tested for either passive avoidance performance or time to emerge into an open field. In contrast to previous findings by others, propranolol was found to markedly potentiate stress-induced increases in latency in these tests. These unexpected results were not due to peculiarities of the behavior measured, the stressors used or the dosage of the drug administered. As propranolol has blocking actions at beta adrenergic and serotonergic receptors, the results raise the possibility that, under some conditions, the response of the noradrenergic and/or serotonergic systems to stress may have anxiolytic or anti-stress effects. Supported in part by grants AFOSR F49620-92-J-0084, MH45265 and MH08618.

590.7

ACUTE STRESS DISRUPTS DEFENSIVE BEHAVIOR IN MICE. D. Quartermain*, E.A. Stone and G. Charbeneau, Depts. Neurology and Psychiatry, New York University Sch. Medicine, New York, NY 10016

Recent experiments with rats in semi-naturalistic environments have indicated that chronic subordination stress impairs risk assessment, i.e., reduces the likelihood that the animal will engage in species typical behaviors to evaluate the location and source of possible danger. The present study was undertaken to determine if acute stress disrupts risk assessment in mice measured in terms of latency to emerge from a dark safe place into a novel, potentially dangerous environment. Groups of male Swiss Webster mice (N=12) were subjected to either immobilization, tube-restraint, unavoidable footshock or exposure to a dominant aggressive mouse. All stressors were administered to individual mice for a duration of 1 hr. Risk assessment behavior was examined 30 min following stress termination. Mice were placed in a small dark enclosed entry chamber which was attached to a large brightly lit open field which could be entered through a small door at the end of the chamber. Latency to emerge (max. 5 min) was determined. Immediately after, animals were observed in the open field for a 5 min period during which time locomotor activity was measured and rearing, grooming and defecation frequencies were recorded. Results showed: 1. Mean entry latency for all stress groups was significantly faster than that of a non-stressed control group. 2. All stressed groups showed significantly less locomotor activity and rearing and significantly more episodes of grooming than non-stressed mice during a 5 min period in the open field. These data indicate that acute stress can disrupt defensive behavior by impairing the animal's ability to respond to potentially threatening stimuli. Supported in part by grants AFOSR F49620-92-J-0084, MH45265 and MH08618.

590.8

THREAT OF ATTACK INCREASES IN VIVO DOPAMINE RELEASE IN PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS. J.W. Tidey* and K.A. Miczek. Department of Psychology, Tufts University, Medford MA 02155.

Exposure to various aversive environmental events appears to activate central and peripheral catecholamine systems. In the present study, we used microdialysis in freely-moving rats to examine whether a "social stressor", namely exposure to an aggressive male rat would specifically increase central dopamine (DA) release in mesocorticolimbic structures. Microdialysis samples from prefrontal cortex, nucleus accumbens and striatum were collected from each rat (1) while in its home cage, (2) while in the empty, soiled cage of a rat which had previously attacked and defeated it, and (3) while in the presence of an aggressive stimulus rat. Samples were compared with those from rats placed into a clean, novel cage. Each test was videotaped and the frequencies and durations of all elements of social and motor behavior were later analyzed. Increased DA release was observed in CSF samples obtained from prefrontal cortex and n. accumbens of rats placed into either a novel cage or the soiled cage of an aggressive rat; further increases were observed in samples obtained while rats were in the presence of the aggressive stimulus rat. These neurochemical responses occurred at the time when the rats displayed increased defensive postural displays, but not at times of heightened motor activity, suggesting that mesocorticolimbic DA may be involved in the initiation of biologically significant behavioral responses to social stress.

590.9

THE ROLE OF NORADRENALINE IN THE MEDIAL PREFRONTAL CORTEX IN THE ELEVATION OF HEART RATE INDUCED BY TAIL PINCH IN THE RAT. D. Funk* and J. Stewart. Center for Studies in Behavioral Neurobiology, Dept. Psychology, Concordia University, Montreal, Canada, H3G 1M8.

The noradrenaline (NA)-containing projections to the medial prefrontal cortex (MFC) are activated in response to stress. The functional role of MFC NA release in the stress-induced activation of the autonomic nervous system (ANS), however, remains undefined. This study examined the effects of microinjections of NAergic drugs into the MFC on a response mediated by ANS activation, the increase in heart rate (HR) induced by tail pinch. Male rats anesthetised with urethane (1.5 g/kg) received 2-10 s tail pinches separated by 5 minutes prior to and after infusions of 10 nmol of the beta adrenergic agonist isoproterenol (ISO) unilaterally into the right MFC. Tail pinch elevated HR, which returned to basal levels in the succeeding 5 minutes. Injection of ISO into the MFC increased baseline HR, and potentiated the increases in heart rate induced by tail pinch. The alpha adrenergic agonist phenylephrine (20 nmol) was without effect on either baseline HR or tail pinch-induced increases in HR. These results suggest that NA released in the MFC, via stimulation of beta adrenergic receptors, plays a facilitatory role in the stress-induced activation of the ANS. Studies are in progress to examine the function of the dopaminergic projection to the MFC in stress responses mediated by the ANS.

590.10

EFFECTS OF CHLORDIAZEPOXIDE ON FOOTSHOCK-INDUCED OVERFLOW OF CEREBRAL NOREPINEPHRINE. Artur H. Swiergiel*, Zhongyou Wei, Yaohui Li and Adrian J. Dunn. Department of Pharmacology, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130-3932.

Synaptic release of cerebral catecholamines is increased during stress. The effects of benzodiazepines on the stress-related release of catecholamines has been varied. We have used *in vivo* microdialysis to examine the effect of chlordiazepoxide (CDP) pretreatment on the footshock-induced release of norepinephrine (NE). Freely moving rats were implanted with microdialysis probes in the medial hypothalamus and the medial prefrontal cortex (PFM). Footshock (60 x 0.1-0.2 mA shocks over 20 min) significantly increased microdialysate concentrations of NE in the first sample collected. The subsequent two samples showed small elevations that were not statistically significant. In both the hypothalamus and the PFM dialysates, NE was significantly augmented over the prefootshock baseline. CDP administration (5 mg/kg ip) had no statistically significant effects on the basal dialysate concentrations of NE, although there was a tendency towards a reduction. CDP administered before the footshock significantly attenuated the dialysate concentrations of NE.

These results suggest that footshock increases the synaptic release of NE in the cortex and hypothalamus, and that this response is attenuated by CDP. The experimental design used cannot determine whether systemic CDP alters the input to LC noradrenergic neurons, or whether benzodiazepines exert a direct effect on the noradrenergic neurons.

Supported by grants from the Air Force of Scientific Research.

590.11

FLUOXETINE TREATMENT ATTENUATES STRESS-INDUCED NOREPINEPHRINE (NE) EFFLUX IN THE HIPPOCAMPUS M.E. Page* and E.D. Abercrombie. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

The noradrenergic locus coeruleus (LC) has long been implicated in various aspects of the regulation of behavioral state. This system is responsive to a wide variety of stimuli, especially novel or threatening cues. Norepinephrine (NE) turnover increases in LC target regions in response to stress. It has previously been shown that presentation of acute stressors increases the synthesis and release of NE in the hippocampus (Abercrombie et al., 1988). Dysfunction of the LC/NE system is thought to contribute to a number of mental health problems including depression, anxiety and panic disorder. Chronic treatment with antidepressants alleviates many of the symptoms of depression and has been shown to alter LC function. However the mechanism of the therapeutic action of these drugs is unclear. It has been demonstrated that serotonin selectively attenuates LC responses evoked by local application of glutamate or noxious sensory stimuli. The effect of acute treatment (2 days; 10 mg/kg/day) with the antidepressant fluoxetine, a selective 5-HT reuptake inhibitor, on hippocampal NE and DOPAC efflux was examined using *in vivo* microdialysis. Because there is no signifcant DA innervation of the hippocampus, DOPAC levels in this region are thought to reflect NE synthesis. Basal levels of NE and DOPAC did not differ between vehicle controls and 2-day fluoxetine-treated animals. Application of a clamp to the rat's tail for 30 min. evoked an 80% increase in extracellular NE in vehicle control animals (n=3) and a 33% increase in animals which received FLU for 2 days (n=5). Tailpinch evoked an 89% increase in extracellular DOPAC levels in vehicle animals compared to a 34% increase in FLU-treated animals. Experiments are presently underway to determine the effects of chronic (14 day) administration of FLU on stress-induced NE efflux. One function of fluoxetine may be to modulate stress-induced responses of the LC-NE system. This work supported by PHS DA08086.

590.12

DIFFERENTIAL EFFECTS OF INESCAPABLE STRESSORS ON LIMBIC ACETYLCHOLINE RELEASE. G.P. Mark*, T.J. Shors, P.V. Rada & B.G. Hoebel. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544

Results from a variety of experimental paradigms have pointed to a cholinergic involvement in the stress response. Recently, analytical techniques have become available to measure ACh release in vivo during exposure to various stressors. In these experiments, microdialysis was used to monitor ACh output every 15 min in the dorsal hippocampus (HIPP), amygdala (AMY), nucleus accumbens (NAcc) and prefrontal cortex (PFC) before, during and after 1 hr of restraint, including a 15 min session of intermittent tail shock (1/min, 1 mA, 1 sec duration) in rats. In response to the stressor, ACh release was significantly increased in the PFC (180%; $p<.01$), HIPP (170%; $p<.01$) and AMY (144%; $p<.05$) but not in the NAcc. In each site tested however, maximal release occurred in the 15 min sample following release from restraint at which point ACh levels reached significance in the NAcc (130%; $p<.05$). These data demonstrate an enhancement of cholinergic activity during application of the stressor in three ACh projection systems (HIPP, AMY, PFC).

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590.13

CHRONIC COLD STRESS ENHANCES THE RESPONSE OF LOCUS COERULEUS NEURONS TO STIMULATION OF NUCLEUS PARAGIGANTOCELLULARIS. M.J. Mana* and A.A. Grace. *Depts of Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260*

We have previously demonstrated that chronic cold stress increases the footshock-evoked response of locus coeruleus (LC) neurons in the anesthetized rat. Using *in vivo* extracellular single-unit recordings, we now demonstrate that this stress-related enhancement of evoked activity also occurs in response to direct electrical stimulation of the nucleus paragigantocellularis (nPGi), the major glutamatergic afferent to the LC. Twenty-four hours after termination of chronic cold stress (5°C for 17-22 days), LC neurons from cold-stressed rats displayed a significant elevation in basal firing rate (mean = 2.7 Hz for Cold Stress v. 1.8 Hz for Controls); these basal rates were significantly higher than we have previously reported and may be related to the implantation of the stimulating electrode into nPGi. There were no differences in the threshold for activation of LC cells by nPGi stimulation (Cold Stress = 190 μA v. Control = 155 μA; 0.3 ms duration, constant current) or in their response latencies (Cold Stress = 0.013 ms v. Control = 0.014 ms). However, the incidence of spike activation by nPGi stimulation was greater in LC cells from the cold stressed rats (Cold Stress = 40 responses v. Control = 34 responses; 50 stimulations at 150% of threshold), as was the incidence of burst firing (Cold Stress = 8 bursts/50 stimulations v. Control = 4 bursts/50 stimulations). In addition, the duration of postactivation spike suppression was shorter for LC cells from cold stressed rats (Cold Stress = 600 ms v. Control = 780 ms). We are currently investigating whether these stress-induced changes in the LC response to glutamatergic excitation from nPGi are due to changes in their sensitivity to glutamate or to a change in some other system which modulates this response.

Supported by USPHS MH 43947 and MRC Canada Postdoctoral Fellowship (MJM).

590.14

AGE-DEPENDENT CHANGES IN 5-HT_{1A} RECEPTORS FOLLOWING FORCED SWIM STRESS AND RESTRAINT STRESS. R. K. Raghupathi* and P. McGonigle Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

This study examined the effect of age on the 5-HT_{1A} receptor response to two stressors, namely, forced swim stress (15 min) and restraint stress (2 hr). Rats were studied 14, 21 and 60 days after birth (n = 6-8 per group). Coronal sections of rat brain were labeled with [^{3}H]-8-OH-DPAT (DPAT) to quantitate 5-HT_{1A} receptors. At 14 days of age, restraint stress did not affect DPAT binding in the hippocampus or cortex. At 21 days of age, neither forced swim stress nor restraint stress affected DPAT binding in the hippocampus or cortex. However, in 60 day old rats, forced swim stress produced a 20-40 % decrease in DPAT binding in the CA1, CA2, CA3 and dentate gyrus subfields of the hippocampus and in the cortex. Restraint stress also produced a significant decrease in DPAT binding, albeit less profound, in the CA2, CA3 and dentate gyrus subfields of the hippocampus, but not in the CA1 region or the cortex. In conclusion: (1) Forced swim stress and restraint stress produce a decrease in 5-HT_{1A} receptor binding in discrete regions of the adult rat brain. These data, in conjunction with the previously described increase in 5-HT_{1A} receptors following adrenalectomy, suggest that the 5-HT_{1A} receptor response to stress is mediated by adrenal steroid hormones. (2) The 5-HT_{1A} response to stress is age-dependent. Unlike 60 day old rats, 14 and 21 day old rats showed no 5-HT_{1A} receptor response to either stressor. There appears to be a period during development when the 5-HT_{1A} receptor system is non-responsive to stress. (3) The 5-HT_{1A} receptor response to stress is stressor-dependent. Forced swim stress produces a more profound and widespread decrease in 5-HT_{1A} receptors, compared to restraint stress. (Supported by USPHS MH 43821 and MH 48125)

590.15

ALTERED EXPRESSION OF mRNAs ENCODING $GABA_A$ RECEPTOR SUBUNITS α1 AND α2 FOLLOWING RESTRAINT OF JUVENILE AND ADULT RATS.

Roberts, Alice A.*, Pleger, Gloria L., Jones, Amy W. and Kellogg, Carol K. Dept. of Psychology, Univ. of Rochester, New York, 14627.

The $GABA_A$ receptor, a multimeric protein complex located on neuronal cell membranes, mediates inhibition in cortical circuits by altering GABA-gated chloride permeability. Cortical $GABA_A$ receptors of male rats functionally respond to acute stressors in young adulthood (P70-90) but not at juvenile (P28) or adolescent (P35-42) ages. Likewise, young adult males exhibit altered behavioral and $GABA_A$ responsiveness to acute stressors based on prior experience, whereas juveniles do not. We have found modest increases (30-40%) in cortical mRNAs encoding α1 and α2 $GABA_A$ subunits immediately after two-hour restraint in young adult, but not juvenile, brains. The present study tracks expression of $GABA_A$ subunit mRNAs in young adult and juvenile brains taken at 0, 2, 4, and 6 hours following two-hour restraint; levels of α1 and α2 mRNAs in cortex, hippocampus, and cerebellum, are determined by RNAse Protection Assay. Our initial results (t = 0) suggest that the impact of experience on cortical $GABA_A$ subunit expression may develop across adolescence in parallel with functional responsiveness of cortical $GABA_A$ receptors to acute stressors. Since juveniles lack experience-dependent behavioral responses to stressors, it may be that altered $GABA_A$ subunit expression underlies inhibitory synaptic plasticity critical for specific behavioral adaptation to challenging environmental stimuli.

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590.16

REGIONAL CHANGES IN DOPAMINE AND SEROTONIN ACTIVATION WITH VARIOUS INTENSITY OF PHYSICAL AND PSYCHOLOGICAL STRESS IN THE RAT BRAIN. T.Inoue*, K.Tsuchiya and T.Koyama. Dept. of Psychiatry., Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

The present study examined whether regional patterns of brain dopamine (DA) and serotonin (5-HT) activation after physical and psychological stress depend on the intensity of that stress. Monoamine concentrations (DA, 5-HT and their metabolites) were measured using high performance liquid chromatography with electrochemical detection in eight brain regions of rats exposed to two different intensities of footshock stress for 30 min (1.5 mA or 2.5 mA) or conditioned fear stress regarded as psychological stress (CFS, after single or repeated footshock). A low level of footshock selectively increased the DA metabolism in the medial prefrontal cortex (mPFC), whereas a high level of footshock increased it in most of the brain regions examined in the present study. A low level of footshock did not increase the 5-HT metabolism in any regions, but a high-intensity shock increased the 5-HT metabolism in the mPFC, nucleus accumbens, and lateral hypothalamus. The increased DA and 5-HT metabolism were especially marked in the mPFC after CFS (24 hours after receiving footshock, the animals were placed in a shock chamber without being given shock) following a single footshock session (2.5 mA). CFS following repeated footshock for 10 days, like footshock per se, increased the DA metabolism in most of the brain regions except for the striatum and increased the 5-HT metabolism in the mPFC, nucleus accumbens and amygdala. These results suggest that regional patterns of brain DA and 5-HT activation after physical and psychological stress depend on the intensity of that stress, although there are some differences between these stress.

590.17

INVOLVEMENT OF 5-HT$_{1A}$ DORSAL RAPHE AUTORECEPTORS IN THE ENHANCEMENT OF FEAR CONDITIONING AND ESCAPE DEFICITS PRODUCED BY INESCAPABLE SHOCK. S. F. Maier*, R. E. Grahn, S. A. Horwood, and K. L. Nichols. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO, 80309.

Exposure to uncontrollable aversive stressors such as inescapable tailshock (IS) lead to a variety of behavioral alterations not produced by controllable stressors. We have recently suggested that some of these effects might occur because IS leads to an intense activation of the dorsal raphe nucleus (DRN), a consequent downregulation of inhibitory somatodendritic 5-HT$_{1A}$ autoreceptors, and a resulting hyper-responsive DRN at the time of later testing. The present experiments tested these possibilities by inhibiting DRN activity either before IS exposure or before behavioral testing 24 hr later. By the above argument both should block the behavioral effects of IS. Male Sprague-Dawley rats were either exposed to 100 unsignalled 1.0 mA tailshocks or were loosely restrained for an equal time period. Testing occurred 24 hr after shock or restraint and involved fear conditioning and shuttlebox escape testing. The 5-HT$_{1A}$ agonist 8-OH-DPAT was infused into the region of the DRN (1 ug in 1 ul) either before the IS treatment or the later testing. 8-OH-DPAT completely blocked the enhancement of fear conditioning and interference with escape behavior produced by IS when administered before IS, and attenuated these effects when given before testing. These results differ from those reported for systemic administrations of 8-OH-DPAT. However, high doses of systemic 8-OH-DPAT may facilitate rather than inhibit 5-HT activity through action at post-synpaptic receptors. Therefore, different doses of 8-OH-DPAT were systemically administered before testing. A dose of 0.01 mg/kg blocked the enhanced escape deficit produced by IS, 0.10 mg/kg attenuated this effect of IS, and 1.0 mg/kg had no effect on escape performance. Conditioned fear, however, was blocked by all doses of 8-OH-DPAT. Support provided by NIH MH-50479.

590.18

THE EFFECTS OF SOCIAL STRESS ON SEROTONERGIC INDICES IN PREFRONTAL CORTEX OF ADULT MALE CYNOMOLGUS MONKEYS. MB Botchin*, JR Kaplan, SB Manuck, JJ Mann. Dept. of Comparative Med., Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27157 and Depts. of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213.

The effects of social stress and social status on monoamine concentrations in the prefrontal cortex were examined in adult male cynomolgus monkeys (*Macaca fascicularis*). Seventy-five animals were housed in five-member social groups for 28 months. Social groups either remained stable in group membership (no-stress condition) or had their group membership reorganized at monthly intervals during months 1-14 (past-stress condition) or during months 15-28 (recent-stress condition). At necropsy, cerebrospinal fluid (CSF) and brain samples were collected and stored at -70° C until analyzed. Frontal pole cortical and CSF monoamines (serotonin [5-HT], dopamine, norepinephrine) and metabolites (5-hydroxyindoleacetic acid [5-HIAA], homovanillic acid, 3-methoxy-4-hydroxyphenethyleneglycol) were assayed using high-performance liquid chromatography with electrochemical detection. Animals exposed to past social stress had significantly lower 5-HIAA ($p < 0.02$) and 5-HT ($p < 0.01$) concentrations in the prefrontal cortex compared to animals in the no-stress condition. Prefrontal cortical 5-HIAA and 5-HT concentrations in the recent stress condition were intermediate between the concentrations in the no- and past-stress conditions. These data suggest that past exposure to chronic social stress may be associated with an enduring and perhaps progressive reduction in serotonergic function in the prefrontal cortex. Such an effect may underlie the behavioral sequelae of stress such as post-traumatic stress disorder.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: PARENTAL/AGGRESSIVE

591.1

PREOPTIC AREA (POA) INFUSIONS OF MORPHINE (MOR) DISRUPT -- AND NALOXONE (NAL) RESTORES -- PARENTAL-LIKE BEHAVIOR (PB) IN JUVENILE RATS (JR). J. Wellman, D. Carr, A. Graham, H. Jones, J.L. Humm, M. Ruscio, B. Billack & C.H. Kinsley* Dept. Psych., U. Richmond, VA 23173.

As in the adult lactating female, opioids disrupt (and NAL restores), PB in JR (~25 days of age). Because the POA regulates the display of PB in lactating females, we examined its PB-role in the JR. At 21 days of age, JR were implanted with bilateral cannulae aimed at the POA using a modified Kopf stereotaxic and extrapolating from adult rat atlases (Paxinos & Watson, 1986; Sherwood & Timiras, 1970), and divided into two groups: Initiation [INIT] and maintenance [MAINT]. On day 25, group INIT received bilateral infusions of either MOR (0.50 ug), saline (0.25 ul), or MOR plus NAL (0.25 ug). 30-minutes later, they were exposed to three 1-6 day-old pups; group MAINT was exposed to pups until they displayed two consecutive days of PB, then infused. MOR disrupted PB in both INIT and MAINT, and NAL restored the behavior to control/saline levels, particularly in males. Maturation within the POA, therefore, may serve to inhibit PB after a certain age. (NSF-9250537 & Keck Foundation)

591.2

PREOPTIC AREA INVOLVEMENT IN PROLACTIN-INDUCED PARENTAL BEHAVIOR IN DOVES. J.D. Buntin* and B.A. Kelleher. Dept. of Biol. Sciences, Univ. of Wisconsin-Milwaukee, Milwaukee, WI 53201.

The role of the preoptic area (POA) in mediating PRL-induced parental responsiveness toward young was investigated in non-breeding doves with previous breeding experience. Birds received microinjections of the axon-sparing excitotoxin ibotenic acid to destroy neuronal cell bodies in the POA (n=13). Sham-lesioned controls (n=8) received POA injections of vehicle. Upon recovery, birds received subcutaneous injections of ovine PRL for seven days, followed by a 2.5 hour test with foster squabs to assess parental responsiveness. Lesioned doves displayed significantly fewer parental feeding invitations, parental regurgitation feeding bouts, and total regurgitation movements than did sham-lesioned animals. As a result, squabs given to sham-lesioned birds gained significantly more weight than squabs given to lesioned birds. A significant negative correlation was obtained between the extent of POA damage induced by ibotenic acid infusion and the amount of parental behavior displayed by lesioned animals. These deficits in parental behavior were apparently a selective effect of POA damage because another PRL-mediated behavior, hyperphagia, was unaffected. These results suggest that the POA is an important component of the neural circuitry underlying the expression of PRL-induced parental feeding behavior in non-breeding ring doves. Because elevated PRL levels are temporally associated with parental activity during the breeding cycle, these results also implicate the POA in the spontaneous display of parental feeding activity that occurs naturally at the time of hatching (supported by NIMH grant MH41447).

591.3

RAT PLACENTAL LACTOGENS: ACCESS TO THE BRAIN DURING PREGNANCY AND CENTRAL STIMULATION OF MATERNAL BEHAVIOR IN RATS. R.S. Bridges*, M.C. Robertson[1], R.P.C. Shiu[1], H.G. Friesen[1], A.M. Stuer and P.E. Mann. Dept. Comparative Med., Tufts Univ. Sch. Veterinary Med., North Grafton, MA 01536, USA and [1]Dept. Physiol., Univ. Manitoba Sch. Med., Winnipeg, Manitoba, R3E OW3, Canada.

Recent research has established a central site of action for the lactogen, prolactin, in the induction of maternal behavior in the rat (*PNAS* 87:8003, 1990). Since plasma levels of other lactogens, i.e. rat placental lactogens (rPLs), are also elevated during gestation, we conducted a series of studies to determine (1) whether rPL-I and rPL-II are present in the cerebrospinal fluid (CSF) during pregnancy, and (2) the effects of central infusions of rPL-I and rPL-II on the induction of maternal behavior. In the first study, CSF samples were collected from days 7 to 21 of gestation using a pull-pull perfusion apparatus (20 μl CSF/min). Significant mitogenic activity measured in a Nb_2 lymphoma cell bioassay was detected from days 12 to 21 of pregnancy (481-595 pg/ml; ovine prolactin standard). Activity in day 12 samples was neutralized equally with antibodies to rPL-I and rPL-II, while activity on day 21 was only reduced by antibodies to rPL-II. The possible behavioral actions of rPL-I and rPL-II were then assessed by infusing rPL-I and rPL-II directly into the medial preoptic area (MPOA) of steroid-primed, bromocriptine-treated, nulliparous rats (see *PNAS* 87:8003, 1990). Latencies of females to respond maternally to foster young were significantly reduced in rPL-I and rPL-II treated rats, e.g. rPL-I animals had response latencies of 2 days compared with 6 days for controls. Thus, rPLs gain access to the CSF during pregnancy and are able to stimulate maternal behavior when infused directly into the MPOA. Supported by PHS Grant HD19789 [RSB].

591.4

THE ROLE OF THE PARAVENTRICULAR NUCLEUS IN THE CONTROL OF MATERNAL BEHAVIOUR IN SHEEP. A.Da Costa*, R.Guevara-Guzman*, S.Ohkura, J.Goode and K.M.Kendrick Dept Neurobiology, The Babraham Institute, Cambridge CB2 4AT, UK; *Dept. Physiology,UNAM, Mexico 04510

Maternal behaviour (MB) in sheep has been shown to be induced by intracerebroventricular (ICV) administration of oxytocin (OT) in non-pregnant steroid primed ewes. In these studies, *in vivo* microdialysis and retrodialysis on free moving animals were used to investigate whether OT release in the paraventricular nucleus of the hypothalamus PVN might be responsible for the induction of MB. *In vivo* sampling showed that OT concentration in the region of PVN at birth ($P<0.05$). Retrodialysis administration of OT in the PVN for 1h induced OT release in the blood. OT 1μM induced MB in 6/8 ewes and OT 10μM 5/8 ewes. The ring structure of OT, tocinoic acid (TOC) induced MB in 5/6 ewes while OT intracerebrally (ICV) and artificial stimulation of the vagina and cervix (VCS) induced MB in 6/8 and 8/9 ewes, respectively. Vasopressin (AVP) 1μM did not induce MB whereas AVP 10μM induced MB in 2/8 ewes. The release of NA increased after the administration of OT 1μM and 10μM ($P<0.05$), TOC 10μM, VCS and OT ICV. AVP 1μM produced a short term increase in the concentration of NA. The release of dopamine (DA) significantly increased after the administration of OT 10μM ($P<0.01$), TOC ($P<0.05$) and AVP 1μM. OT and VCS produced increases in DA but only VCS achieved significance ($P<0.05$). The release of aspartate (ASP) decreased after OT 1μM and 10 μM ($P<0.05$), whilst the behavioural tests increased it. The release of glutamate (GLU) decreased after the administration of OT 1μM ($P<0.01$), followed by an increase during the behavioural test. TOC, OT ICV and VCS did not produce significant changes in GLU in these experiments. GLU also decreased after AVP 1μM and 10μM. The release of GABA was unaltered after the administration of 1μM of OT, but significantly increased during the first behavioural test ($P<0.05$). The release of GABA increased immediately following the administration of AVP and OT ICV. Conversely, neither the administration of TOC nor the VCS produces any effect in the release of GABA in the PVN. These studies provide evidence for a positive action of OT in on its own release, peripherally and centrally, with the consequent induction of MB. This potentiation might be mediated through the neurochemical changes in the monamines and/or aminoacids.

This work was financed by JNICT, Portugal and MAFF, UK.

591.5

EXCITOTOXIC LESIONS OF THE VENTRAL BED NUCLEUS OF THE STRIA TERMINALIS DISRUPT MATERNAL BEHAVIOR IN RATS. M. Numan* and M.J. Numan. Dept. Psychology, Boston College, Chestnut Hill, MA 02167.

Lesions of the medial preoptic area (MPOA) severely disrupt maternal behavior in rats, but these lesions also usually damage the ventral part of the bed nucleus of the stria terminalis (VBNST). Other work has shown that Fos expression increases in both the MPOA and VBNST of maternal female rats. Finally, anatomical work has suggested that it may be VBNST efferents to the brainstem, rather than MPOA efferents, which are important for maternal behavior.

The present work directly tests the importance of the VBNST. Fully maternal postpartum rats received one of the following: bilateral injections (3 ug/.1 ul) of the excitotoxin, N-methyl-D-aspartic acid (NMDA) into either the VBNST or the dorsal BNST, or bilateral injections of the inactive stereoisomer, N-methyl-L-aspartic acid, into the VBNST. Only animals that received NMDA injections into the VBNST showed severe deficits in maternal behavior.

These results suggest that VBNST efferents are important for maternal behavior. Additional work is iontophoretically applying PHA-L to the VBNST in order to examine its major projection routes and termination sites.

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591.6

CHOLECYSTOKININ (CCK) AND THE REGULATION OF MATERNAL BEHAVIOR. P.E. Mann*, L.L. Felicio, J.D. Sturgis, A.M. Stuer and R.S. Bridges. Comp. Med., Tufts Univ. Sch. Vet. Med., N. Grafton, MA 01536.

Recent work in our laboratory has established that concurrent intracerebroventricular (icv) infusions of CCK-8 with β-endorphin prevents the disruptive effects of β-endorphin on the maintenance of maternal behavior in lactating rats (Felicio et al., 1991). CCK has also been reported to stimulate the onset of maternal behavior in virgin rats; Linden et al. (1989) found that chronic pretreatment with CCK-8S (i.p.) for 12 h stimulated maternal behavior within 2 hours of exposure to pups. The goal of the present series of experiments was to further examine the role of CCK in the induction of maternal behavior and characterize CCK's involvement in ongoing maternal behavior. In a set of studies evaluating the role of CCK in the onset of maternal behavior, we were unable to stimulate maternal behavior in virgin Sprague-Dawley rats treated chronically with CCK-8 (i.p. infusions) or in steroid-primed, ovariectomized, virgin rats given icv infusions of CCK-8. Moreover, chronic infusions of CCK-8 into the lateral ventricle of primigravid rats late in pregnancy also failed to stimulate maternal behavior. In contrast to the apparent lack of involvement of CCK in the onset of maternal behavior, ongoing maternal behavior appears to be regulated in part by CCK. When lactating rats were infused icv on day 5 postpartum with either 25 or 125 μg of proglumide, a nonspecific CCK antagonist, lactating rats displayed longer latencies to first crouch over their young, an effect similar to the inhibitory response found after central infusions of β-endorphin (Mann et al., 1991,1992). Together, these data suggest to us that CCK-8 is more strongly involved in the regulation of ongoing maternal behavior than in the induction of maternal behavior in the rat. Further studies are needed to determine sites and modes of CCK action. Supported by PHS Grants DA04291 and HD19789 [RSB].

591.7

THE DISTRIBUTION OF PROLACTIN BINDING SITES IN THE BRAIN OF BROODING SALAMANDERS. L.P. Mangurian*, K.J. MacArthur, R.L. Smetana and D.C. Forester. Department of Biological Sciences, Towson State University, Towson, MD 21204.

Prolactin (PRL) influences reproductive behaviors and has been shown to stimulate maternal behavior in rats and doves. The reproductive behavior of amphibians, such as the water drive prior to oviposition, is also influenced by PRL. *Desmognathus fuscus* females exhibit egg brooding behavior. This study was designed to investigate the possibility that prolactin may influence brooding behavior by examining their brain for potential prolactin sensitive areas. The localization of PRL target areas was carried out using in vitro autoradiography. Frozen brain sections were incubated with ^{125}I ovine PRL alone (total binding) or with a 500 fold excess unlabeled oPRL (non-specific binding). Specificity was assessed using excess unlabeled ovine luteinizing hormone (oLH). A strong autoradiographic reaction was observed in the choroid plexus, the anterior hypothalamus and other areas of the brain. Excess PRL caused a significant reduction ($p < .001$) in the binding of ^{125}I-PRL while oLH had no effect. These results support a possible role for PRL in regulating some aspect of behavior in brooding *D. fuscus* females.

Supported by TSU Faculty Research Grant (LPM) and TSU Student Research Grants (KJM and RLS).

591.8

FOS IMMUNOREACTIVITY IN THE HYPOTHALAMUS ASSOCIATED WITH PATERNAL BEHAVIOR IN THE PRAIRIE VOLE, *M. OCHROGASTER*. B. Kirkpatrick*, D. Litman, J.W. Kim. Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, 21228.

A number of studies have implicated the paraventricular nucleus of the hypothalamus (PVN) in the onset of maternal behavior in rats. However, we found no increase in Fos immunoreactivity associated with male or female parental behavior in the PVN in the prairie vole, a species in which both males and females spontaneously exhibit parental behavior prior to sexual maturity.

To clarify the role of the hypothalamus in paternal behavior, we examined cellular activation in areas of the hypothalamus other than the PVN, following exposure to a pup in male prairie voles. Fos immunoreactivity was quantified in suprachiasmatic, ventromedial, dorsomedial, and premammillary nuclei, and the anterior and posterior hypothalamic areas. Compared to controls exposed to a novel olfactory stimulus, males exposed to pups showed an increase in several discrete areas. These results suggest hypothalamic areas other than the PVN may be involved in the control of paternal behavior in this species.

591.9

QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF D1 AND D2 DOPAMINE RECEPTORS IN RAT BRAIN ACROSS PREGNANCY. J.C. Bakowska[1], Ian Creese[2], J.I. Morrell[2]*. [1]Institute of Animal Behavior, [2]Cnt Mol. & Behav. Neuroscience, Rutgers University, Newark NJ 07102.

Previous work has suggested that dopamine may be a neurotransmitter involved in some aspects of maternal behavior in rats (Phys & Behav.48:211). In addition, treatment of females with pharmacological doses of estrogen, a hormone important in late pregnancy, increased the level of D2 dopamine binding in the striatum (Brain Res.442:349, 1988). We hypothesized that the naturally occurring physiological and endocrine events of pregnancy may alter the level of the D1 and D2 dopamine receptors in a regional specific manner. Using quantitative autoradiography (D1 receptors: 1.0 nM 3H-SCH-23390; D2 receptors: 1.0 nM 3H-spiperone), brains from pregnant females (day 2 and 21), non-pregnant females, and males were examined. As predicted from previous work, even in pregnant females, in most brain regions D1 receptors were present in greater density than D2 receptors. The highest levels of D1 and D2 binding were in striatum, nucleus accumbens, and olfactory tubercle. Moderate to low levels of D1 binding were found in zona incerta, thalamus and hypothalamus, and low levels of D2 binding were found in olfactory bulb and hypothalamus. In some brain regions, pregnancy did not alter the D1 or D2 receptor levels; in other brain regions receptor binding was significantly different in females on day 2 compared to day 21 of pregnancy. For example, d21 females had substantially lower levels of D1 receptors in lateral and medial striatum than 2 day pregnant females. Preliminary results also show that late pregnant females had modestly lower levels of D2 receptors in anterior striatum, nucleus accumbens and olfactory tubercle. These results may provide clues to the brain regions that are most important for dopamine mediation of maternal behavior in rats. Supported by HD 22983 to JIM.

591.10

EFFECTS OF FOOD RESTRICTION ON MONOAMINE CONCENTRATIONS IN HYPOTHALAMIC NUCLEI OF THE CHICKEN BRAIN. L. Moons, Y. Qu and F. Vandesande*. Lab. of Neuroendocrinology, Zoological Institute, Naamsestraat 59, 3000 Leuven, Belgium, Europe.

Intensive selection for increased muscle growth in meat-type chickens has led to a decrease in the reproductive performances. Early quantitative food restriction has become the standard industrial procedure to improve the reproductive efficiency of the meat-type parent stocks. The neural control of both the feeding and the reproductive behavior seems to happen through identical neurotransmitters, catecholamines and serotonin. In order to determine whether food restriction alters in some way the concentration of monoamines in hypothalamic nuclei -assumed to be involved in the control of reproduction-, *ad libitum* fed and food restricted female meat-type chickens were killed at the ages of 4, 10, 16 and 22 weeks. A quantitative determination of the concentration of biogenic amines was performed in ten different micropunched hypothalamic brain areas. The amines L-DOPA, dopamine, norepinephrine (NE), epinephrine and serotonin and their major metabolites were separated on a C-18 HPLC column and determined by electrochemical detection. In most hypothalamic areas studied and for most neurotransmitters determined, no concentration difference, resulting from food restriction, was found. However, food restriction did affect the amount of certain biogenic amines in a few hypothalamic nuclei. The most striking difference occurs in the median eminance, where the concentration of NE is largely increased in food restricted animals. These quantitative data provide an excellent basis for further research on the possible role of catecholamines and indolamines in the altered feeding and reproductive behavior in the meat-type chicken.

592.1

NPY, SS, AND GAD MOLECULAR LAYER SPROUTING IN HUMAN EPILEPTIC HIPPOCAMPI. G.W. Mathern, T.L. Babb, J.L. Leite, J.K. Pretorius, and J. Engel, Jr.*, UCLA School of Medicine, Los Angeles, CA 90024-1769.

Patients with Hippocampal Sclerosis (HS; n=18), Mass Lesions (Mass; n=9), or autopsies were compared for differences in; 1) percentage of hilar neurons immunoreactive (IR) for NPY, somatostatin (SS), and GAD, 2) patterns of fascia dentata (FD) IR-sprouting, and 3) densities of FD IR-sprouting. Results were: 1) NPY-IR hilar neurons decreased in an equal proportion to all hilar neurons (p=0.47), SS-IR neurons were significantly decreased in HS in excess of all hilar neurons (p=0.03), and GAD-IR neurons were not significantly different (p=0.26). 2) Outer molecular NPY-IR decreased in HS compared to Mass (p=0.0002), was unchanged in SS-IR (p=0.37), and was increased in the inner molecular layer with GAD-IR (p=0.007). 3) The patterns of IR-sprouting between HS and Mass were significantly different (p=0.001). This supports the notion that peptide hilar counts and molecular layer synaptic reorganization differ among peptides and between patients with HS and Mass pathogenic mechanisms. Supported by NS 02808 and KO8-NS 01603.

592.2

THREE HUMAN CASES OF STATUS-EPILEPTICUS-INDUCED BRAIN DAMAGE. D.G. Fujikawa* and H.H. Itabashi. Neurology Service, VA Med. Ctr., Sepulveda, CA and Depts. of Neurology and Pathology, UCLA Sch. of Med., Los Angeles, CA U.S.A.

Although status epilepticus (SE)-induced brain damage is well-documented in animal models, human cases of neuronal damage caused strictly by SE have been difficult to document because of confounding systemic factors or a history of pre-existing epilepsy. It has been hypothesized that excessive release of the neurotransmitter glutamate is responsible for the SE-induced neuronal necrosis. We report 3 patients who died 11-27 d after the onset of focal motor SE, in whom hypotension, hypoxemia and hypoglycemia do not complicate the interpretation of results. Two of the 3 patients had no prior seizures and no known cause of their SE. The third patient, who had leptomeningeal carcinomatosis, had 1 seizure 2 months before the onset of SE. The duration of SE was 2.5 h-3 d. EEGs showed unilateral temporal lobe sharp-wave discharges in 1 and independent temporal lobe sharp-wave discharges bilaterally in the other 2. There was widespread neuronal loss in the hippocampus (CA1-3 and hilus), amygdala, piriform cortex, dorsomedial thalamic nucleus and cerebellum (Purkinje cells), with scattered neuronal loss in the cerebral cortex. In 2 of the 3 cases the damage was unilateral and occurred on the side in which seizure discharges were most prominent. The regional distribution of the damage in these 3 cases was similar to that found in animal models of limbic SE: it occurred primarily in limbic structures with high densities of glutamate receptors. (Supported by the Dept. of Veterans Affairs.)

592.3

RELATIONSHIP BETWEEN INTERICTAL EEG SPIKE AND NEURONAL SYNCHRONY AND FUNCTIONAL LIMBIC PATHWAYS. A.L. Velasco, C.L. Wilson*, B.W. Colder, M.H. Shomer, E.J. Behnke, S.U. Khan, and J. Engel, Jr. Depts. of Neurology, Anatomy, Neurosurgery, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

In order to investigate the prevalence of neuronal synchronization in specific pathways of the human hippocampal formation and amygdala, EEG spikes, single and multiple cell action potentials were recorded in patients with surgically implanted intracranial depth electrodes required for diagnostic studies. MRI guidance was used to place electrodes bilaterally in amygdala, anterior and middle hippocampus, entorhinal cortex, and parahippocampal gyrus. Bundles of 9 microwires were used for extracellular unit recording. In one group of patients interictal EEG spikes were monitored continuously for 6 to 30 hr and bipolar spike detection records were obtained at each recording site using EEG spike detection software. Spike frequency distributions (spikes/min) were plotted over the entire period and crosscorrelations between recording sites were computed. Although correlations varied widely, a direct relationship was found between spike rate and the probability of a significant positive correlation between specific anatomical pathways, including the perforant, longitudinal associational, and retrohippocampal pathways. In a second group of patients, microelectrode recordings 5 to 30 min in duration were obtained from a total of 295 cells and subjected to crosscorrelation analysis between 2 to 8 simultaneously recorded cells. Of 93 possible within-bundle unit correlations, 49% were significant, and of 516 possible between-bundle correlations dependent upon anatomical connections, 25% were significant. Both showed a greater proportion of significant correlations in the temporal lobe where seizures originated. Supported by NS 02808.

592.4

BURST DISCHARGE CONTRIBUTION TO NEURONAL SYNCHRONY IN EPILEPTIC HUMAN TEMPORAL LOBES. B.W. COLDER*, C.L. WILSON, R.C. FRYSINGER, L. CHAO, R.M. HARPER AND J. ENGEL. Dept. of Anatomy and Cell Biology, Dept. of Neurology, and Brain Res. Inst., UCLA School of Med., Los Angeles CA 90024.

Synchronized neuronal burst discharge is hypothesized to initiate ictal events. If so, inter-ictal neuronal synchrony in epileptogenic structures may include a higher percentage of burst action potentials (burst AP's). We therefore wished to examine the relative contributions of isolated and burst AP's to synchronous interactions both ipsilateral and contralateral to seizure onset in patients with complex partial seizures. Spontaneous inter-ictal single cell activity was recorded from the hippocampus, amygdala, and other temporal lobe structures of patients undergoing chronic depth electrode implantation to localize seizure onset. When cross-correlation histograms between pairs of simultaneously recorded cells contained a significant central peak, implying a synchronous interaction between those cells (n=70 ipsilateral, n=93 contralateral), the proportion of burst AP's contributing to that peak was calculated for each cell. Bursts AP's showed a strong trend towards a proportionally greater contribution to synchronous interactions contralateral to the side of seizure onset. The correlation between the likelihood of a neuron to discharge bursts and the contribution of burst AP's to synchronous interactions involving that cell was stronger in the contralateral hemisphere (r =.72) than the ipsilateral (r =.27). Our results suggest that in the inter-ictal state, regions that commonly initiate seizures are less likely to contain synchronously bursting neurons. Supported by NS 02808.

592.5

NETWORK PROPERTIES OF CARDIAC-RELATED SINGLE CELL DISCHARGE IN EPILEPTOGENIC HUMAN TEMPORAL LOBE. R.C. Frysinger*, B. Colder, C. Wilson, J. Engel and R.M. Harper. Depts. of Anatomy and Cell Biology, Neurology, and the Brain Research Institute, UCLA Sch. of Med., Los Angeles, CA 90024.

Modulation of neuronal discharge by the cardiac cycle is relatively common in temporal lobe structures. The proportion of cardiac-related cells in amygdala is markedly higher ipsilateral to seizure onset, suggesting an interaction of these cells with epileptogenesis. We examined 314 spike trains from 19 patients with epilepsy undergoing depth recordings of mesial temporal EEG. Bilateral recordings were performed in amygdala, hippocampus, anterior and posterior perihippocampal cortex, and transitional cortex. From 3 to 20 spike trains were recorded simultaneously from each patient, permitting direct evaluation of the nature and number of cell interactions. Spike trains were characterized on the basis of cross- and auto-correlograms, with inhibitory and excitatory phenomena recorded as the proportion of possible interactions. Cardiac-modulated cells were more likely to participate in networks, and were strongly associated with inhibitory interactions. In amygdala, this relationship was strikingly dependent on the side of seizure onset. Cardiac-related amygdala cells on the contralateral side participated in inhibitory interactions, and were likely to "drive" cells in other structures. On the ipsilateral side, cardiac dependency was associated only with a bursting pattern of discharge. This difference may result from a loss of regional inhibitory connections to cardiac-dependent cells. Supported by NS 02808.

592.6

***IN-VIVO* PAIRED-PULSE STUDY OF INHIBITION IN THE HUMAN HIPPOCAMPUS.** K.P. Vives, D.D. Spencer, and G. McCarthy.* Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510 and Neuropsychology Lab., VA Medical Center, West Haven, CT 06516.

Paired-pulse stimulation of the perforant pathway has been used in animal models of epilepsy to examine the status of inhibitory processes in the dentate granule cells; relative facilitation of the granule cell population spike has been observed following experimental treatments which destroy inhibitory interneurons in the dentate hilus[1]. We report the intraoperative use of the paired-pulse paradigm in eighteen patients undergoing anteromedial temporal resection for medically intractable seizures. During the resective surgery, after removal of the lateral neocortex, multicontact depth electrodes with 1 mm contact spacing were placed freehand through the dentate gyrus and into the perforant path. Four contact strip electrodes were also placed on the hippocampal alvear surface and on the entorhinal cortex. Stimulation to the perforant path was performed utilizing single pulses and paired pulses with interpulse intervals (IPI's) of 20, 40, 60 100 and 200 msec. In the initial 10 patients, PSPs were recorded without population spikes. Technical improvements have allowed us to record clear population spikes in six of the last 8 patients. Inhibition was quantified in each patient by taking the ratio of the PSP or spike amplitude elicted at each IPI to that measured after a single pulse. In an on-going study, these measures are compared to pathological studies of the excised hippocampus, other *in-vivo* and *in-vitro* electrophysiological studies, and patient outcome. (Supported in part by an American Heart Assoc. Fellowship and NIH Grant NS30619)

[1]Sloviter RS, Hippocampus, 1:41-66, 1991.

592.7

DISTRIBUTION OF CALCIUM-BINDING PROTEINS PARVALBUMIN, CALBINDIN D-28K, CALRETININ, AND PERINEURONAL NETS IN THE HUMAN EPILEPTIC HIPPOCAMPUS. C. Eickhoff[1], I. Blümcke[1], M.R. Celio[2], O.D. Wiestler[1]*.[1] Dept. of Neuropathology, University of Bonn Medical Center, 53105 Bonn, Germany. [2] Dept. of Histology and General Embryology, University of Fribourg, 1705 Fribourg, Switzerland.

Neuronal vulnerability during epileptic seizures has been associated with an increase in intracellular free calcium concentration. Hippocampal neurons which contain the calcium-binding proteins (CaBPs) parvalbumin (PV), calbindin D-28k (CB) and calretinin (CR) are, therefore, interesting candidates for studies on the morphological and neurochemical alterations in human temporal lobe epilepsy (TLE). PV-immunoreactive (PV-ir) neurons are also covered with different extracellular matrix molecules forming the perineuronal nets (PN's) and are surrounded by astroglial processes. PN's may protect these neurons from an excess release of excitatory neurotransmitters during seizure activity. We studied the distribution of PV-ir, CB-ir, and CR-ir neurons in hippocampus (HC) specimens obtained from patients with TLE. Autopsy brains were used as controls. The immunohistochemical staining patterns were correlated with the following histopathological changes: (1) HC without histological alterations; (2) HC with focal lesions or tumors; (3) HC with Ammonshorn sclerosis (AS); (4) HC with both, focal lesions/tumors and AS. In general, the neuronal loss observed in TLE-patients was associated with a decrease in CaBP-ir neurons. However, among the surviving neurons, many *PV-ir* and *CB-ir* cells were covered with PN's. In some patients, *CR-ir* showed an increase in neuropil staining in the dentate gyrus and CA 3 region, as well as "de-novo" granule cell staining. None of the CR-ir cells were covered with PN's. We conclude that neurons expressing PV and CB, which were surrounded by PN's, were less vulnerable to degeneration in TLE. In addition, CR may serve as an interesting marker for activated and reorganizing neuronal systems in human TLE.
Supported by the German BMFT Helmholtz-fellowship for I.B.

592.8

GABA-A ALPHA AND BETA RECEPTORS AND GAD-IR AXON SPROUTING IN HUMAN HIPPOCAMPAL EPILEPSY. T.L. Babb*, G.W. Mathern, J.P. Leite, and J.K. Pretorius. UCLA School of Medicine, Los Angeles, CA 90024-1769.

Resected human hippocampi (HC) with Hippocampal Sclerosis (HS; n=6) or little HC cell loss (e.g. lateral mass lesions; n=2) were compared for GABAergic (GAD-ICC) fascia dentata (FD) molecular layer (ML) axon sprouting and "matching" changes in postsynaptic GABA-A receptor subtypes alpha and beta. GABA axon densities in the FD-ML were weakly correlated with cell losses in the HC (p=0.07) and FD (p=0.07; i.e. reactive synaptogenesis). The densities of FD-ML receptors for GABA-A alpha (p=0.28) and beta (p=0.85) did not correlate with neuron loss (i.e. HS). There was a trend for the GABA receptors subtypes to have similar densities in the FD-ML (p=0.08) suggesting that both the alpha and beta types were similarly affected in epileptic FD. Finally, the GABA axon patterns in the FD-ML scored semi-quantitatively, showed varying densities and distributions across the inner through outer molecular layers, and these did not predict GABA-A alpha (p=0.15) or beta (p=0.70) densities, suggesting that in epilepsy there may be heterogeneous remodeling of receptor subtypes that do not "match" to aberrant presynaptic terminals. Supported by NS 02808, NIH-FIC, CNPq (Brazil) and K08-NS 01603.

592.9

MORPHOLOGY OF GRANULAR NEURONS IN THE HUMAN HIPPOCAMPUS. G. von Campe, P. Rai*, D. D. Spencer, N. C. de Lanerolle. Yale University School of Medicine, Section of Neurosurgery, New Haven, CT 06510.

Examination of hippocampi removed from patients with medically intractable temporal lobe epilepsy (TLE) has shown considerable neuronal reorganisation within the seizure focus. Granule cells and their mossy fiber axons contain the opioid peptide dynorphin, which in normal hippocampi is not expressed in the molecular layer. In patients with mesial temporal sclerosis, a dense band of staining is observed in the inner molecular layer, suggesting collateral sprouts from granule cells into the molecular layer. To directly examine the presence of such collaterals from granule cells we studied the morphology of individual cells by using an intracellular injection technique. Paraformaldehyde (4%) fixed hippocampi were sliced into 80-100 µm thick sections on a Vibratome. Individual neurons were iontophoretically injected (at 2 nA for 5-15 minutes) with Lucifer Yellow (3-5% aqueous solution) under UV epiillumination. Selected filled cells were then photoconverted by UV irradiation in the presence of diaminobenzidine (DAB) to produce a permanent Golgi-like appearance. In patients with mesial temporal sclerosis at least two morphological types of granule cells were found. One type was spindle shaped with a single branched apical dendrite and a single axon-like process extending from the opposite end toward the hilus. The other type of granule cell has a more rounded cell body with two to several apical dendrites and a single basal axon. In several neurons, fiber-like processes were observed to arise from the basal to lateral region of the cell body, and after a short course toward the hilus, they curved back up toward the molecular layer. Such processes may be the morphological basis of the dynorphin immunoreactivity observed in the inner molecular layer of hippocampi from patients with temporal lobe epilepsy. Supported by NS06208.

592.10

ANALYSIS OF HUMAN EPILEPSY-ASSOCIATED GENE EXPRESSION BY MESSENGER RNA DIFFERENTIAL DISPLAY. H. Xie, M. L. Brines, J. H. Kim*, N. C de Lanerolle, Sections of Neurosurgery, Neuropathology, and Neuroendocrine Program, Yale University School of Medicine, New Haven, CT 06510

Human diseases (including temporal lobe epilepsy; TLE) are usually associated with alterations in patterns of cellular gene expression. These changes in gene regulation may be the consequence or the cause of the underlying diseases. Detailed study of the alterations in patterns of gene expression could provide insight into the etiology of the disease. The objective of this study is to identify genes which are differentially expressed in one subgroup of epilepsy characterized by typical mesial temporal sclerosis and associated hippocampal reorganization (MTLE), compared to another subgroup characterized by an extrahippocampal temporal or neocortical mass lesions (MaTLE group). We used mRNA differential display methodology, which involves the reverse transcription of mRNA into cDNA and the use of PCR with random primers to amplify the cDNA signals. We applied the differential display methodology to two pairs of hippocampal specimens obtained from patients operated to control TLE. More than eighty different primer pairs were used for the PCR differential display, which statistically should cover almost all mRNA species. Fifty two uniquely displayed bands were identified and the corresponding DNA fragment extracted from the dried sequencing gels. Five of the 52 bands have been cloned and sequenced to date. Four of them have no matches in the database Genebank, which indicates that those sequences may represent novel mRNA species yet to be characterized. The fifth cDNA fragment is 320 bases long, with a 70% homology (in a 150 bp overlap region) to the invertebrate transposase gene mariner. Our preliminary PCR, Northern, and Southern blot data indicate that this 320-base sequence is represented in the normal human genome. However, the level of expression in normal humans is reduced compared to epilepsy patients. Further characterization of this gene is in progress. We conclude that mRNA differential display is highly efficient in identification of disease specific genes and in the analysis of gene expression alterations in human epilepsy brain tissue. Supported by NS 27081.

592.11

Distribution of AMPA Receptor Subunits in the Hippocampal Formation of Temporal Lobe Epilepsy Patients. E. Lynd-Balta*, W.H. Pilcher, and S.A. Joseph. Division of Neurosurgery, University of Rochester Medical Center, Rochester, N.Y., 14642.

Glutamate is the principal neurotransmitter identified within the intrinsic circuitry of the hippocampal formation. It is hypothesized that glutamate plays a central role in excitotoxic injury associated with temporal lobe epilepsy. In this report we have addressed the organization of glutamate receptor subunits within this hippocampal circuitry by immunocytochemical localization of the AMPA selective subunits GluR1 and GluR2/3 in relation to patterns of cell loss in patients with temporal lobe epilepsy.

Hippocampal tissue was resected during surgery from patients with medically intractable temporal lobe epilepsy. Blocks of tissue were placed in 4% paraformaldehyde with 0.2% picric acid. Serial sections were cut either on the vibratome or freezing microtome and stored in a cryoprotectant solution. Tissue was processed immunocytochemically using antibodies generated against the GluR1 subunit and the GluR2/3 subunits.

The localization of these subunits is distinct in the hippocampus. The GluR1 subunit is restricted to fibers of the molecular layer of the dentate gyrus. In contrast, the GluR2/3 subunits' distribution is widespread. Surviving pyramidal cells in all CA subfields stain positively for GluR2/3. In addition, immunoreactive neurons are also present throughout the granule cell layer of the dentate gyrus. Knowledge of the distribution of selective glutamate receptor subunits can elucidate the anatomical substrates by which excitotoxic injury may proceed in temporal lobe epilepsy. Supported by NIH grant NS21323.

592.12

HIPPOCAMPAL NMDA RECEPTOR NR1 SUBUNIT EXPRESSION IN EPILEPTIC HUMAN HIPPOCAMPUS.
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NMDA receptors, subtype of glutamate receptors, have been shown to play an important role in the genesis of seizures. Their expression is associated with increased neuronal excitability in temporal lobe epilepsy (TLE). We examined the changes in the prevalence of the NR1 subunit mRNA of the NMDA receptor in frozen-thawed sections obtained from temporal lobe resections in patients with intractable TLE (n=12). In situ hybridizations using a labeled oligonucleotide recognizing the NR1 subunit mRNA of the NMDA receptor were used to generate autoradiograms. Optical density measurements were performed in different hippocampal subfields. Our results show significant increase in NR1 subunit expression in dentate gyrus (DG), CA1, and CA3 areas of the hippocampal formations from patients suffering from intractable TLE as compared to control hippocampal tissue obtained at autopsy. The increased expression of NMDA receptor subunit mRNA in epileptic hippocampi is in apparent contrast with a previous report showing a decrease in total NMDA receptor/channel binding. This may represent a preferential expression of certain subtypes of NMDA receptors that may explain the hyperexcitability and participate in the genesis of seizures in patients with TLE.
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592.13

IMMUNOHISTOCHEMICAL DISTRIBUTION OF NMDA- RECEPTOR (R1), AMPA- RECEPTOR (R2), AND KAINATE (R5/6/7)-RECEPTOR SUBUNITS IN HUMAN EPILEPTIC HIPPOCAMPUS. I. Blümcke*, C. Eickhoff, H.K. Wolf, O.D. Wiestler. Dept. of Neuropathology, University of Bonn Medical Center, Sigmund-Freud Str. 25, 53105 Bonn, Germany.

Glutamate - receptors and receptor - mediated excitoxicity have recently been associated with the pathogenesis of human temproal lobe epilepsy (TLE). Using monoclonal antibodies, which selectively recognize the *N-methyl-D-aspartate (NMDA) R1* -receptor, the *α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) R2*-receptor and the *kainate R5/6/7*-receptor subunits (kindly provided by J.H. Morrison), we have examined the immunohistochemical distribution of these glutamate receptor isoforms in the hippocampal formation (HC) of patients with intractable TLE. Normal human HC-specimens obtained at autopsy were used as controls. Histopathologically, the specimens were classified into 4 categories: (1) HC without histological alterations (7%); (2) HC with focal lesions, e.g. ganglioglioma, low-grade glioma, dysembryoplastic neuroepithelial tumors, hamartoma or vascular malformations (21%); (3) HC with Ammonshorn sclerosis (AS; 54%); (4) HC with both, focal lesions and AS (18%). The analysis of the regional and cellular distribution of NMDA R1, AMPA GluR2, and kainate GluR5/6/7 immunoreactivity showed that the vast majority of neuronal cellbodies and dendrites of the granule and pyramidal cell layers exhibited immunoreactivity for all three proteins. We found no evidence for compensatory upregulation or selective loss of receptor expression in the HC of patients with chronic TLE. A decrease in or loss of immunoreactivity was closely correlated with the overall neuronal loss. So far, there was only one patient with a subtle increase in NMDA R1 staining in the polymorph layer of the dentate gyrus and in the stratum lucidum of CA3. However, this data may indicate that the glutamate receptor subunits are not directly involved in the pathogenesis of human TLE.

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592.14

A HSV-1 VECTOR EXPRESSING GLUR6 RESULTS IN EPILEPTIFORM DISCHARGES BY EITHER NMDA-DEPENDENT OR NMDA-INDEPENDENT MECHANISMS. P.J. Bergold*, P. Casaccia-Bonnefil, H. Shen, Z.-X. Liu, A. Stelzer, and H.J. Federoff^. Department of Pharmacology, SUNY- Health Sciences Center at Brooklyn, NY, NY 11203; ^Department of Medicine, Albert Einstein Sch. of Medicine, NY, NY, 10461

Epileptiform discharges *in vitro* or limbic seizures *in vivo* are readily induced by transduction of hippocampal neurons using HSVGluR6, an HSV vector expressing a fully edited kainate receptor subunit. Discharges appear within 2.5 hours after localized gene transduction to the CA3 region of cultured hippocampal slices. We have previously demonstrated experimental control of the number of transduced cells using HSVLac, an HSV-1 vector that expresses the reporter gene β-galactosidase. We presently investigated how may transduced cells were needed for discharge initiation. Discharge initiation was effectively blocked with the non-NMDA receptor antagonist, CNQX (50μM), regardless of the number of HSVGluR6-transduced cells. The NMDA receptor antagonist, DL-APV, (100μM), blocked discharge initiation in cultures containing fewer than 10 transduced cells. In cultures containing greater than 400 transduced cells, discharges appeared despite the presence of APV. These data suggest that discharges were induced by a small number of HSVGluR6-transduced neurons in addition to non-transduced CA3 neurons recruited by an NMDA-dependent mechanism. If sufficient CA3 neurons are transduced, NMDA receptors are not needed.

592.15

GENE TRANSFER INTO HUMAN HIPPOCAMPAL SLICES WITH AN ADENO-ASSOCIATED VIRUS VECTOR. A. Freese*, M.J. Kaplitt, M.J. O'Connor, W.M. O'Connor, and M.J. During. Div. Neurosurgery, U. Penn., Philadelphia, PA; Div. Neurosurgery, Graduate Hosp., Philadelphia, PA; Lab. Neurobiology, Rockefeller U., New York, NY; Div. Neurosurgery, Yale Univ., New Haven, CT.

The transfer of genetic information into hippocampal cells in primary culture, slice preparations and test animals is yielding significant, new information about the physiology and pathology associated with the hippocampus. In addition, gene transfer into the hippocampus provides hope for gene therapy in diseases affecting the hippocampus, including some forms of epilepsy.

We have recently developed an adeno-associated virus (AAV) vector containing the Lac Z gene, encoding a bacterial ß-galactosidase, under control of a cytomegalovirus Immediate Early promoter. This vector and appropriate controls were injected into hippocampal tissue derived from human patients undergoing temporal lobectomies for medically intractable seizures. Five hundred micron thick slices were maintained in artificial CSF @ 37°C for 5 hours, fixed, and then 30 μm thick slices were generated from the larger slices and X-gal staining was performed to ascertain the presence of the bacterial ß-galactosidase. In those slices exposed to the AAV vector with the Lac Z gene, significant ß-galactosidase activity was observed in cells surrounding the injection tract. No staining was seen in controls. The implications for gene transfer into the human hippocampus and other CNS locations are addressed.

592.16

NEURONAL GLUTAMATE RECEPTORS AS AUTOANTIGENS: I. EPILEPSY AND ENCEPHALITIS. S.W. Rogers[1,2*], P.I. Andrews[3], L.C. Gahring[1,2], T. Whisenand, K. Cauley[4], B. Crain[3], T.E. Hughes[5], S. Heinemann[4], and J.O. McNamara[3]. [1]VA-GRECC, Salt Lake City, UT, 84148; [2]Univ. Utah Med. Cntr., Salt Lake City, UT, 84112; [3]Duke Univ. Med. Cntr., Durham, NC 27710; [4]The Salk Institute for Biological Studies, LaJolla, CA 92307; [5]Yale University Sch. Med., New Haven, CT. 06510.

Rasmussen's encephalitis is a childhood disease of intractable focal seizures and characteristic inflammatory histopathology in the affected brain hemisphere. Two rabbits injected with a bacterial fusion protein expressing a portion of the glutamate receptor subunit, GluR3, were observed to develop seizures and early histopathological changes similar to those observed in Rasmussen's encephalitis. To test the hypothesis that an autoimmune response to GluR3 may be associated with Rasmussen's encephalitis, the sera from affected patients and age and sex matched controls were examined for immunoreactivity towards GluR subunits using Western blot analysis and transfected cells. Rasmussen patients with active disease were found to have circulating IgG antibodies to GluR3. In a therapeutic trial of one patient, removal of circulating GluR3 antibodies by plasmaphoresis correlated with a reduced rate of seizure and improved cognitive function. Our results suggest that Rasmussen's encephalitis consists of an autoimmune component which includes autoreactive antibodies towards glutamate receptors of the CNS.

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592.17

NEURONAL GLUTAMATE RECEPTORS AS AUTOANTIGENS: II. PARANEOPLASTIC DISEASE. J. Greenlee[1,2], L.C. Gahring[1,2], R.E. Twyman[2], J.H. Petajan[2*], and S.W. Rogers[1,2]. [1]SLC VA and GRECC, Salt Lake City, UT, 84148 and [2]Univ. Utah School of Medicine, Salt Lake City, UT 84112.

Paraneoplastic (PN) cerebellar degeneration is a remote complication of systemic cancer. This disease is characterized by loss of Purkinje neurons, variable loss of granule cells, and occasional perivascular lymphocytic infiltrates. High titer anticerebellar antibodies are common. We have examined the sera of 16 patients with Type 1 or Type 2 PN disease for autoimmune reactivity to glutamate receptor (GluR) subunits using both Western blot and immunohistochemical analyses. We identified one patient with Type 1 paraneoplastic disease whose serum exhibited immunoreactivity to GluR5. Unexpectedly, immunohistochemical analyses of mouse brain sections with serum from the same patient exhibited highly specific regional staining of the lateral septum, bed nucleus of the stria terminalis, central nucleus of the amygdala, entire hippocampus, and Purkinje cells of the cerebellum. Staining of this pattern persisted to dilutions of 1:20,000. This pattern of expression is strongly suggestive of the immunohistochemical pattern for GluR1 (e.g., Rogers, et al. JNS 11:2713, 1991). These results suggest that this patient may harbor autoantibodies to more than one GluR subunit. This is the first report that autoimmune reactivity towards GluR subunits can occur in PN disease, and that this differs from the anti-GluR3 immunoreactivity observed in Rasmussen's encephalitis (see abstract by Rogers et al.) suggesting that various GluR subunits may serve as autoantigens in neurodegenerative disease.

572.18

NEURONAL GLUTAMATE RECEPTOR ANTIBODIES — POTENTIAL EXCITOTOXINS? L.C. Gahring, R.E. Twyman, J.M. Eichten, S.N. Sudweeks, L. Jackson, J.R. Baringer*, and S.W. Rogers. Programs in Neuroscience and Human Molecular Biology & Genetics, Univ. of Utah School of Medicine and VA-GRECC, Salt Lake City, UT 84112

Two rabbits injected with bacterially prepared protein of neuronal glutamate receptor (GluR) subunit, GluR3, developed seizures and pathology similar to those observed in Rasmussen's encephalitis (see abstract Rogers et al). Our initial studies have focused on rabbit immune serum and the mechanisms of antibody action. Results indicate that at least two epitopes exist in the region of aminoacids 245-457 of GluR3 near the presumed extracellular glutamate binding site. Immunohistochemical examination on mouse brain reveal patterns of GluR3 expression consistent with in-situ hybridization studies. These antibodies also identify a small subset of morphologically similar cells in live mouse (E14) cortical neurons in culture. Whole cell electrophysiological studies of these neurons indicate that GluR3 antibody rapidly and reversibly activates non-NMDA sodium currents and that these neurons also have kainic acid inducible currents. Antibody evoked currents were blocked by CNQX and currents were not elicited by other sera. Single cell RT-PCR analysis of these neurons identifies the presence of GluR3 and additional GluR subunits. These results indicate that antibodies to GluR3 have agonist properties on unique cultured neurons containing putative GluRs. This suggests the possibility that autoimmunity targeted against specific epitopes on GluR containing neurons may contribute to neurotoxicity in conditions such as epilepsy, neurodegenerative diseases and paraneoplastic syndromes.

592.19

CHANGES IN HIPPOCAMPAL GLUTAMATE/AMPA RECEPTORS IN EPILEPTIC HUMAN HIPPOCAMPUS

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Changes in glutamate receptor properties in the limbic system have been proposed to participate in the increased neuronal excitability observed in human temporal lobe epilepsy (TLE). We examined the changes in binding properties of hippocampal glutamate/AMPA receptors using quantitative ligand binding autoradiography in frozen-thawed sections obtained from temporal lobe resection in patients with intractable TLE. As observed in rat hippocampal sections, preincubation of human hippocampal sections (n=12) at 35°C resulted in a large decrease in ^{3}H-AMPA binding as compared to sections preincubated at 0°C. Preincubation at 35°C in the presence of calcium (2 mM) resulted in a large increase in binding as compared to the absence of calcium. Sections from epileptic patients exhibited an increase in binding following preincubation at 35°C in the absence of calcium and no change after preincubation in the presence of calcium as compared to control tissue obtained at autopsy. Our results indicate that TLE is associated with a decrease in synaptic receptors and an increase in non-synaptic (possibly cytoplasmic) AMPA receptors.
Thus, the changes in AMPA receptor binding are more complex than previously reported, and the increase in functional AMPA receptors with calcium at physiological temperature, may contribute to the hyperexcitability in epileptic human hippocampus.
Supported by grant IBN 9110377 from NSF (MB)

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS II

593.1

POTENTIATION OF AUDIOGENIC SEIZURES BY TMPP AND PTZ IN NAIVE ADULT FISCHER-344 AND SPRAGUE-DAWLEY RATS

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Trimethylolpropane phosphate (TMPP) and pentylenetetrazol (PTZ), two potent convulsants thought to antagonize the $GABA_A$ inhibitory system, potentiated audiogenic seizures in naive adult Fischer-344 and Sprague-Dawley rats. Doses of TMPP (0.0125-0.05 mg/kg i.p.) insufficient to produce observable behavioral convulsions or EEG response nevertheless potentiated wild running fits or wild running fits followed by tonic seizures 30 min. after the initial administration. TMPP doses (0.10-0.20 mg/kg i.p.) sufficient to effect observable behavioral response and EEG paroxysms reliably potentiated wild running fits followed by severe tonic-clonic seizures in 80%+ of animals tested. Time to initial seizure response following onset of the audiogenic stimulus was linearly dose dependent. In some animals a single TMPP administration potentiated an audiogenic seizure response when animals were acoustically stimulated up to three months later. PTZ (5.0-25.0 mg/kg i.p.) potentiated audiogenic seizures in naive adult Sprague-Dawley rats 30 min. following the initial dose. This result was unexpected, and may reflect the use of a mixed frequency (8,000 Hz predominant frequency, 110 dB for 60 sec.) audiogenic stimulus as opposed to the traditionally used bell or pure tone. The results are discussed in terms of possible neurological mechanisms of action.

593.2

DIFFERENTIAL SUSCEPTIBILITY OF BALB/c, SWISS-WEBSTER AND DBA/2 MICE TO INTRAHIPPOCAMPAL γ-ACETYLENIC GABA.

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Intrahippocampal application of the "indirect" excitotoxin γ-acetylenic GABA (GAG) (240 nmol) causes a unilateral loss of hippocampal CA1 and CA3 neurons but no behavioral seizures in rats (Exp. Neurol. 124:184, 1993). Unilateral GAG injections were now made into the hippocampus of 10-week old Balb/c, Swiss-Webster and DBA/2 mice. Animals were killed 3 days later, and their brains were analyzed by light microscopy. Mortality, behavioral convulsions and hippocampal neurotoxicity differed substantially among strains (DBA/2 >> Swiss > Balb/c). 360 nmol GAG had virtually no effect in Balb/c mice and led to seizure-related death in 30% of Swiss mice. In contrast, 90 nmol GAG caused 50% mortality in DBA/2 mice. Convulsions developed by 3-5 hrs post injection and often progressed to status epilepticus and tonus/death. Hippocampal necrosis was seen in all (and only in) animals which had status epilepticus. Neuronal damage occurred bilaterally in area CA1 and often also in area CA3. Granule and hilar cells degenerated mostly ipsilateral to the GAG infusion. In DBA/2 mice treated with 90 nmol GAG, the anticonvulsant valproic acid (2 x 350 mg/kg) and the NMDA antagonist CGP 40116 (20 mg/kg) totally abolished seizures and nerve cell death. In mice, therefore, GAG-induced hippocampal neurodegeneration seems to be seizure-related and mediated via NMDA receptors.

Supported by grant NS 16102.

593.3

ENHANCED KAINATE SENSITIVITY IN THE HAN-WISTAR RAT.

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We are studying a mutant Han-Wistar (HW) rat strain which displays a pattern of hippocampal degeneration (CA3 pyramidal cells) and mossy fiber sprouting similar to rat models of epilepsy (i.e., kainate-injected, kindling). We first studied the sensitivity of 40 day old mutants and littermate controls (n = 20 pairs) to kainate injections (7-9mg/kg). Bipolar electrodes were implanted in the dorsal hippocampus and EEG recordings were made 3-5 days later. Prior to kainate injections, both mutants and controls did not show any abnormal paroxysmal discharges. After injection, behavioral and electrographic seizures occurred earlier (15-30 mins) and were more intense in the mutant. The mutants' seizures resembled the tonic-clonic type, while the controls exhibited short duration, high amplitude, paroxysmal discharges. Kainate injection was lethal to 50% (10/20) of the mutants but only 5% (1/20) of the controls. Another set of kainate-injected pairs (n = 3) was analyzed by immunohistochemistry for the presence of c-Fos, a gene regulating protein which becomes elevated in epileptic cells. Mutant hippocampus, which showed no differences in c-Fos expression prior to kainate treatment, exhibited a 30-40% increase in c-Fos compared with controls. To determine a molecular basis of sensitivity, we examined the expression of non-NMDA glutamate receptor mRNA in the HW hippocampus by ^{35}S-labeled in situ hybridization. We observed a two-fold increase in GluR2 mRNA in all regions of the mutant hippocampus, suggesting an enhanced kainate sensitivity in the mutant. We propose that the HW model, because of its kainate sensitivity, can be used to understand the complex relationship between cell degeneration and hippocampal reorganization in epilepsy.

593.4

PROCONVULSIVE ACTION OF PERIPHERAL-TYPE (OR MITOCHONDRIAL) BENZODIAZEPINE RECEPTORS IN EI AND 'NERVOUS' MUTANT MICE.

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We have recently reported that Ro 5-4864, a specific agonist for the peripheral-type (or mitochondrial) benzodiazepine receptor (PBR), can efficiently induce seizures in EI mice, an animal model of epilepsy (Nakamoto et al., Soc. Neurosci. Abstr. 18, 380, 1992). To further characterize the proconvulsive action of PBR, we have examined the 'nervous' mutant mouse, which develops a selective degeneration of cerebellar Purkinje cells with abnormal mitochondria. Ro 5-4864 at a dosage of 20 mg/kg or higher (i.p.) induced seizures consistently in nervous mice, whereas in their controls (heterozygous littermates) seizures could be induced at a lower dosage of 10 mg/kg. In binding assay for [^{3}H]Ro 5-4864 in brain homogenates, the binding of [^{3}H]Ro 5-4864 was reduced more than 50% in cerebella of nervous mice when compared with controls. In contrast, no significant changes in [^{3}H]Ro 5-4864 binding were observed in forebrains between nervous and control mice. There was no apparent alteration in receptor affinity for [^{3}H]Ro 5-4864. The results suggest that PBRs in the cerebellum could contribute to seizures as those in the forebrain.

593.5

KAINIC ACID-INDUCED SEIZURES IN RATS: DOES ROUTE OF ADMINISTRATION MAKE A DIFFERENCE? G.T. Golden*, N.K. Krishna, G.G. Smith, P.F. Reyes, T.N. Ferraro. Department of Veterans Affairs Medical Center, Coatesville, PA 19320 and Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107

Parenteral administration of drugs can result in differential rates of absorption. The glutamate agonist kainic acid (KA) is usually administered systemically by either the intraperitoneal (ip) or subcutaneous route (sc) to produce acute limbic seizures in rats and is used as a model for temporal lobe epilepsy. In the present study, KA was systemically administered by ip, sc or intramuscular (im) routes to seizure sensitive Fisher 344 rats (F344) and seizure resistant Long Evans rats (LEH) to determine if route of administration differentially effected seizure parameters. A total of 54 F344 and 54 LEH adult rats received one of three KA doses (adjusted to a neutral pH) by the ip, sc or im route. Behavior was monitored for 30 minutes before and for 240 minutes after injection of KA. The number of animals demonstrating behavioral seizures, number of animals exhibiting status epilepticus, behavioral seizure scores, latency to first seizure and latency to status epilepticus were compared for the three parenteral injection routes. Results showed trends for rats with sc injections to have shorter latencies to first seizure and to status epilepticus, for im injected rats to have the longest and ip injected rats intermediate latencies. There was also a trend for ip and im injected rats to have higher seizure scores than sc injected rats. However, there were no statistically significant differences between the three routes of parenteral administration on any of the seizure parameters measured. Seizure response variability due to route of administration and strain differences will be discussed. Supported by the Department of Veterans Affairs.

593.6

THALAMIC-TRIGGERED SEIZURES. G.A. Cottrell*, H. Gnanasekeram and W.M. Burnham. Bloorview Epilepsy Laboratory, Dept. Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Past research has shown that the midbrain and hindbrain can produce convulsive seizures when subjected to high levels of stimulation. The present experiment was designed to investigate convulsive behavior patterns elicited from the thalamus. Male Long-Evans rats were implanted with single bipolar electrodes using standard stereotaxic techniques. High-intensity electrical stimulation was administered to 14 thalamic nuclei, at 3 anterior-posterior planes. The posterior nuclei of the thalamus produced a generalized convulsion which was tonic or tonic-clonic, depending on the intensity of the stimulation. These convulsions resembled maximal electroshock seizures. Stimulation of the anterior and middle nuclei of the thalamus produced bilateral forelimb clonus, sometimes preceded by contralateral forelimb clonus. These seizures were never tonic, even at high stimulation levels. The data suggest that the thalamus is capable of producing two separate convulsive patterns, one purely clonic and the other tonic-clonic.

This research was supported by the Bloorview Epilepsy Program Grant and the Medical Research Council of Canada.

593.7

CHANGES OF DOPAMINERGIC NEUROTRANSMISSION AFTER KAINIC ACID-INDUCED BEHAVIOURAL ALTERATIONS. H.Baran¶ Dep. of Pediat., University of Vienna, 1090 Vienna, Austria.
Injection of kainic acid (KA,10 mg/kg,s.c.) in the rat results in the development of characteristic epileptic syndroms. We analysed dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in amygdala/pyriform cortex, hippocampus, septum, and frontal and parietal cortex 10, 20, 30, 60, and 120 min after KA. At 10 and 20 min the marked increase of DA was found in amygdala/pyriform cortex (+61% and +57%), in hippocampus the DA content was reduced (-25% and -50%) and 30 min after KA in both regions the DA content was normalized. At 1 and 2h the increase of DA was measured in hippocampus (+63% and +86%). In septum DA was decreased (-30%) 10 min after KA, increased (+50%) 20 min after, and was however decreased at 1 and 2h (-53% and -64.2%). NA content was reduced (-20 %) in parietal cortex 10 and 20 min after KA, in hippocampus and parietal cortex 30 min after, and in all regions examined (ca.-50%) 2h after. 5-HT was mildly but significantly lowered 2h after KA. In summary, the induced changes of DA content in the brain after KA indicate notable regional circuits of DA-ergic neurotransission, which may play pivotal function in the induction of epileptic activity.
This study has been performed at the Inst. of Biochem. Pharmacol. Univ. Vienna. Supported by Austria Research Fund 5647 and Charlotte-Buehler Fund H00043-MED to H.B..

593.8

CITRULLINE INCREASE DURING TEMPORAL LOBE SEIZURES SUPPORTS THE ROLE OF NITRIC OXIDE FORMATION IN EPILEPSY: AN *IN-VIVO* MICRODIALYSIS STUDY. M.H. Shomer, C.L. Wilson, N.T. Maidment, E.J. Behnke, A.L. Velasco, J. Engel Jr., and I. Fried*. Depts. of Neurology, Anatomy, Psychiatry, Neurosurgery, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Seizure initiation and spread involving glutamate (GLU) activation of the NMDA receptor may be mediated by Ca^{2+}/ calmodulin-dependent conversion of nitric oxide (NO) and citrulline (CIT) from arginine by nitric oxide synthase (NOS). Using *in vivo* microdialysis, Sorkin (Neuroreport, 1993, 4: 479-482) has shown an NMDA-evoked increase in the levels of CIT and GLU which is blocked by the NOS inhibitor L-NAME. Our hypothesis is that NO is involved in the cascade of events underlying seizure genesis in human epilepsy. Due to its gaseous nature, NO can diffuse to surrounding presynaptic terminals, increasing levels of cGMP and release of transmitter resulting in seizure genesis and propagation. In patients with intractable complex partial epilepsy who require diagnostic studies prior to temporal lobectomy, intracranial probes containing microelectrodes and microdialysis membranes were used to record EEG, single unit activity, and amino acid levels in the hippocampal formation, amygdala and entorhinal cortex. We have observed up to 5-fold increases in glutamate and citrulline during 3 out of 4 complex partial seizures in two patients, and a significant correlation in levels of glutamate with citrulline ($r=.71$, $p<.001$) both ictally and interictally. These increases provide evidence from the human temporal lobe in support of a role for NO in seizure genesis. Supported by NS 02808.

593.9

CHANGES IN CEREBRAL BLOOD FLOW PRECEDE ELECTROGRAPHIC SEIZURE DISCHARGES. J. M. Germano*, L. Guarino, J. B. Bederson. Department of Neurosurgery, Mount Sinai School of Medicine, New York, (NY) 10029.

Treatment of epilepsy depends on the identification of the epileptogenic focus. Minimally invasive methods for this identification are sought. The aim of this study is to compare the use of intracranial and depth electrode EEG recording versus laser doppler flowmetry (LDF) in detecting the onset of seizure activity. Adult Sprague Dawley rats were anesthetized with chloral hydrate, intubated, ventilated and kept anesthetized with halothane. Intracranial electrodes and LDF probe were placed epidurally; depth electrodes were stereotactically implanted in the hippocampus. Seizures were induced with kainic acid (20 mg/kg i.p.). A blood flow increase greater than 200% of baseline was seen in 4 of 5 rats prior to electrographic discharges recorded from depth and intracranial electrodes. These findings suggest that LDF should be further investigated as a diagnostic modality in the evaluation and study of epilepsy.

593.10

Possible Mechanism of the Seizure Attenuating Effects of Vagus Nerve Stimulation. S.E. Krahl*, R.A. Browning, K.B. Clark, & D.C. Smith. Departments of Psychology and Physiology, Southern Illinois University, Carbondale, Illinois 62901.

Vagus nerve stimulation (VS) is a new strategy for the treatment of intractable epileptic seizures. The mechanism(s) by which VS attenuates seizures, however, have not been elucidated. The present study examined the role of the locus coeruleus (LC) in this phenomenon.

Experiment 1 investigated the effects of VS on LC single-unit activity in urethane-anesthetized female rats. Using extracellular electrodes, the activity of single LC neurons was monitored before and after VS. VS was found to significantly increase the firing rate of neurons located in the dorsal LC, while having no significant effect on subcoerulear cells. These results indicate that VS can affect LC activity, and that this effect may be regionally specific.

Experiments 2 and 3 were designed to answer two main questions: 1) were the parameters used in Experiment 1 relevant to those used for the prevention of seizures and, 2) would inactivation of the LC reduce the anticonvulsant effects of VS? In Experiment 2, female rats were either depleted of norepinephrine via a bilateral infusion of 6-OHDA into the LC (6-OHDA/VS), received a sham infusion (Sham/VS), or did not receive surgery (NonOp/VS). Two weeks later, these rats were implanted with vagus nerve electrodes. The next day they were given VS, during which they were subjected to a corneal maximal electroshock seizure (MES) test. The seizure severities of the Sham/VS and NonOp/VS groups following VS were significantly reduced as compared to pretest severities, demonstrating the effectiveness of VS as an anticonvulsant treatment. Seizure severities of the 6-OHDA/VS group following VS, however, did not differ significantly from pretest values, indicating that the LC may modulate the anticonvulsant effects of VS.

In Experiment 3, female rats had cannulae implanted in the LC, through which either lidocaine or saline was infused. Shortly thereafter, the rats were given VS and an MES test. In agreement with the results of Experiment 2, VS caused a significant reduction in seizure severity in those rats with an intact LC (saline infusion), but had little effect on those whose LC was reversibly inactivated (lidocaine infusion). These results further suggest that the LC may modulate the anticonvulsant effects of VS.

594.1

PREDICTORS FOR POST-ISCHEMIC SEIZURES IN LONG-EVANS RATS. K. H. Reid*, V. Iyer, A. Schurr, M. T. Tseng, J. J. Miller. Depts. of Anatomical Sciences & Neurobiology, Neurology, Anesthesiology, and Pathology, University of Louisville, Louisville KY 40292.

After global ischemia, rats tend to develop sound-triggered seizures which resemble those seen in genetically epilepsy-prone rats (Reid et al., FASEB J. 8:A660, 1994). We sought to maximize the fraction of post-ischemic rats which developed audiogenic seizures through an evaluation of 5 possible predictors of post-ischemic seizures in 60 rats. All rats received 7 minutes of chest compression under ketamine anesthesia at a skull temperature of 35°C. Resuscitated rats were tested for sound-induced seizures 24 hours later. The predictors evaluated were **blood glucose, skull temperature, time to first spontaneous respiration, time to first EEG activity, and peak blood pressure during reperfusion.** Receiver operating characteristic curves indicated that the best single predictor was the duration of isoelectric EEG; this had an accuracy value of 0.79. Combinations of predictors did not significantly improve this value, in part because we tried to hold all parameters constant so that the range of measured values was narrow. Supported in part by Alliant Community Trust Fund 9210.

594.2

CHANGES IN SEIZURE PATTERN WITH VALPROATE THERAPY IN POST-ISCHEMIC AUDIOGENIC EPILEPSY IN LONG-EVANS RATS. V. Iyer, K. H. Reid, A. Schurr, L. A. Carr*, M. T. Tseng and J. J. Miller, Depts. of Neurology, Anatomical Sciences & Neurobiology, Anesthesiology, Pharmacology & Toxicology, and Pathology, University of Louisville, Louisville, KY 40292.

Long-Evans rats subjected to cardiac arrest from chest compression tend to develop audiogenic seizures. (Reid et al., FASEB J. 8: A660, 1994). This may be a useful model for human generalized epilepsy. To validate this model, we compared the effect of 200 mg/kg valproate i.p. in 20 post-ischemic rats with 10 saline-injected controls. Each rat was tested 30 times; during 10 successive tests valproate/saline was injected one hour prior to the seizure test. Valproate consistently reduced the number of seizures induced by sound. In addition, the seizures which did occur were briefer and less complex than those seen pre-treatment or in saline-treated controls. Seizure suppression sometimes continued after drug treatment was stopped, suggesting a possible anti-epileptogenic effect. These effects indicate that this model may be useful in evaluating drug therapy for generalized tonic-clonic seizures. Supported in part by Alliant Community Trust Fund 9210.

594.3

SEQUENTIAL CHANGES IN HIPPOCAMPAL CA1 REGION OF THE POST-ISCHEMIC SEIZURE PRONE RATS. M. T. Tseng*, K. H. Reid, V. Iyer, A. Schurr, and J. J. Miller, Depts. of Anatomical Sciences & Neurobiology, Neurology, Anesthesiology, and Pathology, University of Louisville, Louisville KY 40292.

Hippocampal CA1 region is known to be susceptible to global ischemia. The time-dependent change in this region of the post-ischemic seizure prone rats following chest compression was studied in 8 seizure prone rats and 2 controls. Rats were anesthetized and perfusion fixed 4 h, 48 h, 7 day, and 6 weeks following a 7 min global ischemia. Brains were removed, fixed for 3 more days, before being vibrotome sectioned at 40 μm for light microscopy or microdissected for electron microscopy processing. Decreased width of the stratum pyramidale and a concomitant increase in glial cell was observed in 7 day and 6 week samples. The early perivascular edema showed resolution through glial cuffing from day 7. Gradual neuronal death and glial migration as well as the presence of neurofilament-rich glia around the micro-vessels were verified by electron microscopy. These results confirmed selective vulnerability of the CA1 pyramidale cells and suggest that gliosis is an important sequela of this global ischemia model. Supported in part by Alliant Community Trust Fund 9210.

594.4

SYNAPTIC FUNCTION AND ITS FACILITATION BY LOW MAGNESIUM IN HIPPOCAMPAL SLICES OF RATS SUBJECTED TO CARDIAC ARREST R.S. Payne, A. Schurr*, V. Iyer, K.H. Reid, M.T. Tseng, J.J. Miller. Departments of Anesthesiology, Neurology, Anatomical Sciences & Neurobiology, and Pathology University of Louisville, School of Medicine, Louisville KY, 40292.

Long Evans male rats were subjected to chest compression-induced cardiac arrest for 7 min. Subsequent audiogenic seizures (AGS), and histopathological changes in the CA1 region of the dorsal hippocampus were observed.

Using a dual interface recording chamber, evoked extracellular potentials in normal artificial cerebrospinal fluid (ACSF) and low Mg^{2+}-induced synaptic facilitation (LMISF) were evaluated in hippocampal slices obtained from rats not subjected to chest compression and compared with slices prepared from chest-compressed animals. CA1 extracellular responses to electrical stimulation of the Schaffer collaterals were recorded from the pyramidal cell body layer using a stimulus strength x2 threshold (~5 V). This stimulus usually evokes a PS with an amplitude of 10 mV or greater. In experiments where slices were perfused with Mg^{2+}-free ACSF, the stimulus strength was reduced just before the perfusion such that the amplitude of the PS did not exceed 3 mV.

Of 90 slices prepared from control rats, 74.4% exhibited a CA1 PS with an amplitude of $\geq$10 mV, 23.3% had an amplitude of 6-10 mV and 2.2% with an amplitude of $<$6 mV. In contrast, of 172 slices prepared from rats that were exposed to cardiac arrest, only 12% exhibited a PS with an amplitude of $\geq$10 mV, 29% showed a PS of 6-10 mV while the majority of the slices (58%) exhibited a PS amplitude smaller than 6 mV. Of the latter, 32% were completely unresponsive. To test for LMISF, slices were perfused with Mg^{2+}-free ACSF. In preliminary experiments such perfusion brought about 178±48% increase in the amplitude of the PS in slices of control rats. In slices of chest-compressed, AGS rats, LMISF was attenuated (95±34%) which disagrees with results obtained from slices of AGS mice, where LMISF was enhanced (Wieraszko & Seyfried, Epilepsia, 34:979,1993).

We conclude that diminished PS amplitude and lack of enhancement of LMISF in slices from cardiac arrest, AGS rats is due to cerebral ischemic neuronal damage.

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594.5

GABA AND SEIZURE INITIATION. K. Emori[1,2*], Y. Minabe[1], C.R. Ashby, Jr.[3] and H. Katsumori[1]. [1]Dept. of Cortical Function Disorder, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187, Japan; [2]Dept. of Neuropsychiatry, Toyama Med. & Pharmaceut. Univ., Toyama 930-01, Japan; [3]Brookhaven National Labo., Upton, NY 11973.

In this study we examined dynamic changes of monosynaptically evoked field potential in dentate gyrus (DG) by paired perforant path stimuli (25ms apart) for analysis of local $GABA_A$ function during low-frequency (2Hz) DG kindling stimulus train using unanesthetized freely moving rats. We also observed extracellular field recording associating with afterdischarge (AD) triggering. During the kindling stimulus train, in all cases AD was triggered prior to occurrence of disinhibition of paired-pulse depression. Furthermore the triggered AD shapes changed with a population spike like component when the disinhibition occurred. By the kindling development, the paired-pulse depression was rather enhanced in the early period of stimulus train and latency until the disinhibition was prolonged. We consider that these findings suggest a possibility that collapse of local GABA mediated inhibition might contribute to seizure propagation rather than its initiation.

594.6

DETERMINISM, CONTROL AND ANTI-CONTROL IN A MODEL EPILEPTIC FOCUS. D.H. Duong, T. Chang, K. Jerger, M.L. Spano, W.L. Ditto and S.J. Schiff*. Department of Neurosurgery, Children's National Medical Center, Washington, D.C. 20010.

Following the recent theoretical prediction that chaotic physical systems might be readily controllable, there has been rapid and successful application of this technique to mechanical systems, electrical circuits, and arrhythmic cardiac tissue. One of the hallmarks of the human epileptic brain during periods of time in between seizures is the presence of aperiodic bursts of focal synchronized neuronal activity known as *interictal spikes*. The high potassium *in vitro* hippocampal brain slice preparation exhibits population burst-firing activity that is in many ways analogous to the interictal spike. We sought to determine whether such neuronal bursting activity was amenable to control.

Transverse slices 400 μm thick were prepared from the hippocampus of 125-150 gm female Sprague-Dawley rats with a tissue chopper, and placed in an interface type perfusion chamber at 32-35 °C. Experiments consisted of combinations of chaos control, periodic pacing, and the inverse of chaos control which we term *anti-control*.

Ninety-one experimental trials were performed on 22 slices from 9 rats. Good control of this neuronal circuit was achieved in 14/52 chaos control trials, 8/19 trials using periodic pulses, and in 5/21 attempts at anti-control.

This is the second attempt at achieving control of a chaotic biological system, and the first attempt in brain. Chaos control has the advantage over overdrive periodic pacing in terms of its ability to identify and track the system's activity over time. In addition, the control of chaos strategy offers the ability to break up periodic behavior with "anti-control". The anti-control method used here employs a minimum number of stimuli needed to prevent periodic behavior. Such techniques may be applicable to human epileptic foci.

594.7

LONG-TERM EVALUATION OF SPONTANEOUS RECURRENT SEIZURES AFTER INTRAHIPPOCAMPAL KAINIC-ACID INJECTION. R.L.M. Rivero, C.M. Kohmann*, D.K. Arashiro, M.S.França, C.D. Domingos, R.V.R. Duran and L.E.A.M. Mello. Depto. de Fisiologia, Escola Paulista de Medicina, 04023-900 São Paulo, Brazil.

After the pioneer work of Cavalheiro (EEG Clin. Neurophysiol., 53:581-589, 1982) regarding the development of spontaneous recurrent seizures (SRS) after intrahippocampal kainate (KA) administration, very little attention has been given to the behavioral aspects of this model of epilepsy. Thus, there is no reference as to how many animals submitted to this procedure develop SRS, or to the frequency of these SRS. In order to answer some of these questions we decided to study this model of temporal lobe epilepsy in more detail. Wistar rats were submitted to an stereotaxic, unilateral, intrahippocampal kainic acid injection (1.25μg/0.5 μl), under anesthesia. After surgery, the rats were visually monitored regarding occurrence of SRS, for 5 days/week, 5h/day on the subsequent days (over 300 days). 75% of the female rats and 84,5% of the male rats developed SRS after the injection. The latency period for the first SRS to be seen was similar, in males (6-188 days) and in females (11-324 days). The average seizures/week/rat was 0.21. Seizure frequency did not increase with time. After 15 weeks of observation, more than 85% of the female rats and more than 60% of the male rats were still showing SRS. Our results indicate that hippocampal injection of KA may not automatically imply in the development of SRS. Our results do not agree with the previous report of SRS remission after 60 days in this model. Seizure remission on this model was a basic tenet for some authors to suggest that mossy fiber sprouting would help to suppress SRS. Supported by: FAPESP, CNPq and FINEP; R.L.M.R., C.M.K. and R.V.R.D. are FAPESP fellows (IC 93/0665-7, 93/4965-5 and 93/0666-3). M.S.F. is a CNPq fellow.

594.8

AFFERENT REGULATION OF THALAMIC OSCILLATIONS IN PETIT MAL EPILEPSY: A MICROPERFUSION STUDY. A. Kandel*, G. Jando, and G. Buzsáki. CMBN, Rutgers University, Newark, NJ 07102

Spike and wave high voltage spindle (HVS) pattern (7-8 Hz) in rodents is an animal model for petit mal epilepsy. These nonconvulsive generalized seizures originate in the thalamus and become bilaterally synchronous in the cerebral cortex through the neuronal interplay between thalamocortical and cortico-thalamic cells. In this study microdialysis probes were placed unilaterally to perfuse CNQX (0.01-1.0mM) and high calcium-ACSF (2.44-39.04mM) into the ventral basal (VA/VL) and reticular (RTN) thalamic nuclei to alter seizure activity in awake non-anesthetized freely-moving rats. This form of drug application greatly reduces repeated handling, which can affect seizure rate, while enabling the infusion of dialysates into discrete brain regions. EEG was recorded before, during and after drug applications with epidural screw electrodes.

High-calcium concentrations did not change overall HVS duration but seizures were limited to the time of calcium perfusion and greatly reduced during normal ACSF perfusion. Calcium infusion outside of the VA/VL, RTN did not exhibit this effect.

CNQX perfusions: 0.01mM created a 2 Hz (delta), 3mV amplitude spiking pattern ipsilateral to the probe while not affecting the contralateral HVS pattern. 0.1mM induced a 2 Hz, 2mV ipsilateral spiking pattern, while suppressing the contralateral HVS or created ipsilateral 1 mV spiking without affecting the contralateral HVS or decreased ipsilateral HVS. 1.0mM suppressed HVS bilaterally but not completely contralateral to infusion. In most cases HVS durations and amplitudes recovered after 24 hours of ASCF infusion.

These experiments futher underline the importance of calcium and the AMPA glutaminergic system in the thalamic oscillations underlying petit mal epilepsy.

594.9

ALTERED ELECTRICAL ACTIVITY IN THE DENTATE GYRUS OF FREELY-BEHAVING RATS AFTER KAINATE-INDUCED STATUS EPILEPTICUS P.R. Patrylo[1]*, G.M. Rose[2], K.L. Heman[2], and F.E. Dudek[1]
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The kainate-treated rat, an animal model of temporal-lobe epilepsy, undergoes an initial period of status epilepticus that can ultimately result in a permanent epileptic state. The aim of the present experiments was to determine the initial effects of kainate-induced status epilepticus on evoked field potentials from dentate granule cells in freely-behaving rats. Chronic *in vivo* recordings were performed during a 2 day period prior to and 1 day after kainate treatment. Rats (n=3) were given multiple kainate injections (IP; 5 mg/kg per hr) for 5-8 hr, and class IV/V seizures were elicited for $\geq$ 3½ hr. Prior to kainate treatment, single stimuli of the perforant path (at maximal intensity) evoked a positive field-potential PSP that lasted for 18-26 ms with one or two superimposed population spikes. Paired-pulse stimulation of the perforant path (interpulse interval= 20 ms; stimulus intensity that produced a half- maximal population spike) showed attenuation or little change of the field PSP and population spike to the second stimulus. One day after kainate-induced status epilepticus, however, single stimuli evoked prolonged field PSPs (duration= 63-75 ms) with multiple population spikes. Paired-pulse stimulation led to facilitation of the field PSP and population spike to the second stimulus. The prolongation of the field PSP and the paired-pulse facilitation suggests that recurrent inhibition was depressed in the dentate gyrus of the kainate treated rats. Although further experiments are necessary, these results suggest that relatively rapid changes in dentate electrophysiology, apparently including recurrent inhibition, occur following a period of kainate-induced status epilepticus. Supported by NIH grant NS16683 and NRSA NS08993.

594.10

MEMBRANE PROPERTIES OF CA_3 HIPPOCAMPAL REGION IN THE GENETICALLY EPILEPSY PRONE RATS. Suneeta Verma-Ahuja, M. Steven Evans, and Terrence L. Pencek*, Department of Surgery, Division of Neurosurgery, and Department of Neurology, Southern Illinois University, School of Medicine, Springfield, Illinois, 62794-9230.

Genetically epilepsy-prone rats (GEPRs) exibit a generalized increase in seizure susceptibility. Several factors indicating an increased excitability in CA_1 region of the GEPR-9s have previously been reported. These included an increased input resistance, reduction in spike frequency adaptation, less current required to elicit an orthodromic EPSP and marked facilitation with repetitive stimuli in GEPRs. We have now recorded membrane properties from 15 neurons in the CA_3 region of Sprague Dawley (SD) rats and five neurons in the GEPRs CA_3 region. The resting membrane potential and action potential amplitude were not different between the two. A significant difference was seen in the spike frequency adaptation in the GEPR neurons. In SD neurons a suprathreshold stimulus of 800 ms elicits a burst of action potentials followed by a prolonged hyperpolarization lasting 600 to 750 ms. No action potentials were observed following the burst in these neurons. In GEPR neurons there was a significant increase in the number of spikes in the burst. This increased firing in the burst was not followed by a hyperpolarization, and action potentials were elicited throughout the 800 ms depolarizing stimulus. This suggests an abnormality in the calcium or calcium dependent potassium conductance.

594.11

EFFECTS OF HYPOXIA AND HYPOXIA/ISCHEMIA ON THE DEVELOPING HIPPOCAMPUS. J. Owens, Jr., C.A. Robbins, H.J. Wenzel, and P.A. Schwartzkroin*. Depts. of Physiology/Biophysics and Neurosurgery, Univ. of Washington, Seattle, WA 98195.

Exposing immature rats to hypoxia evokes epileptiform activity which is dependent upon both age and oxygen concentration (Ann. Neuro. 29:629-637). Although perinatal hypoxia leads to increased long term seizure susceptibility, morphological damage has not been demonstrated (Epilepsia 33:971-980; Life Sci. 37:1597-1604). We have attempted to identify hypoxic conditions which will produce short-term hippocampal damage in order to study the possible functional abnormalities resulting from such trauma.

Rats representing 3 age groups (P8-P12, P15-P17, and P20-P27) were exposed to either 15 minutes of hypoxia on 3 successive days or a single 60 minute treatment. Silver degeneration and Nissl staining revealed no difference between treated and control animals at 1, 4, or 7 days following treatment. Fifteen minute hypoxic exposures (on P8-P10 or P9-P11) did not significantly affect CA1 field potentials or intrinsic properties, but may have altered fast IPSP conductance in hippocampal slices prepared 1, 4, or 7 days after the third treatment.

The immature brain has been reported to be vulnerable to a combination of hypoxia and ischemia. We found that, whereas neither hypoxia nor unilateral carotid ligation alone produced neuropathology, the combination of the two reliably produced neuronal damage (see also Ann. Neuro. 9:131-141). In P8-P12 rats, unilateral carotid ligation followed by 60 minutes of hypoxia induced light terminal degeneration in the terminal field of the mossy fibers (the stratum lucidum in CA3) as well as apparent transient cell damage in the pyramidal cell region.

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594.12

CORTICAL MICROGYRIA ARE EPILEPTOGENIC IN VITRO. K.M. Jacobs*, M.J. Gutnick, and D.A. Prince. Dept. Neurology & Neurological Sciences, Stanford Univ. Medical Center, Stanford, CA 94305.

Cortical malformations, thought to be the result of abnormal neuronal migration, are commonly found in the brains of epileptic patients. Clinical data suggest that seizures in one form of malformation, microgyria, are initiated from the sites of structural abnormality, although mechanisms underlying epileptogenesis in the maldeveloped cortex are unknown. We used a method for preparing experimental microgyria in rats described by Dvorak and Feit (1977), in which a freezing probe is applied to the skull of pups at P0 or P1, for 5 sec. The area of the microgyrus could be seen *in vitro* in coronal 400 μ slices obtained from P12-P120 rats. Intracortical electrical stimulation was used to assess intracellular and field potential responses within the microgyria and adjacent neocortex. Superficial lamina responses to deep lamina stimulation both online and horizontally-distant were recorded. At sites 4 mm or greater from the microgyrus, field potential responses were similar to those seen in control neocortex, consisting of a short latency negativity lasting 8-12 msec in control and up to 20 msec in experimental hemispheres. When stimuli were applied closer to the microgyrus, evoked activities consisted of similar early events, followed by polyphasic field potentials with a latency of 20-100 msec and duration of 100-500 msec. The short latency events were graded with stimulus intensity, but the late polyphasic activity appeared as an all-or-none response that could be abolished with higher intensity stimulation or stimulus frequencies above 0.2 Hz. Preliminary intracellular recordings showed that the polyphasic field potentials were associated with long-duration, large amplitude, complex synaptic responses. Some superficial neurons were unusual in that they generated high frequency bursts of action potentials during intracellular depolarization, atypical for neurons in layers II-III. Results suggest that hyperexcitability (epileptogenesis) occurs in this model of focal microgyria, perhaps due to circuit reorganization in the region of the malformation. Additional experiments will determine whether excitatory, inhibitory, or modulatory synaptic activities are affected in the area of the malformation. Supported by NIH grants NS06477, NS12151 and NS07280.

595.1

INTERNEURONS ARE PREFERENTIALLY INJURED AFTER SPONTANEOUS SEIZURES IN THE PILOCARPINE MODEL OF CHRONIC SEIZURES. L. Covolan and L.E.A.M. Mello*. Depto. de Fisiologia, Escola Paulista de Medicina, 04023-900 São Paulo, Brazil.

It is still a question of much debate whether single epileptic seizures can cause cell loss. Despite the clinical impression that epilepsy in general is a progressive disorder, experimental evidence is not conclusive on this point. Recently, it has been shown electrically-induced afterdischarges of less than 2 minutes may induce structural impairments in neurons (Soc. Neurosci. Abst. 1992, 18:553). Here we evaluated whether spontaneous seizures would lead to similar impairments. Chronic spontaneous recurrent seizures were induced with pilocarpine (320 mg/kg, i.p.). Animals were sacrificed from 1 to 6 h either after single or multiple seizures. A Golgi-like sensitive silver-impregnation procedure (J.Comp.Neurol. 1990, 296:654-673) was used to reveal injured neurons. Silver-impregnated dark neurons were never found in control animals or in epileptic animals that had no behavioral seizures in the 8 h prior to sacrifice. After spontaneous seizures (injured) dark neurons were mostly interneurons and were present in hippocampus (CA1 stratum radiatum), amygdala, piriform cortex and other limbic structures. Animals with multiple seizures had a higher number of dark cells than animals with single seizures. Our findings suggest that even single generalized spontaneous tonic-clonic seizures can induce long-lasting morphological changes. Our results favor the idea that epilepsy is a progressive disorder where one seizure begets the next. Supported by: FAPESP, CNPq and FINEP; L.C. is a CNPq fellow.

595.2

SELECTIVE HILAR NEURON LOSS AND PATHOPHYSIOLOGY IN THE HIPPOCAMPAL DENTATE GYRUS BY SUICIDE TRANSPORT OF VOLKENSIN. R.S. Sloviter* and J.H. Goodman. Helen Hayes Hosp., W. Haverstraw, NY 10993 and Depts. of Pharmacology and Neurology, Columbia Univ. NY, NY 10032

It has been hypothesized that inherently vulnerable dentate mossy cells excite inhibitory neurons and thereby maintain normal dentate network inhibition. Accordingly, the loss of mossy cells has been postulated to underlie the permanent granule cell disinhibition and hyperexcitability caused by experimental status epilepticus. In order to address this hypothesis, we utilized the retrogradely transported toxic lectin volkensin to destroy the commissurally-projecting mossy cells selectively by a mechanism not involving seizures. Volkensin (5ng per site) was injected into two sites in the dorsal dentate gyrus. Two months later, granule cell inhibition and excitability were evaluated on the uninjected side. Two types of responses were seen. One group (n=17) exhibited large amplitude population spikes in response to low frequency (0.1Hz) perforant path stimulation, and strong recurrent inhibition at higher stimulus frequencies (1-3Hz). Histologically, these rats exhibited destruction of the injected hippocampus, but relatively normal dentate structure on the uninjected side. The other group (n=15) exhibited similar large amplitude spikes on the uninjected side in response to low frequency stimulation, but multiple granule cell population spikes with little or no recurrent inhibition at higher frequencies. These rats consistently exhibited extensive hilar neuron loss. Immunocytochemical staining revealed that despite the loss of most hilar neurons, hilar somatostatin, neuropeptide Y, parvalbumin, calretinin, and GABA-immunoreactive populations had survived. Degeneration staining 1-2wk after volkensin revealed impregnated hilar cells and terminals in the inner molecular layer characteristic of mossy cell degeneration. These results indicate that the pathophysiology produced by prolonged perforant path stimulation is replicated by the selective loss of hilar mossy cells. The gift of volkensin by Prof. F. Stirpe, Univ. of Bologna, is gratefully acknowledged. Supported by NINDS grant NS18201.

595.3

AXONAL ARBORIZATIONS OF BIOCYTIN FILLED CA1 PYRAMIDAL CELLS FROM HYPEREXCITABLE SLICES OF KAINATE TREATED RATS. Y. Perez, F. Morin, I. Jutras, C. Beaulieu and J.-C. Lacaille*. Center for Research in Neurological Sciences and Departments of Physiology and Pathology, University of Montréal, Montréal, Qc, Canada H3C 3J7.

Following hippocampal kainic acid (KA) lesions, CA1 pyramidal cells become hyperexcitable. To examine if sprouting of CA1 pyramidal cells contribute to this epileptiform activity, the axonal arborizations of intracellularly-marked pyramidal cells have been compared in control and hyperexcitable slices of KA treated rats. Hippocampal slices were obtained from Sprague-Dawley rats 2-4 weeks after bilateral intraventricular injections of KA (0.65 µg/µl). Hyperexcitable slices were identified using extracellular field potentials (bursts $\geq$ 2 population spikes). Cells were impaled in control (unoperated animals) or hyperexcitable slices with microelectrodes containing biocytin (1%) in 1M K-acetate. Synaptic responses were recorded and biocytin was injected intracellularly. Slices were subsequently fixed, cut on a vibratome and processed for visualization of biocytin with the standard ABC method. Axons from pyramidal cells that could be followed from the soma to the alveus were drawn with a camera lucida. In cells (n=10) from control slices, the axon branched on average once (mean branch points 0.9 ± 1.0) before entering the alveus and then coursing toward the fimbria or subiculum. In cells (n=7) from hyperexcitable slices, the local axonal arborizations in stratum oriens and the alveus were more elaborate, with an increased number of branches (mean branch points 2.7 ± 1.8) observed in the oriens layer. These preliminary results suggest that following KA lesions CA1 pyramidal cells develop more extensive local axon collaterals and this sprouting may contribute to the epileptiform activity.

(Supported by the MRC, FRSQ, FCAR and the Savoy Foundation)

595.4

LAMINAR ANALYSIS OF NEURONAL REORGANIZATION IN THE HIPPOCAMPUS OF PILOCARPINE CHRONIC EPILEPTIC RATS. R.V. Duran*, D.M. Finch, A.K. Tan and L.E.A.M. Mello. Depto. de Fisiologia, Escola Paulista de Medicina, 04023-900 São Paulo, Brazil, and Brain Research Institute, UCLA, Los Angeles, CA 90024, USA..

Descriptions of changes in the neuronal morphology in the brains of epileptic patients and of experimental models of epilepsy have been received with some skepticism, since some authors suggested that those findings could be based on artifacts. In this work, we took special cares such as *in vivo* perfusion and use of a chronic epileptic model in which the convulsive agent was administered systemically in order to avoid artifacts. Rats (Sprague-Dawley, males, adults) with chronic spontaneous recurrent crises were obtained by inducing *status epilepticus* (SE) with pilocarpine (320 mg/kg, i.p.). Between 60 and 100 days after SE, stereotaxic injection of HRP in neurons of the dorsal hippocampus was carried out under chloral hydrate anesthesia and 4 to 8 hours later, the animals were perfused and their brains histologically processed. We confirmed that loss of dendritic spines, appearance of varicose-like swellings and bizarre distortions of hippocampal pyramidal dendrites is a consistent finding in epileptic tissue. The cellular changes of the epileptic rats were never found in the controls ones. Besides the generalized gross degeneration, there were changes in the size and in the form of dendritic spines. Moreover, the spines, were reduced or even absent in the dendrites of CA1 pyramids just in *stratum radiatum*. Our results confirm the described morphological changes as true markers of the epileptic condition and indicate that such alterations have a specific laminar distribution pattern which is relevant in understanding the epileptic brain circuitry. Supported by NIH Grants NS 23074 and NS 16721, The NIH Fogarty Center; and FAPESP, CNPq and FINEP (Brazil). R.V. Duran is a FAPESP fellow (93/0666-3).

595.5

PARTIAL LOSS OF HIPPOCAMPAL CA1 NEURONS AND TRANSIENT IMPAIRMENT OF WATER MAZE LEARNING INDUCED BY SYSTEMIC ADMINISTRATION OF KAINIC ACID. K. Obata* and H. Asada, Lab. of Neurochemistry, Natl. Inst. for Physiol. Sci., Okazaki 444, Japan.

Long-term changes in animal behavior and hippocampal tissue were investigated on the male Wistar rats in which limbic seizures were induced at the age of five weeks by subcutaneous injection of kainic acid in order to clarify any plastic changes following neuronal loss. When status epilepticus was limited to 1-3 hours by diazepam, neuronal loss was observed in the CA1 pyramidal layer of the dorsal hippocampus. Morris water maze learning (with a hidden platform) and passive avoidance learning were impaired within two weeks after the seizure. However, the rats learned these tasks well when tested 1-2 months later. A small number of neurons survived in the lesioned pyramidal layer and extended their dendritic branches obliquely and tortuously. Synaptophysin/SVP38 immunostaining and cholinesterase histochemistry ware not changed in the lesioned CA1, suggesting that some synapses and afferent fibers were well maintained. Astrocytes proliferated there and some of them immunoreacted on anti-GABA and anti-glutamate decarboxylase (GAD) antibodies. On the other hand, when the seizures continued over 5 hours without diazepam, the lesion was more extensive and permanent. Neuronal loss was observed in CA1-4 and the subiculum and the hippocampus was atrophied. The rats did not recover from the impaired water maze learning.

595.6

INJECTION OF AMINOOXYACETIC ACID INTO THE RAT ENTORHINAL CORTEX CAUSES POSTSYNAPTIC NEURONAL DAMAGE IN THE HIPPOCAMPUS: A POSSIBLE MODEL OF TEMPORAL LOBE EPILEPSY. **F. Du*, T. Eid and R. Schwarcz. Maryland Psych. Research Center, Baltimore, MD 21228.**

Injection of 75 µg aminooxyacetic acid (AOAA) into the rat entorhinal cortex (EC) produces acute behavioral seizures and selective neuronal loss in layer III of the region (Neurosci. Lett., 147:185, 1992). Since the EC provides the major excitatory input to the hippocampus, we examined the hippocampi of rats receiving an entorhinal AOAA injection using Nissl staining and heat shock protein (HSP) immunohistochemistry. HSP expression has been considered a specific marker of excitation-induced neuronal stress. Rats were sacrificed after 15 or 24 hours, or 5 days. After 5 days, neurodegeneration and gliosis were readily observed in Nissl-stained sections in the subiculum and CA1 field of the ventral hippocampus ipsilateral to the injection. Neurons expressing HSP immunoreactivity (-i) were detected ipsilaterally as early as 15 hours following the injection, mainly in the subiculum, CA1 and in the hilus. This distribution pattern of HSP-i neurons remained similar at 24 hours and 5 days after AOAA treatment. These results indicate that injection of AOAA into the EC can produce over-excitation and degeneration of postsynaptic neurons in the rat hippocampus. AOAA injection into the EC in rats may therefore provide a novel model for the study of temporal lobe epilepsy. Supported in part by USPHS grant NS 16102.

595.7

THE DENSITY OF DENTATE GRANULE CELL SPROUTING IN HEAT-INDUCED SEIZURES IN THE RAT IS RELATED TO THE NUMBERS OF SEIZURES EXPERIENCED. W. Jiang, D. D. spencer, P. Kontur*, and N. C. de Lanerolle. Section of Neurosurgery, Yale Univ. School of Medicine, New Haven, CT. 06510

The sprouting of mossy fiber collaterals into the inner molecular layer (IML) of the dentate gyrus has been observed in patients with temporal lobe epilepsy (TLE) (Brain Res. 1989, 495:387). Patients that show such reorganization are often found to have a history of febrile seizures. The relationship of the degree of sprouting to seizure history has remained speculative in humans because of the difficulty of obtaining accurate information on the subject. Rat pups experiencing heat-induced seizures also show sprouting. These seizures are tonic-clonic seizures (lasting 30 sec to 5 min) and not status epilepticus, thus resembling seizures in TLE patients. The relationship of the number of such seizures to the degree of sprouting was examined in this experimental model. Variable numbers of seizures (1, 6, 12, 24) were induced in different experimental groups of rat pups beginning at 22 days of age. Seizure induction was by exposure of a rat for 4 minutes to water at 45°C. Consequent seizures were induced every fourth day in the multiple seizure groups. The core body temperature of the rat rose from approximately 38°C to 44°C on exposure. Sprouting was observed by the Timm stain. No sprouting was found in the IML of the dentate in the one seizure group. In the six seizure group Timm stain positive particles were limited to the tips and angle of the dentate blades in the IML. Sprouting into the IML was seen in the 12 seizure group, sprouting being stronger at the tips and angle of the blade than in the six seizure group. In the 24 seizure group there was considerably increased sprouting which extended in the IML throughout the entirety of the dentate gyrus, with most intense stain at the tips and dentate blade angle. These results support the hypothesis that sprouting is the result of the seizures, and more seizures produce more sprouting. Supported by NS 06208.

595.8

CORTICAL CONOTOXIN BINDING DIFFERENTIATES EPILEPTIC AND NON-EPILEPTIC MICE. S.K. Jensen, A.F. Burroughs, F. Matsuo* and M.J. Litzinger, Laboratory of Applied Neurobiology, Department of Pediatrics and *Neurology, University of Utah, Salt Lake City, UT 84132

Esplin et al. (Epilepsia,1994) have suggested that N-type presynaptic voltage sensitive calcium channels (VSCC) are different in epileptic (DBA/2J) and non-epileptic (C57/Bl) mice. Binding with the presynaptic calcium channel probe ω-conotoxin, believed to mark N-type voltage sensitive calcium channels (VSCC), was used to illustrate differences in synapse formation between these two mouse types. Whole brain ω-conotoxin binding showed the pre - eye opening postnatal day (PND 8) C57/Bl mouse brain to have fewer binding sites than its epileptic counterpart, the DBA/2J mouse. Post - eye opening (PND 16) C57 mouse have significantly more binding sites than the DBA mouse.

The purpose of this study was to show that the majority of binding in the whole brain studies came from **cortical** VSCC development. Regional dissections were preformed as previously described (Litzinger et al., J. Child Neurology, 1994). Preliminary data indicates that the cortex is responsible for the developmental binding differences seen in the whole brain preparations from these two different mouse types. Cerebellar and diencephalon-brainstem binding remains relatively unchanged. This data suggests that pre-synaptic VSCC's in the cortex are potentially linked to the seizure succeptability described by Esplin. For discussion of functional differences see Burroughs and Litzinger, this section.

595.9

PREPRO-TRH mRNA EXPRESSION IN LIMBIC BRAIN REGIONS FOLLOWING KAINIC ACID-INDUCED SEIZURES IN THE RAT. S.A. Richardson*, A. Dutt, and A. Winokur. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Induction of seizure activity utilizing a variety of techniques including electroconvulsive shock (ECS) and amygdaloid kindling has consistently been reported to produce elevations in thyrotropin releasing hormone (TRH) concentration and increases in prepro-TRH mRNA expression in discrete brain regions. Studies in our laboratory have shown that induction of seizures by kainic acid administration , which activates limbic pathways preferentially, has a more pronounced and prolonged effect on limbic TRH concentrations. To further elucidate the neurochemical basis of the effects of seizure activity on TRH neuronal systems, in situ hybridization methods have been employed to study systemic kainic acid administration on the expression of prepro-TRH mRNA in limbic brain regions. Male Sprague Dawley rats (180-200g) received a single subcutaneous injection of either kainic acid, 12mg/kg, in 0.9% NaCl, or vehicle. Separate groups of rats (n=4) were sacrificed by decapitation at 6, 24, and 48 hours, and 4, 7, and 14 days post-seizure. Rat brains were sectioned at 10um and processed for in situ hybridization using a ^{35}S-labeled riboprobe to detect TRH prohormone mRNA. Significant increases in proTRH mRNA at 6 and 24 hours were observed in the hippocampus, amygdala, piriform cortex, entorhinal cortex, and the reticular thalamic nucleus following a single stage 5 seizure. In the hippocampus, prepro-TRH mRNA grains were most dense in the dentate gyrus region. After reaching a peak within 24 hours, message levels in these areas gradually declined and reached baseline by 14 days. These findings provide further support for a role of TRH neuronal systems in seizure modulation.

595.10

LONG TERM UP-REGULATION OF OXYTOCIN MESSENGER RNA EXPRESSION IN RAT PVN FOLLOWING KAINIC ACID INDUCED SEIZURES Q.Sun, S.Pretel*, C.D. Applegate, D.Piekut Departments of Neurobiology and Anatomy, and Neurology, University of Rochester, Rochester, NY 14642

The paraventricular nucleus of hypothalamus (PVN) is the neuroendocrine center for stress-related responses. It is known that the CRF neurons of the PVN have an important role in regulating stress responses of the CNS. Vasopressin and oxytocin (OX) neurons of PVN are traditionally associated with the regulation of CNS responses to disturbances of osmolality. However, these neurons might also be involved in CNS responses to other stressful stimuli. Our laboratory has previously shown that the Fos protein was expressed in OX neurons of PVN in both electrical and kainic acid induced seizures, indicating that these neurons were activated by the stress stimulation induced by seizures. To further characterize the involvement of OX in response to seizures, OX mRNA in rat PVN was examined with quantitative in situ hybridization in kainic acid induced seizure. Fully generalized seizures were induced by injection of kainic acid (17 mg/Kg in 1 ml PBS, i.p.); rats were kept for different survival times such as 1.5 hour, 1 week, 2 weeks, 3 weeks and 4 weeks. In situ hybridization was performed on frozen sections with a [^{35}]S-labeled oligonucleotide probe, complementary to bases 247-294 of the OX gene (Ivell and Richter 1984; generously provided by DR. S. Young, NIH). Levels of mRNA were measured by densitometery analysis of film autoradiography using the NIH IMAGE program after the in situ hybridization. The results show that the density of OX mRNA labeling in PVN of seizure animals was more than 20% higher than that of control animals at all time points of survival. The increase of OX mRNA occurred as early as 1.5 hour following the first stage 5 seizure and remained elevated as long as 4 weeks. These results demonstrate that OX neurons of PVN respond to seizure by up-regulating their mRNA expression. They also demonstrate that this response represents a long term effect of seizures on magnocellular PVN neurons. Supported by NIH grant NS 18626

595.11

DEVELOPMENTAL CHANGE IN PENTYLENETETRAZOL-INDUCED *c-fos* mRNA EXPRESSION IN THE RAT BRAIN

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Employing Northern blot analysis, we examined in the rat the postnatal development of c-fos mRNA expression induced by systemic administration of pentylenetetrazol(PTZ). Subcutaneous injection of PTZ (50mg/kg) induced after one hour a high level of c-fos mRNA in the neocortex at 23 and 49 days, but not at 8 and 14 days, postpartum as compared with the saline-injected control animals. The induction increased gradually from day 8 to 23 and dramatically from day 23 to 49. In contrast, c-fos mRNA was nearly undetectable in the hippocampus of both groups throughout day 8 to 49. Induction of the proto-oncogene c-fos is considered to be a marker of increased neuronal activity. The present results thus indicate that neocortical neuronal circuits responsive to the drug may alter remarkably between day 23 and 49.

595.12

NEUROANATOMICAL CONCORDANCE OF ABNORMAL TYROSINE HYDROXYLASE AND c-FOS mRNA EXPRESSION DURING FOCAL MYOCLONIC EPISODES IN THE MOUSE MUTANT TOTTERING (*tg/tg*). E.J. Hess* and M.E. Williams. Department of Neuroscience & Anatomy, The Pennsylvania State University College of Medicine, Hershey Medical Center, Hershey, PA 17033.

Tottering (*tg*) is an inherited autosomal recessive mutation in the mouse that results in a triad of neuropathologies including spike and wave discharges, ataxia and focal myoclonus. The spike and wave discharges and ataxia have been extensively characterized, however the myoclonus expressed by these mice has been difficult to study because these seizures have no obvious electrical correlates. We have previously identified the neuroanatomical substrate(s) of myoclonus in tottering mice using the expression of the immediate early gene (IEG) c-*fos* to chart the progression of abnormal nervous system activity. *In situ* hybridization revealed myoclonus-induced c-*fos* mRNA expression only in the cerebellum, pontine nuclei and faintly in motor cortex with the highest levels of expression in the cerebellum. We have refined these results and have found that c-*fos* expression in the cerebellum occurs in the more medial aspects of the cerebellum and is not induced in Crus I or Crus II. Thus, these seizures appear to delimit functionally distinct regions of the cerebellum. We have also previously observed ectopic expression of the catecholamine synthetic enzyme tyrosine hydroxylase (TH) in the Purkinje cells of these mutants (Neuron, 6:123). Interestingly, TH expression is also restricted to the medial regions of the cerebellum. In fact, *in situ* hybridization on back to back sections of the cerebellum revealed that c-*fos* induction during a myoclonic episodes occurs in granule cells adjacent to Purkinje cells abnormally expressing TH. These results suggest that not only is the cerebellum important in the generation and maintenance of myoclonus, but it appears that very specific cerebellar regions are affected by the mutation. Additionally, these results suggest that the abnormal Purkinje cells may directly contribute to the phenotypic expression of the tottering mutation. Supported by a Klingenstein Fellowship.

595.13

FOS IMMUNOLABELING FOLLOWING KINDLED PARTIAL SEIZURES ELICITED FROM DIFFERENT BRAIN SITES. J. Nierenberg*, C.D. Applegate, J.L. Burchfiel & D.T. Piekut. Departments of Neurobiology & Anatomy and Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

Neural circuits activated by kindled partial seizures were studied using FOS immunolabeling techniques to better understand the temporal and spatial organization of kindling epileptogenesis. Male rats received kindling stimulation through electrodes placed in either the amygdala (AM), olfactory bulb (OB) or septal nucleus (SN) until they exhibited the earliest signs of motor seizure (e.g., facial clonus; kindling stage 3). Tissue from these animals showed increased FOS-like immunoreactivity (FLI) in specific brain regions when compared with tissue sections from similarly treated rats kindled to stage 2 (or earlier). Animals kindled to stage 3 consistently expressed FLI in the basolateral/lateral amygdala and in the piriform cortex (PC). Data from our laboratory suggest that the PC is part of the core neuroanatomical circuitry underlying AM, OB and SN kindling. Onset of clonic chewing behavior also correlated with FOS labeling in a region of agranular insular cortex which is involved with the cortical control of rhythmic jaw movements in the rat. A specific laminar labeling pattern in the ipsilateral PC was consistently observed in animals showing stage 3 (or higher) seizure, irrespective of the stimulation site. These and other data indicate that FOS immunostaining is a useful tool for detecting kindling-induced reorganization in discrete neural circuits. Implications for understanding the functional neuroanatomy underlying the transition to motor seizure behavior in kindling will also be discussed.

595.14

ACTIVATION OF CRF-EXPRESSING NEURONS IN HYPOTHALAMUS FOLLOWING EPILEPTIC SEIZURES. D.T. Piekut*, B. Phipps, S. Pretel and C. D. Applegate. Depts. of Neurobiology and Anatomy, and Neurology, University of Rochester, Rochester, NY 14642.

Activation of the pituitary-adrenal axis is clearly a response to several stress paradigms and the CRF-containing neuron of the paraventricular nucleus (PVN) of hypothalamus is the dominant component in the regulation of pituitary ACTH. Accompanying the behavioral manifestations of epileptic seizures are changes in neuroendocrine and vital autonomic functions; yet the effects of epileptic seizueres on hypothalamic neurons are essentially unknown. The immunocytochemical detection of the FOS protein was used to identify neurons activated following generalized epileptic seizures elicited in the kainate injected and kindled Sprague-Dawley rats. A substantial number of activated (FOS expressing) neurons was observed in specific parvocellular components of the PVN in both seizure models; little FOS immunolabeling was seen in the control animals. Dual immmunolabeling procedures and combined immunocytochemical and in situ hybridization methodology have demonstrated that a substantial percentage of activated parvocellular PVN neurons express the CRF protein and the CRF mRNA. Few CRF-containing neurons localized in other brain sites (i.e. amydala, bed nucleus of stria terminalis) express FOS following epileptic seizures. The results indicate that a specific population of hypothalamic CRF-containing neurons are activated following seizure elicitation. This study provides new insights into the effects of epileptic seizures on the central control of the pituitary-adrenal axis in an adaptive response to this paradigm of stress. Supported by NIH grant NS18626

595.15

$GABA_A$ RECEPTOR FUNCTION IN THE THALAMUS AND CORTEX IS ALTERED IN RATS WITH EXPERIMENTAL ABSENCE-LIKE SEIZURES. O. Carter Snead III* and P. K. Banerjee Div. Neurol. Childrens Hospital Los Angeles, Dept. Neurol. Univ. Southern California, School of Medicine, Los Angeles, CA 90027

Both clinical and experimental absence seizures are exacerbated by GABAergic agonists. We investigated $GABA_A$ receptor activity during the course of absence-like seizures induced by γ-hydroxybutyric acid (GHB) in rats using receptor autoradiography. Rat brain sections obtained at different time intervals during GHB-induced seizure activty were subjected to [^{3}H]flunitrazepam and [^{35}S]TBPS binding in the presence or absence of the neuroactive steroid, alphaxalone (ALP, 3mM) or GABA (3mM). At the onset of GHB-induced seizures a transient decrease in [^{35}S]TBPS binding in the thalamus (approx. 25-35%) and cortex (approx. 10-15%) associated with a brief loss of ALP-induced increase in [^{35}S]TBPS binding was observed which returned to basal levels 10 min after the onset of seizures. [^{3}H]Flunitrazepam binding rose in midline thalamic structures (by 30%) and cortex (by 15%) throughout GHB-induced absence seizures. There was a selective loss of ALP-induced enhancement of [^{3}H]flunitrazepam binding in cortex, but not thalamus during the course of seizure activity. These results suggest that $GABA_A$ receptor activity is modified in a subtle fashion during experimental absence seizures induced by GHB. Whether this is the cause or effect of the experimental absence seizures is not clear.

595.16

PARVALBUMIN- AND CALBINDIN-POSITIVE NEURONS ARE SURROUNDED BY SPROUTED MOSSY FIBERS IN THE GRANULE CELL LAYER. T. Kotti, R. Miettinen and P. Riekkinen Sr*.: Dept. of Neurology and A.I. Virtanen Institute, Univ. of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, FINLAND.

Sprouting of the mossy fibers into the supragranular region of the dentate gyrus is a wellknown regenerative process after epileptic insult. No consensus has been achieved so far about whether new circuitries formed as a result of sprouting are excitatory or inhibitory in nature. This study investigated this issue by characterizing the target cells for sprouted mossy fibers using a combination of immunofluorescent and Timm stainings. Two months after the induction of mossy fiber sprouting by kainic acid injection, rats were perfused transcardially with Na-sulfide solution followed by buffered paraformaldehyde. Hippocampal sections were first immunostained for parvalbumin (PV) or calbindin D-28k (CaBP) using FITC labelled antibodies. Then they were stained using Timm sulfide-silver histochemical method. Analysis at the conventional epifluorescent and at the confocal laser scanning microscope revealed that the Timm-positive mossy fibers were in close contacts with the PV- and CaBP-immunopositive neurons within the granule cell layer, through which the sprouted mossy fibers were spreading in the kainic acid injected rats. These results suggest that the PV- and CaBP-containing cells are the targets for the sprouted mossy fibers. Therefore if PV is present in the inhibitory nonpyramidal cells and CaBP in the granule cells sprouting results in formation of both inhibitory and excitatory circuits. However, it should be noted that we cannot rule out the possibility that the CaBP-positive neurons which were found to be surrounded by mossy fibers in the granule cell layer are not CaBP-containing nonpyramidal cells, and thus inhibitory in nature.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS—KINDLING

596.1

GENETICALLY FAST VERSUS SLOW KINDLING RAT STRAINS: COMPARISON OF DIFFERENT KINDLING SITES. M.E. Kelly and D.C. McIntyre*. Psychology Dept., Carleton University, Ottawa, Ontario, Canada, K1S 5B6.

Previously we introduced (*Epilepsia* 1989, **30**, 652) two strains of rats that were selectively bred for their amygdala excitability and disposition to kindling (called FAST and SLOW strains). The SLOW rats exhibited local afterdischarge (AD) durations that were 3 times shorter than the FAST rats throughout all stages of amygdala kindling, and they required 6 times more amygdala stimulations than the FAST rats to develop their first stage-5 convulsion. When kindled from the dorsal hippocampus, however, the SLOW strain exhibited local ADs that were similar to the FAST strain, but they required 2 times more hippocampal stimulations than the FAST strain to reach their first stage-5 convulsion. By comparison, kindling from the frontal cortex resulted in the immediate appearance of forelimb clonic convulsions in both strains, although the local ADs associated with these convulsions were shorter in the SLOW compared to the FAST strain.

These data indicate that the original selection of the two strains for high and low amygdala excitability, associated with fast and slow kindling rates, was relatively specific to the amygdala, since kindling sites in the dorsal hippocampus and frontal cortex showed strain effects that were very different from the amygdala. Further study of the kindling characteristics from each of these 3 different anatomical sites in these two strains may help clarify the functional circuitry involved in the convulsive generalization of limbic kindled seizures.

596.2

BRAINSTEM SEIZURES IN AMYGDALA-KINDLED GENETICALLY EPILEPSY-PRONE RATS (GEPR's). L.L. Coffey, M.E.A. Reith, N.-H. Chen and P.C. Jobe*, Dept. of Basic Sciences, University of Illinois College of Medicine, Peoria, IL 61656.

It is known that repeated exposure to audio-stimulated brainstem seizures in GEPR's results in EEG and convulsive patterns characteristic of forebrain seizures (FBSs). Conversely, kindling of FBSs by repeated electrical limbic stimulation increases the susceptibility to electroshock-invoked brainstem seizures, but does not induce behavioral expression of brainstem seizure activity in non-epileptic rats (NER's) under commonly used conditions. Only sporadic occurrence of brainstem seizures reportedly occurs with limbic kindling in audio-susceptible Wistar rats. In the present study, FBSs were kindled in GEPR's displaying class 9 audiogenic seizures (GEPR9's). A paradigm of daily electrical amygdala stimulation was employed. In comparison with NER's, the current required for afterdischarge was lower in GEPR9's, and kindling to severe (Stage 5) FBSs developed more rapidly. Kindling development was intermediate in seizure-naive (SN) GEPR9's which were not subjected to audio-screening as were GEPR9's. Following the FBS, behavioral expression of brainstem seizure activity (on the average class 6-7 on the same scale used to classify GEPR's) occurred in the majority of GEPR9's and SN GEPR9's but in none of the NER's. The occurrence of these seizures within the kindling process had a random nature and was not related to kindling-dependent FBS progression. The results suggest that the spread of amygdala seizures activates brainstem seizure circuitry in epileptic animals but not in NER's. The lack of difference in brainstem seizure activity between GEPR9's and SN GEPR9's is consonant with the view that the determining factor is the genetic predisposition rather than the preexposure to audiogenic seizures delivered during the screening protocol.

596.3

ANTECEDENT TONE PRESENTATION SIGNIFICANTLY DELAYS RATE OF AMYGDALA KINDLING T. D. Hernandez*, L.A. Warner, P.J. Kahler and A.E. Kline. Department of Psychology, University of Colorado, Boulder, CO 80309

The degree to which external stimuli contribute to seizure occurrence is an important issue. Can external events elicit the aberrant neuronal activity that precipitates a seizure? Alternatively, can certain external events cue the organism to an impending seizure and result in a compensatory response that is in effect "anti-convulsant"? While several studies have addressed these issues (Yoshii & Yamaguchi, 1963; Pinel et. al., 1973; Wyler & Heavner, 1979; Janowsky et. al., 1980; Mostofsky & Myslobodsky, 1982; Myslobodsky et. al., 1983; Freeman & Mikulka, 1986) the results have been mixed. Consequently, we conducted the present study in an attempt to clarify these issues. Twenty-seven male Long-Evans hooded rats were chronically implanted with an electrode in the right amygdala and randomly assigned to one of two groups. The *Tone* group was presented with a 2-second tone beginning immediately prior to and overlapping with the daily kindling stimulus, while the *No Tone* group was kindled without the tone. Presentation of the tone significantly delayed the rate of amygdala kindling. All animals in the *No Tone* group reached a Stage 5 kindled seizure by the 12th daily stimulation, while all animals in the *Tone* group responded with a Stage 5 seizure by the 22nd daily stimulation. These data indicate that the antecedent presentation of a mild auditory cue has a profound inhibitory effect on the rate of kindling.

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596.4

CHEMICAL KINDLING OF EEG EPILEPTIFORM BUT NOT BEHAVIORAL SEIZURE RESPONSE IN TWO RAT STRAINS. J. Rossi III* G.D. Ritchie, M.S. Buring, C. Ademujohn, J. Smith, T. Morton and M. Langley. Naval Medical Research Institute Detachment (Toxicology) and GeoCenters, Inc., Wright-Patterson AFB, OH 45433-7903.

Adult Fischer-344 and Sprague-Dawley rats were given tri-weekly i.p. administrations of the convulsant trimethylolpropane phosphate (TMPP) for eleven weeks. The doses selected had been previously shown to: (a) produce EEG epileptiform activity without behavioral consequence; (b) produce both EEG paroxysms and observable sub-clinical seizure activity; or (c) produce EEG paroxysms and clinical seizures, without lethality. Dose levels were adjusted to accommodate differences in TMPP sensitivity previously observed between the two rat strains. While repeated administrations of TMPP led to significant increases in EEG epileptiform activity over that observed with the initial dose, no kindling of behavioral seizure response was observed. This increase in frequency and duration of the EEG paroxysms persisted for up to three weeks following the final drug administration, even though TMPP has been shown to clear 99.9%+ from major body tissues within 24 hours. Rats that had never exhibited more than forequarter myoclonic seizures during the kindling procedure responded with full-blown audiogenic seizures (wild running with generalized tonic-clonic seizures) when exposed to a 110 dB auditory stimulus up to three months after the final dose of TMPP. This result appears to be related to the phenomenon of sensitization currently being considered in investigation of such ailments as Multiple Chemical Sensitivity (MCS), Sick Building Syndrome (SBS), Chronic Fatigue Syndrome (CFS) and Persian Gulf Syndrome (PGS).

596.5

HYPO- OR HYPERTHYROIDISM DOES NOT AFFECT AMYGDALA KINDLING-INDUCED THYROTROPIN-RELEASING HORMONE (TRH) mRNA INCREASES IN LIMBIC STRUCTURES. S.Y. Kim* and J.B. Rosen Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

Our laboratory has demonstrated that TRH mRNA is dramatically increased following amygdala kindling in limbic structures including dentate gyrus, and pyriform, entorhinal and perirhinal cortices, but not in the paraventricular nucleus of the hypothalamus(PVN). The regulatory mechanisms of kindling-induced TRH mRNA elevation are not known. Since thyroid hormone regulates TRH mRNA in the PVN, we investigated whether kindling-induced TRH mRNA elevations in limbic regions are also regulated by thyroid hormone. Rats were treated with 0.05% propylthiouracil(PTU) (equivalent to ~30 mg/kg/day) or 4 μg/ml triiodothyronine(T_3) (equivalent to ~500 μg/kg/day) in drinking water for 10 days before kindling and throughout the kindling procedure. Rats were sacrificed 4 hours after a fully kindled seizure. The efficacy of oral treatment of PTU and T_3 was confirmed by Nothern blotting hybridization of pituitary thyroid-stimulating hormone(TSH) ß mRNA. TSH ß mRNA was significantly increased by PTU and decreased by T_3, but unaffected by kindling. In addition, in situ hybridization showed that PTU increased and T_3 decreased TRH mRNA in the PVN while kindling had no effect on TRH mRNA in the PVN. In contrast, kindling significantly increased TRH mRNA in dentate gyrus, pyriform, entorhinal and perirhinal cortices regardless of PTU or T_3 treatment. The findings demonstrate that unlike its effects on hypothalamic TRH mRNA, thyroid hormone manipulation does not alter baseline TRH mRNA expression in extrahypothalamic areas and does not affect TRH mRNA expression following amygdala kindling. These data suggest that hypothalamic TRH mRNA and seizure-induced TRH mRNA in limbic structures are regulated by different transcriptional mechanisms.

596.6

EXTENDED KINDLING RESULTS IN SPATIAL LEARNING DEFICITS IN THE RAT. **J.Th. Rick*, S. Cammisuli, C.J. Reid, M.P. Murphy, M. Michaels, J. Ferbinteanu, and N.W. Milgram. University of Toronto, Scarborough Campus, 1265 Military Trail, Division of Life Sciences, Scarborough, ON, Canada M1C 1A4.**

Kindling is a model of epilepsy in which repeated electrical stimulation of various forebrain structures leads to the progressive development of motor seizures. Impaired cognitive functioning, sometimes seen in epileptic patients, has not been observed in kindled animals except when tested shortly after a seizure. Previous kindling studies have reported no long-term deficits in spatial learning, but stimulation was terminated after fewer than 10 class five seizures (Racine Scale). However, more seizures may be required before cognitive deficits arise. In the present study, a total of 12 male Long-Evans hooded rats were stimulated 1-3 times daily through electrodes implanted either in the amygdala (N=5) or perforant path (N=7) until they became spontaneously epileptic or received 300 stimulations. Twelve additional animals with matched electrode placements served as controls. Kindled rats experienced up to 290 class four or greater seizures and 5 of these subjects experienced spontaneous motor seizures. Ten days after the termination of kindling, the rats were given 10 trials per day for 3 days in the Morris water maze. Kindled rats required more trials to reach the learning criterion (3 consecutive trials with escape latencies under 20 s) than controls ($p<0.0003$). The number of major seizures (class 4 and up) was a significant predictor of poor performance ($p<0.003$). These results suggest that extended kindling can lead to a persistent spatial memory deficit in the rat.

596.7

DOES CA1 KINDLING IMPAIR SPATIAL MEMORY IN THE MORRIS WATER MAZE? T.H. Gilbert*, L.L. Armitage, D.K. Hanneson, R.K. McNamara, & M.E. Corcoran. Dept. of Psychology, University of Victoria, POB 3050, Victoria, BC, Canada, V8W 3P5.

The hippocampus has long been known to play an important role in spatial learning, in that interference with hippocampal functioning can produce deficits in tasks that involve spatial learning. Leung (1989) reported that kindling of hippocampal field CA1 disrupts performance in the radial arm maze and that this disruption was caused primarily by hippocampal afterdischarges (ADs) in the absence of behavioral seizures. We asked whether CA1 kindling would disrupt spatial learning in the Morris water maze.

We used two procedures: (1) seizures were kindled with stimulation of CA1 prior to training in the maze (acquisition); and (2) seizures were kindled following maze training, and then retesting in the maze occurred after kindling (retention). In both cases, experimental rats received one daily CA1 stimulation until 3 consecutive generalized seizures were evoked, or until a predetermined number of ADs was reached without eliciting generalized seizures (partial kindling). The rats were tested (or retested) in the maze 24 hrs after the last seizure or last AD.

We found that CA1 kindling failed to disrupt maze performance during either acquisition or retention. The results were similar for both the kindled and partially kindled groups, which demonstrated acquisition and retention performance comparable to that in nonkindled controls. These findings are inconsistent with previous results suggesting that memory deficits occur during or after hippocampal kindling and may indicate that deficits are task-specific.

(Supported by NSERC)

596.8

AMYGDALOID KINDLING BY PENICILLIN IN THE RAT. GLOBAL AND RESTRICTED CORTICAL AREA TOPOGRAPHIC MAPPING OF INTERICTAL SPIKING: EFFECTS OF NALOXONE AND ENKEPHALINS. A. Fernández Guardiola*, R. Fernández-Mas, and A. Martínez. Inst. Mex. Psiquiatría, México 14370, D.F. and Fac. Psicol. UNAM.

A single topical dose of Penicillin (Pn) induces a dose dependent "massed" amygdaloid kindling. Interictal spikes (1-3 cps) appear in 1 or 2 minutes after Pn administration and last for several hours. 20 Wistar male rats urethane anesthetized where used. A cannula-electrode was stereotaxically implanted in the left temporal lobe amygdala, and a bipolar electrode was placed in the right amygdala. For cortical recording and mapping, a 16 epidural isometric electrode (stainless steel) array was implanted covering the whole dorsal cortex (frontal, rolandic, temporo parietal and visual). Mapping of restricted cortical areas was performed with a moveable 4x4 electrode matrix covering 16 mm^2.

After a 10 min control recording Pn doses ranging from 50 to 200 IU / 1 μl were microinjected in the baso-lateral amygdaloid nucleus. Naloxone was IP delivered (0.5-1.0 mg/Kg). D-Ala-Met and D-Ala-Leu Enkephalins were topically delivered to the same amygdala (10 μg/μl). Amygdaloid Pn biphasic spikes with a variable latency and progressive enhanced frequency and amplitude were observed during a period of 35 min. After a plateau (1-3 Hz), a frequency decrease appeared. Spiking frequency and amplitude diminished in about 45 min. (200 IU). Some animals displayed brief (5 to 10 sec) focal or generalized seizures during this period . Naloxone increased the interictal spike frequency and amplitude, and occasionally induced seizures. Both enkephalins inhibited Pn spiking when delivered after Pn. When delivered before Pn, the enkephalins depressed the interictal Pn spiking or avoided the Pn effect.

We conclude that endogenous opioids inhibit the Penicillin GABA receptor action and that this inhibition is blocked by Naloxone.

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596.9

THE TIMING OF PERMANENT ALTERATIONS IN SEIZURE SUSCEPTIBILITY DURING KINDLING. J.L. Burchfiel* and C.D. Applegate. Comprehensive Epilepsy Program, Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642.

When does kindled seizure development become permanent? It is well known that kindling is permanent after stage 5, but are permanent changes induced at earlier stages. Specifically, does each successive afterdischarge induce permanent alterations through the kindling process or does the process become permanent only after some threshold is reached. We designed an experiment to choose between these two possibilities.

Adult Sprague Dawley rats were kindled from the septal nucleus. Different groups of animals received 6, 9, 12, 15, 18 or 21 stimulations, respectively. Then the animals were not stimulated for 4 weeks. After the hiatus, animals were again stimulated until they reached stage 5 seizures.

If each afterdischarge induces a permanent increase of seizure susceptibility, then the hiatus should not affect subsequent kindled seizure development. One would predict that: (1) the total number of trials to reach stage 5 should be the same for each group, and (2) the number of post-hiatus trials should be inversely related to the number of pre-hiatus trials for each group.

The outcome was incompatible with these predictions, suggesting a lack of permanent alteration of seizure susceptibility during early kindling trials. We conclude that there is a threshold for permanent changes during kindled seizure development. Our data are most consistent with this threshold occurring during the transition from stage 2 to stage 3.

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596.10

LATERAL OLFACTORY TRACT IS THE OPTIMAL SITE FOR KINDLING T.I. Netoff(+), W.J. Freeman(+), E. Marg(++)*(spon.). (+) MCB-Neuro,(++) School of Optometry, University of California at Berkeley, Berkeley, CA 94720

In the late 1960s Graham Goddard discovered the phenomena of kindling, and that stimulating the amygdala produced kindling the fastest. For years this remained undisputed until recently when it was discovered that stimulation in the deep layers of the pyriform cortex (PC) caused kindling to occur even more rapidly. This lead us to look at the lateral olfactory tract (LOT) as a stimulus site. We have found that kindling here requires fewer stimulus sessions to bring an animal to stage 5 kindling than stimulating in the amygdala. The olfactory bulb (OB) provides the background excitation for the PC through the LOT. Stimulus of the LOT is more efficient than directly stimulating the PC in two ways: 1) the stimulus in the LOT projects broadly to the PC stimulating a large portion of the PC directly and not just a small area around the electrode; 2) the stimulus in the LOT stimulates the OB antidromically as well, thus altering the dynamics of the limbic system very efficiently. The other change we have made from the standard kindling model is the stimulus duration and intensity. The stimulus is much milder (0.1-0.2 ms duration, 60 Hz, 10-15 V) and impedance has been matched to the LOT so that the largest evoked potentials in the OB and PC are obtained. However, the stimulus requires a longer train (6-10 sec) to elicit a seizure.

596.11

THE KINDLED STATE, BRAIN DAMAGE AND SEIZURE SUSCEPTIBILITY. Craig D. Applegate* and Gary M. Samoriski. Comprehensive Epilepsy Program and Dept. of Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

Several studies suggest that the permanent, kindling-induced increase in seizure susceptibility is not static, but continues to evolve over time. Our studies support this interpretation, and further indicate that implantation of chronically indwelling electrodes significantly contribute to increased seizure susceptibility. Male, C57BL mice were stereotaxically implanted with electrodes into the olfactory bulb and following a 4 day recovery were kindled to a criterion of 6 consecutive stage 5 seizures. Seizure susceptibility to flurothyl (HFE) was tested 2 and 30 days following the last seizure. Both implanted mice tested at the same postsurgical intervals and unimplanted mice served as controls. Kindling significantly lowered HFE thresholds to generalized seizure 2 and 30 days post-kindling in comparison with both control groups. Seizure expression differed at these timepoints, however. Only 1/9 seizures were characterized by tonic manifestations at 2 days, whereas 8/9 exhibited tonic seizures at 30 days post-kindling. Electrode implantation did not significantly alter HFE thresholds at either time point, but significantly altered seizure expression such that 7/7 exhibited tonic seizures at 30 days, but 0/7 at 2 days. Data suggest that damage caused by implantation of chronically indwelling electrodes significantly lowers the threshold for tonic seizure expression.

GP	N	HFE(2)	SZ Type	HFE(30)	SZ type
Kindled	9	301+14	Clonic	264+15	Tonic
Implanted	7	403+28	Clonic	401+42	Tonic
Control	16	442+25	Clonic	422+93	Clonic

HFE(day)=flurothyl test-latency to generalized seizure (sec+SE)
SZ type=seizure type: clonic-forelimb clonus; tonic-fore/hindlimb tonus

596.12

MINIMUM REQUIREMENTS FOR GENERALIZED SEIZURE-INDUCED NEURAL REORGANIZATION INDEPENDENT OF CONTINUED SEIZURE INDUCTION. G.M.Samoriski* and C.D.Applegate. Program in Neuroscience and Comprehensive Epilepsy Program, University of Rochester School of Medicine, Rochester,NY 14642.

We have previously reported that in response to multiple flurothyl-induced generalized seizures, a permanent decrease in the latency to the motor event results. Predominantly clonic seizure behaviors are observed during the course of flurothyl sensitization. However, following a 4 week seizure-free period a significant proportion of animals expressed predominantly tonic seizure behaviors. The goal of this study was to determine the minimum number of seizures necessary to initiate the seizure-free behavioral progression and to characterize the time frame for its development. In the first experiment male C57BL/6J mice experienced 1,2,4,6 or 8 flurothyl-induced generalized seizures separated by 24 hrs. Following a seizure-free period of 28 days the mice were again challenged. Whereas 6 trials were sufficient to induce a permanent decrease in the latency to the sustained generalized event, 8 trials were necessary to effect a significant shift in the behavioral progression. Tonic seizure behaviors were observed in 88% of the animals having had 8 generalized seizures compared to 0%,17%,33% and 33% of animals having had 1,2,4 or 6 events, respectively. In order to define the minimum seizure-free period sufficient to cause the behavioral progression to be manifest, mice were tested 1,2,and 3 weeks following the last of 8 seizures. Generalized tonic seizures were prevalent following 2 (67%), 3 (50%) and 4 (88%) weeks, but not 1 (17%) week, of a seizure-free interval. These data provide a framework for which to explore the mechanisms of generalized seizure-induced neural reorganization that is independent of continued seizure induction.

596.13

DIFFERENTIAL CHANGES IN THE ACTIVITIES OF MULTIPLE PROTEIN KINASE C SUBSPECIES IN THE HIPPOCAMPAL-KINDLED RAT. Kazufumi Akiyama*[1], Mitsuhiro Ono[1], Kimiko Tsutsui[2] and Shigetoshi Kuroda[1]. [1]Department of Neuropsychiatry and [2]Third Department of Anatomy, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, JAPAN.

In previous studies we demonstrated that the membrane-associated protein kinase C (PKC) activities in the right and left hippocampus (HIPP) of rats kindled from the left HIPP increased significantly 4 weeks and 4 months after the last seizure compared with those in matched control rats (Daigen et al, Brain Res 545:131-136, 1991; Kohira et al, Brain Res 593:82-88, 1992). In this study, we investigated the long-lasting effect of HIPP-kindling on the membrane-associated activities of PKC subspecies in the bilateral HIPP one and 4 weeks after the last generalized kindled seizure had occurred. The membrane-associated activities of PKC subspecies were found to be subject to differential regulation. The activity of the α-subspecies was unchanged, whereas the respective activities of the β- and γ -subspecies in the kindled group increased significantly, compared with the controls, one week (21%, $P<0.0001$ for the β-subspecies, and 23%, $P<0.001$ for the γ-subspecies) and 4 weeks (19%, $P<0.02$ for the β subspecies, and 19%, $P<0.05$ for the γ subspecies) after the last seizure. There were no significant differences in cytosolic PKC activity between the control and kindled groups for any subspecies examined at either time after the last seizure. These results suggest that activation of the PKC β- and γ-subspecies may play an important role in the enduring seizure susceptibility associayed with kindling.

596.14

ACETAZOLAMIDE, A CARBONIC ANHYDRASE INHIBITOR, ACTS AS A PROCONVULSANT IN KINDLED RATS. L.J. Burdette* and M.J. Lotharius. Dept of Neurology, Graduate Hospital, Philadelphia, PA 19146

Seizure activity alters extracellular pH which has been shown to influence NMDA and $GABA_A$-mediated currents. Acetazolamide inhibits the reversible hydration of CO_2 by carbonic anhydrase, resulting in more pronounced alkaline shifts during synaptic transmission. The possibility that pH modulation of synaptic transmission may be affected by repetitive seizure activity (kindling model) was tested by comparing input/output (I/O) and paired pulse depression (20 and 200 ms interpulse intervals) functions recorded from the dentate gyrus of kindled rats before and after acetazolamide (10-20 mg/kg, ip) administration. Rats were kindled daily by perforant path stimulation (200-800 µAmps, 0.1 ms, 5 Hz, 9 sec) until 5 consecutive generalized motor seizures were elicited. Acetazolamide had no influence on the kindling-induced potentiation of I/O functions or of $GABA_A$-mediated early paired pulse depression. A dose-dependent decrease was observed, however, in late paired pulse depression. The 5 Hz train duration required to elicit an afterdischarge (AD) also was significantly reduced by acetazolamide. We previously have shown that a failure of late inhibition precedes AD initiation, suggesting that the proconvulsant action of acetazolamide in kindled rats may result from the depression of late inhibition. Indirect support of a similar proconvulsant mechanism for NMDA also has been provided in earlier studies. Together with evidence that NMDA currents are increased at alkaline pH, these findings suggest that kindling may enhance the sensitivity of NMDA-mediated currents to alkaline shifts in the extracellular space, leading to a decrease in late inhibition and enhanced seizure susceptibility. Supported by NIH MH45961 to LJB.

596.15

KINDLING-INDUCED POTENTIATION OF THE EPSP BUT NOT THE POPULATION SPIKE IS BLOCKED BY MK-801. M.E. Gilbert* and C.M. Mack. ManTech Environ. Tech., RTP, NC, 27709.

Kindling produces potentiation of excitatory and inhibitory transmission in the dentate gyrus. It is well recognized that NMDA antagonism retards development of limbic kindling, but its effects on synaptic potentiation have been equivocal. In the present report a crossover design was employed in which kindling stimulation was delivered to the perforant path once daily for 10 days, 30 min following 0 or 1 mg/kg MK-801. Following a rest period, the dose groups were reversed. MK-801 blocked kindling-induced potentiation (KIP) of the EPSP and late paired pulse inhibition (IPI=250 ms). Significant KIP of the PS occurred in both groups by the 4th afterdischarge (AD). Although continued stimulation resulted in suppression of the PS in controls, MK-801 produced an additional augmentation by the 10th AD that declined to control levels during the rest period. This suggests that the potentiation of the PS was transiently augmented above control levels by MK-801. When dose conditions were reversed, MK-801 treated animals demonstrated an increase in PS amplitude above the maximum achieved under saline conditions. The data indicate a dissociation in the NMDA dependence of KIP of the EPSP and PS. Potentiation of the EPSP and late inhibition during kindling, however, is strongly suppressed by NMDA antagonism.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID IX

597.1

DOWN-REGULATION OF AMYLOID PROTEIN PRECURSOR BY ANTISENSE OLIGONUCLEOTIDES REDUCES NEURONAL ADHESION TO LAMININ. E.J. Covison[1,2] G. L. Barett[2] P. F. Bartlett[2] K. Bailey[1] K. Beyreuther[3] and C.L. Masters.*[1] 1. Dept. Pathology, University of Melbourne, Vic, 3052, Australia. 2. Walter & Eliza Hall Institute for Medical Research, Parkville, Vic, 3052, Australia. 3. Center for Molecular Biology, University of Heidelberg, D-69120, Germany.

The Amyloid Protein Precursor (APP) is known as the source of βA4 amyloid, which forms plaques in brains of Alzheimer's disease patients. APP forms a complex of alternately spliced membrane bound and secreted glycoproteins and is a member of a larger APP super-family. APP isoforms are widely expressed, and in the brain products lacking exon 7 (the KPI domain) and containing exon 15 are most prevalent. The normal function of APP and its role in neurite extension and synaptogenisis is not yet clear.

We are investigating the function of APP in neurons using phosphothiate antisense oligonucleotides. We find by cell adhesion assay that, after 24-60 hours, P2 mouse dissociated DRG neuronal cultures exposed to antisense APP oligonucleotides are less adherent to laminin ($p<0.001$) than cultures which are exposed to nonsense oligonucleotides of matched base composition. This change in adherence is not observed on other substrates (plastic, poly-lysine, fibronectin, and matrigel). An effect on neuronal survival, neurite outgrowth, or neurite branching is not observed but this may be due to a 'lag time' of APP down-regulation by the antisense oligonucleotides. Although neuritic networks are formed 16 hours after plating, the maximal effect on reduction in adhesion is after 48 hours, coincident with the patchy expression of APP on the surface of neurons These data are consistant with a role for APP as a mediator of medium-term adhesion of neurons to extracellular matrix, and provide support for the specificity of an interaction of APP with laminin.

597.2

LOCALIZATION OF THE AMYLOID PRECURSOR PROTEIN (APP) AT SYNAPTIC SITES OF THE RAT BRAIN. M. Shimokawa-[1], K. Yanagisawa-[2], H. Nishiye-[3], T. Suzuki-[4], J. Ikeda*-[5], T. Yokota-[1], M. Yamada-[1], T. Kobayashi-[1], S. Ishiura-[6] and T. Miyatake-[1]. [1]Dept. of Neurol., [5]Dept. of Physiol. Chem. Tokyo Med. & Dent. Univ. Tokyo 113, [2]Institute for Brain Res. Univ. of Tokyo, Tokyo 113, [3]Dept of Physiol. Juntendo Univ., Tokyo 113, [4]Dept. of Biochem., Nagoya City Univ. Med. Sch. Nagoya 467, [6]Institute of Molec. and Cellular Biosci. Univ. of Tokyo, Tokyo 113, Japan.

Alzheimer's disease is a progressive neurodegenerative disorder characterized by extracellular amyloid β/A4 protein (Aβ) deposits and the loss of neurons and synapses. Aβ is a 39-42 amino acid peptide derived from the larger membrane-associated glycoprotein, termed amyloid precursor protein (APP). APP is expressed in most mammalian tissues, especially in neurons, and it shows a high degree of evolutionary conservation. But the functional significance of APP has been subject to conjecture. To define the potential role of APP in the brain, we investigated the localization of APP at synaptic sites. We purified synaptic plasma membrane (SPM), synaptic vesicles (SV) and the post synaptic densities (PSD) from Sprague-Dawley rat brain, and examined them by immunoblot analysis using two specific antibodies against N-terminal or C-terminal of APP (gifts from Dr. Yasuo Ihara, the University of Tokyo). We now present that 105kDa full-length APP is localized at SPM, and that PSD contains C-terminal truncated APP. Immunoreactivity of APP was drastically decreased in SV. These data indicate that APP may have roles in physiological synaptic activity.

597.3

AMYLOID ß PROTEIN PEPTIDE (25-35) STIMULATES THE ACCUMULATION OF ALZHEIMER AMYLOID PROTEIN PRECURSOR VIA TAU PROTEIN KINASE I IN CULTURED HIPPOCAMPUS NEURON. A. Takashima*[1], H. Yamaguchi[2], K. Ishiguro[1], K. Noguchi[1], T. Hoshino and K. Imahori[1]. *[1]. Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, [2]. College of Medical Care and Technology, Gunma University, 3-39-15 Showa-Machi, Maebashi, Gunma 371, Japan*

Pathological changes of Alzheimer's disease (AD) are characterized by cerebral cortical atrophy as a result of degeneration and loss of neurons. Typical histological lesions include numerous senile plaques composed of deposits of ß-amyloid (Aß) and neurofibrillary tangles consisting predominantly of ubiquitin and highly phosphorylated tau proteins. The exogenous application of synthesized amyloid ß protein (Aß) induced the neurotoxicity in the cultured hippocampus. Recently, we found that some proteins induced by Aß treatment leads to a programmed cell death in cultured hippocampus, and that tau protein kinase I (TPK I) played a role in the Aß induced neuronal death. Now we tried to identify the protein which was altered by Aß treatment in the cultured hippocampus neuron. The level of amyloid protein precursor (APP) in cytoplasm increased by 10 folds of control level in response to exogenous application of Aß peptide (25-35). The pretreatment of TPK I antisense oligonucleotide inhibited the Aß induced APP accumulation and neuronal death. These results were interpreted as that TPK I regulated the increased APP accumulation in cytoplasm due to Aß treatment, and that the increased APP accumulation in cultured hippocampus neuron might contribute to a process of Aß induced neuron death.

597.4

ECTO-PROTEIN KINASE IN BRAIN NEURONS: A TARGET FOR ALZHEIMER' B-AMYLOID PEPTIDES. M. V. Hogan*, Z. Pawlowska, H-A. Yang and Y.H. Ehrlich. CSI/IBR Ctr. Dev. Neurosci., CUNY at Staten Island, NY 10314 and the Biol. Doc. Prog. of CUNY Grad. Sch.

Ecto-protein kinase (ectoPK) can serve as a direct target for neuroactive factors that operate extracellularly. To study the effect of amyloid peptides on this activity, we have used primary neuronal cultures prepared from the telencephalon of 7-day chick embryos. The endogenous substrates of ectoPK on these neurons were identified as proteins with MW of 116K, 105K, 67K, 17K, 13K and 12K. The surface phosphorylation of the 12K and 13K proteins is catalized by an ectoPK with the specificity of PKC, and is maximal during de-novo neurite outgrowth. The β-amyloid peptide 1-40, when used at neurotoxic concentrations, inhibited the phosphorylation of the 12K and 13K surface proteins (over 80% inhibition of the phosphorylation of the 13K protein by 0.1μM β-amyloid peptide 1-40). At the same concentration, the β-amyloid peptide 1-28, that does not have neurotoxic effects, did not inhibit this phosphorylation. On the other hand, the neurotoxic β-amyloid peptide 25-35 selectively inhibited the phosphorylation of the 12K and 13K proteins by ecto-PKC, at the same concentration range (0.1-25μM) in which it produces neurodegeneration in cultured neurons. At nanomolar, neurotrophic concentrations, β-amyloid 25-35 stimulated this activity. Thus, the direct regulation of *extra*cellular- PKC can mediate both the neurotrophic and neurodegenerative actions of β-amyloid peptides. Supported by HD28788

597.5

FURTHER ANALYSIS OF THE BRAIN Na^+/Ca^{2+} EXCHANGER IN ALZHEIMER'S DISEASE. R.A. Colvin*, N. Davis, A. Wu, C.A. Murphy, and J. Levengood. Department of Biological Sciences, Ohio University College of Osteopathic Medicine, Athens, OH 45701

The Na^+/Ca^{2+} exchanger was characterized in plasma membrane vesicles derived from frontal and temporal cortex and cerebellum of control and Alzheimer's disease (AD) frozen postmortem tissues. Na^+/Ca^{2+} exchange activity was defined as the change in vesicular Ca^{2+} content seen after Na^+ loaded vesicles were diluted into choline buffer. The time course of changes in Ca^{2+} content after dilution was similar in all three regions of control brain. In AD brain, both frontal and temporal cortex vesicles showed elevated Ca^{2+} content, most evident as an increased peak Ca^{2+} content at 2 minutes. The AD cerebellar cortex time course was similar to control and did not show an elevated peak at 2 minutes. No differences were seen in the passive permeability to Ca^{2+} when comparing plasma membrane vesicles prepared from control and AD brain. Vesicles from the frontal and temporal cortex of AD brain showed increases in the Vmax of the initial velocity of Ca^{2+} uptake when compared to control brain, whereas, the cerebellum did not. There were no significant effects of AD on the Km for Ca^{2+} activation of the initial velocity. Ca^{2+} influx measured during the rise in vesicular Ca^{2+} content was elevated in vesicles from AD temporal cortex when compared to control. Two known inhibitors (exchange inhibitory peptide and dichlorobenzamil) of the cardiac Na^+/Ca^{2+} exchanger inhibited the human brain exchanger equally well in control and AD vesicles. An antibody to the cardiac exchanger was used to determine the molecular weight of the human brain Na^+/Ca^{2+} exchanger. The molecular weight determinations from western blots showed identical molecular weights of 100-110 kDa in both AD and control vesicle preparations. The data suggest that increased Na^+/Ca^{2+} exchange activity in AD brain is related to the causative factors of neurodegeneration and lends support to the "Ca^{2+} hypothesis of AD".

597.6

THE INHIBITORY EFFECT OF ß-AMYLOID PEPTIDE ON RAT BRAIN NA/CA EXCHANGE. Anfan Wu, Robert A. Colvin and Venugopal Janapati*. Dept. Biological Sciences, Ohio University College of Osteopathic Medicine, Athens, OH 45701 USA.

These studies were performed to determine the effect of addition of Alzheimer's disease ß-amyloid peptide (ß-AP) on Na^+/Ca^{2+} exchange activity. Na^+/Ca^{2+} exchange activity was measured in plasma membrane vesicles (PMV) purified from rat brain. The effect of synthetic peptide ß-AP_{25-35} and scrambled (ß-AP_{sc}) were examined. PMV were preincubated with each peptide for 15 minutes(37°C) in buffer containing 137 mM NaCl, 10 mM HEPES(pH 7.4), and protease inhibitors. Ca^{2+} uptake was initiated by diluting PMV 20 fold with buffer containing either 137 mM NaCl or 137 mM cholineCl and $^{45}CaCl$. Ca^{2+} uptake was terminated by addition of 5 mM $LaCl_3$ and rapid filtration. ß-AP_{25-35} inhibited Na_i^+-dependent Ca^{2+} uptake with an IC_{50} of 800 μM (concentration measured in the preincubation buffer) but it had no effect on the Na_o^+-dependent Ca^{2+} efflux. When ß-AP_{25-35} was added in the dilution buffer instead of during the preincubation step, the same inhibitory effect was observed. No inhibitory effect was seen when ß-AP_{sc} was tested under both conditions. The inhibitory effect of ß-AP_{25-35} could not be overcome by increasing Ca^{2+} concentration. Analysis of unidirectional flux for Ca^{2+} during the initial phase of rapid uptake or after a stable plateau had been obtained showed that 800 μM ß-AP_{25-35} inhibited both Ca^{2+} influx and efflux with the same efficacy (approximately 50 % inhibition) during both time periods. These results suggest that the inhibitory effect of ß-AP_{25-35} is irreversible, and that it inhibits both Na^+/Ca^{2+} exchange and Ca^{2+}/Ca^{2+} exchange activity by directly interacting with the exchanger.

597.7

AMYLOID BETA-PEPTIDE (AβP) POTENTIATES A CALCIUM CURRENT IN NIE-115 NEUROBLASTOMA CELLS. Lydia Shaienko*, Theodore S. Donta and Robert M. Davidson. Neuroscience Program, Department of Periodontology and the Travelers Center on Aging, University of Connecticut Health Center, Farmington, CT 06030.

One of the hallmarks of Alzheimer's disease (AD) is the presence of extracellular senile plaques composed primarily of aggregated amyloid β-peptide (AβP) surrounded typically by dystrophic neurites. It has been hypothesized that some aggregate form of AβP manifests its toxicity on neurons by forming membrane channels thereby destabilizing neuronal Ca^{2+} homeostasis. We have used patch-clamp recording techniques to study the effects of AβP (1-40) on cation currents in differentiated mouse NIE-115 neuroblastoma cells (Davidson et al., 1994). In whole-cell recordings, incubation of cells with AβP for 24 h significantly increased the median peak inward current from -201.8 pA to -352.0 pA, and shifted the voltage at peak current (V_{peak}) and that of current activation (V_{act}) towards more positive potentials. For untreated cells, median V_{peak} was 1.7 mV and V_{act} was -28.9 mV vs. 10.5 mV and -24.7 mV in AβP-treated cells. (Incubation with the reverse sequence AβP(40-1) did not produce significant changes in the amplitude or kinetic behavior of inward current). At the single channel level, AβP added to the pipette increased the open probability of cation-conducting ion channels. As determined by cell viability counts, AβP(1-40) had neurotoxic effects; within 24 h, addition of AβP reduced the number of cells by more than 50%. It is suggested that the neurotoxic effects of AβP may be mediated by its ability to form cation channels *de novo* and/or alter the activity of cation channels already present in the cell membrane. We are in the process of developing a liposomal model system with a lipid composition similar to that of the neuroblastoma cell to analyze AβP's membrane perturbation properties.

597.8

ELECTROPHYSIOLOGICAL STUDY OF AMYLOID β PROTEIN ACTIVITY IN RAT CORTICAL NEURONS. K. Furukawa*, M.P. Mattson, and N. Akaike Sanders -Brown Research Center on Aging and Department of Anatomy and Neurobiology, University of Kentucky, Lexington KY 40536.

The effect of amyloid β-protein 25-35 (AβP) was examined in neurons freshly dissociated from rat cortex using the nystatin perforated patch-clamp technique. AβP at concentrations higher than 10^{-8} M induced an irreversible slow inward current associated with an increase in membrane conductance at a holding potential of -50 mV. The time lag until the appearance of the effect of AβP shortened concentration-dependently, whereas the increase in membrane conductance did not. When the extracellular Na^+ and Cl^-, and intracellular K^+ were replaced by equimolar NMG, isothionate$^-$, and Cs^+, respectively, the membrane conductance and the reversal potential were not affected. Even when Ca^{2+} was removed from the extra- and intra-cellular space by using an Ca^{2+}-free external solution and an internal solution including BAPTA (20 mM), the effect of AβP did not alter. It is suggested that AβP elicits an inward current either by forming membrane pores as suggested by Arispe et al., or by causing dysfunction of ion channels as suggested by our recent finding that AβP can damage ion-motive ATPases by a free radical-mediated mechanism (see Mark and Mattson, this meeting).

597.9

AMYLOID β-PROTEIN DISRUPTS Na-K-ATPase ACTIVITY IN CULTURED RAT EMBRYONIC CORTICAL NEURONS. R.J. Mark*, S.A.Walker, and M.P. Mattson. Sanders-Brown Center on Aging and Dept. of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536

Amyloid β protein (Aβ) is a major component of the plaques that are a hallmark of Alzheimer's Disease. A large literature has shown that Aβ can be neurotoxic, but little has been published on the mechanism through which Aβ kills neurons. Here we report that treatment of embryonic rat cortical cells in culture with Aβ (1-40) or Aβ (25-35) inactivates the ouabain sensitive ATPase. Cultures were exposed to Aβ (10μM-200μM), membranes were prepared, and ATPase activity was assayed in 96-well microtiter dishes using a method adapted from Rohn et al (Biochem. Pharm. 46:525-34). We found that there is a reduction in the Na-K-ATPase activity with no reduction in the non-ouabain sensitive, non-calcium dependent ATPase activity. Co-treatment of cultures with Vitamin E (50μg/ml) blocks the Aβ reduction in ATPase activity, indicating that mechanism of impairment is mediated by free radicals. By a seperate assay we also find no reduction in the activity of the plasma membrane Na/Ca exchanger at concentrations of Aβ that are neurotoxic. We are currently examining the contribution of impairment of Na-K-ATPase activity by Aβ to loss of calcium homeostasis and cell death.

597.10

PROPERTIES OF CHLORIDE CHANNELS IN PC12 CELLS INDIRECTLY MODULATED BY ALZHEIMER'S DISEASE B-AMYLOID. R.J. Pearce*, R. Fukuyama, G. Ehrenstein, S.I. Rapoport and Z. Galdzicki. LNS, NIA and CNB, NINDS, NIH, Bethesda, MD 20892.

A pathological feature of Alzheimer's disease (AD) is the formation of plaques in the parenchyma and vasculature of the brain. β-Amyloid peptide (β-AP) is one of the defining components of these plaques.

We have previously shown from cell-attached patch recordings that β-AP (1-40) increases the activity of channels inherent to PC12 cells (Pearce et al, 1994, Biophys. J. 66:A430). We have now managed to characterize these channels further. Under conditions of symmetrical choline chloride (120 mM) and high calcium (10 mM $CaCl_2$), in excised patches, we could resolve two main conductance levels of 100 and 300 pS. Both had reversal potentials (RP) around 0 mV under these conditions. Exchanging the major cation on the extracellular face to Na^+ had no significant effect on the RP. However, reducing the chloride to 30 mM from 150 mM, by replacement with acetate resulted in a positive shift in the RP, irrespective of whether choline or Na^+ was the major cation. This implicated chloride as the charge carrier for this channel, but the shift was less than expected indicating poor selectivity.

The fact that both conductances were seen to be effected in the same way by these ion substitutions and that both levels were invariably seen in any one patch, means it is likely that we are observing one channel which can have differing conductance levels.

The mechanism by which β-AP or patch excision increases the activity of this channel remains unclear. A possible mechanism may be linked to the ability of β-AP to interfere with calcium homeostasis. Alternatively, disruption of cytoskeletal components linked to the channel may be involved.

597.11

NO APPARENT EFFECT OF β-AMYLOID PEPTIDES ON M1 MUSCARINIC RECEPTOR BINDING OR FUNCTION. C.M. Reever and D.D. Flynn*, Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

While accelerated β-amyloid (βA) deposition has been suggested to play an early and critical role in the pathogenesis of Alzheimer's disease (AD), the precise cellular actions of βA are unclear. The complexity of βA actions are evidenced by the opposing neurotrophic and neurotoxic actions which are dependent on peptide concentration and the presence of soluble vs. fibrillar forms. On the basis of limited sequence homology to substance P and tachykinin peptides, βA has been suggested to interact with tachykinin receptors and function as an allosteric modulator of the ionotropic NMDA receptor. Substance P-related peptides also appear to function as G protein antagonists, uncoupling receptors from G protein-mediated responses. Cortical M1 muscarinic receptor (MR) high-affinity agonist binding and receptor-stimulated, G protein-mediated phospholipase C activity are diminished in AD, resulting from an apparent receptor-G protein uncoupling. In order to test the hypothesis that βA may function as a MR-G protein "uncoupler" in AD, we have assessed the effects of amyloid peptides on MR binding and functional activity. βA peptides (1-40, 25-35; 0.01-100 μM) had no effect on binding of the muscarinic antagonist, [^{3}H]-NMS, to membranes from CHO cells transfected with the m1 receptor, when incubated *in vitro* for up to 18 hr at 4° C or for 2 hr at 25° C, or in culture for 20 hr. Agonist binding as well as basal and carbachol-stimulated [^{35}S]GTPγS binding also were unaffected by peptide under all conditions tested. These findings suggest that βA does not antagonize MR-G protein interaction and that MR dysfunction in AD is not a consequence of excessive βA accumulation. (Funded by NS19065 & the Alzheimer's Association)

597.12

BETA-AMYLOID AMPLIFIES CALCIUM SIGNALLING AND INFLUENCES PHOSPHOINOSITIDE HYDROLYSIS IN CENTRAL NEURONS FROM THE ADULT MOUSE H. Hartmann, A. Eckert, D. Watson,*+ F.T. Crews+ and W.E. Mueller. Central Institute of Mental Health, Dept. Pharmacology, 68159 Mannheim, Germany, +Dept.Pharmacology, College of Medicine, University of Florida, BOX 100267, JHMHC, Gainesville, Florida 32610-0267.

The role of β–amyloid (βA) in Alzheimer's disease and its cellular mechanisms of action are still unclear. Based on observations that βA influences neuronal calcium homeostasis in cultured neuronal cells we investigated the effect of βA on KCl-induced enhancement of free intracellular calcium (Ca_i) and on phosphoinositide- (PI) hydrolysis in dissociated brain cells from adult female NMRI mice. For measurement of Ca_i cell aggregates were loaded with fura-2 AM (10μM) and fluorescence measured with a spectrofluorometer. For determination of PI-hydrolysis cell aggregates were loaded with ^{3}H-myo-inositol and stimulated with the agonist in the presence of LiCl (10mM). Preincubation with βA fragment 25-35 at concentrations <0.05μM resulted in a marked amplification of the KCl-induced calcium response. This effect was also observed with fragment 1-40, whereas fragment 1-28 or 12-28 did not affect the response. The amplification was restricted to depolarization with lower KCl concentrations whereas maximal stimulation (KCl 80mM) was not affected. Preliminary data suggest that βA fragment 25-35 (10nM) enhances KCl (20mM) and carbachol (1mM)-stimulated PI-hydrolysis. A more detailed characterization of these mechanisms will be presented. In conclusion, these findings indicate a small but consistent destabilizating effect of βA on the neuronal calcium homeostasis in adult neuronal cells, which may also result in increased activity of the Phospholipase C system. This will not only lead to an increased vulnerability of neurons to other neurotoxic stresses (e.g. ischemia, glucose deprivation, EAA), but will also influence neuronal signal transduction.The latter mechanism may contribute to memory loss and may also influence amyloid precursor metabolism.(Supported by P0I AG 10485).

597.13

IMPAIRED OXIDATION INDUCED BY THIAMINE DEFICIENCY CAUSES β-AMYLOID PRECURSOR PROTEIN ACCUMULATION IN RAT BRAIN. N. Calingasan[1]*, S. Gandy[2], H. Baker[1], K.-F. R. Sheu[1], S. G. Greenberg[1] and G. Gibson[1], [1]Cornell University Medical College at Burke Medical Research Institute, White Plains, NY 10605 and [2]Cornell University Medical College, New York, NY 10021.

β-amyloid, a fragment of the amyloid precursor protein (APP), accumulates in the brains of patients with Alzheimer's disease (AD) or Down's syndrome. As in AD, thiamine deficiency (TD) is characterized by selective cell loss, decreased thiamine-dependent enzymes and memory deficits. APP increases in the brain in animal models of central nervous system injury. The current study examined ultrastructural changes as well as β-amyloid and $APP^{645-694}$ immunoreactivity in brains from thiamine-deficient animals. Rats received thiamine-deficient diet *ad libitum* and daily injections of the thiamine antagonist, pyrithiamine. Three, 6 and 9 days of TD did not damage any brain region nor change APP immunoreactivity. However, 13 days of TD produced pathological lesions in the thalamus, mammillary body, inferior colliculus and some periventricular areas. Electron microscopy revealed swollen, degenerating neurites with accumulations of abnormal mitochondria, tubulovesicular and filamentous structures in the thalamus. While β-amyloid immunocytochemistry, thioflavine S staining and electron microscopy failed to show fibrillar β-amyloid, APP accumulated in perikarya and aggregates of swollen, dystrophic neurites along the periphery of the lesion in the thalamus. Immunoblotting of the thalamic region around the lesion confirmed increased immunoreactivity of APP holoforms. In addition to the thalamus, APP-immunoreactive neurites were scattered in the mammillary body, medial geniculate and medial vestibular nuclei. In the inferior colliculus, increased perikaryal immunostaining occurred in neurons surrounding necrotic areas. Regions without apparent pathological lesions showed no alteration in APP immunoreactivity. Thus, the oxidative insult from TD alters APP metabolism. This model may be useful in defining the role of APP in the response to brain injury, and in clarifying the relationship between oxidative changes and altered APP metabolism, both of which occur in AD. (Supported by NIH Grant MH-48325).

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SYMPOSIUM. THE CELL BIOLOGY OF SECRETION VESICLES. K.J. Skinner, NIDA/NIH (Chairperson); J.E. Rothman, Memorial Sloan Kettering Cancer Ctr.; R. Kelly, Univ. of Calif., San Francisco; M. Wightman, Univ. of North Carolina, Chapel Hill; A. Fox, Univ. of Chicago.

Different types of vesicles may mediate slow and fast neuronal communication, thus contributing to significant behavioral differences. This symposium will explore the cell biology associated with these two signalling pathways. J.E. Rothman will discuss intracellular protein transport, focusing on the molecular mechanisms associated with vesicle docking, activation, and fusion. R. Kelly will present results from studies investigating secretory vesicle recycling after exocytosis. Post-fusion endocytotic steps will be reviewed, as well as endosomal differences and signal sequences which sort proteins to vesicular targets. M. Wightman will describe studies using carbon-fiber microelectrodes to resolve release of catecholamines from individual vesicles. Kinetic differences in release between synaptic and dense core vesicles will be discussed, as well as evidence related to release mechanisms from dense core vesicles. A. Fox will consider the concept that differential coupling of specific channels may contribute to different rates of transmitter or hormone release, focusing on results suggesting that different Ca^{2+} channels regulate secretion depending upon the level of electrical activity of the cell.

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SYMPOSIUM. NEUROPEPTIDE REGULATION OF BRAIN DEVELOPMENT. E. DiCicco-Bloom, UMDNJ/Robert Wood Johnson Med. Sch. (Chairperson); J.P. Schwartz, NINDS, NIH (Co-chairperson); S. Denis-Donini, CNR Ctr. Cytopharmacol. & Biology Dept., Milan; I. Gozes, Sackler Sch. of Med.

Studies suggest that neuropeptides regulate the component processes of neural ontogeny, including proliferation, differentiation, and survival, as well as neurotrophin function and behavioral maturation. Peptides and receptors are expressed transiently in specific regions, and act as auto-/paracrine or trans-synaptic signals. The complexity of peptide biosynthesis, transport and metabolism provides greater opportunities for signal integration over time and space, characterisitics well-suited for governing long-term processes. E. DiCicco-Bloom will define roles of PACAP in regulating neurogenesis of diverse populations: sympathetics, cerebellar granule cells, and cerebral cortex. PACAP regulates all three principal mechanisms governing stable population growth including 1) neuroblast division 2) survival of dividing precursors, and 3) promotion of long-term survival via *trk* regulation. S. Denis-Donini will describe the role of afferent neuron CGRP in regulating differentiation of dopaminergic olfactory neurons. CGRP protein and mRNA, which are developmentally regulated, appear at highest levels in afferent terminals, suggesting local peptide synthesis. J.P. Schwartz will focus on somatostatin and enkephalin ligand-receptor systems, which are expressed transiently during postnatal cerebellar ontogeny. Normal development depends on a balance of actions, since SOM stimulates differentiation whereas ENKs are inhibitory, acting via diminished astrocytic NGF expression. I. Gozes will describe hybrid and lipophilic VIP agonists/antagonists that are tissue specific and distinguish pathways mediating neurotropism and neurotransmission. Antagonists in vitro inhibit neural division and produce neuronal death, whereas disruption of VIP by antagonists or transgenic methods, leads to deficits in behavior, circadian rhythms, sexual performance, and learning/memory. Thus, neuropeptides affect diverse brain regions. Interactions between peptides, as well as with classical transmitters or neurotrophins, greatly expand possible integrative signals mediating long-term developmental processes.

602.1

PARKINSONIAN AND NORMAL ACCURACY IN A POINTING TASK WHEN TASK INFORMATION IS REDUCED. W. A. Hening*[1,2], M. Rolleri[1], and J. Gordon[3]. Neurology Depts., VAMC[1], Lyons, NJ & UMDNJ-RWJohnson Med Sch[2], New Brunswick, NJ; and Ctr for Neurobiology & Behavior[3], Columbia Univ, NY, NY

We previously reported that well-trained Parkinsonian subjects can show normal accuracy in a simple planar pointing task even if they are deprived of information about their unfolding trajectories and knowledge of the results of their movements (Neurosci Abstr 18:935). It has been often suggested that Parkinsonian subjects require such information to sustain normal performance. However, such dependence might be due to task complexity or to the need to learn an unfamiliar transform. We therefore tested four groups of subjects: practiced normals (PN, N=3), practiced Parkinsonians (PP, N=1), unpracticed normals (UN, N=3), and unpracticed Parkinsonian subjects (UP, N=4). After a brief practice at making movements to match four computer screen targets requiring 8 to 32 cm excursions of the draped arm over a digitizing tablet, subjects were required to respond to randomized targets in two experimental blocks. In the first, they saw the target throughout each trial, were given instantaneous feedback, and received knowledge of results. In the second, none of this information was provided. Both NP and PP subjects sustained accuracy when deprived of information, but neither UN nor UP subjects did so: both groups undershot the larger target distances, the UP subjects much more so (for the largest target, UP, 47.5% vs. UN, 27.3%). We conclude that Parkinsonian patients have a relative inability to master a sensorimotor transform and perform under reduced information conditions rather than an absolute inability to do so.

This research supported by the Dept. of Veterans Affairs Medical Research Council.

602.2

PARKINSONIAN DOMAINS AND RISK OF DEATH IN A COMMUNITY POPULATION. D.A. Bennett*, L.A. Beckett, A.M. Murray, D.A. Evans. Rush Alzheimer's Disease Center, Chicago, IL 60612

Previous work suggests a relation between parkinsonian signs and increased mortality in older persons. This study investigates the association between individual parkinsonian domains and mortality among persons in a geographically defined community population. 3622 persons (80.8%) of residents over the age of 65 had a structured interview in their homes. 467 persons (70.8% of survivors) identified by a stratified random sampling technique underwent a uniform neurologic examination including the presence or absence of 3 measures of gait disturbance, 3 of bradykinesia, 4 of rigidity, and 2 of tremor. A parkinsonian domain was defined as the presence of at least half of the signs within the domain. Proportional hazards models were used to compare the risk of death among persons with and without each domain. After adjusting for the effects of age and sex, the overall relative risks of death and 95% confidence intervals were: gait disturbance 2.36 (1.83-3.04), bradykinesia 1.72 (1.32-2.24), rigidity 1.02 (0.79-1.32) and tremor 1.12 (0.86-1.47). Of the four parkinsonian domains, gait disturbance and bradykinesia are most strongly associated with mortality.

Supported by NIA AG10161, AG05362, AG06789

602.3

DECREASED [123I] ß-CIT SPECT STRIATAL UPTAKE CORRELATES WITH DISEASE SEVERITY IN IDIOPATHIC PARKINSON'S DISEASE J. Seibyl*, K. Marek, E. Smith, B. Sandridge, B. Fussell, S. Zoghbi, R. Baldwin, P. Hoffer, R. Innis Departments of Diagnostic Radiology, Neurology, and Psychiatry, Yale University School of Medicine, New Haven, CT 06510 USA

Our previous studies have utilized SPECT to demonstrate decreased [123I] ß-CIT striatal uptake in idiopathic Parkinson disease patients (PD). The present study extends this preliminary work by examining 1) the test-retest reproducibility of two SPECT measures (ratio specific striatal uptake:nonspecific uptake [V3"] and striatal uptake expressed as the percentage of injected dose) in healthy subjects and, 2) striatal binding using these measures in PD patients of varying disease severity. **Methods**: For the test-retest reproducibility study 7 healthy subjects (age range 19-74) had SPECT brain scans at 18, 21, and 24 hr following the intravenous injection of 10 mCi of [123I] ß-CIT. Repeat SPECT scans were performed 7-14 days following the first examination. For the PD severity study, a total of 26 idiopathic PD patients (age range 41-78, Hoehn-Yahr stages I-V) had SPECT scans 18, 21,and 24 hr after i.v. injection of 10 mCi of [123I] ß-CIT. The two measures were age-corrected based on our healthy control population and correlated with Hoehn-Yahr and total Unified Parkinson's Disease Rating Scale (UPDRS) scores. **Results**: The healthy subject study demonstrated excellent test-retest reproducibility of both outcome measures with a mean 6.8 and 6.7% differences in V3" and percent striatal uptake, respectively. In the PD study binding abnormality was correlated with both Hoehn-Yahr ratings and total UPDRS score for striatal V3" (r^2= 0.345 p= 0.0016 and r^2=0.408 p= 0.0004, respectively) and percent striatal uptake (r^2=0.33 p= 0.0081 and r^2=0.463 p= 0.001, respectively). These data suggest both [123I] ß-CIT SPECT outcome measures have a high degree of within-subject reproducibility and are strongly correlated with disease severity in idiopathic PD.

602.4

[I-123]-ß-CIT BINDING IN THE HUMAN BRAIN: SPECT STUDIES IN PARKINSONIAN PATIENTS AND NORMAL CONTROLS

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The cocaine analog 2-ß-carbomethoxy-3-ß-(4-iodophenyl)-tropane (ß-CIT) is a ligand which binds with high affinity to DA and 5HT transporters. In its [123I] labeled form it can be used for SPECT. Previous studies have shown that the binding of this ligand in the striatum is specific to the DA transporter, whereas binding in the brainstem seems to be due to the 5HT transporter.The purpose of the present study is to characterize the uptake of ß-CIT in the human brain, to compare the findings in normal controls with a group of PD patients and to correlate SPECT measurements with clinical findings. SPECT was performed in 11 controls and 37 patients with PD. 111 to 185 MBq of [I-123] ß-CIT were administered i.v. and images obtained at regular intervals after injection (2, 4, 16, 20 an 24 hrs) with a three-headed scintillation camera (Siemens Multispect). Regions of interest were drawn in the striatum, the brainstem and the cerebellum and the results expressed either as target - cerebellum (specific binding) or as ratio target /cerebellum. Binding kinetics showed a maximum of specific binding in the striatum after 20 hrs, whereas maximal binding in the brainstem was reached at around 4 hrs. Results in 37 patients with PD clearly demonstrate the loss of dopaminergic nerve endings especially in the putamen with a marked reduction of the striatal/cerebellar ratio after 20 hours (controls 8.7±1.7, PD 4.4±1.1, mean±SD). In patients with hemiparkinson (n=15) a clear and highly significant side to side difference is found with a reduction of striatal ßCIT binding which is more marked contralateral to the clinically affected side, but also reduced in the ipsilateral striatum . A good correlation is found between the severity of clinical symptoms according to Hoehn and Yahr and the reduction of ßCIT binding (p=0.0001). These data suggest that PD can be clearly diagnosed with this ligand, the dopaminergic cell loss quantified and hopefully a preclinical or very early diagnosis made possible. Such studies might also open the way for a better evaluation of neuroprotective strategies in PD.

602.5

SPECT STUDIES WITH ß-CIT IDENTIFY DOPAMINE GRAFTS.

D. E. Redmond, Jr.*, E.O. Smith, R. H. Roth, J. D. Elsworth, J.R. Taylor, J.R. Sladek, Jr.†, R.B.Innis, J.P.Seibyl and P.B.Hoffer. Yale Univ. Sch.Med., New Haven CT. 06510; †Chicago Med. Sch, N.Chicago, IL 60064

^{123}I-ß-CIT (2ß-carbomethoxy-3ß-[4-iodophenyl]tropane) binds dopamine (DA) transporter sites with high affinity and may assess DA-related behavioral function better than techniques using ^{18}F-fluorodopa. SPECT studies, obtained 18 hours after ^{123}I-ß-CIT administration in African green monkeys, correlated with anatomical regional averages of postmortem DA concentrations (0.91, n=13, p<0.001) and with a detailed score of parkinsonism (-0.81,n=13, p<0.001).

We obtained SPECT scans 18 hours after ^{123}I-ß-CIT administration in African green monkeys before and twice after they received intrastriatal grafts of fetal mesencephalon. Percent of administered ^{123}I-ß-CIT dose was determined in a region of interest including the striatum and a circumferential background region.

Developing grafts were identified by ^{123}I-ß-CIT SPECT *in vivo* and confirmed by autoradiography and tyrosine hydroxylase immunohistochemistry. Over a 7 month period, mean changes in per cent administered ^{123}I-ß-CIT dose in 4 untreated normals and in 6 non-transplanted MPTP-depleted monkeys were insignificant. Four grafted monkeys with robust histologically confirmed grafts showed mean increases of 70% ±22 (SD). The largest increases corresponded with histological evidence of the most robust grafts. Three monkeys (identified as transplanted for the purpose of blinding the analyses) either had no surviving grafts or were sham operated. They showed no change in striatal uptakes (-12% ±27 [SD]) from their study before transplantation.

SPECT imaging with CIT appears to provide an *in vivo* measurement of the integrity of striatal dopamine systems comparable to direct *in vitro* measurements of DA levels and DA transporters and to detect changes induced by dopamine-producing grafts. *Supported by NS20432, Axion Research Foundation, St. Kitts Biomedical Research Foundation, Strichman Medical Equipment, and RSA MH00643 to DER.*

602.6

ANTIOXIDANT ENZYMES IN AN ANIMAL MODEL OF PARKINSON'S DISEASE C. Thiffault*, R. Quirion and J. Poirier. Douglas Hospital Research Centre, Dept. of Pharmacology and Therapeutics, McGill Centre for Studies in Aging, McGill University. Montreal, Que, Canada H4H 1R3

MPP$^+$, a metabolite of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), induces an irreversible Parkinson's disease (PD) like syndrome. MPP$^+$ toxicity involves the inhibition of complex I activity in the mitochondrial respiratory chain. Similar respiratory deficits are reported in PD. In addition, free radicals are thought to play a role in the mechanism of MPP$^+$ toxicity. They can induce lipid peroxidation leading to an alteration in calcium homeostasis and subsequent neuronal death. The major antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GLU-PX) and catalase (CAT). Lipid peroxidation and SOD activity were found increased in PD brains. To date, it is not clear whether alterations in antioxidant enzymes are responsible for the increase in lipid peroxidation and/or respiratory chain inhibition reported in PD. L-deprenyl was used to up-regulate SOD activity in the striatum (ST) and substantia nigra (SN) of mice. Our results indicate that chronic administration of l-deprenyl (10mg/kg, every 2 days), MPTP (3X20mg/kg, every 2 hrs) and l-deprenyl/MPTP induce a three-fold increase in the activity of SOD in the ST of C57BL/6 mice. No concomitant increases in GLU-PX or CAT is observed while striatal lipid peroxidation was decreased. Although an increase in CAT activity and a reduction in SOD and GLU-PX activity were detected in the SN, no significant change in lipid peroxidation was observed. First, our results indicate that a mild alteration in the activity of antioxidant enzymes does not result in an increase in lipid peroxidation. Second, inhibition of the respiratory chain by MPP$^+$ leads to an adaptive increase in SOD activity. We postulate that an alteration in SOD activity in PD is likely the result of respiratory chain inhibition. *Supported by FCAR studentship.*

602.7

DOPAMINE-DERIVED DIHYDROXYISOQUINOLINE INDUCES PARKINSONISM TO RAT M. Naoi*[1], W. Maruyama[2], P. Dostert [3], D. Nakahara[4], Y. Hashizume[5], [1]Dept. Biosciences, Nagoya Inst. Technol., Nagoya, [2] Dept. Neurol. Nagoya Univ. Sch. Med., Nagoya, [3]Farmacia-Carlo Erba, Milan, Italy, [4]Dept Psychol. Hamamatsu Univ. Sch. Med. , Hamamatsu, and [5] Inst. Med. Sci. Aging, Aichi Med. Univ. , Nagakute, Japan

The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine suggests that endogenous and xenogenic neurotoxins may cause cell death of dopamine neurons in the substantia nigra and thus induce in the Parkinson's disease in humans. As neurotoxin candidates, dopamine-derived 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines (DHTIQs) have been examined on their effects on dopamine neurons. Out of DHTIQs, 1-methyl-DHTIQ (salsolinol, 1-MeDHTIQ) is methylated to 1,2(N)-dimethyl-DHTIQ in the brain, which was found to be oxidized into 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion (1,2-DiMeDHIQ$^+$). 1-MeDHTIQ and its derivatives were injected in the striatum of adult male Wistar rat, and the biochemical, behavioral, and pathological changes were examined. Both (R) and (S)enantiomer of 1-MeDHTIQ and 1(S),2-DiMeDHTIQ did not induce any behavioral change by a single administration. 1(R),2-DiMeTIQ elicited behavioral changes to the rat; akinesia, twitching, postural abnormality and rigidity were detected. The behavioral changes were transit; they disappeared several hours after the injection. Three day after the injection, the rat was sacrificed and DHTIQ derivatives and monoamines were analyzed by HPLC-electrochemical and fluorometric detection. Biochemical analysis showed that 1(R),2-DiMeTIQ was detected in the striatum, and larger amount of the oxidized product, 1,2-DiMeIQ$^+$ was identified in the substantia nigra in addition to the striatum. Dopamine, noradrenaline, an their metabolites were found to be reduced in the striatum and the substantia nigra. The injection of 1,2-DiMeIQ+ induced massive necrosis in the rat brain. Using a human dopaminergic neuroblastoma SH-SY5Y cells, only 1(R),2-DiMeDHTIQ was confirmed to be taken up to the cells by dopamine-transported, among dihydroxy-isoquinoline derivatives. These results suggest that 1(R),2-DiMeDHTIQ may be neurotoxic to dopamine neurons.

602.8

NICOTINE PREVENTS EXPERIMENTAL PARKINSONISM IN RODENTS

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We have demonstrated that diethyldithiocarbamate (DDC) enhances MPTP toxicity in mice. This combined treatment induces a more marked depletion of striatal dopamine (DA) and a severe loss of tyrosine hydroxylase (TH) positive perikaria in Substantia Nigra pars compacta (SNpc) (Corsini et al., Eur. J. Pharmacol.:119,127; 1985). This represents a reliable model of experimental parkinsonism in rodents on which neuroprotective molecules can be evaluated. For instance, it is now well established that non-competitive NMDA antagonists (MK-801, dextromethorphan) provide a complete protection on DDC enhancement of MPTP toxicity.
In the present study we used this model in order to evaluate the effect of nicotine, on experimental parkinsonism. (-) Nicotine (1mg/Kg, s.c.) administered three times (90 and 30 min before and 30 min after MPTP), completely prevented both the marked depletion of striatal dopamine and the severe loss of TH positive perikaria in SNpc induced by DDC+MPTP. Nicotine provided this protective effect without reducing striatal MPP+ levels measured at several time intervals after MPTP administration.
Despite previous contrasting findings, our data suggest that nicotine could be responsible of the reduced prevalence of Parkinson's disease among smokers.

602.9

ELEVATION OF NEURONAL MAO-B ACTIVITY IN A TRANSGENIC MOUSE MODEL DOES NOT INCREASE SENSITIVITY TO THE NEUROTOXIN MPTP.

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To examine whether expressing high levels of monoamine oxidase (MAO-B) activity abberently in neurons results in increased sensitivity of dopaminergic neurons to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 8 week old transgenic mice expressing high neuronal levels of MAO-B were compared with age-matched nontransgenic littermates following i.p. injections of 30 mg/kg body weight of the protoxin. Levels of striatal dopamine (DA) and its metabolite 3,4 dihydroxyphenylacetic acid (DOPAC), as well as tyrosine hydroxylase (TH)-immunopositive cell numbers in the substantia nigra (SN) were compared 1 week later between transgenics and controls. No difference was found in any of these parameters, indicating that high neuronal MAO-B levels does not cause increased sensitivity to MPTP, and therefore neither conversion of MPTP to its active form, 1-methyl-4-phenyl pyridium (MPP+) by MAO-B nor MPP+ uptake by the dopaminergic transporter are likely to be the rate-limiting step in the toxicity of this compound.

602.10

AAV-MEDIATED TRANSFER OF TYROSINE HYDROXYLASE AND AROMATIC AMINO ACID DECARBOXYLASE GENES INTO THE PRIMATE BRAIN: A DIRECT GENE THERAPY APPROACH TO PARKINSON'S DISEASE

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AAV is a defective human parvovirus which has several major advantages for gene transfer in the CNS including non-pathogenicity, targeted integration and stable expression in post-mitotic cells including neurons (Kaplitt et al., Soc. Neurosci. Abstr., 1994). Moreover, AAV vectors are devoid of viral genes and a novel packaging method to generate helper-free stocks is established. This vector system may have application therefore for gene therapy of neurological disorders. The human tyrosine hydroxylase (form II) cDNA was truncated to 1.1 kb by deletion of the N-terminal regulatory region and a FLAG epitope added (FLAG-THΔ). FLAG-THΔ together with the human aromatic amino acid decarboxylase gene was packaged into AAV using the emc virus IRES sequence to from a bicistronic construct (AAVthIRESaadc). AAVthIRESaadc-transduced HEK 293t cells released dopamine into the medium at high concentrations (3.1±1 ng/10^6 cells min^{-1}). AAVthIRESaadc was stereotactically injected into the partially denervated caudate of MPTP-treated African green monkeys. 10 days following injection, two animals were sacrificed and expression of the vector encoded TH was determined using a FLAG antibody. FLAG-IR neurons were observed around the injection sites. These data suggest that AAV can transduce neurons in the primate brain *in vivo*. If dopamine release can be increased by this strategy AAVthIRESaadc may have utility as a direct gene therapy approach to Parkinson's disease.

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602.11

Adeno-associated virus (AAV) vectors yield safe delivery and long-term expression of potentially therapeutic genes in the adult mammalian brain M.G. Kaplitt[1,*], P. Leone[2], A. Freese[3], X.Xiao[4], D.W. Pfaff[1], K.L. O'Malley[5], M.J. During[2]. [1]The Rockefeller University, New York, NY. [2]Yale University School of Medicine, New Haven, CT. [3]University of Pennsylvania School of Medicine, Philidelphia, PA. [4]University of North Carolina, Chapel Hill, NC. [5]Washington University School of Medicine, St. Louis, MO.

AAV vectors are non-pathogenic, integrating DNA vectors in which all viral genes are removed and helper virus is completely eliminated. Previous gene transfer studies have used either recombinant viruses which retain viral genes or defective HSV vectors in which residual helper virus cannot be removed. To evaluate the AAV system in the post-mitotic cells of the mammalian brain, a vector containing the lacZ gene (AAVlac) was injected into rat striatum, hippocampus and substantia nigra. lacZ DNA and gene expression were detected up to 3 months post-injection. A second vector expressing human tyrosine hydroxylase (AAVth) was injected into the denervated striatum of unilateral 6-hydroxydopamine-lesioned rats. TH immunoreactivity was observed in striatal neurons and glia for up to 4 months. There was significant behavioral recovery in lesioned rats treated with AAVth versus AAVlac controls. A vector expressing both TH and aromatic acid decarboxylase (AADC) has been created, and the efficacy of this vector in lesioned rodents in comparison with AAVth is under investigation. Safe and stable gene transfer into the denervated striatum may have potential for the genetic therapy of Parkinson's Disease.

603.1

DEVELOPMENTALLY SPECIFIC RNA EDITING IN AMPA RECEPTOR SUBUNITS GluR-B, -C, -D H. Lomeli[1], T. Melcher[1], T. Höger[2], A. Bach[2], G. Köhr[1]*, M. Higuchi[1], R. Sprengel[1], H. Monyer[1], and P.H. Seeburg[1]. Center for Molecular Biology[1] (ZMBH), University of Heidelberg, and BASF Inc.[2], Ludwigshafen, Germany

In glutamate-operated receptor channels of the non-NMDA type in central neurons several functionally critical channel residues not encoded by the genes for the channel subunits are introduced via nuclear RNA editing. This process operates on the primary gene transcripts to substitute single adenosines for guanosines. Positional selectivity and efficiency of RNA-editing appear to rest on a double-stranded RNA structure formed by the to-be-edited exonic sequence and a complementary sequence in the proximal intron [Higuchi et al., Cell 75, 1361; 1993]. Detailed cDNA analysis has now revealed that AMPA receptor subunits GluR-B, C and -D share a position either occupied by arginine (gene-encoded) or by glycine, the latter residue being introduced by site selective adenosine to guanosine RNA editing. As for the Q/R site editing of GluR-B, editing at this newly-identified site is mediated by an intronic sequence element present in the GluR-B, -C, and -D genes but is absent in the GluR-A gene. A PCR-supported analysis in brains of staged rats showed that RNA-editing at the new site progresses in extent from approximately 10% at E14 to >80% by P12. Furthermore, single-cell PCR analysis suggests that individual subunits expressed in one cell can be edited to different degrees.

603.2

RNA EDITING AND Ca^{2+} PERMEABILITY OF THE HIGH-AFFINITY KAINATE RECEPTOR IN EMBRYONIC CHICK α-MOTONEURONS. D.Lowe* and D.O. Smith. Neuroscience Training Program, University of Wisconsin , Madison, WI 53706.

The high-affinity kainate (KA) receptor subunit GluR6 undergoes RNA editing at two sites in TM1 and at one site in TM2. To determine the extent of this editing in α-motoneurons, RT PCR-generated cDNA encoding GluR6 from embryonic chick motoneurons and an embryonic chick cDNA spinal cord library were subcloned into M13mp18RF-DNA. In the recombinant plaques, the nucleotide sequences in the TM1 and TM2 regions were then determined. All (n=33) of the transcripts were fully edited. Since homomerically expressed GluR6 is more permeable to Ca^{2+} when fully edited, we measured motoneuron Ca^{2+} permeability during KA application in either high-Na^+ or high-Ca^{2+} extracellular solution and with intracellular Cs^+. In whole-cell recordings, we found that 1-mM KA activated comparable currents in both solutions and that the reversal potential is more positive in the Ca^{2+}/Cs^+ solution than in the Na^+/Cs^+ solution. Edited GluR2 is expressed in these neurons, and AMPA (1-mM) does not activate Ca^{2+} permeability. We conclude, therefore, that the edited form of GluR6 may determine the Ca^{2+} permeability of KA receptors in chick motoneurons. Supported by NIH grant NS13600.

603.3

REGULATION OF N-METHYL-D-ASPARTE RECEPTOR SUBUNITS EXPRESSION IN CEREBELLAR NEURONS. B. Lambolez, E. Audinat, S. Charpak, J. Rossier* and F. Crépel, IAF CNRS, 91198 Gif/Yvette, France; Lab. Neurobio. CNRS, 91405 Orsay, France and Dpt. Physiol. CMU, 1211 Genève 4, Switzerland.

The N-methyl-D-aspartate (NMDA) subtype of glutamate receptors is an oligomer formed of different subunits named NR1 and NR2A, B, C and D the combination of which determines the channel functional properties. We have used rat cerebellar slice cultures to study the involvement of spontaneous activity and extracerebellar inputs on the changes of NR2A-C expression observed during the postnatal development of granule and Purkinje cells. The functional properties of the NMDA receptors and the expression of the NR2A-C mRNAs were correlated in single neurons by coupling patch-clamp recording and reverse transcription followed by PCR. Granule cells grown in standard culture conditions expressed mainly NR2A mRNAs when examined after15 to 40 days in vitro. Consistent with this observation, their responses to NMDA were poorly reduced by 3 μM ifenprodil, a non-competitive antagonist which discriminates NR2A and NR2B subunits in expression systems. The NR2C subunit, abundantly expressed in vivo by adult granule cells, was only rarely detected in slice cultures even when excitatory synapses were formed between granule cells and mossy fibers originating from co-cultured brainstem explants. In cerebellar cultures which had been chronically exposed to tetrodotoxin during 15 to 35 days in vitro to reduce spontaneous activity, granule cells expressed predominantly NR2B subunits (as it is the case only during the first two postnatal weeks in vivo) and their responses to NMDA were largely inhibited by 3 μM ifenprodil. These results provide evidence that the expression of NR2A and B subunits is regulated through an activity-dependent mechanism leading to the formation of NMDA receptors with different pharmacological properties. Finally, in none of the experimental conditions described above, could we detect any NR2 subunit in Purkinje cells, although NR1 was consistently expressed. This was in agreement with the absence of NMDA responses.

603.4

EFFICIENT FUNCTIONAL EXPRESSION OF NMDA RECEPTORS REQUIRES HETEROMERIC ASSEMBLY. A.M.J. VanDongen*, M.W. Wood, and H.M.A. VanDongen. Dept of Pharmacology, Duke Univ Med Cntr, Durham NC27710.

NMDA receptors are ligand gated ion channels, thought to consist of five subunits surrounding a central ion conducting pore. The NR1 subunit forms functional homomeric channels following expression in *Xenopus* oocytes. The four isoforms of the NR2 subunit only form functional channels when co-expressed with NR1. In contrast, expression of NR1 subunits in mammalian cells (HEK293, CHO, PC12) does not result in functional channels. Also, NR1 mRNA is found at high levels throughout the brain, even in neurons which do not contain functional NMDA channels (i.e. cerebellar Purkinje cells). We have optimized expression levels of NMDA receptors by removing the 5' untranslated region (5'UTR) of the ε1 cDNA clone, which encodes the mouse isoform of NR2A. Where expression of NR1 alone results in 50 nA of glutamate induced current (at -80 mV), co-expression of NR1 with this shortened cDNA construct (ε1-Δ5UT) resulted in 50 μA of glutamate current, enhancing functional expression 1000-fold. The original full length ε1 enhanced functional expression only 10-fold, suggesting that the 5'UTR hampers efficient translation of this NR2 subunit in oocytes. The absence of the 5'UTR also boosted the *in vitro* translation of ε1 in a reticulocyte lysate system, where the shortened construct resulted in a much higher yield of a 180 kD protein. Western analysis using a NR1 specific monoclonal antibody demonstrated that the same amount of NR1 protein was present in oocytes expressing NR1 alone or NR1+ε1-Δ5UT. This result rules out an effect of the NR2 subunit on the translation efficiency of NR1. Preliminary results using noise analysis suggested that the homomeric NR1 channels have a normal single channel conductance. Immunohistology and protease protection assays are currently being used to determine the localization of NR1 subunits in homomeric and heteromeric assemblies.

603.5

GENERATION OF ANTIBODIES SPECIFIC FOR PHOSPHORYLATED NMDA RECEPTORS. W.G. Tingley and R.L. Huganir*. Department of Neuroscience, H.H.M.I, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

A variety of neuronal processes, including synaptic plasticity, synaptogenesis, and excitotoxic neuronal damage, involve N-methyl-D-aspartate (NMDA) receptors. NMDA receptors in the brain are thought to be composed of multiple subunits, including at least one NR1 subunit and one or more NR2 subunits. A variety of studies have suggested that NMDA receptors are regulated by protein phosphorylation. We have shown previously that protein kinase C (PKC) directly phosphorylates NR1 near its C-terminus, in a region regulated by alternative splicing. For this study, we have generated multiple anti-phoshopeptide antibodies recognizing the NR1 protein only when it is phosphorylated. Immunocytochemical and immunoblot experiments with these antibodies indicate that cAMP dependent protein kinase (PKA), like PKC, phosphorylates NR1 near its C-terminus. However, PKA and PKC phosphorylate different serine residues within this region. In addition, phosphorylation in this region appears to regulate the subcellular distribution of NR1 protein expressed in HEK-293 cells. These antibodies should prove useful for monitoring the phosphorylation state of NR1 during a variety of neuronal processes, in vivo and in vitro, including the induction and maintenance of LTP in the hippocampus.

603.6

Biochemical and Topological Analysis of the NMDA Receptor

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We are interested in the structure function relationship of the NMDA receptor that is thought to be important in learning, memory and several neurological disorders. We want to analyze the properties of distinct subunit compositions by biochemical and electrophysiological methods in a defined artificial system.

Fourteen regio specific antibodies have been produced to characterize the NMDA receptor subunits NR1, NR2A and NR2C. Eight stable HEK 293 cell lines expressing single NMDA receptor subunits (NR1a, NR1b, NR2A and NR2C) or subunit combinations (NR2A/1a, NR2A/1b, NR2C/1a, NR2C/1b) were generated.

The double transfected cell lines showed distinct morphology and were sensitive to glutamic acid in the medium. We found a strong upregulation of NR1 protein expression when the NR2 protein was present, whereas the NR2 protein amount was similar in the presence or absence of NR1. In this defined artificial system, in which standard promotors are driving the receptor gene transcription, the observed upregulation should be posttranscriptionally. These findings could be explained by an increased degradation of single subunits or a translational regulation of NR1 protein expression.

The transmembrane topology of the NR2C subunit was investigated with antibodies specific for the N-terminal (NR2C-A), putative internal loop (NR2C-D) and C-terminal (NR2C-E) sequences. Anti NR2C-A and anti NR2C-E were able to detect the receptor protein on the surface of the NR2C or NR2C/1a transfected HEK293 cell lines. Anti NR2C-D stained the NR2C subunit only in punctured or triton permeated cells. These results are direct experimental evidence that the four transmembrane domain model suggested first for the ionotropic glutamate receptor is correct. Models suggesting a cytosolic localization of the entire C-terminal domain are not in agreement with these results.

Another approach for the direct protein chemical characterization of the NR1b subunit is based on its overexpression in the baculovirus system. The recombinant protein is probably present in inclusion bodies and found at a concentration of approximately 1 mg per liter of culture medium.

603.7

TOPOLOGY OF GLUTAMATE RECEPTORS: NEW INSIGHTS FROM N-GLYCOSYLATION SITE EPITOPE-TAGGING. M. Hollmann, C. Maron, and S. Heinemann*. Mol. Neurobiol. Lab., Salk Institute, La Jolla, CA 92037.

The transmembrane domain (TMD) topology of glutamate receptors (GluRs) is incompletely understood. Although GluRs are commonly modeled after the nicotinic acetylcholine receptor (nAChR), no direct evidence is available for the presumed four TMD model with extracellular N- and C-termini and an ion channel-forming TMD II, and even the assignment of the four hydrophobic domains has been debated (see Hollmann & Heinemann, *Annu. Rev. Neurosci.* 1994,17:31-108).

We set out to obtain experimental evidence for extracellular or intracellular localization of the various domains of the GluR1 protein expressed in *Xenopus* oocytes. We used site-directed mutagenesis to introduce N-glycosylation consensus sites (N-X-S or N-X-T) at various positions throughout the receptor protein. N-glycosylation sites were created in the N-terminal and C-terminal domains as well as in those domains located in between hypothetical TMDs. Mutant GluR1 proteins expressed by *Xenopus* oocytes were analyzed in SDS gel shift assays, for an increase in apparent molecular weight caused by the attachment of carbohydrate side chains. GluR1 was visualized on Western blots of SDS gels using polyclonal anti-GluR1 antisera (kindly provided by Bob Wenthold and Richard Huganir). Upward shifts of mutant receptors vs. wild type receptor were interpreted as evidence for the extracellular localization of the introduced N-glycosylation site.

The results of the gel shift assays demonstrate that the N-terminus of GluR1 is indeed extracellular as assumed in all models proposed to date, while the C-terminus is intracellular. Our data also indicate that the domain between TMD III and TMD IV, which generally was assumed to constitute a large intracellular loop, is actually extracellular. The topology in between TMD I and TMD III is currently under investigation.

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603.8

IDENTIFICATION OF A CaM-KINASE II REGULATORY PHOSPHORYLATION SITE IN NON-NMDA GLUTAMATE RECEPTORS. J.L. Yakel*#, P. Vissavajjhala, V.A. Derkach, D.A. Brickey & T.R. Soderling. Vollum Institute, OHSU, Portland, OR 97201.

Glutamate receptor (GluR) ion channels are colocalized in postsynaptic densities with CaM-kinase II, an enzyme which has previously been shown to phosphorylate and strongly enhance non-NMDA GluR currents in cultured rat hippocampal neurons (McGlade-McCulloh et al., 1993, *Nature* **362**:640). We wanted to extend these findings by demonstrating the regulatory effect of CaM-kinase II on expressed non-NMDA (i.e. AMPA-type) GluRs, and to identify the regulatory site phosphorylated by CaM-kinase II. In this study, the intracellular application of activated CaM-kinase II into cultured mammalian 293 cells expressing GluR6 or into *Xenopus* oocytes expressing GluR1 enhanced the GluR current activated by kainate. This effect of CaM-kinase II was further investigated in the *Xenopus* oocyte/GluR1 system which gave a 1.5-fold increase in kainate current with no effect on the sensitivity of the receptor to kainate. When Ser627, which is located in the major intracellular loop of GluR1, was mutated to Ala, the expressed mutant no longer responded to the CaM-kinase II-induced enhancement of kainate current but was normal with respect to current-voltage relationship and kainate sensitivity. Since all non-NMDA GluR ion channels have a Ser in an analogous position, this regulatory phosphorylation site may be of general importance in enhancing postsynaptic responsiveness as occurs in long-term potentiation.

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603.9

IBOTENIC ACID ACTIVATES A Ca^{2+} MODULATED CHANNEL IN CEREBELLAR PURKINJE NEURONS. L. M. Nowak*, C. Poulopoulou, H. Bao, and D. Paupardin-Tritsch. Dept. of Pharmacology, Cornell Univ., Ithaca, NY 14850.

Ibotenic acid acts as an ionotropic (NMDA and nonNMDA) and metabotropic excitatory amino acid agonist in vertebrate brain. In mouse cultured Purkinje neurons, cells which do not respond to NMDA, ibotenate evokes relatively small inward currents at negative membrane potentials compared to AMPA and kainate. In whole cell recordings ibotenate-activated currents were observed to increase in magnitude when the extracellular Ca^{2+} concentration ($[Ca^{2+}]o$) was increased from 1 to 10 mM, suggesting that these channels are modulated by Ca^{2+} ions. To determine which of the nonNMDA channels might underlie this phenomenon, the effects of changing $[Ca^{2+}]o$ on ibotenate- activated single channel currents were examined in outside-out patches from Purkinje neurons. Recording pipettes typically contained 140 mM CsCl, 4 mM ATP-Mg, 10 mM Hepes-K (pH 7.2) and 10 mM EGTA/1 mM Ca^{2+}, but occasionally 2 mM BAPTA/0.1 mM Ca^{2+} was used. In the standard extracellular solution (150 mM NaCl, 2.8 mM KCl, 1 mM CaCl2, 10 mM Hepes-Na (pH 7.2)), ibotenate opened channels with slope conductances of ~20, 30 and 50 pS that reversed polarity near 0 mV. Of these, the 30 pS channel observed in more than 90% of patches from Purkinje cells, was studied in different $[Ca^{2+}]o$ from 1 mM, to between 5 and 110 mM while substituting for Na^+. In 5 mM $[Ca^{2+}]o$ the open probability (Po) of ibotenate channels increased 1.5 to 2-fold. Changing from 1 to 110 mM $[Ca^{2+}]o$ (150 mM to 0 Na^+), Po increased 6-fold at –60 mV with relatively little change in channel conductance, the result being between a 3.5 and 5.5-fold increase in the net flux through the channels. In time-averaged single channel records the mean current at –60 mV approximately doubled by increasing $[Ca^{2+}]o$ from 1 to 10 mM; a similar result was obtained in whole cell recordings. These observations were made by increasing $[Ca^{2+}]o$, however, the possibility remains that the increase in Po is due to an increase in $[Ca^{2+}]i$.

603.10

ODORANT INDUCED PHOSPHORYLATION OF INOSITOL 1,4,5-TRIPHOSPHATE RECEPTOR IN OLFACTORY RECEPTOR NEURONS. *T.Ingi* J.P. Steiner and G.V. Ronnett. Johns Hopkins University School of Medicine, Department of Neuroscience, Baltimore, MD 21205.*

Both the cyclic adenosine 3', 5'-monophosphate and phosphoinositide second messenger systems are involved in olfactory signal transduction, which occurs in the cilia of olfactory receptor neurons. In olfactory receptor neuron, inositol 1,4,5-triphosphate (IP_3) receptor is located to the surface plasma membrane of olfactory cilia and play roles in regulating calcium flux at the surface membranes. To explore its involvement in odorant signal transduction, the phosphorylation properties of IP_3 receptor were studied in cultured olfactory receptor neuron. Odorant remarkably enhanced IP_3 receptor phosphorylation with its peak at 2 minutes after its stimulation. Further exposure of odorants induce dephosphorylation to the previous level. This result indicates the modification of IP_3 receptor function in the late phase of odorant signal transduction. Dose-response curve revealed the maximal odorant effect at the concentration of 100 nM. These results may provide a mechanism whereby desensitization occurs in odorant perception.

603.11

HETEROMERIC OLFACTORY CYCLIC NUCLEOTIDE-GATED CHANNELS: A NEW SUBUNIT THAT CONFERS INCREASED SENSITIVITY TO CAMP. J. Ji, J. Bradley, N. Davidson, H. Lester* and K. Zinn. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

One mechanism of olfactory signal transduction involves the opening of cyclic nucleotide-gated (cng) channels. Cng channels in rat olfactory neurons are activated by cAMP in the low μM range and are outwardly rectifying. The previously cloned channel (rOCNC1), however, shows much lower cAMP sensitivity and very weak rectification when expressed in 293 cells. We have cloned another cng channel subunit (rOCNC2) from a rat olfactory epithelium cDNA library. rOCNC2 encodes a protein of 575 amino acids with 51% identity to rOCNC1. It does not form functional channels when expressed alone. When rOCNC2 is coexpressed with rOCNC1, however, the resulting conductance is characterized by an outward rectification stronger than that of rOCNC1; and by a cAMP sensitivity close to that of the native channel. Extracellular Ca^{2+} and Mg^{2+} block the inward current at rOCNC1 channels more strongly than at rOCNC1/rOCNC2. In divalent-free conditions, the openings of rOCNC1 channels are uninterrupted for tens of ms, while those of rOCNC1/rOCNC2 channels are flikery, especially at negative membrane potentials. This flikery behavior resembles the native channel. *In situ* hybridization demonstrates that the two subunits are coexpressed in olfactory receptor neurons. These results indicate that the native olfactory cng channel is likely to be heteromeric, and that the cng channels in olfactory neurons are structurally and functionally analogous to those in rod photoreceptors.

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603.12

ELECTROPHYSIOLOGICAL PROPERTIES OF LIGAND-GATED ATP RECEPTOR IN RAT AUTONOMIC NEURONES AND VAS DEFERENS SMOOTH MUSCLE. A. Surprenant*,B .Khakh and C.Gill Glaxo Institute for Molecular Biology, Geneva, Switzerland, CH 1228

P2X purinoceptors exist in autonomic neurones and many vascular and visceral smooth muscle where they can mediate fast synaptic transmission by activating a cationic receptor-channel complex. We used whole-cell recording techniques to compare properties of this receptor in rat nodose and SCG neurones as well as in isolated rat vas deferens smooth muscle. Fast-flow application (0.5 - 2 s duration) of ATP, αβmeATP and 2MeSATP evoked rapid inward currents in all cells; marked desensitization of the current was observed in smooth muscle but not in neurones. Calcium permeability was significant in vas deferens and nodose neurones ($P_{Ca}/P_{Na} \approx 5$) but minimal in SCG neurones. Inward rectification of the current was observed in all cells but was most pronounced in vas deferens and SCG. In all neurones, agonist potency was 2MeSATP ≥ ATP > αβmeATP. Half-maximal effective concentration (EC50) of ATP was about 1 μM for nodose and vas but 40 μM for SCG; αβmeATP was a full agonist in nodose (EC50 = 3 μM) but acted as a partial agonist in SCG. Properties of the ATP response in nodose and vas deferens are very similar those observed for the P2X receptor recently cloned from rat vas deferens (Valera et al., this meeting). The different pharmacological and physiological properties of the ATP response in SCG neurones suggests the presence of a distinct receptor subtype.

603.13

STIMULATION OF [^{3}H]SEROTONIN RELEASE IN THE CEREBRAL CORTEX VIA NMDA AND NON-NMDA RECEPTORS AND INTERACTION WITH PRESYNAPTIC INHIBITORY AUTO- AND HETERORECEPTORS. M. Göthert*, K. Fink, C. Böing and V. Schmitz. Inst. Pharmacol. Toxicol., Univ. Bonn, Reuterstrasse 2b, 53113 Bonn, Germany.

The aims of the present investigation were to provide evidence that in rat and guinea-pig cerebral cortical slices, [^{3}H]serotonin ([^{3}H]5-HT) release can be stimulated via glutamate receptors of the NMDA and non-NMDA type and that this release is modulated via presynaptic auto- and heteroreceptors. The slices were preincubated with [^{3}H]5-HT in the presence of maprotiline (in order to avoid false labeling of noradrenergic neurons) and superfused with Krebs' solution (Mg^{2+}-free in the experiments with NMDA). NMDA (0.1-1 mM) stimulated the overflow of [^{3}H]5-HT from slices of both species in a manner sensitive to blockade by 0.3 μM tetrodotoxin (TTX), 1.2 mM Mg^{2+}, 3-100 μM CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid; a competitive NMDA receptor antagonist), 100 nM dizocilpine, 10-100 μM 5,7-dichlorokynurenic acid, 100-300 μM arcaine and 0.1-1 μM ifenprodil. Kainic acid (KAI; 0.01-1 mM) also stimulated [^{3}H]5-HT overflow. The KAI-evoked overflow in the presence of 1.2 mM Mg^{2+} was not affected by CGP 37849 and arcaine, but was inhibited by 0.3 μM TTX and 100 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; an antagonist of non-NMDA receptors). 5-Carboxamidotryptamine (1-100 nM; a 5-HT_1 receptor agonist) and norepinephrine (1 and 10 μM) inhibited the NMDA-evoked overflow; these responses were antagonized by the 5-HT receptor antagonist methiothepin and the α_2 receptor antagonist idazoxan, respectively, which given alone facilitated [^{3}H]5-HT overflow. The A_1 purinoceptor antagonist DPCPX (10 nM; 8-cyclopentyl-1,3-dipropylxantine) also increased the overflow. In conclusion, 5-HT release in the cerebral cortex is stimulated by NMDA and non-NMDA receptors. The NMDA-evoked release is modulated via presynaptic 5-HT, α_2 and A_1 receptors which under the conditions applied are activated by the endogenous agonists.

CALCIUM CHANNELS VI

604.1

α2-ADRENERGIC INHIBITION OF CALCIUM CURRENTS IN CHICK SYMPATHETIC NEURONS INVOLVES PHOSPHOLIPASE A2 AND ATYPICAL PROTEINKINASE C. S.Boehm*, S.Huck, M.Freissmuth. Depts. of Neuropharmacology and Pharmacology, University of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria.

Autoinhibitory regulation of noradrenaline release from chick sympathetic neurons depends on α_2-adrenergic inhibition of N-type calcium channels via pertussis toxin-sensitive $G_{o/i}$ proteins. The present study investigates the signalling cascade between G proteins and calcium channels.

A role of protein kinase C (PKC) in neurotransmitter modulation of calcium channels is generally indicated by transmitter-analogous effects of phorbol esters or diacylglycerol analogues. However, neither 4ß-phorboldibutyrate (PDB, 3μM) nor oleoylacteylglycerol (OAG, 30μM) affected whole-cell calcium currents (I(Ca)). Nevertheless, inclusion of a pseudosubstrate peptide inhibitor of PKC (PKCI 19-36, 10μM) in the pipette progressively reduced the inhibitory action of the α_2-adrenergic agonist bromoxidine (10μM) upon I(Ca). When neurons were dialyzed with PKCI for 10min, the bromoxidine inhibition was 6.8% (n=7) as compared to 32.7% (n=9) under control conditions. This effect of PKCI was specific for PKC as neither an inactive analogue peptide nor a peptide inhibitor of protein kinase A impaired the α_2-adrenergic reduction of I(Ca). The PKC inhibitors staurosporine (1μM) or calphostin C (1μM) reduced the α_2-adrenergic inhibition of I(Ca), but neither a 24h pretreatment with PDB nor acute application of PDB altered the bromoxidine effect, observations consistent with a role of atypical PKCs. At least one of these phorbol ester- and OAG-insensitive, but fatty acid-stimulated isoforms was detected by immunoblots, namely PKC ζ.

Accordingly, arachidonic acid (AA) and linoleic acid (100μM) diminished I(Ca) in a partially reversible manner by 52.9% and 30.7% (n=6), respectively, and intracellular PKCI reduced these values of inhibition to 23.3% and 14.2% (n=6). Moreover, application of AA (30μM) occluded a subsequent inhibition of I(Ca) by the α_2-agonist bromoxidine. In order to identify a role of endogenous AA, cellular lipids were analyzed by thin layer chromatography after incubating neurons with [^{3}H]AA. These experiments revealed a bromoxidine-induced AA formation, and 7,7-dimethyl-(5,8)-eicosadienoic acid (10μM), an inhibitor of phospholipase A_2 (PLA_2), reduced the inhibition of I(Ca) by bromoxidine.

Our results delineate the following signalling cascade in chick sympathetic neurons; α_2-adrenoceptor → $G_{o/i}$ → PLA_2 → atypical PKC (possibly PKC ζ) → N-type Ca^{2+} channels.

604.2

ACTIVATION OF PKC SELECTIVELY UP-REGULATES BOTH α_{1E} AND α_{1B} CALCIUM CHANNELS. A. Stea*, T.W. Soong, S.J. Dubel, W.J. Tomlinson, and T.P. Snutch. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1Z3.

The modulation of Ca channels plays critical roles in a number of physiological processes. We have tested the effects of Protein Kinase C (PKC) activation on four classes of cloned neuronal calcium channels. Activation of endogenous PKC in Xenopus oocytes by application of phorbol esters (PDBu and/or PMA) caused a pronounced increase in the Ba currents from expression of the α_{1B} (N-type) and α_{1E} (Soong et al., Science 260: 1133-1136) calcium channels. In contrast, both the α_{1C} (L-type) and α_{1A} (Q-type) channels were unaffected by PKC stimulation. Application of 100 nM PMA to oocytes expressing either α_{1E} or α_{1B} caused a rapid (within 2-5 min) increase in the whole-cell Ba current to between 125 and 150% of control levels. There appeared to be no significant shift in the voltage dependent properties of the Ba currents up-regulated by PMA. The increase in the Ba current during PMA application was blocked by the addition of the PKC inhibitor staurosporine (5 - 10 μM) and was prevented by the addition of the inactive form of PMA (4-α-PMA). The increase in current due to PKC activation was dependent upon coexpression of the α_1 subunits with a β subunit. Up-regulation of the Ba current was prevented by the chelation of intracellular calcium by injection of BAPTA (2-5 mM final conc.). In addition to the increased whole-cell current, a pronounced slowing of inactivation of α_{1E} was observed with PMA application. These results provide direct evidence for the selective modulation of α_{1E} and α_{1B} calcium channels by PKC and support the notion that the differential regulation of Ca channels may play a significant role in neuronal excitability.

604.3

THE G PROTEIN, G_{13}, MEDIATES INHIBITION OF VOLTAGE DEPENDENT-CALCIUM CURRENT BY BRADYKININ. M.A.Wilk-Blaszczak*, W.D.Singer, S.Gutowski, P.C.Sternweis and F. Belardetti. Dept. of Pharmacol. Univ. of Texas Southwestern Med. Center, Dallas, TX, 75235.

In neuroblastoma x glioma hybrid cells (NG108-15) bradykinin (BK) has dual modulatory effects on ionic currents, it activates a K^+ current, $I_{K,BK}$, and inhibits $I_{Ca,V}$. Both actions are mediated by pertussis toxin (PTX)-insensitive G proteins. Two antisera, raised against peptides representing the C-termini of the α subunits of $G_{q/11}$ and G_{13}, recognized polypeptides of the expected size in membranes from NG108-15 cells. In whole-cell patch-clamp recording, intrapipette perfusion of the antibodies against $G_{q/11\alpha}$ blocked only activation of the $I_{K,BK}$, suggesting that at least two distinct G protein pathways transduce the effects of this transmitter (Neuron 12,109, 1994). In contrast, the anti-$G_{13\alpha}$ antibodies attenuateded inhibition of $I_{Ca,V}$ by BK by 76% (n=22). Perfusion of non-immune serum (n=30), and antibody preincubated with the antigenic peptide (n=9), did not affect the action of BK. The same antibody was also without any effect on inhibition of $I_{Ca,V}$ by Leu-enkephalin (n=10) and activation of $I_{K,BK}$ by BK (n=11). G_{13} may play a general role in PTX-insensitive inhibition of $I_{Ca,V}$.

604.4

UP- AND DOWN REGULATION OF P-TYPE CA^{2+} CHANNELS BY TWO DIFFERENT METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES. Y.M. Lu and T. Knöpfel*.Department of Molecular and Cellular Biology, CNS Research, Ciba, CH-4002 Basle, Switzerland

P-type Ca^{2+} channel activity in rat cerebellar Purkinje cells was enhanced by quisqualate and (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) and decreased by L-2-amino-4-phosphonobutyric acid (L-AP4). Enhancement of w-Aga-IVA-sensitive inward current by 5 µM quisqualate (6 of 9 cells) and by 50 µM (1S,3R)-ACPD (6 of 8 cells) averaged 34.3 ± 9.3 % and 22.2 ± 8.1 %, respectively. The maximal inhibition by L-AP4 amounted to ~ 42 % (18 of 18 cells) and a half maximal inhibition (EC_{50}) was obtained at 48.5 ± 14 µM.

This modulation of P-type Ca^{2+} channels required the presence of either GTP or GTPγS in the internal solution and were irreversible when using GTPγS. These results show that activation of metabotropic glutamate receptors can modulate P-type Ca^{2+} channels through a G-protein-mediated mechanism. The agonist profile together with available data on the expression pattern of mGluRs in Purkinje cells suggests that the enhancement and depression of P-type Ca^{2+} channel activity are mediated by subtype mGluR1 and subtypes mGluR4/7, respectively.

604.5

CALCIUM CHANNEL MODULATION BY LECTINS. A. Golard*+. Howard Hughes Medical Institute, Columbia Univ. P&S, New York, NY 10032

Calcium currents were measured in dissociated chick sympathetic ganglia using the whole cell patch clamp technique. Lectins (1 μM) were applied by local superfusion. All lectins tested reversibly inhibited Ca currents. Two types of inhibition were observed: a speeding of inactivation (SI) and a voltage-dependent inhibition (VDI) with slowing of the activation kinetics. When GTP-γ-S is substituted for GTP, VDI is irreversible, while SI is still reversible. GDP-β-S largely suppresses VDI, but has little effect on SI. These results indicate that G-proteins are involved in VDI, not SI. Thus, VDI may be due to activation of G-protein-coupled receptors, while SI may be a direct effect on the channel. Lectins with high affinities for N-acetylglucosamine or α-D-glucose preferentially induce VDI, while lectins with α-D-galactose affinity produce SI. Differential effects of concanavalin-A and succinyl-Con-A indicate that SI may be due to capping.

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604.6

MODULATION OF CALCIUM CHANNELS AND CONTRACTION BY EXTRACELLULAR HEPARIN IN FROG SKELETAL MUSCLE FIBERS. J.A. Sanchez*, M.C. Garcia, H. Cruzblanca, J.M. Farias and M. Martinez. Department of Pharmacology, CINVESTAV-IPN, A. P. 14-740, Mexico D.F. 07000.

Heparin binds with high affinity to the DHP receptor of skeletal muscle (Knaus et al. JBC, 265:156, 1990). Our experiments investigate the action of heparin on isometric tension, Ca signals, DHP-sensitive Ca currents (I_{Ca}) and charge movement. **Methods**: 1.- Single muscle fibers from frog (*Rana montezumae*). Cells were loaded with Rhod 2 AM (2-10 μm) and fluorescence signals were obtained with a photodiode. Tension was simultaneously measured. 2.- Voltage-clamp experiments in cut fibers using the triple Vaseline gap technique. **Results**: Heparin (100 μg/ml) reversibly potentiates twitch, tetanic tension and Ca signals by ca. 50%. Heparin blocks I_{Ca} movement by 30% and shifts the current-voltage relation towards more negative potentials by 15 mV. Heparin makes activation of I_{Ca} more rapid at all potentials and slows down the deactivation during tail currents. Double pulse experiments indicate that the kinetics of I_{Ca} is dependent on the frequency of stimulation as shown by others. Heparin further accelerates this process. Half-time of activation increases by 13 ms during the second pulse in control experiments and by 22 ms in the presence of heparin. Heparin blocks charge movement by 30 %. These results suggest that the potentiating effect of heparin may be related to the kinetic effects on Ca channels in skeletal muscle.

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604.7

LEMS IgG DECREASES AND ALTERS Ca^{2+} CURRENTS IN MOUSE MOTOR NERVE TERMINALS. D.O. Smith*, M. Conklin, P. Jensen, and W.D. Atchison. Dept. of Physiology, University of Wisconsin, Madison, WI 53706 and Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Lambert-Eaton Myasthenic Syndrome (LEMS) is an autoimmune neuromuscular disorder that results in decreased release of ACh, presumably due to disrupted function of motor-nerve Ca^{2+} channels. To determine the direct effect of LEMS autoantibodies on Ca^{2+} currents (I_{Ca}) in mammalian motor nerve terminals, we measured presynaptic I_{Ca} in mouse diaphragm during phrenic nerve stimulation. Mice were injected daily for 30 days with 1.5 mls of serum derived from patients with LEMS or from disease-free individuals. As expected for LEMS, curarized end-plate currents (EPCs) elicited at 20 Hz were not depressed, while those evoked at 40 Hz were facilitated. EPCs from control mice were depressed during repetitive stimulation. At 0.5-Hz stimulation, I_{Ca} was 57 ±14% (n=10) less in LEMS mice, but no differences were seen at frequencies ≥2 Hz. Nifedipine, which normally has no effect on I_{Ca} at mammalian motor nerve terminals, blocked I_{Ca} in the LEMS-treated animals, but ω-conotoxin GVIA reduced I_{Ca} by 41 ±13% (n=10) in control animals only. We conclude that LEMS serum alters the normal characteristics of the channels, thus decreasing I_{Ca} and rendering them dihydropyridine sensitive. Supported by NIH grant ES05582.

604.8

EVIDENCE FOR ALTERED REGULATION OF NEURONAL CALCIUM SIGNALLING IN DIABETES MELLITUS J.W. Wiley*, K.E. Hall, and B. Dzwoneck. Div. of Gastroenterology, Dept. of Int. Med., U. of Michigan, Ann Arbor, MI 48109.

We have previously shown that diabetes mellitus (DM) is associated with increased high-threshold, voltage-activated calcium currents in dorsal root ganglion (DRG) neurons (Soc. Neurosci. 19: 1762, 1993). In the present study we examined the possibility that calcium regulation is altered in capsaicin-sensitive DRG neurons. Two groups of Wistar Bio-Bred (BB) rats were used: i) adult male non-diabetic controls (C), and ii) matched diabetics (D), DM duration 12-32 wks treated with insulin to maintain serum glucose 350-450 mg%. Studies were performed on acutely dissociated DRGs. Cytosolic calcium ($[Ca^{2+}]_i$) was measured using the Fura-2 method in 20-35 μm neurons. KCl (50 mM) caused a significantly ($p<0.05$) greater increase in $[Ca^{2+}]_i$ in D (basal 42±3 nM; post KCl 162±12 nM; n=12) than C (basal 35±5 nM; 84±15 nM; n=8). Capsaicin (Cap; 1 μM) also increased $[Ca^{2+}]_i$ to a significantly greater degree in D (133±15 nM; n=17) than C (78±13 nM; n=17). Approximately 80% of neurons responded to Cap. Treatment with the kappa opioid agonist dynorphin A (Dyn A; 1 μM) reduced the KCl-mediated increase in $[Ca^{2+}]_i$ by 73% and 33% in C and D respectively (n=8, $p<0.05$), an effect which was pertussis toxin-sensitive. High-threshold calcium currents were recorded in comparably-sized cells, using the whole cell variant of the patch-clamp method. Currents were evoked from a holding potential of -80 mV to a clamp potential of 10 mV. Dyn A (1 μM) followed by 10 μM ω-Conotoxin GVIA (ω-CgTX, N-type antagonist) were administered by pressure ejection from blunt pipettes. The percentage inhibition of peak calcium currents caused by Dyn A was significantly less in D (8±2%; n=10) than C (25±5%; n=11; $P<0.05$). ω-CgTX decreased C currents by 49±3%, D by 26±6%. Following ω-CgTX, no further inhibition by Dyn A was observed (C 6±5%; D 5±5%); therefore, the decrease in the effect of Dyn A was not simply due to a decrease in the proportion of N-type current. These results suggest that inhibitory G protein modulation of calcium channels may be decreased in DM, resulting in enhanced calcium influx during depolarization.

604.9

ROLE OF TWO PUTATIVE ENDOGENOUS NEUROMODULATORS DBI $_{17\text{-}50}$-TTN AND ANANDAMIDE IN THE REGULATION OF Ca^{2+} INFLUX IN IMMUNOCOMPETENT CELLS. A.Berkovich*, A.Guidotti, E.Costa. FGIN,Georgetown Univ.,Washington D.C.20007.

Macrophages (MP) and monocytes (MC) express peripheral type receptor for both benzodiazepines (PBR) and cannabinoids (PCR). TTN (DBI $_{17\text{-}50}$), a processing product of rat Diazepam Binding Inhibitor (DBI) and 4'clorodiazepam (4'CD), a PBR specific ligand, have been reported to increase the level of cytokines, INT-1β and TNF-α and their mRNAs in MP and MC cells[1]. Anandamide, an endogenous cannabinoid receptor ligand acts as an antiinflammatory and immunosuppressive agent[2]. Here we studied whether TTN, 4'CD and anandamide, acting on their specific receptors, regulate Ca^{2+} transport through the plasma membrane of rat lymphocytes and mouse macrophage cell line (ANA-1).

We have investigated whether storage regulated Ca^{2+} channel function that is stimulated by depletion of Ca^{2+} intracellular stores is controlled by PBR or PCR. This was assessed by Fura-2 fluorescence quenching with Mn^{2+} in cell with depleted Ca^{2+}stores by a 30 min preincubation in Ca^{2+} free medium. Incubation with TTN and anandamide resulted in inhibitory action on Ca^{2+} influx. However the inhibitory potency of TTN and 4'CD (full inhibition at 1 μM) was lower than that of anandamide (full inhibition at 50nM). These results suggest that PBR and PCR agonists can inhibit Ca^{2+} influx in immunocompetent cells and therby modify their genomic expression.

[1] Taupin,V. Mol.Pharmacology 43:64-69.1993.
[2] Devane,W. TIPS.15:40-41.1994.

604.10

STATE-DEPENDENT MODULATION OF NEURONAL CALCIUM CURRENTS A.Sculptoreanu, A.Figourov, W.C.de Groat*. Dept. of Pharm., Univ. Pittsburgh, Pittsburgh, PA. & Div. of Urology, Lady Davis Institute for Med. Res., SMBD-Jewish General Hospital, McGill Univ., Montreal, Quebec.

Neuronal calcium currents were studied in primary cultures of major pelvic (MPG) and dorsal root (DRG) ganglia from the adult rat using patch clamp techniques. We reported previously that synaptic transmission and neuronal integration may depend on state-dependent modulation of calcium channel function and the degree of cAMP-dependent kinase activity. The aim of this project was to further characterize the mechanism of state-dependent modulation of calcium channels. Neurons isolated from the MPG's express predominant dihydropyridine-sensitive, L-type calcium channel currents that are enhanced by 8Br-cAMP, whereas neurons isolated from DRG's express large ω-conotoxin sensitive, N-type calcium channel currents that are depressed by 8-Br-cAMP. The state-dependent modulation of L-type calcium channels by concurrent voltage dependent activation and phosphorylation of the channels predicts that they would be facilitated by high frequency, short duration stimuli. To test this hypothesis we compared the effect of a single 200 ms conditioning pulse and trains of 5 ms pulses at frequencies 10, 20, 50, 100 and 200 Hz also 200 ms in duration. A clear frequency dependence of L-type calcium channel facilitation could be demonstrated in MPG neurons. In DRG neurons, predominant N-type calcium channels were inhibited although in some neurons L-type calcium channels were unmasked. We propose that potentiation of neuronal calcium channel currents is due to state-dependent phosphorylation and may contribute to the facilitation of transmitter release after high frequency nerve discharges. This type of facilitation may be involved in the enhanced neural control of urogenital function by MPG pathways in pathological conditions such as spinal cord injury. (Support from JGF to AS and AF and skillful assistance of Ms. V. Dumitru is acknowledged.)

604.11

MODE SHIFT OF N-TYPE Ca^{2+} CHANNEL BY THE AUXILIARY SUBUNITS α_2 AND β Minoru Wakamori* and Yasuo Mori Dept. of Pharmacol. and Cell Biophys., Univ. of Cinti., Cinti, OH 45267-0575

ω-Conotoxin-GVIA-sensitive N-type channels are inhibited by various neurotransmitters. Modulation is probably mediated by G-proteins which alter equilibrium among diverse functional behaviors. To gain a clear structural insight into functional diversity of N-type channels, we have investigated the electrophysiological properties of recombinant N-type channels composed of various combinations of the pore-forming subunit α_1 (BIII) and the two additional subunits, α_2 and β, in *Xenopus* oocytes. Whole-cell measurements in 40mM Ba^{2+} showed that the BIII α_1 subunit alone elicited ω-conotoxin-sensitive HVA Ba^{2+} current, with a time-to-peak (t_p) and decay time constant (τ_h) of 16 and 215ms, respectively, at +20 mV. Modulation of the BIII channel kinetics by β subunits was strikingly different from that observed for the L-type channels, i.e., both t_p and τ_h are increased in BIII (21 and 477ms) but decreased in L-type. The β subunits shifted the current-voltage relationship in a hyperpolarizing direction by 8mV as was observed for the L-type channels. Surprisingly, the effects of the α_2 subunit contrasted with and antagonized the effects of the β subunits (t_p=9 and τ_h=144ms for BIII+α_2; t_p=10 and τ_h=248ms for BIII+$\alpha_2\beta$). Single channel analysis revealed different patterns of opening for BIII, BIII+α_2, BIII+β, and BIII+$\alpha_2\beta$, suggesting that different subunit combinations of calcium channel complexes give rise to different modes of gating dependent upon the association/dissociation of the subunits.

604.12

FUNCTIONAL COUPLING OF N-TYPE CALCIUM CHANNELS AND CALCIUM ACTIVATED POTASSIUM CHANNELS IN NEURONS. P. Sah* The Neuroscience Group, Department of Physiology, Faculty of Medicine, University of Newcastle, Newcastle, NSW 2308. AUSTRALIA

It has previously been shown that neurones of the dorsal motor nucleus of the vagus (DMV) express voltage-gated calcium and sodium channels. Calcium influx during the action potential leads to a long lasting afterhyperpolarisation (AHP) which regulates the discharge frequency of these neurones. In this study I have examined the properties of the calcium current supplying calcium to activate the AHP in these neurones. Whole cell recordings were made from DMV neurones in transverse slices of rat brainstem. Calcium currents were measured by using Cs^+ as the main internal cation, blocking sodium currents with tetrodotoxin (1 μM) and using barium (2.5 mM) as the permeant cation. 'Calcium current(s)' were activated when the neurone was stepped to membrane potentials more positive than -50 mV from a holding potential of -60 mV. There was no evidence for a low voltage activated calcium current (T- type). The calcium current had a smooth current-voltage relation and was completely blocked by cadmium (100 μM) and nickel (1 mM) ions. Approximately 40 % of the calcium current was blocked by ω-conotoxin (200 nM), about 20 % of the current was blocked by high concentrations of nifedipine (10 μM). The remaining current was effectively blocked by cadmium. The calcium current was insensitive to agatoxin (250 nM). In current clamp, application of ω-conotoxin slowed action potential repolarisation and blocked 70% of the calcium-activated potassium current underlying the AHP. The remaining current was blocked by cadmium. Nifedepine has no effect on the action potential or the AHP. These experiments indicate that in DMV neurons N-type calcium channels are preferentially coupled to the calcium-activated potassium channels underlying the AHP. These channels are known to be SK-type calcium-activated potassium channels and are thought to be located close to the calcium channels which supply calcium ions for activating them (J. Neurophysiol. 68,2237). Thus, N-type calcium channels are co-localised with SK-type calcium-activated K channels in DMV neurons.

VISUAL SYSTEM DEVELOPMENT AND PLASTICITY

605.1

DEVELOPMENT OF DIRECTIONAL SELECTIVITY IN TURTLE RETINA: PHYSIOLOGY AND MODEL. N. M. Grzywacz* and E. Sernagor. Smith-Kettlewell Inst., 2232 Webster St., San Francisco, CA 94115.

Directional selectivity requires a spatially asymmetric mechanism to mediate the larger responses to preferred- than to null-direction motions. What developmental processes mediate the breaking of symmetry in the retina? To study this development, we recorded extracellularly from turtle retinal ganglion cells during embryogenesis (Stages 22-26), the first 40 days post-hatching, and in adults. In particular, recordings were made of the spontaneously recurring bursts of spikes in immature retinas and of the responses to edges of light moving in sixteen directions. The bursts occurred from Stage 22 until 2-4 weeks post-hatching. Light responses began at Stage 23, and early on, polar plots of the responses to motion were highly anisotropic, including orientation and directional selecivity. However, orientation selectivity reached a peak incidence at hatching, while directional selectivity disappeared only to reappear in adults (after 40 days post-hatching). Receptive-field sizes and the incidence of isotropic cells stabilized 2-4 weeks post-hatching.

We built and tested with computer simulations a biophysical model to account for the development of directional selectivity. In this model, the early anisotropy of the retina reflects immature, polarized dendritic layout, with directional selectivity being incidental to the dendrites' cable properties. As dendritic processes grow and branch, embryonic directional selectivity disappears, but orientation selectivity is maintained by a Hebbian process, which reinforces some of the early anisotropy. Later in development, the emergence of a specialized inhibitory synapse onto the dendritic inputs to some orientationally selective cells, transforms them onto weak directionally selective cells, which are then reinforced by a Hebbian process.

In conclusion, the increase in dimensions of dendritic trees, their degree of polarization, and the spontaneous bursts of spikes do not appear to account directly for the development of directional selectivity. Rather, it seems to require visual experience and/or the late establishment of a specialized inhibitory synaptic drive.

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605.2

ROLE OF EARLY SPONTANEOUS ACTIVITY AND VISUAL EXPERIENCE IN SHAPING COMPLEX RECEPTIVE FIELD PROPERTIES OF GANGLION CELLS IN THE DEVELOPING RETINA. E. Sernagor* and N.M. Grzywacz. Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

Receptive field properties of adult retinal ganglion cells are well documented, but little is known about their development.

To study how such properties develop, extracellular recordings of activity from turtle retinal ganglion cells were made during embryogenesis (Stages 22-26), the first 40 days post-hatching (PH), and in adults. From Stage 23, the cells fired in spontaneous recurring bursts and responded to light. Polar plots of the responses to motion were highly anisotropic in early embryonic cells (including orientation and immature directional selectivity). More than 40% of embryonic cells exhibited multi-axes anisotropy, and only 6% were statistically isotropic. The incidence of the anisotropic cells gradually decreased throughout development. The incidence of isotropic cells and the excitatory receptive field diameters of all ganglion cells gradually increased during development and their maturation coincided with the disappearance of the spontaneous bursts, at 2-4 weeks PH, when hatchlings were raised in normal light conditions.

If, on the other hand, hatching turtles were deprived of visual experience, all ganglion cells remained spontaneously active, at least during the first month PH (they were not investigated beyond that period). At the same time, their excitatory receptive field areas expanded by 82%. Responses to motion of these dark-reared ganglion cells were 30% more isotropic than in normal hatchlings.

These results show that mature spatio-temporal receptive-field properties of retinal ganglion cells emerge from initially highly anisotropic properties, which may reflect an immature, polarized dendritic layout. Embryonic spontaneous bursts and visual experience seem to play opposite roles: spontaneous activity may induce and constantly stimulate dendritic outgrowth in the developing retina, whereas exposure to light first accelerates and then terminates these processes.

Supported by NIH grant EY 08921.

605.3

AXONAL BRANCH DYNAMICS: AN *in vivo* EXAMINATION OF THE APPLICABILITY OF HEBB'S HYPOTHESIS TO AXON MORPHOLOGY. S. Witte* and H.T. Cline. Dept. of Physiology, University of Iowa, Iowa City, IA and Cold Spring Harbor Labs, Cold Spring Harbor, NY.

We sought to examine the possibility that there is a structural correlate to Hebb's hypothesis. A high degree of dynamism, or addition and retraction of branches, occurs in retinotectal arbors in the developing frog as these arbors seek to establish and maintain contacts with topographically appropriate targets. The refinement of retinotectal topography can be blocked by interfering with NMDA receptor activation. One mechanism for the refinement of the topography may be that temporally correlated inputs from neighboring ganglion cells activate the NMDA receptor, and this activation leads to the structural stabilization of the branches on which these synapses are located. Branches having synapses with uncorrelated activity would not be stabilized and these structures would be broken down.

We examined the role of NMDA receptor activation in branch stability by 1) using APV to block NMDA receptor activation, and 2) exposing animals to strobe lighting to produce universally correlated activity. Single DiI labeled retinotectal axons were imaged in living *Xenopus laevis* tadpoles using a Noran laser scanning confocal microscope. After taking an initial image, tadpoles either swam in control solution, 100 uM APV solution, or control solution with strobe lighting. Four more images were taken at half hour intervals while the treatments continued.

Treatment with 100 uM APV caused a 33% increase in the total number of new branches normalized for initial branches (n=10, p=0.057). Branch retractions and final branch numbers tended to be greater in the APV treated group. Strobe treatment did not affect total branch additions or retractions, but there was a trend toward increased branch lifetime, i.e. branches that first appeared at the second observation did not retract as quickly in the strobe treated group. Increased branch additions in APV treated arbors may indicate that these arbors are less stable, while activation of the NMDA receptor appears to have increased arbor stability.

605.4

INHIBITION OF NITRIC OXIDE SYNTHASE DISRUPTS ON/OFF SUBLAMINATION IN THE FERRET LATERAL GENICULATE NUCLEUS. K. S. Cramer* and M. Sur. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Retinogeniculate axons in the ferret segregate into eye-specific layers during the first two postnatal weeks. Axons from on-center or off-center retinal ganglion cells further segregate into sublaminae by four postnatal weeks in a process dependent on postsynaptic NMDA receptors (Hahm et al., *Nature* 351: 568, 1991). During this period, the lateral geniculate nucleus (LGN) of the ferret transiently expresses NADPH-diaphorase, a nitric oxide synthase (Cramer et al., *Soc. Neurosci. Abst.* 19: 891, 1993). Nitric oxide may act downstream of the NMDA receptor as an intercellular retrograde messenger during hippocampal synaptic plasticity (e.g., Schuman and Madison, *Science* 254: 1503, 1991).

To investigate whether nitric oxide has an analogous role in the development of on/off sublaminae, we blocked nitric oxide synthase during the third and fourth postnatal weeks using an arginine analog, which competitively inhibits the enzyme at the arginine binding site. Ferrets received daily intraperitoneal injections of N-nitro-L-arginine (L-NoArg). Control animals received the inactive isomer N-nitro-D-arginine methyl ester (D-NAME) or L-NoArg together with L-arginine. L-NoArg treatment resulted in reduced NADPH-diaphorase staining compared to controls or normal ferrets. Retinogeniculate sublamination was assessed using intraocular injections of WGA-HRP at postnatal day 24. The extent of the LGN containing visible sublaminae was scored quantitatively. Animals treated with L-NoArg had significantly less sublamination than both control and normal animals. These results suggest that nitric oxide is involved in the segregation of retinal afferents into on/off sublaminae in the LGN.

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605.5

RAPID ACQUISITION OF DENDRITIC SPINES BY VISUAL THALAMIC NEURONS AFTER BLOCKADE OF NMDA RECEPTORS. M. Rocha* and M. Sur. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139; and Institute of Biophysics, Federal University of Rio de Janeiro, 21941, Brazil.

The NMDA subtype of glutamate receptor plays a critical role in the development of retinogeniculate afferents and postsynaptic target cells in the ferret lateral geniculate nucleus (LGN) (Hahm et al, 1991; Rocha et al, 1991). Here, we have examined the time course of dendritic changes of LGN cells in vitro after NMDA receptor blockade. Horizontal slices containing the thalamus were obtained from 28 ferret kits between postnatal day 5 (P5) and 45, and maintained alive in a slice chamber. LGN cell dendrites were labeled by DiI crystals placed into the LGN, and imaged on-line using laser confocal microscopy. Segments of dendrites (n=318) were imaged again after 2 and up to 7 hours of perfusion with control solution, d-APV (50-100 μM), or NMDA (10-100 μM). At P5-6, dendrites often gave rise to short side branches in control and treated slices. Acquisition of dendritic spines occured after P7 and increased following d-APV perfusion ($p<0.05$). From P14 to P20, blockade of NMDA receptors led to a five-fold net increase in spine acquisition ($p<0.001$). Changes in spine acquisition declined after P21, and were unaffected by d-APV perfusion. NMDA treatment had no significant effect when compared to control conditions at all ages. Thus, NMDA receptors regulate dendritic spine acquisition in LGN cells mainly during the third postnatal week, a period when retinogeniculate axons segregate into on and off-center sublaminae. These results suggest that rapid structural changes in postsynaptic cells contribute to the formation of specific retinogeniculate connections.

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605.6

ROLE OF THE MYELIN-ASSOCIATED NEURITE GROWTH INHIBITOR NI-35/250 IN THE TERMINATION OF CRITICAL PERIOD PLASTICITY IN KITTEN VISUAL CORTEX

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During a restricted period in postnatal development the thalamo-cortical circuitry of the mammalian visual system is malleable for activity-dependent changes of the interconnectivity. As neurite growth and synapse formation contribute to visual cortical plasticity the termination of the critical period may be due to a reduced growth permissiveness of the tissue. We have shown in an in vitro assay, where chick embryonic cortical neurons are cultured on cryostat sections of unfixed cat visual cortex sacrificed at different postnatal ages, that neurite growth is significantly impaired on tissue from kittens which have passed the critical period. This is paralleled by the appearance of the myelin-associated growth inhibitor NI 35/250 in the visual cortex. In order to test whether there is a causal relation between the reduced growth permissiveness and the appearance of NI 35/250 we treated the substrate sections with the monoclonal antibody IN-1, which has been shown to neutralize the growth inhibiting effect of NI 35/250. Controls included untreated sections and sections preincubated with the oligodendroglia-specific antibody O1.

IN-1 preincubation lead to a significant increase in the percentage of neurite-bearing cells and the neurite length of individual cells cultured on sections from more mature visual cortex. Both effects were most pronounced on the heavily myelinated infragranular cortical layers. O1 pretreatment revealed only minor effects on neurite growth. The data show that the developmental reduction of growth permissiveness of cortical tissue and possibly the termination of the critical period is due to the expression of the myelin associated neurite growth inhibitor NI 35/250.

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605.7

POSTNATAL DEVELOPMENT OF NMDA RECEPTOR-MEDIATED, NITRIC OXIDE DEPENDENT RELEASE OF NEUROTRANSMITTERS IN THE CEREBRAL CORTEX. M.J. Friedlander*, C. Gancayco, and F. Hester. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294 USA.

Depolarization or activation of NMDA receptors in cortical synaptosome preparation from adult animals causes a calcium dependent release of glutamate and norepinephrine which in the case of NMDA receptor activation requires nitric oxide production (Montague, Gancayco, Winn and Friedlander, Science, 263, 973-977, 1994). Moreover, induction of synaptic potentiation in adult cortex requires NMDA receptor activation, although in neonates it is independent of NMDA receptor activation (Harsanyi and Friedlander, Soc. Neurosci. Abstr., 370, 24, 1993). Thus, we evaluated the development of NMDA receptor mediated release of neurotransmitter in cortex. Cortical synaptosomes were prepared from guinea pigs at various postnatal ages (0, 5, 10, 15, 20, 25, 30, 35 and 70 days, n=3 animals at each age). Release of endogenous glutamate was measured with a luminometric assay and release of exogenous ^{3}H-norepinephrine was measured with scintillation counting. Synaptomes were contin-uously washed with HEPES buffered saline (HBS) and stimulated with either a 50mM potassium depolarizing stimulus or 100μM NMDA either with or without a 30 minute pre-incubation with the nitric oxide synthase (NOS) blocker, L-Nitro-Arginine. Fractions of neurotransmitter release were expressed as the percentage of available transmitter and normalized to the HBS controls. Calcium-dependent depolar-ization-induced release of both glutamate and norepinephrine remained constant and independent of NOS activity at all ages. However, NMDA receptor-mediated release of both neurotransmitters did not occur until 10-15 days of age, with glutamate release showing a second increase in response between 30 and 70 days of age. At all ages when NMDA receptor-mediated release occurred, it was completely inhibited by NOS block-ade. These results suggest that the NMDA receptor-NO system is immature in the neonate, in agreement with our neurophysiological studies.

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605.8

A CRITICAL PERIOD FOR INDUCTION OF LONG-TERM DEPRESSION IN VISUAL CORTICAL LAYER IV NEURONS

S. M. Dudek, F. W. Hester*, and M. J. Friedlander. Neurobiology Research Center, Univ. of Alabama at Birmingham, AL 35294.

Activity-dependent weakening of excitatory synaptic transmission has been hypothesized to play a dominant role in the mechanisms that mediate ocular dominance plasticity in the developing visual cortex. We have taken advantage of an *in vitro* model of synaptic weakening to examine whether visual cortical layer IV neurons express a developmentally dependent long term depression (LTD) corresponding to critical period plasticity. We have shown previously (Dudek & Friedlander, Soc. Neurosci. Abstr. 19: 894,1993) that LTD could be induced in a subset of immature guinea pig and kitten visual cortical neurons. However our efforts to characterize the developmental expression of this effect have been hampered by the prevalence of IPSP-like hyperpolarizing potentials that contaminate the excitatory postsynaptic potentials (EPSPs); LTD is not induced in neurons exhibiting these hyperpolarizing responses. We have extended our previous findings to include experiments where we have attempted to overcome the effects of this synaptic inhibition. Conventional intracellular recordings were made from layer IV of slices from mature (>45 days old) and immature (< 8 days old) guinea-pig visual cortex and cat (day 1 through adult) striate cortex. In some cases, a mixture of the selective blocker of chloride channels, 4,4'-dinitro-stilbene-2,2'-disulfonic acid (DNDS), cesium acetate, and/or biocytin was included in the electrode. While blockade of inhibition appears to restore the ability to induce LTD in immature animals, it has no effect on the (lack of) induction of LTD in layer IV neurons from adults. These data suggest that while inhibitory synaptic potentials can influence the induction of LTD, their presence does not likely represent a subset of neurons incapable of supporting LTD.

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605.9

INDUCTION OF SYNAPTIC POTENTIATION IN NEONATAL VISUAL CORTEX DOES NOT REQUIRE POSTSYNAPTIC ACTION POTENTIALS, NMDA RECEPTOR ACTIVATION OR NITRIC OXIDE PRODUCTION K. Harsanyi * and M.J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, AL 35294, USA.

Low frequency pairings of postsynaptic depolarizing pulses with presynaptic activation causes potentiation of postsynaptic potentials (PSPs) in mature visual cortex (Fregnac, Smith, Burke and Friedlander, J. Neurophysiol. 71, 1403-1421, 1994). We previously showed that this type of synaptic plasticity is more reliably induced in the neonate (85% of cells vs. 40% of cells - Harsanyi and Friedlander - Soc. Neurosci. Abstr., 370, 24, 1993). In the present study, we evaluated the mechanism underlying the reliable induction of this form of plasticity in the neonate. Intracellular recordings were made from supragranular neurons in 500 µm thick slices of guinea pig visual cortex (from 5 to 60 postnatal days). Low frequency (0.1 Hz) low intensity (<30% threshold) stimuli were applied to cortical layer 6 with intracellular application of depolarizing pulses (x=+2.0nA, 80 ms, 60 pairings). In the youngest animals (5-28 days), 85% (n=34/40) of such pairings led to synaptic potentiation (>30% for > 10 minutes after the pairing). Application of the NMDA receptor blocker, D-APV (50 µM) did not block induction of synaptic potentiation in this age group (n=11/11). The depolarizing pulses applied to the postsynaptic neurons are effective at inducing synaptic potentiation even during action potential blockade by intracellular application of QX 314, the fast sodium current blocker. Application of the nitric oxide synthase (NOS) blocker, L-Nitro-Arginine at 100 µM for 60-120 minutes did not block synaptic potentiation (n=11/14). Thus, subthreshold postsynaptic depolarization via activation of non-NMDA receptors, without NO production is sufficient to induce potentiation in the neonatal visual cortex.

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605.10

REDUCED SYNAPTIC PLASTICITY IN VISUAL CORTEX SLICES OF α-CaM-KII KNOCKOUT TRANSGENIC MICE. A. Kirkwood*, A. Silva and M.F. Bear. Department of Neuroscience, Brown University, Providence, RI 02912 and Ctr. for Learning and Memory. Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724

Recent work indicates that synaptic plasticity induced in vitro in the superficial layers of visual cortex and in the CA1 region of the hippocampus share remarkably similar mechanisms of induction. Several lines of evidence have implicated calcium-calmodulin- kinase II (CaM-KII) in the induction of long term potentiation (LTP) in the hippocampus. We have used a knockout transgenic mouse line that lacks the α subunit of CaM-KII to address the possible role of CaM-KII in the induction of LTP in the visual cortex.

Synaptic responses to stimulation of layer IV were recorded extracellularly in layer III. To induce LTP we used theta burst stimulation (TBS) which previous work has shown to yield robust synaptic potentiation in rat visual cortex. LTP was measured as the increase in field potential amplitude 20 min. after TBS. When TBS was applied to slices from wild type animals it resulted in significant LTP (24.2 ± 4%, 34 slices from 6 mice). In contrast, when it was applied to slices from mutants, TBS resulted in little or no potentiation (2.2 ± 1.0%, 44 slices from 7 mice). Furthermore, while potentiation of ≥ 20% was observed after TBS in 50% of the control cases, 20% LTP was observed in only 5% of the mutant cases.

The dramatically reduced capability of the mutant visual cortex to sustain LTP strongly suggests that in the adult neocortex, like in the CA1 region of hippocampus, CaM-KII is crucial for the formation of LTP. Work in progress is aimed at examining (1) if there is a deficit in LTD in the mutant visual cortex, and (2) whether the deficit in LTP applies to the immature as well as the adult visual cortex.

605.11

REGULATION OF LONG-TERM POTENTIATION IN VISUAL CORTEX BY AGE AND VISUAL EXPERIENCE. M.F. Bear* and A. Kirkwood, Department of Neuroscience, Brown University, Providence, RI 02912

Binocular connections in the visual cortex are modified by sensory experience, but only during a "critical period" of early postnatal life. These modifications may employ mechanisms similar to those responsible for long-term synaptic potentiation (LTP) in slices of visual cortex. LTP of synaptic responses in layer III can be readily elicited by theta-burst stimulation (TBS) of layer IV in slices prepared from adult animals. However, we and others have found that (in the absence of $GABA_A$ antagonists) LTP can be induced by white matter (WM) stimulation only in slices prepared from immature animals. Thus, like naturally-occurring synaptic plasticity, LTP after WM stimulation is restricted to a finite period of postnatal life, which in the rat lasts about 5 weeks. One interesting aspect of the "critical period" for modification of binocular connections is that it can be extended by rearing animals in complete darkness. The aim of the present study was to see if LTP in visual cortex also is regulated by the history of visual experience.

Slices of visual cortex were prepared from 4-5 week-old Sprague-Dawley rats that had been reared either in complete darkness or in a standard lighted environment. Synaptic responses to WM and layer IV stimulation were recorded extracellularly in layer III. In slices from control rats TBS of the WM had little effect (5.9 ± 3.0% increase in the field potential amplitude at 20 min. after conditioning, n = 7 rats), while TBS of layer IV induced substantial LTP (26.4 ± 6.6%, n = 12 rats). In contrast, in slices from dark reared rats LTP was evoked equally well by TBS of the WM (20.0 ± 3.3%, n = 7 rats) or of layer IV (24.2 ± 4.1%, n = 8 rats).

The present results indicate that the history of visual experience can remarkebly affect the proba-bility and magnitude of LTP in visual cortex following WM stimulation. Thus LTP evoked by WM stimulation in vitro resembles experience-induced plasticity observed in vivo in two respects: both forms of plasticity can be induced only for a short "critical period", and this critical period can be prolonged by the absence of visual experience.

605.12

NEONATAL ENUCLEATION PREVENTS THE FORMATION OF A NORMAL OCCIPITAL CORTICOCOLLICULAR PROJECTION IN RATS. G. Prusky*, Department of Psychology, The University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

In rats, corticocollicular axons arising from lateral-posterior visual cortex develop a restricted terminal zone in rostral superior colliculus (SC) from an initially-diffuse projection during the first two weeks of postnatal life (Jepp and Prusky, Soc. Neurosci. Abs., 19, 455, 1993).

I investigated the role of the retina in this process by either enucleating rats at birth, before axons from the visual cortex normally arrive in the SC, or at postnatal day (P) 15, after order in the corticocollicular projection has been established. The distribution of DiI-labelled corticocollicular axons in the SC was then examined through development.

The corticocollicular axons of neonatally enucleated animals initially extended caudally, past their topographically-appropriate position in rostral SC, as far as its caudal border. By P15, there was a significant reduction of mistargeted axons in the caudal half of the SC, but axons in the rostral half of the SC arborized widely without forming a restricted terminal field. This pattern remained unchanged in animals that survived as late as P30. In contrast, the corticocollicular axons of animals, enucleated at P15 and viewed on P30, had clearly defined terminal zones that were indistinguishable from normal P30 controls.

These data suggest that during the first two weeks of postnatal life, a regional factor in the SC, that is independent of the presence of the retina, participates in corticocollicular map formation. However, the establishment of a precisely ordered corticocollicular projection is dependent upon normal retinal function.

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CHEMICAL SENSES

606.1

NOVEL ENHANCER TRAP MUTANT REVEALS DIVERSITY OF OLFACTORY FUNCTION IN *DROSOPHILA MELANOGASTER*. Adrienne E. Dubin *, John Molina, Haleh Razzaghi, Brett Fenson & Greg Harris.Dept. Biol.,San Diego State Univ.,San Diego,CA 92182.

Similarities between olfactory systems in vertebrates and insects suggest that the general rules underlying olfaction are conserved. We have taken a genetic approach to identify genes involved in olfaction in *Drosophila*. Enhancer trap line HD1 expresses the reporter gene *lacZ* encoded by a P-element inserted near position 64 in a subset of cells in the antennal third segment (the main olfactory organ). HD1 flies have no detectable olfactory defects, however after excision of the transposon, at least 2 of 62 independent lines revealed a dominant impaired sense of smell to the potent odorant ethyl acetate (EtAC) as assessed in a behavioral assay and electroantennogram recordings. The percentage of excision line 15A flies indifferent to EtAC in the behavioral assay (12±5% (mean±S.E.M.); n=7) was significantly higher than controls (68±5%; n= 7; $p<0.005$). EtAC applied at 10^{-3} dilution for 500 ms from a cotton-plugged puffer pipet caused a 4-fold decreased response in 15A (1.7±0.2mV; n=22) compared to HD1 (6.9±0.7mV; n=17; $p<0.005$) and another excision line (15B: 7.6±0.5mV; n=18; $p<0.005$). Responses to benzaldehyde, propioniate, butanol, isobutanol, acetone, acetic acid were unaltered. The maximum response to EtAC was decreased with no change in the EC_{50}. Responses to short aliphatic chain acetates were most affected (compared to controls): 25% (EtAC), 41% (PropylAC), 54% (EtAcetoAC), and ~100% (ButylAC and tert-ButylacetoAC). A molecular analysis of the mutation is underway. Supported by AHA, California Affiliate 92-275 and NSF BNS-9022216.

606.2

HETEROLOGOUS EXPRESSION OF AMILORIDE-SENSITIVE SODIUM CHANNEL SUBUNITS AND AN ALTERNATIVELY SPLICED FORM IN TASTE TISSUES. X-I. Li.* and S.H. Snyder, Dept. of Neuroscience. Johns Hopkins Medical School. Baltimore, MD 21205

The transduction of sodium salt taste is mediated by voltage-independent, amiloride-blockable epithelial sodium channels (ASSC) in taste cells . Recent molecular cloning studies have revealed that an amiloride-sensitive epithelial sodium channel isolated from rat colon consists of three homologous subunits (α, β and γ) (Canessa et al Nature 367: 463,1994). Utilizing polymerase chain reaction and Northern blot analysis, we have identified the presence of all three subunits and an alternatively spliced form of α subunit of ASSC in rat tongue epithelial tissues that contain taste receptor cells. The heterologous expression of α, β and γ subunits in taste tissues appear to be distinct from that in lung and kidney. In taste tissues, α and β subunits of ASSC are equally expressed and the-γ subunit is undetectable by Northern analysis. In lung and kidney, however, the expression levels of β and γ subunits are about same whereas the expression of the-α subunit is the greatest. The expression levels and tissue distribution of the alternatively spliced form of the-α subunit of ASSC are similar to that for the native α subunit: higher in lung and kidney and lower in taste tissues. Preliminary studies on transfected cells expressing ASSC cDNAs showed that α subunits, but not β and γ subunits, were able to bind [^{3}H]-phenamil, an amiloride analog. The heterologus co-expression of ASSC subunits may contribute to tissue specific functions of ASSC such as salty taste.

606.3

ODORANT INDUCED CURRENT BUMPS IN ISOLATED OLFACTORY RECEPTOR CELLS FROM THE SALAMANDER. S. Firestein*, C. Picco and A. Menini. Dept. of Biol., Columbia Univ.,NY USA, and Istituto di Cibernetica e Biofisica., C.N.R, Genova, Italy.

We have previously shown that vertebrate olfactory receptor cells have narrow dynamic ranges and low affinities for certain odorants. Dose-response relations studied in the presence of different concentrations of cineole, isoamyl acetate or acetophenone for an exposure time of 1.2 sec were well fitted by the Hill equation with Hill coefficients greater than 1 and K1/2 values between 3 and 100 μM. Although the K1/2 concentration for these odorants was in the micromolar range, the cellular threshold for odorant detection could be significantly lower. To investigate this possibility we exposed a single cell to low odorant concentrations for longer periods of time. In a cell with a K1/2 of 10 μM as measured from dose-response curves with the odorant cineole, high gain recordings showed isolated current bumps induced by 500 nM of the same odorant. These bumps had a bell like shape with amplitudes of 1-10 pA and average durations of 300-500 ms.

Olfactory receptor neurons can therefore detect odorant concentrations lower than previously shown. They may accomplish this by sampling over longer periods and integrating information over time. Thus a cell expressing a braodly tuned low affinity odor receptor may regain sensitivity at the expense of temporal resolution, an apparently sensible trade-off for olfactory neurons. The observed small current bumps could be caused by the binding of a single odor molecule to a receptor, as in photoreceptors it is possible to observe quantum bumps caused by the absorption of a single photon.

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606.4

SALAMANDER OLFACTORY RECEPTOR NEURON ODOR RESPONSES DEPEND UPON STATES ESTABLISHED BY PRIOR STIMULUS PRESENTATIONS. R.C. Gesteland*, P. Farmer and M. Overway. University of Cincinnati Medical Center, Cincinnati, OH 45267-0521.

Voltage-sensitive dyes observed with a confocal microscope allow the activity of each of hundreds of neurons to be observed as odorous stimuli are sequentially presented. The results of experiments on the salamander show that different cells can respond in different ways to a stimulus, that the responses are strongly dependent upon prior stimulus presentations, and that the difficulty in obtaining reliable and repeatable responses in microelectrode studies is not the result of experimental insult but in fact characterizes the code for odor identity and intensity. In a typical experiment, the first stimulation evokes mostly depolarizing responses from a large fraction of the cell population. Subsequent presentations of the same stimulus and other stimuli are as likely to hyperpolarize or evoke no response as to depolarize. Stimuli are presented as vapors at low concentrations for 1 sec with 5 min between presentations, responses remain vigorous throughout the experiment, and all neurons respond similarly to changes in external [K^+]. Several different membrane ion channels have been identified in patch-clamp studies. It is likely that different cells have differing numbers of these channels and that the duration of activation can be long-lasting. The response of a cell to a stimulus will depend upon the activation states of the various channel types resulting from prior odor experience.

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606.5

OLFACTORY TRANSDUCTION IS INTRINSICALLY NOISY. Graeme Lowe & Geoffrey H. Gold* Monell Chemical Senses Center, Philadelphia, PA.

The high sensitivity of olfaction suggests that olfactory receptor cells reliably detect single odorant molecules. Discrete events, presumably triggered by single odorant molecules, have been observed in insect pheromone receptor cells, but analogous events have not been reported in any vertebrate species. We sought to resolve single molecular responses in rat by recording the transduction current in dissociated olfactory receptor cells. When cells were exposed to threshold concentrations of one of the odorants: menthone, 2-isobutyl-3-methoxypyrazine, isoamyl acetate or 2-hexylpyridine, the whole-cell current displayed pronounced low frequency (< 10 Hz) fluctuations which might represent the summation of single molecular responses. However, fluctuations of similar amplitude and frequency spectrum were also observed in the absence of odorants by inhibiting basal phosphodiesterase activity with IBMX, or by photolytic release of cyclic AMP. Thus, the odorant-induced fluctuations represent the basal, or intrinsic, noise of olfactory transduction, not the activation of receptor proteins by odorants. In unstimulated cells this noise is suppressed by the threshold inherent in the cyclic-AMP activated current. Our data suggest that for many common odorants, single odorant molecules are not reliably detected by mammalian olfactory receptor cells.
Supported by NIDCD Grant DC00505.

606.6

MINERALOCORTICOID (TYPE I) RECEPTOR-LIKE IMMUNOREACTIVITY IS ASSOCIATED WITH BOWMAN'S GLANDS IN THE MAMMALIAN OLFACTORY MUCOSA. J.D. Foster[1*], R.C. Kern[2], and D.Z. Pitovski[1]. Depts. of Otolaryngology - Head and Neck Surgery, [1]Wayne State University, Detroit, MI 48201 and [2]Northwestern University, Chicago, IL 60201.

The location of mineralocorticoid (type I) receptor-like immunoreactivity in mouse, hamster, and guinea pig olfactory mucosae was determined by the use of immunocytochemistry. Within the olfactory mucosae of all three rodent models, acinar cells (found in the glandular portion of Bowman's glands) and duct cells (found in the ductal portion of Bowman's glands) were positive for the mineralocorticoid receptor as revealed by both immunofluorescence and the ABC immunoperoxidase procedures. The distribution of mineralocorticoid receptor-like immunoreactivity was uniform throughout the cytoplasm of the acinar and duct cells of Bowman's glands. The respiratory regions of the nasal mucosa did not appear to be immunoreactive for the mineralocorticoid receptor. There was no immunocytochemical staining when the antibody to the mineralocorticoid receptor was omitted from the primary incubation medium.

There is evidence that Bowman's glands and their ducts are involved in both the secretion of glycoproteins and the secretion of electrolytes and water, processes which are apparently controlled by neurotransmitters (e.g., adrenergic and cholinergic nerve endings) and peptide hormones (e.g., VIP and LHRH). Since mineralocorticoids, such as aldosterone, have been shown to be involved in fluid and electrolyte regulation in other systems, the localization of mineralocorticoid receptor-like immunoreactivity to cells of Bowman's glands and ducts suggests that mineralocorticoids may play a similar role in the olfactory mucosa.

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606.7

LOCALIZATION OF SUPEROXIDE DISMUTASES IN HUMAN OLFACTORY MUCOSA: TRENDS RELATED TO AGE AND ALZHEIMER'S DISEASE. M.L. Getchell*, A.P. Kulkarni, N.S. Rama Krishna, and T.V. Getchell. Depts. of Physiology and ENT Surgery, Univ. of Kentucky Coll. of Med., Lexington, KY 40536.

Mn and CuZn superoxide dismutases (SODs) are enzymatic scavengers of superoxide free radicals. Mitochondrial MnSOD and cytosolic CuZnSOD were localized immunohistochemically in olfactory mucosa obtained at autopsy from 19 subjects, 11 males and 8 females, ranging from 24 to 98 years of age. Four patients were classified as young/middle aged (under 60 years old), 6 as old (over 60 years old), 4 (69-98 years old) as "successfully aged", and 5 (52-90 years old) had pathologically confirmed Alzheimer's disease (AD). Postmortem intervals (PMIs) ranged from 2-22 h. In young/middle aged subjects, intense immunoreactivity was observed in olfactory receptor neurons, sustentacular and basal cells in the olfactory epithelium and in Bowman's glands, olfactory nerves, and vascular endothelium in the lamina propria. Comparing young/middle aged and old, there was an age-related decrement in the overall intensity and distribution of immunoreactivity that was most pronounced in olfactory receptor neurons, basal cells, and endothelium. Old subjects often had restricted foci of intense epithelial immunoreactivity. In the "successfully aged," large regions of immunoreactivity as intense as that in young/middle aged subjects were intermingled with smaller regions of reduced intensity. Subjects with AD generally exhibited the most intense staining that was distributed like that in the "successfully aged." These differences were not related to PMI; the 3 youngest subjects had the longest PMIs, 3 old subjects had short PMIs; age- and AD-related trends were observed in subjects from the different groups matched for age and PMI. High levels of SODs in the young/middle aged and "successfully aged" may reflect the higher cellular viability of their olfactory mucosae, while high levels in the AD subjects may be related to oxidative stress associated with the disease process. Supported by NIH grants DC 01715 (MLG) and DC 00159 (TVG).

606.8

NMDA Blockade Partially Reverses Paired-Pulse Depression in the Turtle Olfactory Bulb

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In the turtle olfactory bulb, primary olfactory neurons are thought to excite mitral/tufted (M/T) cells by stimulating both NMDA and non-NMDA glutamate receptors (Berkowicz *et al.*, J. Neurophysiol. 71, 1994). It has been suggested that the non-NMDA receptors allow M/T cells to respond rapidly to incoming odor information while the NMDA receptors might provide a means for the short-term temporal integration of this information. We have examined this idea by optically monitoring electrical activity in the isolated turtle olfactory bulb stained with the voltage-sensitive dye, RH 155. The figure below shows optical responses recorded from the external plexiform layer evoked by paired-pulse stimulation (500 ms separation) of the olfactory nerve before (thin trace) and after (thick trace) addition of the NMDA antagonist, AP-5 (100 μM), to the bathing solution. As can be seen, NMDA blockade partially reverses depression of the second (test) response but has little effect on the initial (conditioning) response.

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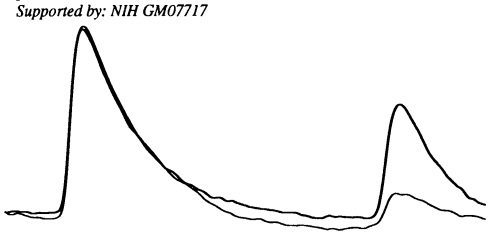

606.9

A QUANTITATIVE PCR STUDY OF $GABA_A$ AND GLUTAMATE RECEPTOR SUBUNIT EXPRESSION IN THE RAT OLFACTORY BULB FOLLOWING PERIPHERAL DENERVATION. P. Bovolin*[1], I. Perroteau[1], R. Ebbers[2], A. Fasolo[1]. [1]Dept. Animal Biology, Univ. of Torino, 10123 Torino, Italy and [2]Dept. Anim. Physiology, Univ. of Nijmegen, Nijmegen, 6525 The Netherlands.

The olfactory system is a remarkable model to study *in vivo* basic phenomena occurring in neuronal plasticity. Olfactory neurons, located in the olfactory mucosa, send their axons to the glomerular layer of the olfactory bulb where they make synapses with several neuronal types. An important consequence of peripheral afferent denervation of the olfactory bulb is a sharp reduction of the neurotransmitter dopamine due to loss of tyrosine hydroxylase (TH) mRNA. In the present study we began to address the question of whether the expression of subunits belonging to various $GABA_A$ and glutamate receptor complexes is modified after unilateral chemical lesion ($ZnSO_4$-irrigation) of the rat olfactory mucosa. Analysis of the olfactory bulb total RNA was performed on 3 groups of male adult rats [(1) untreated, (2) saline- and (3) $ZnSO_4$-irrigated rats], sacrificed 12 days post-lesion. Both competitive polymerase chain reaction (PCR) assays and semiquantitative PCR experiments were employed to compare the relative abundance of mRNAs in the 3 groups. Measurements of TH mRNA were used as an indication of the effectivencss of the lesion. In $ZnSO_4$ irrigated rats we found a remarkable reduction of both the γ_{2L} and γ_{2S} $GABA_A$ receptor subunit mRNAs, while we observed only a slight increase in the NMDR1b mRNA splicing form and no significant change in the NMDR1a and in the GluR1 mRNAs. Our findings suggest that the expression of at least some $GABA_A$ receptor subunits in the olfactory bulb are under transynaptic regulation. Since it has been recently demonstrated that glutamate-immunoreactivity is present in olfactory neuron terminals (Sassoè-Pognetto et. al., Neuroreport, 1993), loss of release of the neurotransmitter glutamate could be responsible of the negative modulation in the expression of $GABA_A$ receptor genes in post-synaptic elements. (Supported by grants from CNR, MURST and CEE).

606.10

OLFACTION AND CHEMICAL INJURY OR SENSITIVITY. Donald L. Dudley† Chemical Injury Research Foundation, Tacoma, WA 98407.

Auditory, visual and upper and lower somatosensory evoked potentials were measured in 20 patients with clinical criteria for chemical sensitivity. Measures were made before and during exposure to chemicals that volatilized and led to complete debilitation.

In general, auditory and visual P3 latencies and N19, P22, P37 and N45 latencies were significantly increased (p= 0.0001). In general, amplitude for the same variables was not significantly changed (p= 0.20). An exception was the auditory P3 amplitude which was significantly decreased (p= 0.0001).

Latency correlations were low or not significant prior to exposure and highly significant during exposure. On the other hand, amplitude correlations were highly significant before exposure and not significant during exposure.

Since olfactory signals are sent to nearly every part of the brain it is thought that this system's use of excitatory amino acids and their precursors for neurotransmission in association with chemicals with six or less carbon fragments that volatilize could lead to brain cell injury and subsequent neuronal changes that are experienced by the patient as the signs and symptoms of chemical sensitivity or injury.

The above will be discussed in association with agonists and antagonists of excitatory amino acids in humans.

606.11

LOCALIZATION OF HUMAN GUSTATORY AND SPEECH CENTERS USING FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI). J. Hirsch*[1,3], R. DeLaPAZ[2,3], N. Relkin[3], J. Victor[3], T. Li[1] and K. Kim[1,3]. Dept of Neurology[1] and Radiology[2], Memorial Sloan-Kettering Cancer Center; Dept of Neurology and Neuroscience, Cornell University Medical College[3], New York, NY 10021.

We present the first direct evidence that the cortical representation of human taste sensations tends to be lateralized to the dominant hemisphere. Fourteen contiguous 5mm coronal slices rostral to a plane passing through the colliculi were imaged on 1.5T GE scanner equipped for echo-planar imaging. Two right handed and two left handed normal volunteers were imaged during baseline and stimulation conditions consisting of 8cc of either water, 1 M salt or 1 M sucrose injected into the mouth. Speech tasks were either alphabetical naming or memorized rhymes and confirmed that the dominant hemispheres for language were contralateral to handedness for all 4 subjects. Tongue movements alone and water conditions served as controls for related activations. A multi-stage single voxel statistical analysis including replication criteria identified active taste regions in the insula and frontal operculum predominantly in the dominant hemisphere. These findings suggest that human cortical gustatory processing is functionally lateralized.

Supported by MSKCC (JH, RD), EY7977 (JV) and CV Starr Foundation (NR).

606.12

A 3-D FUNCTIONAL MRI DEMONSTRATION OF BRAIN STRUCTURES INVOLVED IN HUMAN OLFACTION. N.F. Ramsey, R. Rawlings, P. Van Gelderen, J. Duyn, C.T.W. Moonen, D.W. Hommer* National Institute of Alcoholism and Alcohol Abuse, NMR *In Vivo* Center, Laboratory of Diagnostic Radiology Research. NIH, Bethesda, MD 20892.

Olfactory processing selectively involves the limbic system. Imaging of limbic areas with fMRI is problematic, due to their location near the base of the skull, close to major blood vessels. We report an olfactory stimulation study involving a 3D Echo-Shifted FLASH sequence and a Gaussian random field based statistical evaluation (Worsley, K. J., Evans, A. C., Marrett, S. et al., J. Cerebr. Blood Flow Metab. 12, 900-918, 1992). Pleasant odors were administered to 10 subjects (Ss). After anatomical scans, 8 stimulation trials were done at 3 minute intervals. Each trial consisted of two consecutive functional scans, with 7 seconds between scans. Olfactory stimulation was switched on at the end of the first scan, and off at the end of the second scan (odor duration 27 secs.). For each subject, data were converted to difference images, obtained by subtracting the unstimulated from the stimulated 3D image within each trial. Trials were then summed, and Ss analyzed individually. Significantly activated voxels were found in structures of the olfactory system, including the amygdaloid complex (5Ss), septal nuclei (3Ss) ventromedial striatum (3Ss), posterior lateral orbitofrontal cortex (4Ss). Very few significant voxels were found outside of the olfactory system. These results indicate that the 3D Echo-Shifted Flash sequence enables *in vivo* measurement of odor-induced signal-intensity changes in human brain and that a random field model is a rigorous method for minimizing false positive results, while preserving statistical power.

PROCESS OUTGROWTH II

607.1

IDENTIFICATION OF NOVEL PHOSPHOLIPID MEDIATORS AND LIPID SECOND MESSENGERS CAUSING GROWTH CONE COLLAPSE AND NEURITE RETRACTION IN PC12 CELLS. G.Tigyi*, A. Sebök‡, J. Szeberényi† Dept.of Physiology & Biophysics, University of Tennessee, Memphis, TN 38163; †Dept.of Biology, Medical Univ. of Pécs, 7624, Hungary.

The endogenous phospholipid lysophosphatidic acid (1-acyl-2-lyso-*sn* glycerophosphate, LPA) causes the remodeling of actin cytoskeleton leading to neurite retraction in NGF-differentiated PC12 cells (Tigyi & Miledi, *J. Biol. Chem.* **267**, 21360-67, 1992). We now show that several other structurally related phospholipids, including the ether-linked hexadecyl-lyso-glycerophosphate (GP), hexadecylacetyl-GP, as well as palmytoylacetyl-GP, palmytoyl-cyclicGP, and sphingosylphosphoryl-choline, all in nanomolar concentrations, cause a rapid retraction of neurites. Interestingly, all these mediators activate oscillatory chloride currents in *Xenopus* oocytes via receptor-mediated activation of the phosphoinositide system. In contrast, PAF, DAG, and a number of other structurally similar lysophospholipids (LPC, LPE) failed to elicit a responses in either cell. LPA action is initiated at a plasma membrane receptor blocked by suramin. Retraction was not inhibited by pertussis toxin, but was sensitive to neomycin, Al^{3+} with F^- ions, and U73122, indicating G protein involvement. LPA-induced neurite retraction was accompanied by activation of phosphoinositide turnover, Ca^{2+} transients and inhibition of adenylylcyclase. However, activation of these very same signaling events was also found following carbachol or bradykinin stimulation, suggesting that an alternative signaling pathway is responsible for neurite retraction. Phorbol esters blocked retraction, yet staurosporine failed to inhibit it in concentrations that inhibit PKC. Although arachidonic acid and prostanoids cause neurite retraction, the action of LPA was not inhibited by indomethacin. Leukotrienes C_4, D_4 and E_4 were also ineffective. ADP ribosylation of the small-molecular-weight GTP binding protein *rho* by C3-toxin also prevented neurite retraction. In contrast, LPA caused cell shape changes in PC12 cells expressing a dominant negative *ras*. Interestingly, the lipid second messengers sphingosine, ceramide (C2 and C8), and sphingosine-1-phosphate also caused neurite retraction. Because LPA was detected in postinjury and posthemorrhagic cerebrospinal fluid, a hypothesis is suggested that LPA-like lipid mediators may alter the regenerative sprouting of axons through a yet unidentified signaling mechanism linked to the receptor-mediated generation of ceramide and sph-1-P. Supported by grants from the NSF (IBN 93-21940), the APA (TB1-9301), and the AHA National Center (A.S.)

607.2

TALIN FUNCTION IS SPATIALLY INTEGRATED IN THE NEURONAL GROWTH CONE. Anne M. Sydor* and Daniel G. Jay. Department of Cellular and Molecular Biology, Harvard University, Cambridge MA, 02138.

Dynamic extension and retraction of filopodia is thought to function in signal detection and force generation needed for directed motility of the neuronal growth cone. To address the role of cytoskeletal proteins in filopodial motility we have used micro-CALI (Chromophore Assisted Laser Inactivation) to functionally inactivate the cytoskeletal protein talin in growth cones of chick DRG neurons in culture. We have found that inactivation of talin in a region of the growth cone results in a loss of filopodial extension and retraction, whereas inactivation of talin over the whole growth cone has no effect on filopodial motility. Micro-CALI inactivates proteins within a 10-15 micron spot by directing the energy of a 620 nm laser beam to the protein via a dye-labeled antibody. Neurons were trituration loaded with malachite green-labeled antibodies to talin and regions of the growth cone were laser irradiated for five minutes. This caused the rate of extension and retraction of filopodia within the irradiated area to drop to zero for one to four minutes; filopodia outside this region showed no change in motility. Irradiation of whole growth cones at the same laser power did not result in any change in filopodial behavior. Laser irradiation of malachite green-BSA loaded controls showed no change in growth cone motility. This provides evidence that talin can act to couple filopodial movement to cytoskeletal dynamics. These data also suggest that the growth cone spatially integrates internal differences in protein function. We believe such spatial integration of protein function could provide the basis for directed growth cone motility and are investigating this possibility.

607.3

CALCINEURIN FUNCTIONS IN NEURITE OUTGROWTH AND DIRECTED GROWTH CONE MOTILITY. H. Y. Chang, F. Rusnak‡, and D. G. Jay*, Dept. Cell. Mol. Biol., Harvard Univ., Cambridge, MA 02138. ‡Section of Hematology Research, Mayo Clinic and Foundation, Rochester, MN 55905.

Calcineurin is a Ca^{++}-calmodulin-dependent protein phosphatase that is most abundant in the brain. Application of specific inhibitor cyclosporin A or FK506 to cultured chick dorsal root ganglia neurons delayed neuritogenesis and inhibited neurite extension, suggesting that calcineurin is involved in both of these processes. On the basis of its potential signaling function and localization in the growth cone, we hypothesized that differential calcineurin activity across the growth cone may direct growth cone motility and guide neurite extension. This idea was tested by microscale chromophore-assisted laser inactivation (micro-CALI) of calcineurin in cultured DRG neurons. Calcineurin phosphatase activity is sensitive to CALI *in vitro*. Chromophore-labeled antibodies against calcineurin were loaded into chick DRG neurons by trituration, and site-directed inactivation of calcineurin in regions of the growth cone was achieved with a laser microbeam. Micro-CALI of calcineurin caused the filopodia and lamellipodia in the irradiated region to retract; growth cone behaviors outside of the irradiated area were not affected. Micro-CALI with chromophore-labeled pre-immune serum had no effect on growth cone motility. Since the instantaneous and regional loss of calcineurin activity causes local retraction, we suggest that the spatial distribution of calcineurin activity regulates growth cone motility.

607.4

NERVOUS SYSTEM ABNORMALITIES RESULT FROM TARGETED MUTATION OF MAP1b. W. Edelmann, P. Costello, P. Davies, L. Roback, B.H. Wainer*, R. Kucherlapati, Depts. of Molecular Genetics, Neurosurgery and Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

For proper development of the brain, an intact complement of cytoskeletal elements is required, including that of the microtubule associated proteins. The microtubule associated protein 1b (MAP1b) is a member of this microtubule network in neurons and glia. MAP1b expression is developmentally regulated and is highest during embryogenesis and the early postnatal period. This expression pattern suggests involvement in the regulation of neurite outgrowth. We targeted a mutation in the MAP1b gene which resulted in embryonic lethality in homozygous mice and a marked phenotype in some heterozygous animals. This phenotype includes blindness, an ataxic gait, a spastic tremor of the hindlimbs and an overall reduction in body weight of 25-50%. Histologically, the phenotype is pronounced in the cerebellum. The principal abnormality is in the configuration of the Purkinje cell bodies and their dendritic arborizations. Immunohistochemical studies with MAP1a and MAP1b specific antibodies show reduced staining in Purkinje cells. No decrease in staining of another cytoskeletal protein, MAP2, was observed. Western blots revealed no change in levels of neurofilament proteins, MAP2 or tau. Additional abnormalities were seen in the hippocampus, olfactory bulbs and visual system. These results demonstrate an essential role of MAP1b in the development of the brain.

607.5

EVIDENCE THAT CAMs STIMULATE NEURITE OUTGROWTH BY ACTIVATING FGF RECEPTORS IN NEURONS. P. Doherty*, E.J. Williams, J. Furness and F.S. Walsh. Department of Experimental Pathology, UMDS, Guy's Hospital, London SE1 9RT.

We have used monolayers of parental 3T3 and 3T3 cells expressing one of three transfected cell adhesion molecules (CAMs) (NCAM, N-cadherin and L1) as a culture substrate for rat cerebellar neurons. A number of lines of evidence suggest that neurite outgrowth stimulated by the above CAMs involves activation of the FGF receptor in neurons. Affinity purified antibodies that bind to the FGF receptor in neurons inhibit neurite outgrowth stimulated by the above CAMs but have no effect on integrin dependent neurite outgrowth or neurite outgrowth stimulated by a variety of other agents. In addition we have found that a soluble L1-Fc chimera is as effective as cell associated L1 in promoting neurite outgrowth. The response to the L1-Fc chimera is fully inhibited by pre-treatment of neurons with antibodies that bind to L1 or the FGF receptor. The response is also associated with an increase in phosphotyrosine on the same set of neuronal proteins, including the FGF receptor, that are phosphorylated following activation of the FGF receptor with basic FGF. We conclude that activation of the FGF receptor, rather than changes in adhesion, underly the neurite outgrowth response to a number of CAMs.

607.6

PERMISSIVE ROLE OF GAP-43 IN NEURITE OUTGROWTH: PRIMARY SENSORY NEURONS AND TRANSGENIC MICE. L. Aigner, F. Botteri, and P. Caroni*. Friedrich Miescher-Institute, P.O. Box 2543, CH-4002 Basel, Switzerland.

The specific association of the growth-associated protein GAP-43 with nerve growth during development and regeneration suggests that it may play a role in this process (Skene (1989) Ann. Rev.Neurosci.12:127). Downregulation of neuronal GAP-43 expression during development frequently coincides with the process of synapse elimination, suggesting that it may also be involved in local process outgrowth and maintenance. To define the role of GAP-43 in neurite outgrowth we have 1) analyzed neurite and growth cone (GC) activity in primary sensory neurons specifically depleted of GAP-43 by an antisense approach (Aigner and Caroni (1993) JCB123:417), and 2) generated transgenic mice that constitutively express chick GAP-43 in adult neurons. Characteristic features of primary sensory neurons regenerating neurites on a PORN/laminin substratum in the absence of GAP-43 included: 1) poorly adhesive GC's with highly dynamic peripheral lamellae and rudimentary central domains; 2) absence of NGF-induced GC spreading or IGF1-induced GC branching; 3) high susceptibility to retraction induced by CNS myelin-derived inhibitors; 4) protection against inhibitor-induced retraction by NGF. Mice expressing GAP-43 in adult neurons (neuron-specific Thy-1 promotor construct) show extensive intramuscular nerve sprouting and a striking motor and behavioral phenotype. Several lines of mice were generated and the phenotype correlated with expression levels of the transgene. Our results indicate that GAP-43 plays an important permissive role in neurite outgrowth.

607.7

GAP-43 IMMUNOREACTIVITY AND AXONAL REGENERATION OF RAT RETINAL GANGLION CELLS. H. Schaden*, M. Bähr+, C.A.O. Stuermer. University of Konstanz, Germany, +MPI Tübingen, Germany.

While more than 90% of the retinal ganglion cells (RGCs) in rats die upon optic nerve section (ONS), a subfraction of those that survive is capable ("competent") of regenerating an axon into a peripheral nerve (PN) graft (Vidal-Sanz et al., 1987). To determine whether re-expression of GAP-43 and the transcription factor c-JUN is specifically found in "competent" RGCs, the relevant antibodies were applied to sections of retinae (i) in rats with ONS only and (ii) in rats which had received a PN graft. RGCs were labeled by the retrograde tracer Fluorogold (FG) applied (i) to the ON to identify surviving RGCs, (ii) to the PN graft to identify RGCs with regenerating axons.

(i) GAP-43-immunoreactivity (Ir) was seen in 22% of RGCs at 5d after ONS and in 2% and 1% at 14 and 21d, respectively, in rats without grafts. (ii) In rats with PN grafts, only those RGCs which were FG-labeled (and thus had regenerated an axon into the graft) exhibited GAP-43, and they represented 3% and 2% at 21 and 28d after surgery, respectively. c-JUN-Ir, however, was found in (i) many more RGCs than GAP-43 and in (ii) at least twice as many RGCs at 21 and 28d, than there were RGCs labeled with FG and exhibiting GAP-43-Ir.

Thus regenerating axons in PN grafts derive specifically from GAP-43 re-expressing RGCs. Appearance of GAP-43 may therefore identify those RGCs that possess the intrinsic property of axonal regeneration or sprouting.

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607.8

REDUCED PRESYNAPTIC ACTIVITY DURING NEUROMUSCULAR DEVELOPMENT PROMOTES FOREIGN MOTOR ENDINGS IN *DROSOPHILA*. J. Jarecki* and H. Keshishian, Genetics and Biology Depts., Yale Univ., New Haven, CT 06511.

Electrical activity plays a role in the development of *Drosophila* neuromuscular synapses. We previously showed that blocking presynaptic (but not postsynaptic) activity during embryonic synaptogenesis promoted inappropriate motoneuron contacts (JJ & HK, Neurosci Abstr, 19:645). In order to follow the development of these ectopic contacts during larval life, temperature sensitive activity mutants affecting Na channels were examined (*para*^{ts1}, *nap*^{ts}, and *sei*^{ts}). Collateral sprouting increased onto muscle fibers 6&7 in the mutant embryos when reared at 34°C during stages 15 &16. (WT: 16% sprouts, n=169 segments; *para*: 25%, n=163, p <.05; *nap*: 29%, n=91, p <.025; *sei*: 28%, n=103, p <.05). By the 3rd instar, an increase in the number of ectopic motor endings was also observed on these fibers in *nap* and *sei* but not *para* larvae. (WT: 5.6% of fibers receiving sprouts, n=89 segments; *para*: 8%, n=110, p >.10; *nap*: 17%, n=76, p <.025; *sei* :17%, n=117, p <.01). About 60% of the ectopic endings arise from the transverse nerve, 30% from endings on the oblique muscle fibers, and less than 5% from endings on muscle fibers 13 or 8. The morphology of the native neuromuscular endings is not changed by the presence of the ectopic synapses. Temperature shifting (37°C 6 hrs/day) during larval development further increased the number of ectopic endings to 30% in *nap* (n=84) and 34% in *sei* (n=104) as opposed to shifted WT animals (6%, n=62) and shifted *para* (2.4%, n=80). Significant increases were not observed in hyperactive mutants such as *eag sh* or *sei*^{2}. The critical period includes both late embryogenesis and the 1st instar. We hypothesize a reduction in synaptic activity in the embryo induces filopodial sprouting, and that in the 1st instar reduced synaptic activity stabilizes them. These results show that synaptic activity contributes to the maintenance of correct neural connectivity.

607.9

KAINATE RECEPTOR ACTIVATION INDUCES F1/GAP-43 mRNA IN DENTATE GYRUS GRANULE CELLS AND SPROUTING OF ITS AXONS, THE MOSSY FIBERS: BLOCKADE BY MK-801 AND PENTOBARBITAL. R. K. McNamara*, P. A. Serrano & A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern Univ. Evanston, IL 60208.

In the adult rat, dentate gyrus granule cells do not express F1/GAP-43 mRNA (Rosenthal et al., EMBO, 6: 3641, 1987). Kainic acid (KA), a rigid glutamate analogue, induces F1/GAP-43 mRNA in granule cells maximally at 24 hr (McNamara et al., Soc. Neurosci., 1993). KA also induces sprouting of granule cell axons, the mossy fibers, into the supragranular layer beginning ~10 d after KA (Nadler et al., Brain Res., 182: 1, 1980). To relate the induction of F1/GAP-43 mRNA in granule cells to subsequent mossy fiber sprouting, adult male Sprague-Dawley rats were injected with: 1) saline 2) KA (s.c., 10 mg/kg), 3) PENT (i.p., 50 mg/kg), 4) PENT then KA 15 min later, 5) MK-801 (i.p., 2 mg/kg), and 6) MK-801 then KA 15 min later. Rats were sacrificed either (A) 24 hr after the last injection and F1/GAP-43 gene expression assessed using quantitative *in situ* hybridization or (B) 30 d later when mossy fiber sprouting was assessed with Timm's stain.

KA alone first induced F1/GAP-43 mRNA in granule cells as well as subsequent mossy fiber sprouting into the supragranular region. Visual inspection revealed no significant cell loss in areas CA3/4 after KA alone. Pre-treatment with PENT or MK-801 significantly attenuated both F1/GAP-43 mRNA induction and supragranular sprouting but, except in one case, did not affect cell loss in CA3/4. In the one rat showing extensive cell loss in areas CA3/4, only minor mossy fiber sprouting was observed. When administered alone, PENT and MK-801 did not affect F1/GAP-43 mRNA, mossy fiber sprouting, or cell survival. It is proposed that induction of F1/GAP-43 expression in granule cells leads to mossy fiber sprouting. This sprouting appears to be independent of extensive cell loss in areas CA3/4. [supported by MH25281-21 to A.R. and Post-doctoral fellowships from NSERC to R.K.M. and NSF to P.A.S.].

607.10

INCREASED SPROUTING OF PRIMARY AFFERENTS IN THE MYELIN-FREE RAT SPINAL CORD. Josef P. Kapfhammer*, Guido Schwegler, and Martin E. Schwab. Brain Research Institute, University of Zürich, August-Forel-Str. 1, 8029 Zürich, Switzerland.

We are interested in defining physiological functions of myelin-associated neurite growth inhibitors. These molecules are involved in the prevention of axonal regeneration in the adult mammalian CNS (Schnell et al., Nature *367*:170, 1994). Previous work has shown that the regional expression of GAP-43, a putative marker for fiber growth and plasticity, is inversely related to the degree of myelination in the CNS of normal rats (Kapfhammer and Schwab, JCN *340*:194, 1994). Furthermore, GAP-43 expression is strongly increased in myelin-free lumbar spinal cords when myelination was suppressed by neonatal X-irradiation (Kapfhammer and Schwab, Eur.J.Neurosci. *6*:403, 1994). These results suggest a role for the inhibitors in restricting sprouting and anatomical plasticity in the normal CNS.

We have now investigated whether the increased expression of GAP-43 in the myelin-free spinal cord is indeed correlated with an increased potential for sprouting of nerve fibers and terminals. Dorsal roots of lumbar segments L2- L4 were cut in myelin-free and normal cords of 8 or 15 day old rats. Sprouting of primary afferents was studied 3 weeks later using thiamine monophosphatase (TMP) histochemistry. This enzyme specifically labels a subclass of spinal cord primary afferents. We found that the sprouting of TMP positive afferents is increased in the myelin-free cords as compared to normal controls. This further supports our hypothesis that CNS myelin and its associated neurite growth inhibitors are involved in the regulation of terminal sprouting and plasticity in the normal CNS.

607.11

AN ANTENNAE-AMPLIFIER MODEL FOR REGULATION OF NEURONAL GROWTH CONE BEHAVIOR

S.B. Kater, P. Dou, L.R. Mills*, R.W. Davenport Colorado State University, Ft. Collins, CO 80523;*Toronto Western Hospital, Toronto, Ontario M5T-2S8.

Neuronal guidance is determined, in part, by the interaction of advancing growth cones with the environmental guidance cues they encounter. Growth cone behavior may change locally at the level of individual filopodial changes, regionally in restricted subdomains of the individual growth cone or extensively with overall changes in outgrowth pattern. This spectrum of growth cone behaviors enables growth cones to guide extending axons and dendrites to their targets. At each level, such morphological changes have been associated with changes in intracellular calcium levels. How the underlying architecture of this second messenger system, i.e. the sources and sinks of calcium, interact and compartmentalize within growth cones to coordinate resultant growth cone behavior has not been directly addressed. Here, in a single system we provide evidence of how such components can be arranged in individual growth cones. In preparations consisting of both isolated filopodia and their parent growth cones, intracellular calcium signals were examined in response to application of excitatory neurotransmitters and the calcium ionophore A23187. The presence of thapsigargin or calcium-free medium revealed that intracellular calcium pools are present and functional within the growth cone proper but not in isolated filopodia. Calcium release from stores can amplify second messenger signals dependent upon calcium influx, as occur in filopodia. The spatial distribution of growth cone organelles, which could serve as morphological correlates to such calcium amplification, were examined. While the large majority of organelles are located in the central core of the growth cone proper, peripheral organelles can be present at the base of a subset of filopodia. Such peripheral organelles could subserve a regional amplification system within growth cones. A model for second messenger regulation of growth cone behavior based on highly sensitive distal antennae, limited peripheral amplifiers and a dominant central amplifier will be presented.

VISUAL CORTEX: STRIATE V

608.1

RULES DETERMINING THE MONOSYNAPTIC CONNECTIONS BETWEEN LGN CELLS AND SIMPLE CELLS IN CAT VISUAL CORTEX. *R. Clay Reid*, Jose-Manuel Alonso and Torsten N. Wiesel.* Laboratory of Neurobiology, The Rockefeller University, New York, NY.

We have studied the rules that determine whether an individual afferent from the lateral geniculate nucleus (LGN) will have a monosynaptic connection with a given cortical simple cell. Our question was: how precisely can the synaptic connections between these two cell types be predicted by their functional properties. Single units were recorded with electrodes in both LGN and cortex. Receptive field position and structure were mapped with white noise visual stimuli. Connectivity between pairs of cells (one LGN cell and one simple cell) were determined with cross-correlation analysis of the spike trains. Narrow peaks with very short latencies were taken as evidence for monosynaptic connections.

The rules of connectivity are very precise. Over 80% of cell pairs were monosynaptically connected when a receptive field was centered over a simple cell subregion of the same sign (*on* or *off*). Connections were never seen when an different sign LGN receptive field was centered over a simple subregion (*on vs. off*). The probability of monosynaptic connection also depended on a good match between the receptive field sizes and the time-course of the responses. In particular, fast LGN afferents were less likely to be connected to a simple cell if the overlapping simple subregion had slower dynamics than the LGN center.

The elongated distributions of the direct *on* an *off* LGN afferents to individual simple cells explains at least part of cortical orientation selectivity. It does not, however, reject an important role of intracortical processes in sharpening this tuning.

(Supported by NIH EY10115 and EY05253, the Klingenstein Fund and Fulbright/MEC).

608.2

COUPLING BETWEEN NEIGHBORING LGN CELLS: POSSIBLE IMPLICATIONS FOR SIMPLE RECEPTIVE FIELDS. *Jose-Manuel Alonso*, R. Clay Reid.* Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

The receptive field of simple cells is constructed largely by the convergence of the aligned input from the lateral geniculate nucleus (LGN). However, intracortical and even intrageniculate interactions could also determine part of the final shape of the receptive field. Here we are presenting evidence for excitatory connections between neighboring LGN cells recorded with the same electrode. Each receptive field was mapped with a pseudo-random dynamic checkboard stimulus (m-sequence), and the spikes were isolated with the Brainwave System. Forty percent of the LGN pairs studied showed strong and narrow peaks with cross-correlation analysis. This percentage was even larger for those pairs with good overlap of the centers (>20%), and well matched size and timing between the receptive fields of the two cells. Asymmetric narrow peaks were seen mainly when the two cells had partially overlapped *on* and *off* receptive fields. Symmetric narrow peaks were more frequent in pairs with partially overlapped either *on/on* or *off/off* receptive fields. If, as we suspect from our geniculocortical study, the connected pairs feed into the same simple cell, this could provide a means to generate a synergistic input that could drive the simple cell to threshold. (Supported by Fulbright/MEC, NIH EY10115 and EY05253, and the Klingenstein Fund)

608.3

VISUALLY EVOKED CALCIUM ACTION POTENTIALS IN SIMPLE AND COMPLEX CELLS IN THE CAT STRIATE CORTEX. *Judith A. Hirsch*, José-Manuel Alonso, and R.Clay Reid.* Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Synapses made by thalamic afferents compose a small minority of those made in layer 4, yet their input largely accounts for the simple receptive field. One means of enhancing excitatory synaptic input is through voltage-dependent calcium conductances in the postsynaptic membrane. We examined the role of these in the construction of visual cortical receptive fields by making whole-cell recordings *in vivo*.

We mapped receptive fields with randomly flashed bright and dark squares (Jones and Palmer, 1987, *J. Neurophysiol.* V58: 1187). Intracellular labeling showed that the simple cells we studied were either spiny stellate or pyramidal neurons. When recordings were made with a conventional internal solution, visual stimulation evoked large, fast action potentials. Intracellular blockade of these sodium spikes with QX-314 routinely revealed, on visual stimulation or current injection, smaller, slower action potentials presumably mediated by calcium. Moreover, we could map receptive fields with the visually driven calcium action potentials as we usually do with sodium spikes. Parallel results were obtained for pyramidal cells with complex receptive fields. We conclude that regenerative calcium potentials, normally eclipsed by the sodium spikes they trigger, may play a significant role in conveying synaptic excitation to the axon hillock. (Supported by NIH EY09593 and a Klingenstein Award to J.A.H., a Fulbright/MEC to J.M.A., NIH EY10115 and a Klingenstein Award to R.C.R. and NIH EY05253 to T.N.Wiesel.)

608.4

SYNAPTIC RESPONSE PROPERTIES OF COMPLEX CELLS IN THE CAT STRIATE CORTEX. *María V. Sánchez-Vives*, Judith A. Hirsch, José-Manuel Alonso and R. Clay Reid.* Laboratory of Neurobiology, The Rockefeller University. New York, NY 10021.

Complex cells in the superficial layers of area 17 often have more transient responses than the simple cells in layer 4 that provide much of their input. We used whole-cell recording *in vivo* to measure the relative role of synaptic excitation and inhibition in the generation of these briefer responses. The stimuli were randomly flashed bright and dark squares each lasting 40ms. Most of the cells we recorded from were pyramids, as identified by intracellular label. In these cells, the stimulus evoked EPSPs were frequently followed by marked inhibition. This inhibition seemed in large part to be mediated by chloride, as it persisted in the presence of QX-314 which suppresses the slow IPSPs mediated by potassium. The strong inhibitory component of the response observed in pyramidal cells suggests that there is a population of inhibitory interneurons driven by the rapidly flashed squares. In fact, we have recorded from a large basket cell that was vigorously excited by these stimuli. Such cells project densely to pyramidal neurons (Somogyi et al., 1983, *Neuroscience* V10: 261). We propose that the inhibition mediated by smooth cells regulates the temporal properties of their target neurons in the superficial layers of the visual cortex.(Supported by F.P.I.(M.E.C.)to M. V.S.V, NIH EY09593 and a Klingenstein Award to J.A.H., Fulbright/M.E.C. to J.M.A., NIH EY10115 and a Klingenstein Award to R.C.R. and NIH EY05253 to T.N.Wiesel.)

608.5

THE NATURE OF INPUTS UNDERLYING SIMPLE CELL DIRECTION SELECTIVITY. D. Ferster* and L.L. Kontsevich
Dept. of Neurobiology, Northwestern Univ., Evanston, IL 60208 and Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

Direction selectivity in simple cells of cat area 17 appears to be based on variations within the receptive field of the time course of the response to visual stimulation. Response latency to flashed stimuli is longer in those parts of the receptive field first encountered by a stimulus moving in the preferred direction. We have explored whether the latency varies smoothly across the receptive field or abruptly in a small number of shifts, and we have characterized the properties of the inputs underlying these variations.

Intracellularly recorded fluctuations in membrane potential evoked by stationary sinusoidal gratings at 8 spatial phases (*Science* **257**:1901) were applied to a singular valued decomposition (SVD). This analysis showed that each of the 8 responses (R_n, where n = spatial phase) could be expressed with up to 98% accuracy as a linear combination of 2 functions, $f_1(t)$ and $f_2(t)$. I.e., $R_n = A_n \cdot f_1 + B_n \cdot f_2$. The coefficients A and B varied nearly sinusoidally with spatial phase of the stimulus grating, indicating that the two types of synaptic input underlying f_1 and f_2 were linear in spatial summation. The SVD does not provide the actual input signals from the two types of synaptic input; f_1 and f_2 are instead linear combinations of those signals. To derive the input signals, a minimization procedure was applied to the even harmonics of R_n. The inputs derived from this procedure were approximately 90° out of spatial phase with one another at the optimal spatial frequency, and delayed with respect to one another by 60 to 80 ms. They were also of unequal strength, the mismatch in strength being greater for cells with lower direction selectivity.

From this analysis we conclude that 1) simple cells receive input from two cell types with different response latencies; 2) the two types are spatially linear but temporally nonlinear; 3) the simple cell sums these inputs in a highly linear fashion.

608.6

Effect of Eye Misalignment on Receptive Field Formation in Realistic Visual Environments.
Harel Shouval, Nathan Intrator†, C. Charles Law, and Leon N Cooper*. Department of Physics and Neuroscience and Institute for Brain and Neural Systems, Brown University, Providence RI 02912.

In this paper we study the ability of cortical cells to concurrently develop orientation selectivity and ocular dominance. We examine whether two proposed learning rules BCM, and a stabilized Hebbian Rule (PCA) can accomplish this aim. In addition we study how initial misalignment affects the binocularity of mature cells. We chose a realistic visual environment, composed of natural scenes that have been preprocessd by center surround filters. Each neuron is exposed to partially overlapping circular regions of the images originating from both eyes. We examine how changing the degree of the overlap, affects the properties of the mature cells. We find that for both learning rules the receptive fields from the two eyes become matched. For the PCA rule, the receptive fields from both eyes are symmetric, and are completely binocular as long as there is an overlap. For BCM neurons on the other hand the receptive fields from both eyes are non symmetric and mature neurons attain varying degree of binocularity.

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608.7

SPATIAL EXTENT OF V1 EXTRA-RF MECHANISMS IN MACAQUE K. Zipser*, T-S. Lee, V.A.F. Lamme, and P.H. Schiller.
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Neurons in macaque area V1 respond more strongly to texture displayed over their receptive fields when that texture appears to be part of a discrete, object-like region of the scene than when the texture appears as part of a homogenous background. Extra-receptive field mechanisms responsible for this effect thus permit V1 neurons to play a role in scene analysis at a scale larger than their receptive field area. Here we used single and multiple cell recording to study the spatial extent of extra-receptive field mechanisms in parafoveal area V1 of the awake monkey. We used texture displays in which a disc-shaped region was delineated from the background by contrast in orientation, brightness, or color of texture. As the monkey fixated, texture displays were presented with the disc region centered on the receptive field of the V1 neuron under study; texture over the receptive field was identical in all cases during recording from a cell. The diameter of the disc was varied between 1.8° and 14.4°, always larger than the receptive field region of cells under study. The resulting response profile to texture displays could be divided into two intervals: an initial burst of activity at texture onset that was similar under all conditions, and a subsequent tonic response period that showed enhanced response for disc versus homogenous texture background. Enhanced response to discs declined monotonically with increasing disc diameter, falling off for discs of diameter 12°, on average. The time course of this modulation generally did not depend on disc size. Experimental controls showed that these effects were not a result of visual attention or small eye movements.

608.8

CONTEXTUAL INFLUENCES ON VISUAL DISCRIMINATION IN HUMANS AND ON RF PROPERTIES OF CELLS IN STRIATE CORTEX OF ALERT MONKEYS. M.K. Kapadia, C.D. Gilbert* and G. Westheimer. The Rockefeller University, New York, NY 10021.

The context surrounding a feature influences both the perception of its attributes and the response properties of cells with receptive fields (RFs) containing the feature. We studied the context dependency of the responses of striate cortical cells in awake, behaving primates and compared these findings with psychophysical measurements of line detection in human subjects.

We compared the minimum contrast necessary to detect a light bar presented alone and in conjunction with a second, suprathreshold bar. The second bar lowered the contrast detection threshold of the first bar by 40%. The magnitude of this effect was greatest when the two bars were colinear, and was reduced as the bars were further separated along their axis of orientation, by displacing them from colinearity, and by changing their relative orientation.

The response properties of some neurons in the primary visual cortex of the alert monkey showed similar dependencies on stimulus configuration, increasing their response to a bar located within the classical RF when an iso-oriented, colinear bar was presented simultaneously in the RF surround. The second bar, when presented alone, elicited either no response or a suppression of background firing. The response facilitation often declined with bar separation and orientation contrast in a fashion similar to that observed in the psychophysical studies. The plexus of long-range horizontal connections in visual cortex provides a likely substrate of these effects by connecting cells with similar orientation preferences in disparate parts of the visual field. (Supported by NIH grant EY07968 and a McKnight Development Award)

608.9

THE RELATIONSHIP OF RECEPTIVE FIELD COVERAGE TO FUNCTIONAL MODULES IN PRIMATE V1. C. E. Landisman*, A. W. Roe, and D. Y. Ts'o.
Division of Neuroscience, Baylor College of Medicine, Houston, TX. 77030. *Rockefeller University, New York, NY. 10021.

We are studying how the different functional modules in parafoveal V1 provide coverage of visual space. In particular, how do ocular dominance columns and color patches provide full representations of visual space, given their discontinuous organization in V1? Are the maps continuous or independent across the different modules? Using a combination of tangential electrode penetrations and a series of perpendicular penetrations guided by optical images of functional activity, we examined visual coverage across ocular dominance (OD) columns and across blobs.

In long tangential penetrations crossing OD columns, we have confirmed the existence of receptive field "jumps" at ocular dominance borders. Within OD columns, we have observed roughly monotonic receptive field progressions. At ocular dominance borders, we have observed jumps between left and right eye progressions, some of which are consistent with the two-steps-forward, one-step-back pattern described by Hubel and Wiesel.

We are particularly interested in coverage by color cells in V1. The occurrence of color selective cells in tangential penetrations is much lower than in perpendicular penetrations made by targeting color regions revealed by optical images. In the tangential penetrations, only about 20 percent of cells encountered were unoriented cells and approximately 15 percent were color selective cells. Only 3 to 4 percent were the classic color-opponent unoriented cells. In targeted penetrations, 50% were color-selective cells and 33% were broad band unoriented cells. Thus, by using the optical images to target a row or a region of color-selective patches, color cells can be located with much greater frequency. We are using this technique to study coverage by the color system.
(Supported by grants GM07524, EY08240, ONR N00014-91-J-1865 and McKnight).

608.10

SPATIAL INTEGRATION AND RESPONSE CORRELATION IN THE CENTRAL VISUAL SYSTEM OF CAT AND MONKEY
A.M. Sillito*, K.L. Grieve, J. Cudeiro~ and J.N. Davis. Visual Science, Inst. of Ophthalmology, Bath St., London EC1V 9EL, UK. ~Health Sciences, University of La Coruña, Spain.

Previously we have shown that when stimulated by moving contours layer VI cells in the visual cortex provide a feedback influence that causes correlated firing in LGN cell pairs with receptive field alignments appropriate to signalling the presence of the contour. This may serve to increase the synaptic effectiveness of their input to layer IV cells in the cortex. The layer VI cells projecting to the LGN also project to layer IV but as yet we do not know how or whether they influence the synchronisation of layer IV cells directly. With this in mind we have examined the way the spatial extent, orientation and direction of motion of a concentric bipartite grating stimulus influences the response and correlated activity of groups of cells recorded in layers II/III, IV and VI. The shuffle corrected cross-correlogram revealed highly significant synchronisation compatible with common mode input in cells with common orientation tuning and overlapping fields. This held for responses obtained throughout their orientation tuning curve. We observed that varying the orientation of the outer stimulus could profoundly modify the response to the inner stimulus in the receptive fields of cells in both cat and monkey striate cortex. This suggested that connections from orientation columns tuned to quite different orientations to those of the cell in question could influence its response. Under these circumstances we found evidence of connectivity between cells of different orientation for specific stimulus configurations only. We suggest that the input from layer VI cells may contribute to the common mode input correlation of the firing of cells with like orientation selectivity whilst other elements of the intracortical circuitry underpin the effects seen under more complex stimulus situations.

608.11

REDUCING BIOPHYSICALLY DETAILED SINGLE CELL MODELS TO SINGLE COMPARTMENT MODELS C. Koch,*[1] Ö. Bernander,[1] R. J. Douglas [2], M.A. Mahowald,[2] (1) Caltech, 139-74, Pasadena, CA, 91125, (2) MRC Anatomical Neuropharmacology Unit, Oxford QX1 3QT, UK.

Simulations of biophysically detailed neurons that explicitly model the dendritic tree and incorporate multiple active conductances are computationally too intensive to be feasible in the study of large networks. We therefore derive several highly simplified cell models in a two step approach. First, an intermediate model is derived that is electrically complex (using a Hodgkin-Huxley like formalism) and spatially simple in that the soma and entire dendritic tree are collapsed into a single compartment. This model preserves the response to somatic input, as described by input resistance, f-I curves, and voltage and current thresholds. In a second step, the intermediate model is reduced to three yet simpler models: Integrate and Fire (I&F), Diode, and Hopfield units. The I&F model preserves the voltage threshold and generates output spikes whenever the threshold is exceeded. The Diode model is based on the observed exponential relationship between the time-averaged somatic potential (including spikes) and the total membrane current. The Diode and Hopfield models preserve the current threshold and generate a continuous output variable that can be thought of as the mean firing rate. These models represent two extreme viewpoints: *pulse* or *temporal coding* assumes that the exact timing of inputs are relevant, while the *mean-field* viewpoint focuses on the average spike rate.

608.12

A STATISTICAL MEASURE OF COOPERATIVE INTERACTIONS WITHIN CORTICAL NEURONAL GROUPS. E.D. Lumer, G. Tononi*, and G.M. Edelman. The Neurosciences Institute, 3377 North Torrey Pines Court, La Jolla, CA 92037.

The neocortex of higher vertebrates is characterized by extensive functional and anatomical compartmentalization at different scales. It has been suggested that, at the scale of a few hundred microns, the cortex is organized into dynamic and cooperative collections of strongly interconnected neurons, referred to a neuronal groups (G.M. Edelman, Neuron, 10:115-125, 1993). Here, we investigate a dynamic criterion for the metastable partitioning of a cortical region into neuronal groups, which is based on a statistical measure of the degree of cooperative firing within a local population of neurons. This measure is the normalized third-order moment, or skewness, of the distribution of spike counts cumulated over a local set of simultaneously recorded neurons and a short time interval. The magnitude of positive skewness of the distribution is indicative of the degree of local cooperativity. We show that this skewness is sensitive to the level of coupling among the recorded neurons by means of detailed computer simulations of cortical regions. Such a measure also discriminates local cooperative interactions from firing biases due to shared inputs and, if applied repeatedly over neighboring spatial positions, it can detect statistical borders between neuronal groups. We shall also consider the relationship of a dynamic cortical partitioning of the kind discussed above to underlying factors, such as the activity-dependent changes in the distribution of synaptic strengths and the origin of the organization of axonal terminals into segregated cortical patches.

608.13

NEURAL COMPLEXITY AND THE RELATIONSHIP BETWEEN FUNCTIONAL SEGREGATION AND INTEGRATION IN THE NERVOUS SYSTEM. **G. Tononi, O. Sporns*, and G.M. Edelman, The Neurosciences Institute, 3377 North Torrey Pines Court, La Jolla, California 92037.**

In brains of higher vertebrates, the functional segregation of local areas that differ in their anatomy and physiology contrasts sharply with their global integration during perception and behavior. We have recently (PNAS 91, 5033) introduced a measure, called neural complexity (C_N), that captures the interplay between these two fundamental aspects of brain organization. We express functional segregation within a neural system in terms of the relative statistical independence of small subsets of the system and functional integration in terms of significant deviations from independence of large subsets. C_N is then obtained from estimates of the average deviation from statistical independence for subsets of increasing size. C_N is shown to be high when functional segregation coexists with integration and to be low when the components of a system are either completely independent (segregated) or completely dependent (integrated). We apply this complexity measure in computer simulations of a primary visual area to examine how some basic principles of neuroanatomical organization constrain brain dynamics. We show that the connectivity patterns of the cerebral cortex, such as a high density of connections, strong local connectivity organizing cells into neuronal groups, patchiness in the connectivity among neuronal groups, and prevalent reciprocal connections, are associated with high values of C_N. The complexity measure has been used in an initial exploration of data from humans obtained by functional neuroimaging.

ISCHEMIA: PROTECTION

609.1

HALOPERIDOL PREVENTS THE INDUCTION OF HSP70 BUT NOT THE ACTIVATION OF MICROGLIA IN POSTERIOR CINGULATE CORTEX FOLLOWING KETAMINE AND PHENCYCLIDINE. F.R. Sharp*, J. Nickolenko, R. Nakki, and S.M. Sagar, Dept. Neurology (V127), University of California, SF, CA 94121.

Phencyclidine (PCP), ketamine (K) and MK801 are non-competitive NMDA receptor antagonists that decrease injury produced by stroke, trauma and seizures. However, these agents produce vacuoles and kill cingulate neurons. We have previously shown that the HSP70 heat shock gene is induced in the pyramidal neurons injured by PCP, K and MK801, that the HSP70 induction is age related, and that anti-psychotics like haloperidol prevent the vacuoles and HSP70 induction in injured neurons (Sharp, J Neurosci Res, 33:605). The present study shows that ketamine also activates microglia around injured and possibly dieing neurons in posterior cingulate of adult rats. This effect is dose related, the most microglia being induced at 80-100mg/kg of ketamine. This effect was also age related, since ketamine did not induce HSP70 or activate microglia in 3, 10 and 20 day old rats, but did induce microglia in progressively older rats. Surprisingly, though haloperidol blocked hsp70 induction produced by ketamine, haloperidol did not block the activation of microglia in posterior cingulate produced by ketamine. The data is interpreted to mean that haloperidol blocks an injury pathway leading to induction of HSP70, but it does not block the injury pathway leading to microglia activation and possibly neuronal cell death produced by ketamine. The microglial activation and possibly cell death produced by ketamine, PCP and MK801 is not blocked by haloperidol alone, but may require multiple compounds.

609.2

LOCAL IMMUNE RESPONSES AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT. G. Stoll, M. Schroeter, S. Jander, and O.W. Witte* Dept. Neurology, Heinrich-Heine-Univ, Düsseldorf, Germany

This study examined infiltration of hematogenous cells after occlusion of the middle cerebral artery in rats. Cryostat sections of the infarcts were stained immunocytochemically for CD5, a pan-T cell marker, lymphocyte subsets CD4 and CD8, and ED1, a marker for macrophages. Besides granulocytes, numerous CD5+ T cells were present from days 2-7. By day 14 and 30, their number had decreased. Subtyping of lymphocytes revealed that CD4+ helper/inducer T cells were rare, while CD8+ cytotoxic/suppressor lymphocytes were abundant. Moreover, CD8+ lymphocytes greatly outnumbered CD5+ T cells indicating the presence of CD5-/CD8+ natural killer lymphocytes. ED1+ macrophages appeared on day 1 and were still present on day 30. As an early event ICAM-1 was upregulated on cerebral vessels.
This study shows that ischemic lesions can lead to lymphocytic infiltration into the CNS. Blocking of lymphocyte-derived cytokines will help to elucidate the functional contribution of immune cells to stroke.

609.3

MICE DEFICIENT IN NEURONAL NITRIC OXIDE SYNTHASE GENE EXPRESSION EXHIBIT DECREASED FOCAL INFARCT VOLUME AND NEUROLOGICAL DEFICIT AFTER MIDDLE CEREBRAL ARTERY OCCLUSION. M.A. Moskowitz*, Z. Huang, P.L. Huang, N. Panahian, T. Dalkara and M. Fishman. Massachusetts General Hospital & Harvard Medical School, Boston, MA 02114

The proposal that nitric oxide and/or its reactant products mediate toxicity in brain remains controversial, in part, due to the use of agents which block NO formation in neuronal, glial and vascular compartments. In mutant mice deficient in neuronal NOS activity, we observed significantly decreased infarct volume (approximately 35%; determined by the TTC method) and neurological deficits 24 hrs after middle cerebral artery occlusion (filament method) as compared to Wild type (SV-129), which could not be accounted for by blood flow (laser-Doppler flowmetry) or vascular anatomical differences (carbon black studies) between mutant and Wild type. Elevations in brain cGMP levels present in the Wild type 30 min. after ischemia were not observed in mutant mice. These data indicate the relevance of NO to ischemic injury and the importance of developing strategies to selectively inhibit the neuronal isoform in stroke and brain injury.

609.4

EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE CONTRIBUTES TO BRAIN DAMAGE IN FOCAL CEREBRAL ISCHEMIA C. Iadecola*, X. Xu, F. Zhang, R. Casey and M. E. Ross. Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

After focal cerebral ischemia there is induction of calcium-independent nitric oxide (NO) synthase (NOS) activity in the infarct (X. Xu et al., Soc. Neurosci. Abstr., 1994). This finding may reflect expression of inducible NOS (iNOS), an enzyme that produces potentially toxic amounts of NO. We sought to determine (a) whether iNOS mRNA is expressed in the ischemic area, (b) the cell type in which iNOS is induced and, (c) whether NO production by iNOS contributes to ischemic brain damage. The middle cerebral artery (MCA) was occluded in rats and animals sacrificed at different time points (6hrs-7days). iNOS expression in the infarct was determined by iNOS mRNA amplification using RT-PCR. NOS catalytic activity was determined using the citrulline assay. In the infarcted brain, but not contralaterally, there was substantial expression of iNOS mRNA that began at 24 hrs, peaked at 36-48 hrs, and started to decline at 4-7 days. iNOS mRNA expression paralleled the time-course of induction of calcium-independent NOS activity in the infarct. iNOS immunoreactivity was seen mainly in polymorphonuclear cells. To determine whether production of NO by iNOS contributes to ischemic damage, rats were treated for 3 days with the iNOS inhibitor aminoguanidine (AG), starting 24 hrs *after* stroke. AG treatment attenuated the stroke-induced iNOS activity and reduced infarct volume by 33±4% (n=10), an effect reversed by co-administration of L- but not D-arginine ($p>0.05$; n=7/group). AG did not affect calcium-dependent brain NOS activity ($p>0.05$). We conclude that iNOS mRNA and enzymatic activity are expressed in the post-ischemic brain. iNOS induction occurs in polys. The finding that iNOS inhibition reduces stroke size indicates that NO synthesis by iNOS contributes to the late stages of ischemic brain damage. iNOS inhibition might be a new avenue for the treatment of ischemic stroke. (Supported by the Sklarow fund, AHA and NIH)

609.5

DIRECT MEASURE OF NITRIC OXIDE IN RAT BRAIN SUBJECTED TO PERMANENT AND TEMPORARY ISCHEMIA. T. Matsui*, T. Nagafuji, K. Tsutsumi, S. Itoh, K. Nagata, T.Asano. Department of Neurosurgery, Saitama Medical Center/School. 1981 Kamoda, Kawagoe, Saitama 350, Japan.

Although the role of nitric oxide (NO) in the pathogenesis underlying ischemic brain damage has remained to be elucidated, our previous results conducted the conclusion that NO might mediate the ischemic cerebral damage. The present study aimed at directly measuring nitric oxide in the ischemic brain, employing middle cerebral artery occlusion (MCAo) and reperfusion in rats. The newly developed microsensor was calibrated in NO containing solution. The NO microsensor (pA), oxygenometer (mmHg) and laser CBF flowmeter (ml/100gm/min) were implanted in the lt. MCA territory. A total of 20 animals were assigned into 4 hour MCAo(saline-[N=5], LNA(N^{ω}-nitro-L-arginine)-treated[N=5]) or 2hour MCAo followed by 2hour recirculation (saline-[N=5], LNA-treated[N=5]). In this setting, the value obtained is not absolute NO concentration but relative to the baseline. The first peak (P1) of NO production, $1.73 \pm 0.19\mu M$, was found at the period of initial 20-30min MCAo. Three and a half hours later another peak was observed (P2, $0.53 \pm 0.08\mu M$). Also, P3 ($0.93 \pm 0.52\mu M$) was induced by reperfusion. LNA inhibited or abolished P1-3. The present results support the hypothesis that overproduction of nitric oxide in the brain subjected to permanent and temporary ischemia might lead to the cytotoxic effects of nitric oxide in the ischemic brain.

609.6

HEAT SHOCK PROTECTS CULTURED CORTICAL NEURONS AGAINST DEPRIVATION OF OXYGEN OR GLUCOSE BUT NOT EXPOSURE TO EXCITOTOXINS. B.J. Snider* and D.W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Induction of heat shock proteins protects cultured cerebellar or cortical neurons against excitotoxic death (Lowenstein et al., Neuron 7:1053, 1991 and Rordorf et al., Neuron 7:1043, 1991). Heat shock also protects astrocyte cultures from oxygen-glucose deprivation injury (Giffard et al., Soc. Neurosci. Abs. 19:1651,1991). Hyperthermic pre-exposure reduces subsequent brain injury in a rat model of focal ischemia (Chopp et al., Neurology 39: 1396,1989).

We studied the effects of heat shock on cultured mouse cortical cells. Cultures were exposed to 44°C for 25-30 min, a treatment sufficient to induce substantial expression of hsp72 in astrocytes as assayed immunocytochemically with a monoclonal antibody (Amersham) and by Northern blot analysis using oligonucleotide probes. These heat shocked cultures did not exhibit reduced neuronal vulnerability to subsequent exposure to either glutamate (10 min, 10 μM - 1mM) or NMDA (24 hr, 3-300 μM). However, heat shocked cultures were modestly more resistant than control cultures to injury induced by glucose or combined oxygen-glucose deprivation. This protective effect was best seen when glutamate receptors were blocked with MK-801 (10 μM) and CNQX (100 μM), and was abolished when cycloheximide was added during the heat stress. Present data support a hypothesis that expression of heat shock proteins reduces hypoxic and hypoglycemic neuronal death by attenuating non-excitotoxic components of injury. Supported by NIH NS 30337.

609.7

FRUCTOSE-1,6-BIPHOSPHATE PROTECTS ATP LEVELS IN HYPOXIC NEONATAL RAT BRAIN SLICES. L. Litt*, M.T. Espanol, J. MacDonald, L-H. Chang, G.A. Gregory, P. R. Weinstein, T.L. James, and P.-H. Chan. University of California, San Francisco, California 94143

In vivo administration of fructose-1,6-bisphosphate (FBP) provides hemodynamic and metabolic protection during ischemia and hypoxia.[1] However, mechanisms of protection have not been elucidated, and, in fact, appear to not work in certain circumstances. We applied *ex vivo* NMR spectroscopy techniques, recently developed for studies of live rat brain tissue[2], to examine if there is direct metabolic protection by FBP in hypoxic (PO_2<5 mm Hg) neonatal (7-day old Sprague Dawley) slices (350μ). Total acquisition time for interleaved $^{31}P/^{1}H$ spectra was 5 minutes. With no pre-treatment, metabolic impairment was seen in ^{31}P spectra obtained at the end of 30 min of hypoxia, at which time the Lactate/NAA ratio was increased by a factor of 3. Pre-treatment with FBP decreased ATP loss and lactate production, and increased the rate of recovery during reperfusion. Post-treatment with FBP did not produce the nearly full recovery found with pre-treatment. In this study protection comes primarily from intracellular effects in the tissue of interest, and not secondarily from physiologic response such as cardiac protection and increased blood flow. Mechanisms of FBP's protection are not understood and additional studies are needed. Hypotheses for FBP protection point to the involvement of calcium homeostasis, reduction of free-radical injury, and increased pentose phosphate pathway. Supported by NIH Grants GM34767, NS 22022, NS14543, NS25372, and RR03841. **References:** (1) Kelleher JA, Gregory GA, and Chan P-H. Neurochem. Methods 19:209-215, 1994. (2) Espanol MT, Litt L, Xu, Y et al. J Cereb Blood Flow Metab. 14:269-278, 1994.

609.8

EXPRESSION OF BCL-2 FROM A DEFECTIVE HERPES SIMPLEX VIRUS 1 VECTOR LIMITS NEURONAL DEATH IN STROKE

Matthew D Linnik*, Peter Zahos, Michael D Geschwind, Howard J Federoff. Marion Merrell Dow Research Institute, Cincinnati, OH 45215-6300, and Departments of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Recent evidence for programmed cell death (PCD) in stroke predicts that expression of genes that prevent PCD should protect neurons after an ischemic insult. To test the hypothesis, a candidate neuroprotective gene, *bcl-2*, previously shown to prevent PCD in other settings, was inserted into a defective HSV-1 amplicon vector under the transcriptional control of the HSV IE 4/5 early promotor (pHSVbcl-2). An identical vector expressing the *E. coli* β-galactosidase gene was used as a negative control (pHSVlac). These vectors were packaged into virus, purified, concentrated and microinjected (approx. 10^5 infectious particles/site) into rat cerebral cortex at 2 sites (+1.7 and -3.3 anterioposterior to bregma; 3.6 mm lateral of midline). The right middle cerebral artery was occluded 24 hrs after the injection and animals were sacrificed 24 hr after the occlusion. Brains were stained with TTC to distinguish viable tissue from infarcted tissue. Protection was assessed by measuring the extent of viable tissue from the longitudinal fissure to the edge of the infarction along a line perpendicular to the midsaggital plane. The extent of viable tissue at the injection sites was 3.47±0.55 mm (pHSVbcl-2) vs 2.65±0.53 mm (pHSVlac) and 3.40±0.62 mm (pHSVbcl-2) vs 2.67±0.5 mm (pHSVlac) for the anterior and posterior injection sites, respectively (mean±S.D., $p<0.05$, n=6 animals per group). There was no difference in viable tissue at a site chosen equidistant between the 2 injection sites. These results suggest that viral vector mediated transfer and expression of *bcl-2* can protect neurons from dying in an acute neurodegenerative disorder.

609.9

INDUCTION OF PROTECTIVE MECHANISMS IN CEREBRAL ISCHEMIA [1]N. Kawahara, [1]C.A. Reutzler, [2]S.J. Wiegand, [2]S. Croll and [1]I. Klatzo*. [1]Stroke Branch, NINDS, NIH, Bethesda, MD 20892 and [2]Regeneron Pharmaceuticals Inc. Tarrytown, NY 10591

Our study is based on serendipitous finding indicating protection of CA1 pyramidal neurons subjected to cardiac arrest cerebral ischemia, when the latter is preceded by the spreading depression (SD), induced 3 days earlier in the cerebral cortex of Sprague-Dawley rats, by local application of KCl. Assays on brain tissue after SD revealed : 1)intense c-Fos expression in the neurons of the cerebral cortex on the side of KCl application and bilaterally in the dentate gyri demonstrable 3 hr after SD, 2)no enhanced HSP-70 expression, 3)conspicuous increase in protein synthesis measured by 3H-leucine incorporation associated with a pronounced reduction in deoxyglucose utilization, both demonstrable in ipsilateral hemisphere 3 days after SD and 4)upregulation of mRNA for the brain-derived neurotrophic factor (BDNF), conspicuously noticeable at 4 hr and 3 days after SD.

Our studies indicate that a sublethal stress, such as SD, is capable of inducing a temporary state of resistance to cerebral ischemia, in which stimulation of protein synthesis and neurotrophic factors may play a significant role.

609.10

CHANGES IN GENE EXPRESSION FOLLOWING MULTIPLE SPREADING DEPRESSIONS. T. L. Hoffman[1], W.B. Macklin[2], W.D. Lust[1], W.R. Selman[1] and R.A. Ratcheson*[1], Lab. Experimental Neurological Surgery, Case Western Reserve Univ.[1], Cleveland, OH 44106 and MRRC, UCLA Medical School[2], Los Angeles, CA 90024

Multiple episodes of spreading depression (SD) prior to a focal ischemic insult have been shown to reduce cell loss, relative to equivalent ischemic controls. The current studies were begun to identify changes in gene expression that might be responsible for the protective effects of SD. Male Wistar rats were subjected to 2h of SD by topical application of 2 M KCl onto the dura, followed by 2, 10 and 24h of recovery time. Cerebral cortical RNA was isolated, and analyzed by Northern blots for changes in known mRNAs. c-fos mRNA was increased in 2h samples and reduced by 10 and 24h, while glial fibrillary acid protein (GFAP) mRNA was increased at 10 and 24h. Thus, these samples contained mRNA that were part of the early response and the long-term response to SD. Differential display of polymerase chain reaction (PCR) products was used to identify mRNAs in these samples that may be increased or decreased during recovery following SD. cDNA libraries of each mRNA sample were generated using $T_{12}MN$ (M=A/T/C/G; N=C/G) primers. ^{35}S-dATP-labelled cDNAs were amplified by PCR, using the same set of primers and a set of arbitrary 10mers. Samples were separated on a DNA sequencing gel, and 8 differentially expressed bands were isolated for further characterization.

609.11

CHARACTERIZATION OF INDUCED ISCHEMIC TOLERANCE IN FOCAL CEREBRAL ISCHEMIA IN RATS

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Background: Brief periods of non-lethal global ischemia have been reported to induce resistance against subsequent lethal ischemic injury in hippocampal neurons. To determine if this phenomenon is relevant to stroke, we developed a rat model of ischemic tolerance in focal ischemia. **Method**: Ninety-four adult rats underwent either sham operation or brief intervals of temporary focal ischemia by occluding the MCA by suture insertion. Twenty-four or 72 hrs later the MCA was occluded by the same technique for 100 min. Infarct volumes were determined 72 hrs after the 100 min ischemic insult. HSP70 protein induction was studied by immunocytochemistry at 24 or 72 hrs after the brief temporary ischemia. Alterations of glutamate receptor subunits 1-5 (GR1-GR5) were studied at these time points by *in situ* hybridization. In additional 12 rats, CBF was measured by the [14C] iodoantipyrine quantitative autoradiographic technique at 30 min of MCAO 72 hrs following either brief ischemia or sham operation. **Results**: Either 30 min of ischemia or three 10 min intervals of ischemia followed by 72 hrs but not 24 hrs of reperfusion significantly ($P<0.05$) reduced infarct volume produced by 100 min of ischemia compared to sham-operated animals. CBF was not significantly different between sham and three 10 min ischemia-treated brain regions where tolerance was shown. HSP70 protein induction in neurons was similar in degree at 24 and 72 hrs after the three 10 min ischemia insults, but HSP70 protein was also induced in glia cells at 72 hrs. GR1-GR5 transcription was decreased at 72 hrs but not 24 hrs after three 10 min ischemia. **Conclusions**: These data indicated that the phenomenon of ischemic tolerance is relevant to focal ischemia and requries greater than 24 hrs to develop. Alterations in cellular expression in HSP or alterations in glutamate receptor subunit expression could underlie the development of ischemic tolerance, while it could not be attributed to differences in collateral blood flow. This model may be useful in studying the molecular mechanisms that determine the inducible cerebral self-defense against ischemic injury.

609.12

CONDITIONED NEUROPROTECTION FROM ISCHEMIC INFARCTION ELICITED BY ELECTRICAL STIMULATION OF THE CEREBELLAR FASTIGIAL NUCLEUS (FN) IN RAT. D.J. Reis, E.V. Golanov, S. Yamamoto, K. Kobylarz and V. Prabhakar*, Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. College, New York, NY 10021.

Electrical stimulation of the FN increases cerebral blood flow (rCBF) but not metabolism (rCGU) (Nakai et al., *Brain Res.* 260:35,1983). Stimulation for 1h also reduces, by ~50% 24h later, the volume of the cerebral infarction produced by occlusion of the middle cerebral artery (MCA) (Reis et al., *J. Cereb. Blood Flow Metab.* 11:810, 1991). Since FN stimulation neither increases rCBF nor reduces rCGU within the salvaged area (Yamamoto et al., *Soc. Neurosci. Abstr.* 19:1056, 1993), protection cannot be due to rematching reduced rCBF with elevated rCGU in the ischemic penumbra. To assess if protection results from an enduring signal, we stimulated FN at various times before occluding MCA. Rats were anesthetized, FN stimulated for 1h (50 Hz, 1s on/1s off, 50 μAmp), and animals returned to their cages. At different times thereafter, they were reanesthetized, MCA occluded and, 24h later, the distribution and volumes of infarctions measured. Controls were sham-stimulated. Stimulation of the FN for 1, 3, 24 or 72h prior to MCA occlusion (n=5/group) decreased infarction volume by 26, 41, 44 and 42%, respectively ($p<0.05$ for each). In 5 rats, rCBF and rCGU in the area of salvage, measured autoradiographically 2h after stimulation for 1h, did not differ between experimental and sham-stimulated controls. We conclude that: (a) stimulation of FN for 1h conditions brain so as to provide long-lasting protection from focal ischemic infarctions; (b) protection is not attributable to changes in rCBF or rCGU; (c) the neuroprotective mechanism may involve rapid transcription of "protective" genes and/or modification of constitutive molecules; (d) pathways exist in brain which function to protect the brain from ischemia.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION VII

610.1

NGF-HIGH-EXPRESSING TRANSGENIC MICE CONTAIN INCREASED NUMBER OF PGP 9.5-IMMUNOREACTIVE FIBERS IN THE VOMERONASAL ORGAN.

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The expression of protein gene product (PGP) 9.5 in extrinsic nerve fibers of the vomeronasal nonsensory epithelium (VN-NE), the neighboring respiratory epithelium (RE), and the associated glands of the vomeronasal organ of the nasal mucosa, was examined immunocytochemically in nerve growth factor (NGF)-high expressing 6-week-old transgenic mice that contained the gene construct of keratin (K) 14 promoter-NGF and in age-matched controls. Increased density of extrinsic PGP 9.5-immunoreactive (IR) fibers was observed in the VN-NE, RE, and the glands of the transgenic mice. The soft palate, whisker pad skin, and tongue of the transgenic mice, which were used as positive controls, all demonstrated a marked increase in the density of PGP 9.5-IR fibers. The numbers of intraepithelial PGP 9.5-IR nerve fibers were significantly greater in the VN-NE (12.8 X control, $P<0.01$) and RE (3.5 X control, $P<0.01$) of the transgenic mouse. Since K14 was expressed by basal cells in the VN-NE and RE, these cells in the transgenic mice presumably contain the NGF transgene, resulting in continuous production and secretion of NGF, which induced significant hyperinnervation in the VN-NE and RE. This hypothesis is supported by the fact that basal cells in the epithelia of positive tissue controls of transgenic mice express both K14 and NGF.

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610.2

MECHANICAL INJURY INCREASES bFGF and CNTF EXPRESSION IN THE RAT RETINA. Rong Wen, Ying Song, Michael T. Matthes, Douglas Yasumura, George D. Yancopoulos, Matthew M. LaVail, and Roy H. Steinberg*. Depts. of Physiology, Ophthalmology, and Anatomy, UCSF, San Francisco, CA 94143, and Regeneron Pharm. Inc., Tarrytown, NY 10591.

It has been shown that mechanical injury to the retina prevents photoreceptors from degenerating in the RCS rat with an inherited retinal degeneration, and in the light-damaged rat retina. These findings indicate that mechanical injury activates intrinsic retinal mechanisms that promote photoreceptor survival. To find out if factors that show neurotrophic activity are involved in injury-induced photoreceptor rescue, we examined the expression of bFGF, CNTF, BDNF, and IGF-1 following mechanical injury. The retina was injured by making an incision through the choroid and RPE that penetrated the subretinal space of each eye of an adult Sprague-Dawley rat. Control animals were without injury. Retinas were taken 0.5, 1, 2, 4, 7, or 10 days post-injury. Northern blot analysis showed marked increases in bFGF and CNTF mRNAs following injury. Compared to controls, expression of bFGF increased by more than 6-fold at 12 hrs post-injury; peaked at 2 days post-injury (more than 7-fold); and was still at a 5-fold level at 10 days post-injury. Expression of CNTF increased more than 2-fold at 1 day post-injury; peaked at 4 days post-injury (about 3-fold); and was still at a 2-fold elevation at 10 days post-injury. *In situ* hybridization showed that both bFGF and CNTF were expressed in the inner nuclear layer and were more concentrated around the wound. BDNF and IGF-1 did not show significant changes in expression. Our results indicate that bFGF and CNTF up-regulate in the retina following mechanical injury, with the bFGF effect being earlier and greater than that of CNTF. We conclude that these two factors are likely to be involved in injury-induced photoreceptor rescue.

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610.3

CDF/LIF mRNA INCREASES FOLLOWING CORTICAL INJURY. N. N. Moayeri, L. R. Banner* and P.H. Patterson. Biology Division, California Institute of Technology, Pasadena, CA 91125.

The neuropoietic cytokine cholinergic differentiation factor/leukemia inhibitory factor (CDF/LIF), can act both as a trophic factor, enhancing neuronal survival, and as a differentiation factor to alter neuronal gene expression. CDF/LIF also plays a role in the response of adult neural tissue to injury. When peripheral nerves are transected, CDF/LIF mRNA is dramatically up-regulated near the site of injury (Banner & Patterson, PNAS, in press). Moreover, the neuronal response to nerve section is much reduced in LIF knockout mice (Rao *et al.*, Neuron 11:1175, '93).

To test whether CDF/LIF expression is regulated in a similar manner after injury in the CNS, surgical lesions were made in adult rat cortex. Using a quantitative RNAse protection assay, we find that CDF/LIF mRNA increases significantly relative to the contralateral cortex and to anesthesia-treated controls. The increase in CDF/LIF expression begins within 6 hrs after injury and reaches a peak at 24 hrs. CDF/LIF mRNA expression returns to baseline values by 7 days post-lesion. Thus, this cytokine is likely to play a role in the response to adult brain injury as it does in the periphery.

610.4

IMPLICATION OF PROHORMONE CONVERTASES PC1, FURIN AND PC5 IN THE PROCESSING OF NGF AND BDNF PRECURSORS IN RATS WITH PILOCARPINE SEIZURES. M. Marcinkiewicz,* T. Nagao,[1] M. Avoli,[1]R. Day, NG Seidah & M. Chrétien. Clinical Research Institute of Montreal, Montreal Quebec H2W 1R7 and [1]Montreal Neurological Institute, Montreal, Quebec H3A 2B8.

Neurotrophins NGF, BDNF, NT-3 and NT-4 are synthesized as large immature prepropeptides and subsequently proteolytically processed to active neurotrophic factors. Their processing enzyme(s) have not yet been conclusively identified. However, the data on the tissue distribution and enzymatic cleavage specificity suggest that one or more of the prohormone convertases including furin, PC1, PC2, PC5 or PACE4 are involved in the maturation of proneurotrophins. To understand at the molecular and neuroanatomical levels the interrelationships between putative prohormone convertases and neurotrophic factors we studied the hippocampus of the rats in which the seizures were induced by pilocarpine. By *in situ* hybridization using ^{35}S-labeled riboprobes we compared the expression patterns of neurotrophins with that of the convertases 1,2,3,12 and 96 hrs after induction of seizures. Consistent with other groups, in the dentate gyrus both NGF and BDNF showed substantial temporary rise of mRNA levels with a peak at two hours. In the dentate gyrus, similar to NGF and BDNF the PC1 level increased strongly, while furin and PC5 displayed rather weak transient augmentation of mRNA level, and finally PC2 and PACE4 did not change much. In the CA1-CA3 regions, there was a maximal transient increase of BDNF two hours after pilocarpine administration. Interestingly, only PC1 showed a parallel pattern of mRNA expression in this region. Based on our results we propose that in the *status epilepticus* PC1, and to lesser extent also furin and PC5 may play a role in processing of pro-NGF and pro-BDNF.

610.5

AUTOCRINE REGULATION OF NEUROTROPHIN SECRETION BY PERIPHERAL GLIAL CELLS. A.Zimmermann*, A.Sutter and U.Stephani. Institut f. Zoologie der THD, Schnittspahnstr.3, 64287 Darmstadt, FRG.

It has been shown in several vertebrate species that peripheral glial cells express receptors for nerve growth factor during development as well as following nerve injury. The functional significance of these glial receptors in the biology of NGF is not understood. Since embryonic glial cells as well as glial cells in lesioned peripheral nerves also express NGF mRNA, we have explored whether NGF would affect NGF (neurotrophin) secretion by these cells.

Glial cells purified by a rapid procedure from chicken embryonic sensory ganglia were cultured in the presence of various concentrations of NGF. After two days conditioned media (CM) were harvested and assayed for the presence of neurotrophic activity in a single neuron bioassay. Anti-NGF antibodies were employed to probe for NGF in the CM.

Glial cells cultured without NGF were found to secrete neurite growth promoting activity which was not inhibitable by antibodies against NGF. However, if the glial cultures were spiked with very low concentrations of NGF ($\leq$ 2pM), NGF like avtivity in the medium rose dramatically (up to 50 fold). This was not observed, if NGF had been added to the glial cultures at 50 times higher concentrations.

It is concluded that the availability of neurotrophins during development may not only be regulated by the target of innervation but also by glial cells which appear to have the capability to 'sense' traces of NGF and respond in an autocrine fashion, finely tuned to the NGF concentrations present extracellularly. An autocrine function of glial NGF receptors can be expected to be of critical importance for morphogenetic aspects of nerve development and in nerve regeneration.

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610.6

NERVE GROWTH FACTOR REGULATES THE EXPRESSION AND KINASE ACTIVITY OF p33^{cdk2} AND p34^{cdc2}. K.J. Buchkovich* and E.B. Ziff, New York University Medical Center and Howard Hughes Medical Institute, 550 First Avenue, New York, NY 10016.

Nerve growth factor (NGF) promotes the survival and differentiation of the pheochromocytoma cell line PC12. The function of NGF as both a survival and differentiation factor may be related to its ability to regulate PC12 cell cycle progression. NGF treatment of PC12 cells leads to a decrease in the percentage of cells in the DNA synthesis phase (S phase) of the cell cycle and an accompanying increase in G2 phase cells. We have investigated the mechanism by which NGF regulates PC12 cell cycling. Specifically, we have analyzed the regulation of two cyclin-dependent kinases, p33^{cdk2} and p34^{cdc2}, which are required for progression through the G1/S and G2/M phase transitions of the cell cycle. NGF treatment lead to a decrease in the steady state levels of p33^{cdk2} and p34^{cdc2}. p33^{cdk2} and p34^{cdc2} form complexes with regulatory molecules known as cyclins. NGF treatment resulted in decreases in the kinase activity of several cyclin-p33^{cdk2} and cyclin-p34^{cdc2} complexes. The decreases in kinase activity could be attributed at least in part to the decreases in the steady state levels of p33^{cdk2} and p34^{cdc2}. The timing of the down regulation of the kinases was dependent on the level of p140^{trk} NGF receptor in the cells. A clonal cell line that overexpressed p140^{trk} and differentiated with accelerated kinetics (BL Hempstead et al, 1992) also down-regulated the kinases with accelerated kinetics. This suggested that the rate of the decrease in p33^{cdk2} and p34^{cdc2} kinase activity was dependent on the level of p140^{trk} receptors and the strength of the NGF signal.

610.7

NERVE GROWTH FACTOR INDUCTION BY OKADAIC ACID IN ASTROCYTES MAY BE MEDIATED BY INTERLEUKIN-1 AND IMMEDIATE EARLY GENE ACTIVATION. S.P. Pshenichkin* and B.C. Wise. Fidia-Georgetown Institute for the Neurosciences and Department of Pharmacology, Georgetown University, Washington, D.C. 20007.

Nerve growth factor (NGF) expression in astroglial cells is regulated by growth factors and cytokines. Previous studies have shown that IL-1 and okadaic acid, a phosphoprotein phosphatase inhibitor, stimulate NGF gene transcription and NGF mRNA stabilization. The onset of okadaic acid activation of NGF gene expression was much slower than that of IL-1 requiring 6-9 h before an increase in NGF mRNA level was evident. This delay suggested that intermediary signals, such as immediate early gene (IEG) induction or increasing IL-1 synthesis may be operative. Okadaic acid treatment (20 nM) of primary cultures of astrocytes stimulated *c-fos* and *c-jun* gene transcription by 60-80%. This response peaked at about 3 h, followed by an increase in *c-fos* and *c-jun* mRNA content peaking at 4 h. The increase in *c-fos* mRNA content lasted longer than that of *c-jun* and after 24 h was about 5-fold higher than control levels. This protracted increase appears to be due to mRNA stabilization. The increase in IEG expression was followed by a 50% increase in NGF gene transcription 24 h after okadaic acid treatment. Okadaic acid also increased by 4-fold the half-life ($t_{1/2}$) of the NGF mRNA as early as 3 h of treatment. This increase was maintained for up to 24 h. Conditioned medium from okadaic acid treated (24 h) astrocytes increased NGF mRNA content following a 3 h treatment of astrocytes (okadaic acid was ineffective at this time), indicating that okadaic acid stimulated the secretion of a factor, perhaps IL-1, that induced NGF expression. Indeed, okadaic acid increased IL-1 mRNA content as early as 3 h of treatment. These results suggest that the multiple actions of okadaic acid on NGF mRNA expression are mediated, in part, through an induction of IL-1 and IEGs.

610.8

FGF-2 AND IL-1ß REGULATE S-100ß GENE EXPRESSION IN CULTURED RAT ASTROCYTES FROM MULTIPLE BRAIN REGIONS. **D.A. Hinkle§*, J.P. Harney#, D.C. Hilt$, P.J. Yarowsky¶, and P.M. Wise#. Depts. of §Physiol. and ¶Pharm., Univ. of Maryland Sch. of Med., Balt., MD 21201, $AMGEN, Thousand Oaks, CA 91320, and Dept. of #Physiology, University of Kentucky College of Medicine, Lexington, KY 40536.**

The dynamic interaction between neurons and astrocytes is critical for the development and maintenance of normal physiological function in the brain. S-100b is a neurotrophic factor which is synthesized in and released by astrocytes, and may therefore play an important role in such interactions. We examined the effects of interleukin-1ß (IL-1ß) and basic fibroblast growth factor (FGF-2) on S-100ß gene expression in enriched rat astrocyte cultures since these factors are known to regulate the expression of other neurotrophic factors in astrocytes. We report that both FGF-2 and IL-1ß regulate the S-100ß gene in astrocytes from the cerebral cortex, hippocampus, and hypothalamus. Total RNA was isolated from cultures, purified, then subjected to the solution hybridization-RNase protection assay using highly specific riboprobes for rat S-100ß and rat cyclophilin, an internal standard which is not regulated by either FGF-2 or IL-1ß. We find that S-100ß mRNA levels decrease 2- to 3-fold relative to controls in response to 24 or 48 hours of treatment with either 10 ng/ml FGF-2 or 10 U/ml IL-1ß alone, and decrease 5- to 10-fold in response to combination treatment at the same doses over the same time period. In contrast, long-term 7-day treatment with FGF-2 increases S-100ß gene expression two-fold over control levels in cerebral cortical astrocytes. Further, over a similar time-course and at the same doses, we find that only FGF-2 suppresses S-100ß mRNA levels in C6 glioma cells, whereas IL-1ß has no effect on gene expression. These data demonstrate that both FGF-2 and IL-1ß regulate astrocyte S-100ß mRNA levels, which may be an important mechanism through which these factors modulate neuron-glia interactions.

610.9

CHARACTERIZATION OF NEUROTROPHIN-3 IMMUNOREACTIVE SENSORY NEURONS IN RAT DORSAL ROOT GANGLIA

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Neurotrophin-3 (NT3) is a member of the NGF family known to support survival of sensory neurons *in vitro* but its physiological roles are not known. We recently reported, using a newly generated antibody, that a subpopulation of rat sensory neurons contained NT3-like immunoreactivity (ir; Zhou & Rush, 1993, Brain Res. 621, 189-199). In the present study, we have characterized NT3-ir neurons by size and segmental distribution, peripheral innervation, transportation and colocalization with low affinity NGF receptor (LNGFR). The size distribution indicated NT3-ir was mainly localized within larger neurons. More NT3-ir neurons were detected in cervical (36% of total) and lumbar (38%) than in thoracic dorsal root ganglia (DRG; 18%). Double-labelling experiments showed that only 12% of neurons positive for LNGFR, colocalized with NT3-ir. The combination of Fluorogold retrograde tracing with NT3 immunohistochemistry demonstrated NT3-ir sensory neurons primarily innervate muscle and cutaneous structure but not viscera. Sciatic nerve transection induced a significant reduction of NT3-ir neurons in lumbar DRG. Finally, NT3-ir accumulated distal to a ligature placed on the sciatic nerve and dorsal roots, suggesting sensory neruons require NT3 from both peripheral and central sources. Thus the characterization of NT3-ir DRG neurons in the present study suggests that large sensory neurons innervating muscle and skin require NT3 for their survival during development and for the maintenance of normal function in the adult.

610.10

REGULATION OF NEUROTROPHIN-3 BY BRAIN DERIVED NEUROTROPHIC FACTOR IN HYPOGLOSSAL MOTOR NEURONS.

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Identification of the cellular location of the neurotrophins has so far proved difficult, primarily due to the lack of suitable antisera and appropriate techniques for neurotrophin immunohistochemistry. Recently we have generated antiserum to peptide sequences of neurotrophin-3 (NT3) which have proved useful for immunohistochemistry (Zhou and Rush, Brain Research, 621:189-199,1993). Purified antibodies from this serum show no cross-reactivity with nerve growth factor (NGF) or brain derived neurotrophic factor (BDNF). Within the brain stem and spinal cord of rat, most reactivity is confined to motor neurons, consistant with in situ hybridization studies for $mRNA^{NT3}$. We now show that unilateral lesion of the hypoglossal nerve leads to the loss of NT3-immunoreactivity (ir) from the ipsilateral hypoglossal nucleus. This is accompanied by a reduction of choline acetyltransferase-ir (ChAT), but an up-regulation of both low affinity nerve growth factor receptor-ir (LNGFR) and NADPH diaphorase activity. However, application of BDNF to the lesioned nerve leads to restoration of the NT3-ir and ChAT-ir, but does not reverse the up-regulated LNGF-ir or diaphorase activity. These findings support the view that BDNF can regulate the synthesis of NT3 via a specific intracellular pathway, thereby providing a mechanism by which neuronal connectivity can be controlled.

610.11

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND NEUROTROPHIN-3 (NT-3) mRNA'S ARE EXPRESSED IN DIFFERENT AVIAN MOTONEURON SUBSETS.

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NT-3 stimulates primary differentiation of cultured avian motoneurons (MN's) (Averbuch-Heller et al, PNA's, in press, 1994), whereas BDNF supports their survival in vivo during the period of programmed cell death (Oppenheim et al, Nature 360, 755, 1992).

We therefore studied the expression of BDNF and NT-3 transcripts in spinal MN's using in situ hybridization of serially sectioned chick embryos aged of 3-to-8 days (E3-8). NT-3 mRNA is present in MN subsets from E3 in brachial segments of the neural tube and from E4 onward in both brachial and lumbar regions..

Expression of BDNF mRNA is first detected on E5 in the brachial area and from E6 also in the lumbar and sacral levels of the spinal cord. No detectable mRNA coding for either of the factors was revealed by the assay in MN's localized to the remaining areas of the spinal cord.

Analysis along the rostrocaudal extent of the limb-innervating segments revealed that MN's expressing BDNF mRNA are located more rostrally than those expressing NT-3 transcripts, with an intermediate zone in which both factors appear to be synthesized. In transverse sections of these overlap zones it was observed that, in general, NT-3-positive MN's occupy the lateroventral part of the enlargements, whereas BDNF-containing MN's localize to more mediodorsal areas. These results suggest that BDNF and NT-3 are synthesized in discrete levels of the spinal cord by distinct MN subsets both along the longitudinal and cross-sectional planes.

We hypothesize that these differentially expressed neurotrophins may serve various functions at different times. They may act locally within the spinal cord on the corresponding MN populations and/or be transported ortogradely to affect development of the respective target muscles. Supported by the Israeli Academy of Sciences, the Dysautonomia Foundation, and by Genentech, Inc.

610.12

A HISTORY OF TRANSIENT CALCIUM ELEVATION HAS LONG LASTING EFFECTS ON THE ABILITY OF FIBROBLAST GROWTH FACTOR TO PROMOTE NEURONAL SURVIVAL.

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This study investigates the potential capacity of transient calcium signals to modify future cellular responsiveness to neurotrophic factors. The simultaneous presence of low levels of depolarization and basic Fibroblast Growth Factor (bFGF), which in and of themselves did not promote survival, acted synergistically to promote survival of E7 chick ciliary ganglion neurons (plated on poly-L-ornithine). This synergistic interaction was dependent on calcium influx. To test whether this synergism could be obtained when the events were temporally separated, neurons were subjected to a 3 hour pulse of elevated extracellular potassium followed by the addition of bFGF. Such treatment produced high levels of survival. In contrast, treatment with either the pulse alone or the continuous presence of bFGF failed to promote survival. This priming effect of depolarization on bFGF was mediated by intracellular calcium because it was completely abolished by the addition of calcium channel blockers and because the magnitude of the priming effect was directly proportional to the pulse induced rise in intracellular calcium. To test the time constant for the temporal summation between the calcium pulse and bFGF, we increased the time interval between the calcium pulse and the addition of bFGF. Our results are consistent with the notion that a brief calcium pulse could have long lasting effects, up to 8 hours after cessation of the depolarizing pulse, on neuronal responsiveness to bFGF. These results imply that brief pulses of increased intracellular calcium can significantly alter neuronal responsiveness to neurotrophic factors and may have significant implications for how neural activity influences neuronal survival.

OPIOID RECEPTORS

611.1

HUMAN DELTA OPIOID RECEPTOR: CLONING, EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION.

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Antinociceptive ligands selective for δ opioid receptor have fewer side effects than ligands selectively acting at μ or κ opioid receptors. Recently, we cloned a human δ opioid receptor for exclusive characterization its ligand requirements. The cloning was based on hybridization screening of several human cDNA libraries. The probe was obtained by PCR from the mouse δ opioid receptor cDNA and hybridized to a clone in a human striatum cDNA library that lacked the 5' end of the ORF. The probe obtained from this clone was used to identify the clone from human temporal cortex cDNA library that contained the missing upstream part of the ORF but ended before the termination codon. These two clones were ligated together using HincII site present in the overlapping cDNA region. It is now shown that the ligated clones encode for the same receptor using southern blot analysis of human genomic DNA. Two probes, one obtained from 5' end of human temporal cortex cDNA clone and second obtained from 3' end of human striatum cDNA clone, gave a band of the same size (about 4.6 kb) while hybridizing to human genomic DNA digested with BamHI. The reconstituted receptor encodes a 372 amino acid protein that has 93% amino acid identity to the mouse δ opioid receptor. The cDNA was expressed in COS-7 cells and its pharmacological profile was partially characterized. These cells express over 1.0 pmole receptor/mg protein when measured by saturation binding with [^{3}H]naltrindole. The δ receptor selective ligands NBT, BNTX, [Glu^4]deltorphin, and pCl-DPDPE all have Ki values under 10 nM while the affinities of the μ and κ opioid receptor ligands CTAP and U-69593, respectively are over 4.0 μM. The cloned receptor is now further characterized with the newly synthesized δ receptor selective, non-peptidic ligands such as analogs of BW 373 and SNC 80 and peptidic ligands such as analogs of DPDPE and deltorphins. Supported in part by NIDA grants.

611.2

δ-OPIOID RECEPTOR IMMUNOREACTIVITY: RELATIONSHIP TO BIOGENIC AMINES AND ENKEPHALIN IN RAT SPINAL CORD AND BRAIN STEM.

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Using antisera directed against the mouse δ-opioid receptor (DOR) the distribution of DOR-like immunoreactivity (-LI) was analyzed in rat spinal cord and brain stem in relation to serotoninergic, noradrenergic, and enkephalinergic neurons. We found DOR-LI in varicosities distributed throughout the spinal cord gray, with highest densities in the superficial dorsal horn, in autonomic regions, around the central canal as well as in the motor nuclei. In the brain stem DOR-immunoreactive (-IR) fibers were found in several nuclei such as midline raphe nuclei, locus coeruleus, parabrachial nuclei, periaqueductal gray matter, interpeduncular nucleus and substantia nigra. A group of DOR positive cell bodies was seen in the laterodorsal tegmental nucleus. All DOR positive cells showed a punctate staining pattern at the cell body level and in primary dendrites, but no plasma membrane staining could be demonstrated. Double labeling for DOR and 5HT revealed that some 5HT-IR neurons in the raphe complex were surrounded by DOR-IR fibers. Also, TH positive neurons in the brain stem were apposed by DOR positive fibers. In the spinal cord a high degree of coexistence was found between DOR and 5HT in the neuropil around motoneurons. In autonomic regions, a low degree of colocalization was seen between DOR and 5HT whereas in the superficial dorsal horn no coexistence was found. No coexistence could be seen between DOR and TH in any part of the spinal cord. In the intermediolateral nucleus a low degree of colocalization was observed between DOR- and enkephalin-IR; however, a close relation, but no coexistence, was observed among such fibers in the spinal cord ventral horn. These data suggest that DORs may affect the activity of descending serotoninergic and noradrenergic neurons by means of modulating the release of neurotransmitters from afferents to these neurons. In addition, presynaptic DORs appear poised to modulate the release of 5HT (and of coexisting peptides) from fibers in the spinal cord ventral horn. However, these receptors appear unlikely to be importantly involved in modulation of the release of catecholamines or enkephalin in the spinal cord, or in modulation of the release of 5HT in the superficial dorsal horn. Supported by NIDA.

611.3

CELLULAR LOCALIZATION OF A μ-OPIOID RECEPTOR (MOR) IN RAT BRAIN AND SPINAL CORD.

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The μ-opioid receptor (MOR) was localized by immunohistochemistry using antisera raised against a synthetic peptide corresponding to the C-terminal of the predicted amino acid sequence for the μ-opioid receptor. The MOR antisera immunoprecipitated I-125 β-endorphin cross-linked membrane proteins with mw's of 55-69 kDa that had been isolated from a cell line stably expressing μ-opioid receptor cDNA. Proteins of similar mw were labeled by MOR antisera using Western blots. In addition, the MOR antisera stained cells transfected with native and epitope-tagged μ-opioid receptors. MOR-like immunoreactivity was frequently observed in neuronal membranes, both in the somatic and dendritic domains. However, in some areas such as in the dorsal horn, axon labeling could also be demonstrated. In the brain MOR-like immunoreactivity showed an excellent correlation with the distribution of mRNA encoding MOR, and also with earlier μ-opioid receptor binding studies. The distribution of enkephalin, the putative endogenous ligand for this receptor, showed a distribution complementary to that of MOR in many areas in the brain and spinal cord. However, double-labeling was not observed. These findings suggest that the antisera to MOR recognize a CNS μ-opioid receptor on somatic and dendritic membranes, however, neurons also exist that transport MOR to the axonal compartment. Supported by NIDA.

611.4

THE HUMAN μ OPIOID RECEPTOR: CLONING, PHARMACOLOGICAL CHARACTERIZATION and MODULATION of INTRACELLULAR SIGNALING by PROTEIN KINASES.

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Three major types of opioid receptors designated δ, κ, and μ, mediate a number of physiological functions including hormone secretion, neurotransmitter release, and respiratory activity. Because opioid drugs with abuse potential such as morphine, methadone, and fentanyl act upon the μ receptor, it is considered the main cellular mediator in the development of tolerance and opioid addiction. We have isolated a human μ opioid receptor cDNA containing an open reading frame capable of encoding a protein of 400 amino acids, with 94% sequence homology to the rat μ opioid receptor. A major effect caused by activation of the μ opioid receptor in brain is the decrease in neuronal membrane excitability. One of the mechanisms for this effect is an increase in K^+ conductance, accomplished by the opening of an inward rectifier, resulting in outward K^+ currents and hyperpolarization of the cell membrane. The human μ receptor demonstrates functional coupling to a recently cloned G protein-activated K^+ channel, when both proteins are expressed in *Xenopus* oocytes. Upon repeated stimulation of the μ opioid receptor, functional desensitization is observed as a reduction in the K^+ current. Both protein kinase C and the multi-functional Ca^{2+}/calmodulin-dependent protein kinase potentiate functional desensitization, suggesting kinase modulation of intracellular signaling mediated by the μ opioid receptor.

611.5

CELLULAR DISTRIBUTION OF μ, δ and κ OPIOID RECEPTOR mRNAs IN HUMAN CNS.

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The cloning and sequence analysis of cDNAs encoding rodent μ, ∂ and κ opioid receptor classes have allowed generation of specific nucleic acid probes to identify and visualize with precision by in situ hybridization the anatomical localization of cells expressing these opioid receptor classes in both mouse and rat brain. We have recently isolated genomic clones encoding the human μ, ∂, and κ opioid receptors. Sequence information derived from these clones allowed us to generate specific cRNA probes targeted to exonic sequences which were used for in situ hybridization to elucidate the anatomical distribution of cells synthesizing the μ, δ and κ opioid receptor mRNAs in human tissue. Cells expressing the three opioid receptor transcripts were found in the major human cortical fields following distinct laminar and expression patterns within the 6 cortical layers. Cells containing the three opioid-receptor messages were also detected in the striatum showing distinct expression levels for each opioid receptor transcript. Additionally, in the caudate-putamen, cells expressing both ∂ and μ mRNAs appear somewhat diffusely distributed whereas cells containing κ mRNA seem to be localized predominantly in clusters, suggesting a possible patch-like distribution for this latter opioid receptor subtype in human caudate-putamen. Co-labelling experiments coupling opioid receptor in situ hybridization to choline acetyltransferase (ChAT) immunocytochemistry to explore preferential patch-matrix distribution of opioid receptors in human caudate-putamen are currently in progress as is the anatomical mapping of other human brain regions.
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611.6

KAPPA-OPIOID RECEPTOR AGONISTS SUPPRESS VOLTAGE-ACTIVATED POTASSIUM CURRENT IN CATH.a CELLS.

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The CATH.a cell line is derived from tyrosine hydroxylase (TH)-positive tumors in transgenic mice. CATH.a cells in culture synthesize norepinephrine (Suri et al, *J. Neurosci.* 13:1280, 1993). In this study we examined the opioid sensitivity of CATH.a cells using whole-cell voltage-clamp techniques. Depolarizing commands from the holding potential (-60 mV) produced sustained outward current (I_K). External tetraethylammonium (25 mM) reduced I_K by 40-60% ($n=10$). Depolarizing steps following a hyperpolarizing pre-pulse (-110 mV) elicited fast, transient outward current (I_A). I_A was selectively blocked by 4-aminopyridine (10 mM; $n=5$). Intracellular dialysis with TEA (25 mM) and CsCl (160 mM) blocked voltage-activated potassium currents ($n=5$).

Morphine (0.5-100 μM) reduced I_K in a concentration-dependent manner (EC_{50}= 3.5 μM, max. inh.= 53%, $n=30$). Naloxone (10 nM) blocked the inhibitory effect of 10 μM morphine ($n=8$). The μ-specific agonist DAGO (2-20 μM; $n=6$) and the δ-specific agonist DPDPE (2-20 μM; $n=7$) produced no effect. U50,488 (0.3-50 μM), a κ-specific agonist, reduced I_K in a concentration-dependent manner (EC_{50}= 2.3 μM, max. inh.= 44%, $n=40$). The κ-specific receptor antagonist nor-BNI (10 nM) blocked the inhibitory effect of 10 μM morphine ($n=6$) or 10 μM U50,488 ($n=7$). Kappa-receptor mediated current reduction was prevented by intracellular dialysis with an inactive form of GDP (GDP-β-S 100-200 μM, 3-5 min., $n=10$). Incubation with pertussis toxin (PTX 500 ng/ml, 24-48 hr; $n=10$) did not eliminate kappa-opioid induced suppression of I_K. These results suggest that opioid suppression of I_K in CATH.a cells is mediated by a kappa opioid receptor coupled to a PTX-insensitive G-protein.

611.7

CLONED KAPPA AND MU OPIOID RECEPTORS COUPLE TO AN INWARD RECTIFIER POTASSIUM CURRENT WHEN EXPRESSED IN MOUSE AtT-20 CELLS.

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The recent cloning of the kappa, delta, and mu opioid receptors allows new techniques to be used to study cellular mechanisms of action of these receptors. Experiments focusing on endogenous opioid receptors have shown that regulation of ionic conductances is an important mechanism by which these receptors mediate cellular events. Recently, we have reported that the cloned kappa opioid receptor stably expressed in PC-12 cells is able to modulate an N-type calcium current. To further examine opioid receptor-ion channel coupling we have generated AtT-20 cell lines which stably express kappa and mu receptors. Whole cell patch clamp recordings demonstrate that both the kappa and mu receptor are able to modulate the activation of an inward rectifier potassium current that has previously been described in this cell line. These effects are selective as both kappa- and mu-mediated activation is blocked by selective antagonists. Having both receptors expressed separately in the same cell line will allow us to compare functional properties of the two receptors, including G protein coupling and desensitization. Supported by DA08951.

611.8

PHYSICALLY SEPARATE EXTRACELLULAR DOMAINS OF THE KAPPA RECEPTOR ARE NECESSARY FOR AGONIST AND ANTAGONIST BINDING.

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The cloned κ and δ receptors display unique pharmacological profiles. Their amino acid sequences are about 60% identical. The areas of the receptors most divergent are the NH_2- and COOH-termini as well as the extracellular loops. We have hypothesized that these extracellular domains have a role in ligand recognition and/or binding. To test this hypothesis, we have constructed chimeric receptors of κ and δ in which the NH_2-termini as well as the second extracellular loops have been exchanged. The chimera containing the NH_2-terminus of the κ receptor and the remainder of the δ receptor (κ/δ) revealed that agonist and antagonist binding to the κ receptor are on separate domains. This chimera bound κ-selective antagonists but not κ-selective agonists. In contrast, binding of both δ-selective agonists and antagonists to this chimera was not dependent on the presence of the δ NH_2-terminus. δ-selective agonists could specifically inhibit cAMP accumulation via the κ/δ chimera, but κ-selective agonists did not. These pharmacological and functional results suggest that the NH_2-terminus of the κ receptor is responsible for antagonist recognition and binding. The second extracellular loop chimeras between the two receptors have shown that this region is important for κ-selective agonist binding but not antagonist binding. The abilities of the κ-selective agonist U50,488 and dynorphin B to bind to the chimeric κ receptor containing the δ second extracellular loop (κ/δ2eloop) were greatly reduced when compared to wild-type κ. In contrast, antagonist binding to the wild-type and chimeric receptors was similar. Furthermore, the abilities of κ-selective agonists at the κ/δ2eloop chimera to inhibit forskolin-stimulated cAMP accumulation was lost. These results clearly demonstrate that κ-selective agonists and antagonists bind to physically distinct regions of the κ receptor. Antagonists bind to the NH_2-terminus whereas selective agonists bind to the second extracellular loop. [Supported by DA08951, MH10485 and the Scottish Rite Foundation.]

611.9

Opiate Receptor Structure/Function Relationships and Molecular Modeling *Christopher K. Surratt[#*], Peter S. Johnson[#+], Akiyoshi Moriwaki[#], Brian K. Seidleck[#], Carrie J. Blaschak[#], Jia-Bei Wang[#], Stacieann Yuhasz[@] and George R. Uhl[#*!]* [#]Molecular Neurobiology Branch, [!] Office of the Director, Intramural Research Program/NIDA, [+]Pharmacology Research Associate Program, NIGMS; [@]Biophysics and [*]Depts. of Neurology and Neuroscience, Johns Hopkins School of Medicine.

μ opiate receptor cDNAs encode poorly conserved N-and C-terminal regions and conserved, charged transmembrane domain residues. Deletion of 64 N-terminal amino acids yields a receptor that binds DAMGO and mediates DAMGO and morphine inhibition of cAMP accumulation at wildtype levels. Deletion of an additional 33 C-terminal amino acids removes DAMGO's ability to inhibit cyclase, while removal of the entire C-terminal tail yields a nonfunctional receptor. Replacement of charged amino acids D114 (TM II) and D147 with alanine, asparagine or glutamic acid drastically reduces agonist binding. Naloxone binding is fully retained in D147N and D147E mutants. Each of 6 substitution mutants for H297 (TM VI) bind DAMGO poorly. Opiate inhibition of cyclase is preserved for D114E, D147E and certain H297 mutants. COS cells expressing mutant receptors can be immunostained by specific polyclonal anti-μ receptor sera. These data are reflected in molecular modeling scenarios for ion, peptide and alkaloid recognition. Charged residues in TM II, III and VI are key for full receptor function, contrasting with N- and distal C-terminal domains, while several mutant receptors revealed intriguing differences between binding and second messenger activities.

611.10

CONSTRUCTION, EXPRESSION AND CHARACTERIZATION OF EPITOPE-TAGGED OPIOID RECEPTORS R. L. Zastawny, S. R. George, R. Briones-Urbina* and B.F. O'Dowd. Addiction Research Foundation and Department of Pharmacology, University of Toronto, Toronto, Ontario, CANADA, M5S 1A8.

We have previously reported the cloning, pharmacological characterization and mRNA distribution of the rat μ, κ and δ opioid receptors. These studies revealed that the receptors have unique pharmacological profile and distributions in the brain suggesting unique roles for each receptor in the CNS. To facilitate further study of these receptors, we have designed μ and κ opioid receptors with extended N-terminals incorporating six histidine residues and a 12 amino acid sequence from the T7 major capsid protein for which a monoclonal antibody is available. The cDNA constructs encoding the receptor fusion proteins were inserted into a mammalian expression vector downstream of a cytomegalovirus (CMV) promoter and transiently expressed in COS cells. Initial characterization of the pharmacological properties of the tagged κ receptors show it to be identical to the the wild-type receptor suggesting the added peptide epitope does not interfere with ligand binding properties. In stable mammalian cell lines expressing these tagged receptors, the histidine-tag will be used to affinity purify the receptors using a nickel chelating affinity column. The stable expression of these tagged receptors in mammalian cells should be useful for functional analysis, biochemical characterization and immunocytochemical localization, to study the post-translational modifications and desensitization of opioid receptors.

611.11

μ OPIOID RECEPTOR EXPRESSION AND DESENSITIZATION IN A STABLY TRANSFECTED CELL LINE. A.Kouvelas, M.A.Scheideler, R.L.Zastawny, J.S. Rasmussen, H.Juhl, Y.Israel*, B.F.O'Dowd and S.R.George. Dept. of Pharmacology, University of Toronto, Addiction Research Foundation, Toronto, Canada M5S 1A8 and Novo Nordisk A/S, 2760 Malov, Denmark.

We have previously reported the cloning, characterization and mapping of the μ opioid receptor (OR) in rat brain. μ OR was transfected into BHK-570 cells using Lipofectin, and geneticin resistant clones screened for specific ^{3}H-bremazocine binding. A subclone (BHK-48) which stably expressed high levels of specific opioid binding was characterized. The pharmacology of the μ OR in the BHK-48 cells was compared to μ OR transiently expressed in Cos-7 cells or natively expressed in F11 cells. In BHK-48 cells, naloxone Kd for the μ OR was 3.0±.85 nM, Ki rank order for antagonists was naltrexone<<naloxonazine<naltrindole. Agonist competition revealed ~80% of receptors in the high affinity state. The Ki of agonists at the high affinity state was morphine≤DAMGO<DADLE<<U 50488<DPDPE. Coupling of the μ OR to adenylyl cyclase in BHK-48 cells was determined by measurement of agonist mediated decreases in cAMP synthesis. Exposure of cells to high concentrations of morphine resulted in a rapid reduction of the apparent density of μ OR by 43%, whereas low concentrations of morphine resulted in in an increase in the apparent density of μ OR by 46%. These results demonstrate that our BHK-48 cell line stably expresses μ OR with appropriate pharmacology, G protein coupling and second messenger linkage. μ receptor activation and desensitization by agonist were accompanied by dose-dependent regulation of detectable receptor numbers.

611.12

QUANTIFICATION OF DELTA OPIOID RECEPTOR BINDING IN HUMAN BRAIN WITH N1'-([^{11}C]METHYL) NALTRINDOLE AND PET. **J.S. Smith, J.K. Zubieta, J.C. Price, J. Madar, J.R. Lever, C. Kinter, H.T. Ravert, R.F. Dannals, J.J. Frost*.** The Johns Hopkins Medical Institutions, Baltimore, MD.

Central opiate-mediated signaling takes place via three main receptor subtypes (mu, delta and kappa). Investigations of their roles in living human subjects with PET has been limited by the lack of specific radiotracers for delta or kappa subtypes. We have recently developed a radiolabelled delta subtype-selective, non-peptide opioid receptor antagonist, N1'-([^{11}C]methyl) naltrindole ([^{11}C]MeNTI); this compound was prepared at high specific activity by C-11 methylation of a naltrindole derivative. Seven healthy subjects (27±2 years old) underwent dynamic PET studies after intravenous injection with 18-20 mCi [^{11}C]MeNTI. 25 scans were obtained over 90 min, with arterial blood sampling and correction for the presence of labelled metabolites. Both graphical analysis and three compartment tracer kinetic models were applied to the data. Using the three compartment model, delivery rate constants (K1) ranged from 0.22-0.28 ml/g/min across brain regions. A constrained (k4=0) yielded values of the binding parameter k3 of 0.008±0.003 in thalamus, 0.022±0.007 in occipital cortex and 0.028±0.005 in putamen. Regional k3 values (basal ganglia > neocortex > thalamus > cerebellum) obtained with these methods were highly correlated (r=0.98) with the known distribution of delta opioid receptors in human brain reported in *in vitro* binding studies. These results support the potential use of [C-11]MeNTI as an agent for the quantification of delta opioid receptor densities in human brain with PET.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID X

612.1

COMPARISON OF A BRAIN PURIFIED METALLOPROTEASE WITH HUMAN CATHEPSINS G AND D: Evaluation of Sequence Specificity Using Peptide Substrates and Regional Selectivity Using Purified Brain APP J. Sonnenberg-Reines*, A.B. Brown, D.M. Tummolo, M. A Spruyt, and J.S Jacobsen Department of Central Nervous System Biological Research, Lederle Laboratories, Division of American Cyanamid Co., Pearl River, N.Y. 10965.

A number of proteases have been proposed as candidates for the activity that cleaves at the amino-terminus of β–amyloid peptide (βAP). No single protease has emerged as possessing all the required properties for β-secretase including sequence specificity, ability to cleave amyloid precursor protein (APP) appropriately, brain localization and correct sensitivity to sequence substitution.

We have recently purified another candidate β-secretase, a 55-60 kDa metalloprotease from rabbit and monkey brain and characterized its activity using a synthetic peptide substrate (SEVKMDAEF) which flanks the N-terminal of βAP. HPLC and amino acid analysis reveal that the major cleavage is between Met and Asp. The enzyme is inhibited by EDTA, EGTA, 1,10-phenanthroline. The pH optimum for this protease is 5.0-6.0, but activity is still retained at physiological pH.

We have compared three candidate proteases (cathepsin D, cathepsin G, and the above metalloprotease) for their ability to cleave, in addition to SEVKMDAEF, peptides which (1) incorporate the Lys-Met to Asn-Leu mutation found in a Swedish family with early onset Alzheimer's disease, (2) contain various amino acid substitutions near the M/D cleavage site, and (3) contain oxidized methionine at the M/D cleavage site. In addition, we have probed these enzymes ability to form carboxy-terminal fragments with a longer purified APP substrate or cell derived PN II. Epitope mapping of these larger substrates and their digestion products were used to localize the site of cleavage to a region near the N-terminus of βAP.

612.2

INCREASED NEURONAL CATHEPSIN D GENE EXPRESSION AND ACTIVE RELEASE OF COMPETENT PROTEASE IN ALZHEIMER DISEASE. R.A. Nixon*, A.M. Cataldo, A.L. Schwagerl, N. Kowall, L.A. Kanaley-Andrews. McLean Hospital, Harvard Medical School, Belmont, MA

We have found that activation of the lysosomal system, evidenced by increased cathepsin gene expression and accumulation of secondary and tertiary lysosomes, is an early marker of metabolic dysfunction in "at-risk" neuronal populations in Alzheimer disease. In quantitative image analyses of a series of 8 AD brains (moderate-severe neuropathology) and 8 age-matched controls immunostained with cathepsin D antibodies under identical conditions, >90% of neocortical perikarya in layers III and V of AD brains contained 2- to 8-fold elevated numbers of secondary and tertiary hydrolase-positive lysosomes in the absence of atrophic, chromatolytic or neurofibrillary changes. *In situ* hybridization analysis showed the expression of cathepsin D mRNA to be increased an average of 2-fold in these cell populations in parallel to hydrolase immunoreactivity ($p < 0.001$). Neurons exhibiting overt atrophy or neurofibrillary changes displayed massive accumulation of hydrolase-positive lysosomes and lipofuscin which were then released into the extracellular space in association with neuritic and cell degeneration. These lysosomal compartments, containing a full spectrum of enzymatically competent hydrolases, persist in the extracellular space specifically in association with deposits of ß-amyloid within both neuritic and diffuse plaques. Cathepsin D content was >3-fold higher in ventricular CSF from AD patients ($n = 35$) than in samples from 26 patients with Huntington's, diffuse Lewy body or Pick's disease ($p < 0.001$), indicating that cathepsin D release from affected neurons in Alzheimer brain is an active ongoing process. Prominent extracellular accumulations of lysosomal hydrolases have thus far been observed only in the brain parenchyma of patients with Alzheimer disease or other conditions where ß-amyloid accumulates. Support: NIH (AG10916).

612.3

LOCALIZATION OF A HUMAN METALLOPROTEASE CAPABLE OF GENERATING POTENTIALLY AMYLOIDOGENIC FRAGMENTS FROM THE AMYLOID PRECURSOR PROTEIN. C.R. Abraham*, K.-J. Conn, M. Pietropaolo, A. Amaratunga, R.E. Fine and B. Meckelein. Departments of Medicine and Biochemistry, Boston Univ. Sch. Med., Boston, MA 02118, and ENRVA Hospital, Bedford, MA 01730.

Alzheimer's disease (AD) is characterized by the deposition of ß-amyloid protein (Aß) in brain parenchyma and blood vessels. The formation of Aß requires its proteolytic release from a larger molecule, the amyloid precursor protein (APP). Recently, we have purified and characterized a protease from human brain which is a candidate for an amyloidogenic APP processing. The enzyme is highly homologous to the rat metalloendopeptidase EC 3.4.24.15. It cleaves a synthetic peptide flanking the N-terminus of Aß at the Met-Asp bond and is capable of generating a 15 kDa amyloidogenic fragment from recombinant human APP *in vitro*.

To characterize the enzyme further, we developed monoclonal antibodies against a 20 amino acid peptide derived from the sequence of the purified protease. These antibodies were used to characterize the tissue distribution and the cellular localization of the enzyme, using immunohistochemistry, immunoprecipitation and Western blots. The enzyme is present in all monkey tissues examined, with highest amounts being found in brain, testis and kidney, organs which also contain high levels of APP. In human brain, neurons were strongly stained. In human cultured cells, the metalloprotease was found in neuroblastoma and kidney cells and their conditioned media, but not in glioblastoma cells. Using a rabbit retina/optic nerve system *in vivo*, we purified by equilibrium sucrose density gradient centrifugation three fractions containing transport vesicles from the optic nerve. The protease colocalizes with APP and ApoE in the same vesicular compartments.

612.4

REMOVAL OF AMYLOID ß PROTEIN BY CULTURED CELLS S. Gillespie*, L. Ho, and S. G. Younkin. Case Western Reserve U., Cleveland, OH 44106

There is increasing evidence that the deposition of amyloid ß protein (Aß) as insoluble amyloid fibrils plays a critical role in Alzheimer's disease (AD). Aß is secreted by normal processing of the amyloid ß protein precursor. Thus the rate of amyloid deposition will depend on (i) the rate at which Aß is secreted, (ii) the rate at which secreted Aß is removed, and (iii) the rate at which insoluble amyloid fibrils are formed at any prevailing concentration of soluble, extracellular Aß. Fibril formation and factors influencing the rate of Aß secretion are currently being intensively studied, but little is known about the mechanism(s) that remove secreted Aß. To assess this issue, we added synthetic $A\beta_{1-42}$ or $A\beta_{1-40}$ to (i) unconditioned medium, (ii) medium removed from cells after 24 hours of conditioning or (iii) cultured cells when fresh medium was added. We then assayed periodically by sandwich ELISA to assess Aß loss. In 24-hour conditioned but not in unconditioned medium, 95% of Aß at 500 fmol/ml was lost in 4 hours. This effect of conditioning was also produced by human neuroblastoma (2C), human embryonic kidney (293), mouse fibroblast (3T3), rat glioma (C6), and mouse neuroblastoma (N2a) cells. Aß loss was attenuated by protease inhibitors of several types suggesting that several different secreted proteases may be involved in the loss of Aß observed in conditioned medium. We are now further characterizing the proteases involved in an effort to determine whether they are likely to be important in removing Aß secreted by CNS cells *in vivo*. Medium conditioned for 24 hours by human neuroblastoma (M17) cells, unlike that conditioned by the other lines, did not cause loss of Aß. Despite this, Aß was rapidly lost when it was added (500 fmol/ml) to M17 cells in fresh medium. Thus this cell line, which is a BE-2 subIcone with a neuronal phenotype, may be able to remove Aß through a mechanism other than protease secretion. Interestingly, human 2C and 293 cells also removed Aß added in fresh medium but 3T3, C6, and N2a cells did not.

612.5

INTRACELLULAR AND EXTRACELLULAR ACCUMULATION OF AMYLOID Aß PEPTIDE BY DIFFERENTIATED PC12 CELLS.
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The mechanisms of amyloid accumulation in Alzheimer's disease are largely unknown, but may be central to the etiology of the disease. Our previous results demonstrated that Aß1-42 accumulates in late endosomes or secondary lysosomes of human fibroblasts and is resistant to degradation. We have extended these investigations to NGF-differentiated PC12 cells, and we found that while Aß1-42 also accumulates in PC12 cells, there are a number of differences from the results obtained with fibroblasts. Immunofluorecent confocal microscopy indicates a dual localization of the accumulated Aß1-42, in intracellular, punctate granules and aggregates of peptide at the cell surface. Unlike fibroblasts, the Aß1-42 on the surface of PC12 cells is largely resistant to removal by trypsin. In contrast to fibroblasts, the shorter amyloid peptides, Aß1-28 and Aß1-40, accumulate in PC12 cells but to a lesser degree than Aß1-42. The accumulated Aß1-42 is stable for at least 3 days while Aß1-28 and Aß1-40 are degraded within 90 min after exchanging the cells into peptide free media. The intracellular Aß1-42 is located in a dense organellar compartment which overlaps the distribution of late endosomes and lysosomes and colocalizes with internalized horseradish peroxidase which is targeted to lysosomes. The internalized Aß1-42 is not sequenceable and highly aggregated, suggesting that it may be subject to modification by the cells. These results indicate the neuronal plasma membrane may nucleate amyloid assembly or otherwise stabilize amyloid aggregates and suggests that a failure to degrade Aß1-42 may be an important component of amyloid accumulation. Supported by NIH GM07311 and NS31230

612.6

DEGRADATION OF THE ALZHEIMER'S DISEASE AMYLOID Aβ PEPTIDE BY A SECRETED METALLOPROTEASE. J.T. Durkin*, S. Murthy, W. Biazzo, R. Siman and B.D. Greenberg. Cephalon Inc., West Chester, PA 19380-4245

The levels of soluble Aβ amyloid peptide in biological fluids depends both on its rate of production and its rate of removal. Human neuroblastoma cells have been shown by several groups to secrete soluble Aβ. Here we show that M17 human neuroblastoma cells also secrete a protease that degrades Aβ. Cells were metabolically labeled with [35-S]Met and the labeled conditioned medium was separated from cells by filtration. The cell-free medium was then incubated on ice or at 37 °C for 24 hours, and Aβ levels were measured by immunoprecipitation, electrophoresis, and phosphorimaging. Media incubated at 37°C contained 60%-90% less Aβ than media incubated on ice. The metalloprotease inhibitors EDTA or *o*-phenanthroline prevented this loss of Aβ, suggesting that this proteolytic activity was a member of the metalloprotease class. Matrix metalloproteases are generally secreted as inactive zymogens that can be activated *in vitro* by the organomercurial compound *p*-aminophenylmercuric acetate (APMA). APMA addition prior to the incubation step resulted in a potentiation of Aβ degradation; however, degradation under these conditions was still inhibited by *o*-phenanthroline and EDTA. Moreover, general inhibitors of the other major protease classes (3,4-dichloroisocoumarin for serine, E-64 for cysteine, and pepstatin A for aspartic) did not inhibit Aβ degradation. This pharmacology demonstrates that M17 cells secrete a protease of the matrix metalloprotease family that degrades soluble Aβ. To the extent that cultured M17 cells reflect processes in human brain tissue, we speculate that inefficient proteolytic removal of soluble Aβ from brain may contribute to the etiology of Alzheimer-type amyloid formation.

612.7

MOLECULAR CHARACTERIZATION OF THE APP/APLP SUPERFAMILY. W. Wasco*, D.M. Kovacs, A. Crowley, K.M. Felsenstein and R.E. Tanzi. Laboratory of Genetics and Aging, Massachusetts General Hospital, Harvard Medical School, Boston, MA and Bristol Myers Squibb, Wallingford, CT.

The Alzheimer-associated amyloid β-protein precursor (APP), which was first identified in 1987, is now known to be a member of an evolutionarily conserved gene family. We have identified and isolated cDNAs encoding two mammalian amyloid precursor-like proteins (APLP1 and APLP2), and APP-like genes have also been identified in both *Drosophila* and *C. elegans*. All of these proteins show extensive amino acid and domain homologies, and like APP, the mammalian APLPs have a number of alternatively transcribed forms. Interestingly, the direct comparisons of the amino acid sequences of the APP-like genes and APP have demonstrated that the amyloidogenic Aβ domain is not conserved in the family and is found exclusively in APP.

For our current set of experiments we have established stably transfected human H4 neuroglioma cell lines that overexpress APP, APLP1 and APLP2. These lines are being used to characterize the subcellular localization of the APLPs and to compare the effects that overexpression of one member of the family may have on the expression and processing of the others. Reagents capable of indisputably distinguishing the members of the APP-like family by immunohistochemical and immunoblot analysis are currently being developed. In addition, we have compared the tissue distribution of alternatively transcribed forms of APP and the APLPs. Finally, we have examined whether the overall expression of the APLP gene transcripts and the specific expression of APLP alternatively transcribed domains (such as the APLP2 KPI and 12 amino acid domains) are developmentally regulated.

612.8

TISSUE DISTRIBUTION, DEVELOPMENTAL EXPRESSION, AND PROCESSING OF APLP2 ISOFORMS IN MOUSE. G Thinakaran*[1,2], HS Slunt[2], and SS Sisodia[1,2]. [1]Department of Pathology, [2]Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Amyloid precursor-like protein 2 (APLP2) is a member of a larger family of proteins that includes amyloid precursor protein and APLP1. APLP2 pre-mRNA undergoes alternative splicing to generate mature mRNAs that encode for at least four APLP2 isoforms. We have employed quantitative reverse-transcriptase polymerase chain reaction assays to analyze the expression of transcripts encoding various APLP2 isoforms in adult tissues and during mouse development. In parallel, the expression of APLP2 polypeptides is being monitored with highly specific anti-APLP2 antibodies.

We have also examined the metabolism of the APLP2-751 isoform in cultured mammalian cells. In transfected CHO, COS-1, and neuroblastoma N2A cells, APLP2 is modified by chondroitin sulfate (CS) glycosaminoglycan. Using site-directed mutagenesis strategies, we have identified the site for the CS modification of APLP2-751. We are presently characterizing the posttranslational processing of the other APLP2 isoforms.

This work was supported by grants from the U.S. Public Health Service as well as the Adler Foundation.

612.9

IMMUNOCYTOCHEMICAL LOCALIZATION OF AMYLOID PRECURSOR-LIKE PROTEIN 2 (APLP2) AND AMYLOID PRECURSOR PROTEIN (APP) IN MOUSE BRAIN. CA Kitt*[1,5], G Thinakaran[1,5], JC Mojekwu[5], RR Reed[2,3], DL Price[1,3-5], and SS Sisodia[1,5]. Departments of [1]Pathology, [2]Molecular Biology & Genetics, [3]Neuroscience, and [4]Neurology, [5]Neuropathology Laboratory, The Johns Hopkins University School of Med., Balto., MD 21205.

APLP2 belongs to a family of homologous proteins that include APP and APLP1. In earlier experiments, we provided strong evidence that several antibodies raised against APP also cross react with APLP2. We now report on the immunocytochemical distributions of APLP2 and APP in mouse brain by using antibodies that are exquisitely specific for either APLP2 or APP. We document that APLP2 and APP have overlapping distributions in cortex and hippocampus; however, the cellular localization and intensity of staining for each of these proteins in olfactory receptors, nerves, and bulbs are unique. APLP2 immunoreactivity is most pronounced in olfactory receptor cells, including cilia, cell bodies, olfactory nerve fibers, and glomeruli in the olfactory bulb. On the other hand, APP immunostaining is most pronounced in the olfactory cilia and receptor cell bodies and in granule cells that surround the glomeruli. APP immunoreactivity is conspicuously absent in olfactory nerves and glomeruli in the olfactory bulb. Notably, a distinct band of mitral cells in the olfactory bulb is intensely stained with APP antibodies. In addition, APP-immunoreactive processes course through most layers of the main olfactory bulb, and dense accumulations of large immunostained puncta are observed in the external plexiform layer. Our studies provide strong support for the view that APLP2 and APP play prominent roles in olfactory receptive processes.

This work was supported by grants from the U.S. Public Health Service as well as the Adler Foundation.

612.10

SIMILAR AND DIVERGENT FEATURES OF ALTERNATIVE SPLICING OF THE AMYLOID PROTEIN PRECURSOR APP AND THE APP-RELATED PROTEIN APLP2. R. Sandbrink, U. Mönning, R. Prior*, C. L. Masters and K. Beyreuther. Zentrum für Molekulare Biologie Heidelberg, Univ. of Heidelberg, Germany.

Among the transmembrane glycoproteins constituting the family of APP-like proteins, APLP2 is the nearest relative of the Alzheimer βA4-amyloid protein precursor (APP). We determined the complete cDNA and amino acid sequence of rat APLP2 comprising 765 residues (1), and compared its tissue-specific expression and alternative splicing to APP gene expression.

In APP, there are three alternatively spliced exons, of which, as we have previously shown, the L-APP mRNA isoforms lacking exon 15 are ubiquitously expressed in non-neuronal cells, but not in neurons (2). In APLP2, we were able to identify two alternatively spliced exons, of which the the KPI-encoding exon is highly expressed in neurons and thus differs in its tissue-specific expression from the KPI-encoding APP exon. While no equivalent to exon 8 of APP was detected in APLP2, there is another alternatively spliced exon with a strikingly similarly regulated expression, that is part of that APLP2 domain most divergent to APP. L-APLP2 mRNA isoforms lacking this exon represent the major part of APLP2 transcripts in non-neuronal tissues, but are only weakly expressed in neurons (3).

Because of the similar regulation of alternative splicing of exon 15 of APP and the described APLP2 insert, and because of structural similarities of the sequences and the predicted secondary structures, a functional relatedness of alternatively spliced isoforms of APP and APLP2 is suggested. Ubiqitous expression of high levels of L-APP and L-APLP2 mRNAs except for neurons indicates an important function in non-neuronal cells, and is remarkable since neurons are the primarily affected cells in Alzheimer's disease.

References: (1) Sandbrink, R., et al. (1994) *Biochim. Biophys. Acta,* in press;
(2) Sandbrink, R., et al. (1994) *J. Biol. Chem.* **269**, 1510-1517;
(3) Sandbrink, R., et al. (1994) *J. Biol. Chem.*, in press.

GENESIS OF NEURONS AND GLIA III

613.1

20-HYDROXYECDYSONE STIMULATES GLIAL-CELL PROLIFERATION DURING A CRITICAL PERIOD IN THE DEVELOPMENT OF THE INSECT BRAIN. SR Kirschenbaum*, M Higgins and LP Tolbert. ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Developmental events that shape the nervous system are often regulated by steroid hormones. During metamorphosis in the moth, *Manduca sexta*, when the larval nervous system is reshaped for adult function, the hormone 20-hydroxyecdysone (20-HE) controls diverse aspects of neuronal differentiation. Little is known, however, about hormonal effects on glial cells. In the antennal (olfactory) lobe of *Manduca*, glial cells are critically important because one type, the neuropil-associated glial cells, forms boundaries for developing olfactory glomeruli as a result of proliferation and migration during metamorphosis. The normal temporal pattern of glial proliferation is similar to the pattern of changing hormone titers, suggesting a regulatory role for hormones. We manipulated hormone titers *in vivo* by injecting 20-HE into the hemolymph at different stages of metamorphic development and monitored glial-cell proliferation, using the incorporation of BrdU as a marker. Hormone injections enhanced glial-cell proliferation during the early stages of metamorphosis when, in normal animals, the hormone titer is rapidly increasing. Later, after stage 6 (of 18 developmental stages), hormone treatment became less effective at enhancing proliferation; by stage 12, when 20-OH titers are near zero, there was no significant stimulation of proliferation. Thus, the ability of cells to respond to hormone is limited to the window of time when, normally, 20-HE circulates in the hemolymph. Two other glial classes, cell-body associated glial cells and perineurial cells also proliferate in response to 20-HE. Our results indicate that glial proliferation in the brain, like neuronal survival and growth elsewhere in the nervous system, is under the control of steroid hormones during metamorphic development.

613.2

NEUROGENESIS AND SYNAPTOGENESIS OF THE DEVELOPING SEPTUM AND AMYGDALA IN THE BRAZILIAN OPOSSUM BRAIN. J. J. Swanson*, J. Iqbal, J. K. Elmquist[1], and C. D. Jacobson. Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, [1]Department of Neurology, Harvard Medical School, Beth Israel Hospital, Boston, MA 02115.

The Brazilian opossum, *Monodelphis domestica* is a small marsupial whose young are born in an extremely immature state with a protracted postnatal period of neurogenesis. We have previously shown that neurogenesis of the hypothalamus continues postnatally. In this study we have used postnatal injection of bromodeoxyuridine (Brdu) and immunohistochemistry to describe neurogenesis of the septum (Spt) and amygdala (Amg). A high proportion of labelled cells were found in the Amg and Spt following Brdu injections at postnatal day (PN) 7 or 8. The number of Brdu labelled cells decreased with increasing age of injection. No Brdu labelled cells were present in the Amg and Spt following injections on PN 12 and 13 respectively. However, a few cells were labelled in the lateral Spt and posterior Amg following PN 13 injection. To correlate neurogenesis with synaptogenesis, immunohistochemical analysis of proteins associated with synaptic vesicles, synaptic membranes, and microtubule-associated proteins during development in the Spt and Amg was conducted. From 1 through 9 PN the Spt and Amg are lacking immunoreactivity for the synaptic vesicle-associated proteins (SVAPs) synaptophysin and synaptotagmin. After 10 PN, there is an increase in immunoreactivity for the SVAPs. Tau-1 (associated with axons) immunoreactivity follows a similar pattern of expression, whereas synaptosomal-associated protein 25 immunoreactivity is not evident until after 11 PN. In contrast, immunoreactivity for map-2 (associated with dendrites) is present in the Spt and Amg from 7 PN on. These results indicate that synaptogenesis begins as neurogenesis is completed in the Spt and Amg of the opossum.

613.3

CELL SIZE REDUCTION DURING NORMAL SPINAL CORD DEVELOPMENT. A. Chen* and R.D. Heathcote. Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201

Regressive phenomena play an important role in the morphogenesis of the vertebrate nervous system. For example, cell death, pruning of neuronal arborizations and synapse elimination are frequently involved in the maturation of neuronal populations. During neural tube formation in the frog *Xenopus laevis*, average spinal cord cell body size underwent two phases of reduction. The initial phase was characterized by a corresponding increase in cell number. To determine if the reduction in cell size was caused by cell division, animals were treated with a mixture of hydroxyurea and aphidicolin (HUA) to block DNA synthesis and consequently cell division. The cells of spinal cords treated at the time of neural tube closure did not decrease in size or increase in number, showing that the first phase of reduction in cell size was the result of cell division. Subsequently, a specific population of catecholaminergic spinal cord neurons underwent a rapid decrease in size. Since catecholaminergic neurons in the peripheral nervous system can divide, we tested the possibility that this second phase of reduction might also be due to cell division. Cell division was blocked by treatment with HUA throughout this period of time, but the catecholaminergic cells continued to decrease in size, showing that the second phase of reduction in cell size was not the result of cell division. In this case cell size reduction could be caused by diversion of cytoplasm to axons and dendrites, elimination of cytoplasmic yolk droplets or cellular secretion.

Decreases in spinal cord cell size can be separated into two phases. During the one to two days following neural tube closure, cell division can account for a marked decrease in the size of spinal cord cell bodies. During the subsequent one to two days, at least some differentiating cells undergo a greater than twofold decrease in size that is independent of cell division.

613.4

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL CHANGES OF MIDBRAIN EPENDYMAL CELLS DURING LIZARD CNS ONTOGENY. M. Monzon-Mayor[1], C. Yanes[2], R. Sturrock[3], J. de Barry[4] & G. Gombos[4]*. [1]Dept. de Morfologia, U. de Las Palmas de G.C., Canary Islands, Spain; [2]Dept. de Biologia, U. de La Laguna, Canary Islands, Spain; [3]Dept. of Anatomy & Physiology, U. of Dundee, UK; [4]Neurobiologie cellulaire, Centre de Neurochimie, Strasbourg, France.

In adult lizard, CNS repair and regeneration processes are apparently carried out by cells originating from specific areas (i.e. sulci) of the ventricular wall. Little is known on these structures and on their embryogenesis in reptiles. We have thus begun a study on the development and differentiation of ependymoglial cells in Gallotia galloti, a lizard indigenous to the Canary Islands.

At the earliest stage of CNS development (E31 to E34) the ventricles are lined with an undifferentiated pseudostratified columnar neuroepithelium where all cells are Vimentin positive. Between E35 and E37, Vimentin immunoreactivity decreases while GFAP and/or Glutamine synthetase immunoreactivity appear and ventricular cells begin to exhibit phenotypic differences. During this period three types of ependymoglial cells become distinguishable: columnar cells, cuboidal cells and cells rich in microtubules, glycogen granules and with an irregular shaped apical nucleus. From E38 to hatching, the regional differences in cell type proportions appear and are maintained in the adult. Columnar cells display long processes terminating subpially; some of these cells apparently mature into radial glia (wich in lizard is present also in adult), others into tanicyte-like cell exhibiting microtubule rich processes. Possibly the processes of the first correspond to the GFAP rich processes while those of the latter correspond to the Glutamine synthetase rich processes.

A comparison with rat ependima development was also carried out.

Studies are now in progress aiming at identifying the loci where neural cell multiply after CNS lesions in the adult lizard.

613.5

HISTOGENESIS OF FERRET SOMATOSENSORY CORTEX. S.C. Noctor*, N. Scholnicoff, S. Pedersen, and S.L. Juliano. Departments of Neuroscience and Anatomy & Cell Biology, USUHS, Bethesda, MD.

Although it has long been established that neocortical development occurs in a rostral to caudal and medial to lateral gradient, the precise time course of events has not been elaborated in many species or regions of cortex. To this end we wished to establish when cells destined for specific layers of ferret somatosensory cortex are generated in relation to those of the well-studied visual cortex and their association to intrinsic cortical connectivity. Timed-pregnant ferrets were injected IV with bromodeoxyuridine (BrDU) on embryonic days (E) from E22-38 and on postnatal days (PND) 1 and 4. Cortical tissue was collected from ferret kits after various survival times, ranging from PND 1-60. As expected, a progressive sequence of inside-out maturation occurred in the genesis of cortical layers. Neurons labeled on E22-26 did not migrate into the cortex and were found in the subplate up to PND 14. Injections of BrDU on E33-35 labeled neurons found in layer 4 in the mature cortex; and injections on E38 labeled neurons found in layer 2. Several features of the developing somatosensory cortex differed from previous observations in the visual system. We observed very few neurons distinctly populating the cortex generated postnatally. Although occasional BrDU positive cells were seen in the cortex after injections on PNDs 1 and 4, they were highly dispersed and not definitively localized in any particular layer. In addition, by PND 1, neurons residing in all cortical layers, including layer 2, were observed in the cortical plate. Therefore, although experiments with fluorescent tracers reveal that many neurons are still migrating to the somatosensory cortex during the first week after birth, elements of the laminar scaffolding are in place at PND 1. These findings confirm the notion of a rostral to caudal gradient in neocortical development and suggest that elements of ferret somatosensory cortex development may occur up to a week prior to visual cortical development.

613.6

LINEAGE-SPECIFIC REGULATION OF NEUROGENESIS: PACAP INHIBITS PROLIFERATION AND PROMOTES DIFFERENTIATION OF CEREBRAL CORTICAL PRECURSORS N. Lu* and E. DiCicco-Bloom. Dept. of Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Med. Sch., Piscataway, NJ 08854

Neurogenetic regulation has been characterized in several distinct lineages, including cerebellar granule and sympathetic precursors. In contrast, mechanisms governing the complex cerebral cortex, potentially composed of multiple neuronal lineages, remain undefined. Do common signals regulate cortical precursors? Pituitary Adenylate Cyclase Activating Peptide (PACAP), the newest member of the VIP family, was previously found to stimulate both neuroblast mitosis and survival. The expression of PACAP and receptors in embryonic cortex led us to define the peptide's role in corticogenesis.

Embryonic day 13.5 rat cerebral cortical precursors, cultured in serum-free medium, exhibited 90% and 99% MAP-2 immunoreactivity at days 1 and 2 respectively, suggesting a virtually pure neuroblast population. In contrast to other lineages, PACAP (10^{-8}) inhibited cortical [^{3}H]thymidine incorporation 50%, reflecting a similar decrease in the percentage of neuroblasts entering the mitotic cycle. Inhibition was observed even in the presence of mitogenic stimulation elicited by serum, insulin, EGF and FGF. Other VIP family members, including PHI, secretin and VIP, were without effects at equimolar concentration, indicating that PACAP activity is highly specific. However, since VIP was inhibitory at higher doses (10^{-6}), the peptides apparently act via PACAP type I receptors.

To begin defining mechanisms, we examined second messenger pathways: cAMP agonists, including forskolin, DbcAMP and IBMX, reproduced PACAP's effects. Further, the peptide elicited a 5-fold increase in cAMP content.

In addition to inhibiting mitosis, the peptide increased trkB immunoreactivity by 30% and neurite outgrowth by 80%, suggesting that PACAP blockade of proliferation induced neuronal differentiation.

In sum, while the PACAP ligand-receptor-cAMP pathway regulates multiple precursors, effects are lineage-specific. Endogenous PACAP may play a critical role in regulating progress of cortical neuroblasts through the ontogenetic sequence.

613.7

EFFECTS OF INTERLEUKINS ON NEURONAL AND OLIGODENDROGLIAL LINEAGES IN EGF-GENERATED CNS STEM CELL CULTURES.

R.E. Gross*, M.F. Mehler, L. Santschi, and J.A. Kessler. Depts. of Neurosurgery, Neuroscience & Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Recent experiments have begun to demonstrate that various neuropoietic cytokines play an important role in survival and differentiation of neural stem and progenitor cells along the three neural cell lineages. We have previously reported that certain interleukins (IL) affect neuronal maturation of immortalized neural stem cells, and in other abstracts implicate a role for cytokines in neural development *in vivo*. Here we extend our studies of the role of ILs in neural development by investigating their effects on non-immortalized EGF-generated neural stem cells.

EGF-dependent neural stem cells were generated from embryonic or adult rodent brains as described by Reynolds & Weiss (1992). Neurospheres were dissociated, washed twice, and cultured on poly-ornithine coated coverslips in serum-free medium (SFM) with TGFα in the presence of various ILs, followed by immunocytochemistry for markers of the three neural cell lineages. Control cultures contained a large number of map1b-positive neurons and a relatively small number of myelin basic protein (MBP) - positive, highly aborized oligodendrocytes. Various ILs (including IL4, IL7, IL9, IL10 & IL12) and G-CSF had a marked stimulatory effect on both the number and length of neuritic branches of a subset of map1b-positive neurons without affecting the total number of neurons generated. Thus, certain neuropoietic cytokines may affect maturation of a set of neurons without influencing survival or proliferation. In contrast, cultures grown in the presence of these cytokines contained four- to five-fold greater numbers of MBP-positive oligodendrocytes, suggesting a role for interleukins signalling through non-gp130 receptors in proliferation, survival or differentiation of cells of the oligodendroglial lineage.

613.8

CELL LINEAGE AND CELL PHENOTYPE IN RAT CEREBRAL CORTEX STUDIED WITH AN ALKALINE PHOSPHATASE (AP) RETROVIRAL LIBRARY.

C. Walsh*[1,2,3], C.L. Cepko[2,3], C. Reid[1,2], and L. Liang[1]. [1]Neurology Department, Beth Israel Hospital, and Programs in [2]Neuroscience and [3]Biological/Biomedical Sciences, Harvard Medical School, Boston, MA 02115.

We have analyzed cell production in rat cortex with a library containing up to thousands of retroviruses distinguishable by polymerase chain reaction (PCR), and encoding AP (Fields-Berry et al, PNAS 89:693,1992). The library was injected at E14-19, followed by histological and clonal analysis by PCR at P15 (Walsh & Cepko, Science 255:434,1992). AP histochemistry revealed normal neuronal morphology, allowing identification of >90% of labeled cells. Earlier injections labeled progressively more neurons in deep cortical layers, consistent with the "inside-out" sequence of neurogenesis. Thus, histological analysis suggests that AP-retroviral labeling is not obviously biased, and appears to sample normal cerebral cortical progenitors.

For clonal analysis, cells from tissue sections were PCR-amplified, successful for ≈45% of AP-positive cells. E15-labeled clones contained single cells (28%), clustered (≤1mm) cells of similar morphology (25%)(cp. Luskin et al, J. Neurosci. 13:1730, 1993), or were widely dispersed (46%)(2.1-6.7 mm, mean=3.8mm). Widespread clones contained mostly neurons of diverse morphology and laminar location and included >75% of E15-labeled cortical neurons. Widespread clones contained 2-4 'subunits' of 1-5 neurons each, spaced at apparent intervals of 2-3 mm, with each subunit morphologically indistinguishable from a clustered clone. Neurons within subunits showed similar morphology and laminar location; distinct subunits in the same clone usually differed in laminar location, suggesting sequential formation. E17-labeled clones contained fewer neurons (mean=1.6, versus 2.2 at E15); widespread E17 clones so far contain no more than two subunits. Our data suggest that clustered clones are produced by stationary progenitors; however, progenitors of clustered clones may be produced by migratory, multipotential cells.

613.9

LINEAGE OF SUBPLATE (SP) AND CORTICAL PLATE NEURONS IN FERRET STUDIED WITH AN ALKALINE PHOSPHATASE (AP) RETROVIRAL LIBRARY.

C. Reid* and C. Walsh[1,2,3]. [1]Neurology Department, Beth Israel Hospital, and Programs in [2]Neuroscience and [3]Biological/Biomedical Sciences, Harvard Medical School, Boston, MA 02115.

Since the ferret allows analysis of early cortical development, we produced an amphotropic retroviral library that encodes AP and contains up to thousands of DNA tags for cell lineage analysis. Cortical cells were labeled by injections at E27-P0, and processed for AP histochemistry and clonal analysis by polymerase chain reaction (PCR) (Walsh & Cepko, Science 255:434, 1992) at P17. As expected, early injections (E27 and E29) labeled deep layer cells, including subplate neurons, in addition to more superficial cells: 27% of neurons labeled at E29 occupied SP, layer VI, and layer V, versus 22% at E33, 9.5% at E35, and 0% at P0. Consistent with the inside-out sequence of neurogenesis, progressively later injections labeled neurons increasingly restricted to layers II and III: 43% after injections at E29 versus 61% at E33, 81% at E35 and 100% at P0. After correction for differences in cell density (Beaulieu and Collonnier, J. Comp. Neur. 279:228, 1989), E29 injections labeled remarkably similar proportions of cells in layers II-V, suggesting that retroviruses label normal cortical progenitor cells in an unbiased manner.

Since many subplate neurons are postmitotic by E27 and E29, they were labeled less frequently than cortical plate cells. Subplate neurons occurred predominantly as scattered cells, usually without associated labeling in the overlying cerebral cortex. Labeled neurons located in the cortical plate occurred as scattered cells or in small clusters after injections at E29, E33, E35, or P0.

Preliminary PCR analysis, limited to cortical plate neurons, indicates that sibling cells, both neurons and glia, occur in loose clusters (≤ 1 mm apart), or can be dispersed over large distances in both the medial-lateral and anterior-posterior axes. Thus, patterns of neuronal cell lineage for ferret cortical plate cells resemble patterns seen in rodents and are consistent with multiple modes of cell migration observed in vitro (O'Rourke et al, Science 258:299, 1992; Fishell et al, Nature 362:636, 1993).

613.10

PROMINENT EXPRESSION OF A TUMOR SUPPRESSOR GENE, APC IN GLIAL CELLS.

Ratan V. Bhat*, Karen J. Axt and Jay M. Baraban. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Tumor suppressor genes act as brakes to control excessive cellular proliferation and therefore could play a key role during development. *Adenomatous polyposis coli* (APC) is a tumor suppressor gene that is mutated in a familial type of colon cancer. In previous studies, we found high levels of APC mRNA during CNS development. Immunoblots of brain extracts confirmed the presence of APC protein (~310 kD). To identify the cell types expressing APC protein during development, we performed immunohistochemical studies on brain sections from E19-P30 rats. Prominent APC staining was present in oligodendrocytes as early as E19 in the brain stem. By P5, immunostaining was observed in oligodendrocytes of cerebellar white matter tracts. APC immunostaining was seen in interfasicular oligodendrocytes in white matter tracts throughout the brain from the 2nd postnatal week into adulthood. APC protein was detected in the cortex in S100 positive astrocytes starting at P7-P10. In the cerebellum, APC staining was seen in the Bergmann astroglial cells and in presumed satellite oligodendrocytes in the granule cell layer. The high levels of APC expression in astrocytes and oligodendrocytes indicate that this tumor suppressor gene may have a role in controlling glial proliferation.

613.11

A BRAIN-SPECIFIC ACTIVATOR FOR CYCLIN-DEPENDENT KINASE 5. L.-H. Tsai* and E. Harlow. Molecular Oncology, MGH Cancer Center, Charlestown, MA 02129.

Cyclin-dependent kinase 5 (cdk5) shares about 60% identity at the amino acid level with both cdc2 and cdk2, kinases that play key roles in cell cycle progression. Despite the fact that cdk5 is similar in structure to cdc2 and cdk2, it displays properties distinct from those kinases. First, cdk5 is expressed in a tissue-specific manner, being highest in adult nervous system and low or undetectable in other tissues. Second, we have detected kinase activity of cdk5 only in post-mitotic cells of neuronal origin in brain. Finally, cdk5 does not form an active kinase complex with the conventional cyclins. We observed a 35 kd protein that was associated with cdk5 from primary neuron cultures containing active cdk5 kinase activity. A cDNA for p35 has recently been cloned. Expression of p35 in human cultured cell lines activated both the endogenously as well as exogenously overexpressed cdk5. Cotransfection of p35 with a dominant negative version of cdk5 in these cells abolished the ability of p35 to activate cdk5. Cdk5 appears to be the only member of the cdk family proteins that is readily activated by p35. Therefore, p35 serves as a specific activator for cdk5. Surprisingly, p35 displays no sequence homology to any existing cyclin molecules. Northern analyses indicated that p35 is only expressed in brain. Among the central nervous system p35 is most highly expressed in the forebrain including cerebral cortex and thalamus, and it is not detectable in the hind brain or spinal cord.

613.12

CELLULAR DISTRIBUTION OF *Dlx2* mRNA IN THE DEVELOPING BASAL GANGLIA. M.Ding, L.Robel, A.J.James, and F.M. Vaccarino*. Child Study Center, Yale University School of Medicine, PO Box 3333, New Haven, CT 06520.

The acquisition of a morphological and functional identity of different areas of the nervous system may be regulated by specific combinations of transcription factors. In the developing mammalian forebrain, the expression of homeobox genes of the *Dlx* family is restricted to the basal telencephalon, the anlage of the basal ganglia. However, the exact cellular location of these genes is presently not known. Cells obtained from the ventricular layer of the basal telencephalon at the embryonic day 13.5 and grown in primary culture express *Dlx1*, *Dlx2*, *Dlx 5* and *Dlx6*, as they do *in vivo*. In contrast, primary cultures of cells from the dorsal telencephalon do not express the *Dlx* genes. The time course of expression of *Dlx2*, *Dlx 5* and *Dlx6* mRNAs peaks at 3-5 days *in vitro* and is protracted during the entire period of neuronal differentiation and maturation. *Dlx2* mRNA, identified by *in situ* hybridization, is localized in young neurons, immunoreactive for the microtubule-associated protein MAP1B, and, to a lesser extent, in more mature neurons, which contain MAP2 immunostaining. Nestin-positive stem cells and GFAP-containing astrocytes never expressed *Dlx2* mRNA. Blocking *Dlx2* gene expression by means of antisense oligonucleotides resulted in lower numbers of MAP1B- and MAP2-immunoreactive cells and in a dramatic change in the morphological characteristics of the cultures. Nestin and GFAP immunoreactivity were unchanged by the antisense *Dlx2* treatment. These data suggest that the expression of genes of the *Dlx* family may control important characteristics of the neuronal phenotype in the developing basal ganglia.

613.13

TREATMENT WITH bFGF INCREASES THE EXPRESSION OF *Otx2*, *Dlx2*, AND *Dlx5* HOMEOBOX GENES IN PRIMARY CULTURES OF CELLS FROM EMBRYONIC DAY 13.5 RAT TELENCEPHALON. L.Robel, M.Ding, A.J.James, A.Simeone, J.F. Leckman* and F.M. Vaccarino. Child Study Center, Yale University School of Medicine, New Haven, CT 06520 USA.

The characterization of the domains of expression of homeobox genes specifically expressed in the mammalian forebrain (*Dlx1, Dlx2, Dlx5, Dlx6, Emx1, Emx2, Otx1, Otx2)* led to the hypothesis of its early regionalization in neuromeres. So far, the role of these homeobox genes in cell specification has not been clearly demonstrated. To address this question, we studied the effect of basic fibroblast growth factor (bFGF) on the expression of these homeobox genes in cultures of cells obtained from embryonic day 13.5 rat telencephalon. In this *in vitro* preparation, stem cells multiply and then differentiate into aspartate-, glutamate- and GABA-containing neurons, as well as in glial cells. We have previously shown that bFGF treatment leads to a 3-fold increase in the number of glutamate-containing neurons. Using an RNase protection assay, we observed an increase in the expression of *Otx2*, *Dlx2* and *Dlx5* at 3 and 5 days *in vitro*, corresponding with active neuronal differentiation in the cultures. There was no such increase at day 12, after completion of the neurogenesis. *Otx2* is expressed in the ventricular zone from E8.5, whereas the *Dlx* genes are expressed in the subventricular and mantel zones within neurons (Ding et al., Neurosci. Abstr. 1994). These results suggest that *Otx* genes mediate the effects of bFGF on neuronal differentiation through a concerted expression of downstream genes, including *Dlx2* and *Dlx5*.

613.14

THE MOLECULAR GENETICS OF NEURONAL GROWTH: CHARACTERIZATION OF THE Tα1 α-TUBULIN PROMOTER IN DEVELOPING TRANSGENIC MICE. A. Gloster*, A. Speelman, J. Toma, E. Chan, and F.D. Miller. Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, Montreal, Canada.

The Tα1 α-tubulin gene is regulated as a function of neuronal growth in both developing and mature mammalian neurons. We have generated transgenic mice carrying a fusion gene comprised of 1,100 nucleotides of the upstream, putative Tα1 promoter region linked to a nuclear β-galactosidase reporter gene. Developmentally, expression of the transgene appears early during embryogenesis and is coincident with neurogenesis. Expression of the transgene is limited to the nervous system, and is first seen in the spinal cord, brain, trigeminal ganglia, facio-acoustic ganglia, and in the heart at E9.5, and in the retina at E12.0. This expression remains high during neuronal morphogenesis, at birth, and is subsequently downregulated following neural maturation, as indicated both by X-gal staining and by Western blot analysis. However, in the adult the olfactory epithelium, a region of ongoing neurogenesis, expression remains elevated. Thus, sequences exist within this promoter region which are responsible for coupling gene expression to neurogenesis and neuronal growth exist during development. We are now deleting regions within this 1.1 Kb fragment of the Tα1 α-tubulin promoter, to see which regions are responsible for conferring tissue and developmental specificity upon the expression of the transgene.

613.15

ANALYSIS OF CIS-ELEMENTS IN THE Tα1 α-TUBULIN PROMOTER D. Rogers*, N. Laferriere#, A. Gloster, D. Brown#, F. Miller. Centre For Neuronal Survival, Montreal Neurological Institute, McGill University, Montreal, Canada. # Dept. Biology, University of Ottawa, Ottawa, Canada.

Tα1 α-tubulin is a neuronal specific isotype of α-tubulin which is expressed as a function of neuronal growth. We have previously utilized 1.1kb of the 5' upstream promoter region to drive the expression of a lacZ reporter gene in transgenic mice. The pattern of expression was found to mimic the spatiotemporal expression pattern of the endogenous gene. Furthermore, we have previously demonstrated that this promoter region is capable of driving lacZ expression in P19 embryonal carcinoma cells which are induced to adopt a neuronal fate using retinoic acid. To characterize the relevant cis-elements involved in the developmental expression of Tα1 α-tubulin, deletion mutants of the Tα1 α-tubulin promoter were generated. Mutant promoters were assayed for their ability to drive lacZ expression in P19 embryonal carcinoma cells which which were induced to adopt a neuronal fate using retinoic acid. The effects of deletion of specific cis-elements on expression of the reporter gene in P19 cells was determined.

613.16

BIRTHDATES AND SURVIVAL AFTER AXOTOMY OF NEUROCHEMICALLY DEFINED SUBSETS OF TRIGEMINAL GANGLION CELLS F.A White* G.J. Macdonald, and R.W. Rhoades Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Trigeminal (V) ganglion cells with different neurochemical phenotypes or different birthdates are affected differently by neonatal axonal transection. This study attempted to determine whether sensory ganglion cell birthdate and neurochemical phenotype were correlated and if these two variables could be related to responses to neonatal axonal transection. Immunocytochemistry, histochemistry, and [^{3}H]-thymidine labelling were used to determine the birthdates of V ganglion cells recognized by antibodies directed against neurofilament protein (NF), calcitonin gene-related peptide (CGRP), substance P (SP), and those that bound the lectin *Bandierea simplicifolia*-I (BS-I). The percentages (normalized to equal 100%) of NF-positive ganglion cells born on E-9.5, 10.5, 11.5, 12.5, 13.5, and 14.5 were 0.9%, 27.3%, 30.3%, 37.0%, 4.3%, and 0.3%, respectively. The respective values for CGRP-positive ganglion cells were 0%, 0.2%, 2.3%, 3.9%, 51.5%, and 42.1%. Those for SP-positive neurons were 0%, 0%, 0%, 19.6%, 65.0%, and 15.4%, and those for BS-I-positive cells were 0%, 2.4%, 10.9%, 7.2%, 70.9%, and 8.6%. NF- and BS-I-positive ganglion cells are significantly more likely to be lost after neonatal axotomy and SP-positive cells are more likely to remain. The percentage of CGRP-positive cells in the V ganglion was not altered by neonatal ION transection. These findings do not indicate a strong relationship between cell birthdate, phenotype, and survival after neonatal axonal damage.

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613.17

ABLATION OF CEREBELLAR ASTROCYTES IN POSTNATAL TRANSGENIC MICE Catherine L. Delaney, Michael Brenner, and Albee Messing* Neuroscience Training Program and Dept. of Pathobiological Sciences, Univ. of Wisconsin-Madison, Madison, Wisconsin 53706 Stroke Branch, NINDS, NIH, Bethesda, Maryland 20892

To study the role of astrocytes during CNS development, a transgene was designed to enable their ablation at selectable times. The transgene consists of the coding region for the herpes simplex virus thymidine kinase (hsv-tk) under the control of the human glial fibrillary acidic protein gene promoter. The hsv-tk is innocuous, but converts the antiherpetic agent ganciclovir to a toxic product that interferes with DNA replication in proliferating cells. Treatment of transgenic mice during the first postnatal week with ganciclovir, with evaluation at 19d postnatal, revealed marked behavioral effects. The animals were ataxic, clasped their feet when suspended, and had difficulty standing upright to obtain food. Histological examination revealed disrupted astrocyte development, particularly in the cerebellum, with marked secondary effects on other cell types. Cerebellar defects included an overall reduction in size and disruption of the normally well defined cellular layers. The radial glia were disordered, and there was an apparent deficient myelination. The molecular and granule cell layers were greatly reduced in size. There was marked depletion of granule cells, and Purkinje cells were ectopically distributed in the molecular layer. These effects were more severe in animals treated 1d postnatal versus treatment at 5d. These results suggest a critical role for astrocytes in cerebellar development.

613.18

POSTNATAL EXPOSURE TO METHYLAZOXYMETHANOL PRODUCES HYPOPLASIA AND REACTIVE GLIOSIS IN THE CEREBELLUM OF DEVELOPING RATS. S. Barone, Jr.[1]*, J.H. Freeman Jr.[2] and M.E. Stanton[2,3] [1]ManTech Environmental Technology, RTP, NC 277091; [2]Dept. Psychology, UNC, Chapel Hill, NC, 27599-3270; [3]Neurotoxicology Div., US EPA, RTP, NC 27711.

In rats, there is dramatic cerebellar growth during the first three postnatal weeks (Altman, 1969, *J. Comp. Neurol., 136*, 269-294). Exposure to antimitotic agents during this period produces neuronal loss and disruption of cellular structure within the cerebellum that varies depending on the day of exposure and dose (Altman & Anderson, 1973, *J. Comp. Neurol., 149*, 123-152). In the present study, we experimentally manipulated cerebellar maturation by exposing neonatal rat pups to the antimitotic agent methylazoxymethanol (MAM) during the period of cerebellar cortical neurogenesis. Pups were given either saline or MAM (20 mg/kg) on PND4 and 7, and sacrificed on days 10, 19, 22, 26, or 33. The neuroanatomical effects of this MAM treatment were examined in sections stained for glial fibrillary acidic protein (GFAP) immunoreactivity and Nissl. Exposure to MAM greatly reduced cerebellar volume, disrupted cellular organization, and induced reactive gliosis. Cell loss within the cerebellar cortex was apparent at all ages assessed. The monocellular structure of the Purkinje cell layer was disrupted in many of the cerebellar lobules. There was also a dramatic reduction the volume of the granule cell and molecular layers. Ectoptic granule cells were seen in the molecular layer of MAM-treated animals. GFAP immunoreactivity increased as a function of age in both dosing groups. At PND26 and 33 there was an increase in GFAP immunoreactivity in the cerebellar white matter in MAM-treated animals. The dosing strategy used in this experiment produced effects that were primarily restricted to the cerebellar cortex. There was little evidence of cell loss or reactive gliosis in other postnatally developing structures such as the hippocampus or cerebellar efferents.

613.19

THE *meander tail* GENE HAS MULTIPLE EFFECTS ON CEREBELLAR GRANULE CELL DEVELOPMENT AND SURVIVAL. K.M. Hamre* and D. Goldowitz. Dept. of Anat. and Neurobiol., Univ. of Tennessee Memphis, Memphis, TN 38163.

The murine genetic mutation *meander tail* (gene symbol *mea*) results in the near total ablation of granule cells in the anterior lobe of the cerebellum. How the *mea* gene results in granule cell reduction remains unknown. Two approaches were used to determine whether the action of the *mea* gene is specific to anterior lobe granule cells. First, we find by estimating granule cell number in the posterior lobe (lobules VIII-X), a small (less than 20%) but statistically significant reduction in the number of granule cells in adult homozygous *mea* mice compared to heterozygous controls. Second, using two types of intraspecies murine chimeras (homozygous *mea* embryos with BALB/C or globin transgenic [GT] embryos) we find a complex effect of the *mea* gene on cerebellar granule cell survival. In *mea*<-->BALB/C chimeras, cells from *meander tail* embryos are labeled immunocytochemically with a strain specific nuclear antigen (Mullen and Chichocki, 1988). In *mea*<-->GT chimeras, GT cells are labeled using in situ hybridization with a probe for the globin transgene construct (Lo et al., 1987). The percentage of *meander tail* cells that contribute to the CNS were estimated in the cerebellar granule cell layer in anterior and posterior lobes as well as in hippocampal and olfactory bulb granule cells, and in cerebellar molecular layer interneurons. Cerebellar granule cells that are genotypically *meander tail* are found in both the anterior and posterior lobes. Additionally, the percentage of cells that are genotypically *meander tail* is equivalent in both anterior and posterior lobes. However, the percentage of *mea* cerebellar granule cells is markedly less than the percentage of *mea* cells found in other neuronal populations including the cerebellar molecular layer interneurons. These results suggest that while the *mea* gene directly affects overall granule cell survival, there are extrinsic factors that permit the successful rescue of genotypically *mea* granule cells in the anterior lobe. Support: NS23475 to DG.

613.20

EPIDERMAL GROWTH FACTOR (EGF), TRANSFORMING GROWTH FACTOR-α (TGF-α) AND BASIC FIBROBLAST GROWTH FACTOR (bFGF) DIFFERENTIALLY INFLUENCE NEURAL PRECURSOR CELLS OF THE MOUSE EMBRYONIC MESENCEPHALON. J. Santa-Olalla and L.Covarrubias*. Departamento de Biología Molecular, Instituto de Biotecnología, UNAM, Apdo. Postal 510-3, Cuernavaca, Morelos 62271, México.

The molecules generally known as growth factors are key elements in the process of neural cell differentiation. In this report, we examined the effects of classical mitogens on neural precursor cells, by culturing mouse cells of the embryonic (13.5 days *post coitum*) mesencephalon and treating them with EGF, TGF-α, bFGF, Nerve Growth Factor (NGF) and Transforming Growth Factor-β (TGF-β). Our initial results show that EGF, TGF-α or bFGF but not NGF nor TGF-β, induced general proliferation of the cultured cells, followed by the formation of colonies. Combinations between these three growth factors suggest that an overlapping cell population is target of EGF, TGF-α or bFGF in promoting colony formation. It is noteworthy, that the number of colonies increased significantly when EGF, but not TGF-α, was used in combination with bFGF. Furthermore, a population that respond only to EGF+bFGF was detected in the dorsal mesencephalon. The colony forming activity of bFGF depended on insulin, but their cooperativity was indirect since we could not observe colony regeneration even in the presence of insulin. Cells obtained from our colonies displayed neuronal and glial morphology and expressed markers of both neurons and astrocytes; nestin, a marker of neural precursor cells, was also expressed in the majority of the colonies. Growth factors also influenced neuronal maturation; the highest neurite outgrowth was obtained from cells derived from bFGF-induced colonies cultured in the presence of EGF+bFGF. These data indicate the existence of neural precursor cells in the embryonic mesencephalon that respond to growth factors.
This Work was supported by DGAPA (IN-201991) and PADEP (030364)

CELL MIGRATION AND DIFFERENTIATION III

614.1

MODULATION OF G PROTEIN ACTIVITY AND INTRACELLULAR CALCIUM AFFECTS THE MIGRATORY BEHAVIOR OF EMBRYONIC NEURONS. A.M. Horgan* and P.F. Copenhaver. Cell Biol. & Anat. L215, Oregon Health Sciences U., Portland, OR 97201.

During embryonic development of the moth, *Manduca sexta*, a population of approximately 300 neurons (EP cells) in the enteric nervous system migrate and extend processes along eight specific muscle pathways to innervate the gut. This motile sequence is precisely regulated such that these neurons begin their migration at 55% of development (1% = 1 hour). The neurons migrate for ~5 hours, travelling up to 250 μm. Process outgrowth then continues after neuronal migration is complete, lasting until ~80% of development. The accessibility of the EP cells and their muscle pathways during development can be used to investigate the regulatory processes that are involved in the control of neuronal motility. In previous work, we showed that one of the heterotrimeric G proteins (Go) is first expressed by the EP cells around the onset of migration. Using extracellular bath applications in semi-intact embryos and intracellular injections of individual neurons, we have examined the effects of compounds known to alter G protein activity on neuronal motility. Injections of stimulatory agents (including GTPγS and mastoparan) caused a reduction in both neuronal migration and process outgrowth, whereas the inhibitory compound pertussis toxin resulted in exuberant filopodial extension in a number of injected cells. Transient exposure to compounds that increase intracellular Ca (including ionomycin and thapsigargin) also caused a substantial inhibition of migration. We are currently exploring the potential interactions between multiple intracellular signalling pathways in the regulation of neuronal motility during embryogenesis. Supported by NSF BNS9010538.

614.2

MIGRATION OF LHRH NEURONS IN OLFACTORY EXPLANTS: EFFECTS OF SERUM-FREE MEDIA, TETRODOTOXIN AND DEPOLARIZATION. S. M. Fueshko* and S. Wray Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

We have previously shown that embryonic mouse olfactory explants maintain large numbers of LHRH neurons which emerge and migrate away from the olfactory pit in directional tracks along a subset of peripherin positive fibers. To continue our study of the mechanisms guiding the neurophilic migration of LHRH neurons, we evaluated the migratory properties of LHRH neurons cultured in serum-free media (SFM). We also examined whether spontaneous electrical and synaptic activity and/or depolarization play a role in the movement of LHRH neurons. Olfactory explants from E11.5 mouse embryos were cultured in SFM for 1-7 days. Immunochemical staining indicated that compared to explants grown in the presence of serum, SFM had no significant effect on LHRH cell numbers (25-50% of the total LHRH population was typically maintained) or on LHRH cell migration as determined by emergence, directionality and distance that cells migrated away from the olfactory pits. Inhibition of spontaneous electrical and synaptic activity by continuous incubation with tetrodotoxin (10^{-6}M) from day 2-7 in culture had no significant effect on either LHRH cell number or the ability of cells to migrate. Similarly, depolarization by incubation with potassium (20 mM) had no quantitative effect on LHRH cell migration. Therefore, we propose that interactions at the cell surface which are independent of spontaneous electrical activity and/or depolarization are instrumental in directing LHRH cell migration. Studies are now in progress to determine whether electrical activity and/or depolarization influences secretion from embryonic LHRH cells.

614.3

GABA and NGF INDUCE CHEMOTAXIS IN CORTICAL NEUROBLASTS. T.N. Behar*, H.T. Tran, and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, Md. 20892

Chemotactic and chemokinetic migration of acutely dissociated embryonic cortical cells derived from 14 to 20 day-old rat embryos (E12-E15) was analyzed *in vitro* using a chemotaxis chamber. Embryonic cells migrated toward γ-amino butyric acid (GABA) beginning at E15, indicating cells generated in the ventricular zone migrate towards GABA. In contrast, NGF-induced migration was not detected until E17, suggesting cells generated in the subventricular zone respond to NGF. A modified checker-board analysis revealed that micromolar GABA exerted a chemokinetic effect on cells (increased random motility), while pM NGF and low GABA concentrations (fM) were predominantly chemotactic, inducing cells to migrate along a chemical gradient. Immunolabeling showed that the majority (>95%) of migrating cells expressed neurofilament protein (NF) and hence, were postmitotic neurons. The chemotropic effects of GABA were mimicked by muscimol and R-baclofen, suggesting GABA mediates motility via both $GABA_A$ and $GABA_B$ receptor proteins. These results demonstrate that both GABA and NGF are capable of directing the migration of newly generated neurons during early cortical development.

614.4

ANTI-IDIOTYPIC ANTIBODIES TO GM1 PROMOTE SCHWANN CELL MIGRATION ON NORMAL AND DENERVATED SCIATIC NERVE. W.D. Matthew*, L.S. Barna and M.J. Riggott. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710

Gangliosides are potent modulators of cell adhesion and migration. They may interact with membrane proteins that act as receptors for growth factors or extracellular matrix molecules. We have generated putative anti-idiotypic antibodies that bind the functional sites of GM1 binding proteins. These antibodies were tested for their ability to recognize Schwann cell antigens and to promote Schwann cell migration on cryostat sections of denervated or normal sciatic nerve. The antibodies recognize antigens on the cell surface of cultured primary Schwann cells and, in Western blots, these antibodies bind proteins at 66, 45 and 21 kD. Cultured primary Schwann cells treated with the antibody respond by protein tyrosine phosphorylation of proteins at 151, 134 and 107 kD. In bioassays of Schwann cell migration, the antibodies enhance migration on denervated sciatic nerve, and one antibody promotes migration on normal nerve. These results suggest that anti-idiotypic antibodies bind to cell surface receptors that are linked to protein tyrosine second messenger systems. Activation of these receptors facilitate Schwann cell migration which is crucial to peripheral nerve development and successful nerve regeneration.

614.5

NEURONAL MIGRATION FROM THE EMBRYONIC NASAL CAVITY TO THE ROSTRAL FOREBRAIN. S.A. Tobet*, T.W. Chickering, I. Kaddis, J.E. Crandall, and G.A. Schwarting, Program in Neuroscience, The Shriver Center, Waltham, MA 02254 and Harvard Med. Sch., Boston, MA 02115.

Following their birth in olfactory placode, gonadotropin releasing hormone (GnRH) containing neurons migrate across the developing cribriform plate and form a dispersed population in the mammalian basal forebrain. To study the migration of these neurons we are utilizing a paradigm to visualize cell migration in vitro, in tissue slices that maintain the structure and contact of the nasal cavity with the forebrain. On day 15 and 16 of gestation rat embryos were dissected, and the heads embedded in agarose and cut by Vibratome into 300 micron slices. Individual cells were randomly labelled by briefly immersing the slices in the carbocyanine dye, DiI. Cells were selected for video microscopic analysis based on their apparent "neuronal" morphologies and location within the known pathway for GnRH cell migration (e.g., Tobet et al., Dev. Biol. (1993) 155:471). The patterns and rates of cell movements were analyzed throughout the rostral-caudal extent of the GnRH cell migration pathway. Characteristics of cell movements differed depending upon location along the pathway. Cell movements in the nasal cavity involved fewer turns than in the basal forebrain. These studies raise the possibility that there are constraints on the ability of cells to move in selective portions of the migration pathway from the nasal cavity to the basal hypothalamus. Supported by PHS grants HD05515, MR Core Grant HD-04147, and the DMR-MA.

614.6

THE VENTRALLY GENERATED "U-SHAPED" GROUP OF CHOLINERGIC CELLS IN EMBRYONIC RAT SPINAL CORD EXPRESS NADPH-DIAPHORASE AND MIGRATE DORSALLY *IN VIVO* AND *IN VITRO*. P.E. Phelps,* R.P. Barber, R. Wetts, and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Most neuronal migration is thought to be directed radially from the ventricular zone (VZ), but members of the "U-shaped" group of cholinergic neurons, first detected around the ventral VZ, appear to move dorsally within the spinal cord between E16-18. Some of these cells translocate relatively long distances to the dorsal horn, and others, only short distances to surround the central canal. Both dorsal horn and central canal cluster ChAT-immunoreactive cells co-express NADPH-diaphorase between E16-18, and thus either marker can label these immature neurons. As a first step in analyzing this dorsal migration experimentally, 300-400 μm slices of E16 cervical enlargement were cultured for periods encompassing *in vivo* migration times. Preliminary data suggested that diaphorase-labeled cells of the "U-shaped" group had moved dorsally in a relatively histotypic pattern before differentiating. Thus, these *in vitro* data provide additional information that the "U-shaped" group of cells have a unique migratory pattern from the ventral VZ into the dorsal horn. *Supported by NIH grant NS 18858.*

614.7

CALCIUM ION INFLUXES THROUGH THE PLASMA MEMBRANE TRIGGER SPONTANEOUS INTRACELLULAR CALCIUM OSCILLATION IN MIGRATING NEURONS. H. Komuro* and P. Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510

Recently, we found that Ca^{2+} influx, regulated by a combination of voltage- and ligand-gated channels, is essential for extension of the leading process and translocation of cell soma during neuronal cell migration (Komuro & Rakic, Science **257**: 806, 1992; **260**: 95, 1993). Furthermore, we reported that migrating neurons exhibit transient elevation of intracellular Ca^{2+} levels (Komuro & Rakic, Soc Neurosci Abstr **19**: 34, 1993). Here, we study possible cellular mechanisms underlying spontaneous intracellular Ca^{2+} oscillation in migrating neurons. Rectangular pieces (100-200 μm) of cerebella from 3-7 day-old CD-1 mice were placed on poly-L-lysine and laminin-coated Petri dishes containing 1 ml serum-supplemented culture medium. After 2-3 day incubation, cells were bathed in serum-free, defined culture medium with 1-3 μM Fluo-3/AM for 30-60 min at 36 °C. After rinsing and an additional 1-3 hr postincubation in defined culture medium, granule cell migration from microexplants was examined using confocal laser scanning microscopy. Changes in the rate of movement and the levels of intracellular Ca^{2+} were directly recorded in single optical sections collected at 1-60 sec intervals for up to 70 min. Migrating granule cells exhibited undulatory movement and spontaneous oscillation of intracellular Ca^{2+} levels at a frequency of 10-20 times per hour. The duration of each elevation lasted 1-2 min; the amplitude as indicated by Fluo-3 fluorescence ranged between 5-30 % of resting levels. Reduction of extracellular Ca^{2+} concentrations eliminated the spontaneous Ca^{2+} oscillation and caused cessation of cell movement. Furthermore, the addition of antagonists to either N-type Ca^{2+} channels or NMDA receptors to the culture medium reduced the amplitude of Ca^{2+} oscillations and decreased the rate of cell migration. These results suggest that spontaneous oscillation of intracellular Ca^{2+} levels may be triggered by Ca^{2+} influx through a combination of N-type Ca^{2+} channels and NMDA receptors. Furthermore, the assembly and disassembly of neuronal cytoskeletal elements associated with migration may be controlled by the periodic increase and decrease in intracellular Ca^{2+} levels. (Supported by NS22807)

614.8

Ng-CAM BLOCKADE RESULTS IN CALCIUM-SIGNALED INHIBITION OF MIGRATION AND SURVIVAL OF NEW NEURONS IN THE ADULT AVIAN FOREBRAIN K. Barami,*[1] M. Nedergaard,[2] V. Lemmon,[3] S. Goldman.[1] Depts. of [1]Neurology and [2]Neurosurgery, Cornell Univ. Med. Col. NYC, 10021, and [3]Neurosciences, Case-Western Res. Sch. of Med., Cleveland, OH 44106.

The avian forebrain generates neurons throughout adulthood, from ependymal/subependymal zone (SZ) precursor cells lying within the ventral surface of the lateral ventricle. New neurons migrate from the SZ along radial guide fibers, a process which permits neuronal insertion into the adult forebrain parenchyma. In the process of identifying ligands which might mediate the neuronal-radial fiber interaction in adulthood, we examined Ng-CAM/8D9 expression by new neurons in HVC, a neurogenic region of the avian neostriatum. In cryostat sections of both adult finch and canary brain, Ng-CAM/8D9 was found throughout HVC and its adjacent neostriatum, but was confined to neurites, with scant expression on neuronal cell bodies. In vivo, new neurons pre-labeled with ^{3}H-thymidine expressed Ng-CAM/8D9 at all stages of their migration. In cultured explants of adult canary SZ, migrating neurons rapidly developed Ng-CAM/8D9 immunoreactivity. In contrast, the ependymoglia and ependymal/radial fibers upon which the neurons migrated were never found to express Ng-CAM/8D9, in vivo or in vitro. Addition of anti-8D9 Fab to these cultures yielded a dose-dependent inhibition of neuronal outgrowth from the parent explants. In addition, neurons already in the outgrowths at the time of antibody addition exhibited process retraction, cell rounding, and in some cases, late death. Migrating neurons responded to anti-8D9 with sustained increments in cytosolic calcium, as viewed by laser-activated confocal imaging of cultures pre-loaded with fluo-3. Thus, the migration of new neurons from the adult avian VZ may require their expression of Ng-CAM/8D9, and their adhesion to a heterophilic ependymal/radial cell receptor. Furthermore, the viability of new neurons may be regulated, directly or indirectly, by Ng-CAM/8D9-activation dependent, calcium-mediated signaling processes.

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614.9

MIGRATION OF LHRH NEURONS IN EARLY HUMAN EMBRYOS: ASSOCIATION WITH NEURAL CELL ADHESION MOLECULES. M. Schwanzel-Fukuda* and D.W. Pfaff (Rockefeller University, New York, USA); P.M.G. Bouloux (Royal Free Hospital, London, England); J.-P. Hardelin and C. Petit (Pasteur Institute, Paris, France).

Luteinizing hormone-releasing hormone (LHRH) neurons originate in the epithelium of the medial olfactory pit and migrate into the brain along a migration route formed by the vomeronasal and terminal nerves. This migration route, rich in neural cell adhesion molecule (NCAM), is formed before the LHRH neurons are first detected. In 28-31 day old embryos, NCAM-immunoreactive cells were seen in the epithelium of the medial olfactory pit and emerged to form a cellular aggregate below the rostral forebrain. Antibodies to a polysialylated form of NCAM (PSA-NCAM) showed no immunoreactivity in the nasal mesenchyme at these ages. In 41-42 day old embryos LHRH-immunoreactivity was detected in some cells among the cords of NCAM cells in the nasal mesenchyme, just outside of the epithelium of the olfactory pit. LHRH and NCAM were not co-localized. Many more LHRH cells were seen in the ganglion terminale of the terminal nerve and along the caudal margin of the cellular aggregate below the forebrain and at the basal lamina of the medial forebrain, caudal to the developing olfactory bulb. A few LHRH cells were seen in the medial basal forebrain and NCAM was present in all parts of the migration route. The PSA-NCAM, in association with LHRH cells, was seen in fascicles in the nasal mesenchyme, in the ganglion terminale, along the caudal margin of the cellular aggregate but not in the cellular aggregate below the rostral forebrain.

614.10

MUTATION OF THE SUBEPENDYMAL LAYER IN THE NCAM-180 DEFICIENT MOUSE AND ITS PHENOCOPY BY ENZYMATIC REMOVAL OF POLYSIALIC ACID. K. Ono*, H. Tomasiewicz, T. Magnuson and U. Rutishauser. Depts. of Genetics and Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

Genetic deletion of NCAM-180 results in a smaller olfactory bulb (OB) and morphological alteration of the subependymal layer (SEL), the latter being the source and migration pathway for OB precursor cells. The mutant mice also showed a nearly complete loss of NCAM-associated polysialic acid (PSA) that is normally expressed in these tissues. The present study was intended to determine the contribution of PSA loss to the mutant phenotype in the SEL. Newborn mice received an intracranial injection of endoneuraminidase N (endo N), which completely and specifically destroys PSA, and the distribution of precursor cells was examined at P1 and P7. At P1, which is prior to appearance of the mutant phenotype in the SEL, endo N injection did not produce any morphological alterations. That is, the precursor cells tended to be positioned in caudal SEL. At P7, however both the mutant and endo N-treated animals showed the same clear perturbation: the number of OB precursor cells remaining in the caudal part of the layer increased from less than 20% to over 50%, and there was a corresponding decrease in cells that have arrived at the OB. Pulse-labeling of the SEL cells at P1 revealed an arrest in migration of cells in the caudal SEL. By contrast, there were no detectable changes in cell proliferation or death. These results provide direct evidence that the NCAM-180 mutation causes a decrease in tangential cell migration in the SEL, and that this perturbation is due to the corresponding loss of PSA.

614.11

MIGRATION OF SPINAL CELLS INTO THE DEVELOPING MENINGES. K. Sharma*, Z. Korade and E. Frank. Department of Neurobiology, University of Pittsburgh School of Medicine. Pittsburgh, PA 15261.

Neuroepithelial cells continue to migrate from the spinal cord into the periphery after neural crest emigration is complete. Cells emigrating at st 25 differentiate into dorsal root ganglion cells and melanocytes. Do spinal cells continue to migrate away from the cord at still later stages? If so, what is the developmental fate of these cells?

Migration of neuroepithelial cells in the spinal cord was studied in st 29-30 chicken embryos. Cells were labeled with DiI injected into the central canal of cultured spinal segments. Labeled cells left the spinal cord through the dorsal and ventral spinal boundary, adjacent to the roof plate and through the floor plate, and migrated into the meningial anlage. To determine which cells in the spinal cord were migrating, we made small injections of fluorescent dextran into the floor plate of st 29 cultured spinal segments. After 12 hours, labeled cells had migrated into the meninges. Further evidence of the fate of these cells was obtained from chimeric embryos in which quail spinal cord was transplanted into chick hosts at stage 25-26. By st 31 to P0, quail cells were present in pia, arachnoid and dura. These cells were not seen in embryos in which neuroepithelium was labeled with DiI at stage 25-27 and embryos allowed to develop for only 24 hr, suggesting that emigration of meningial cells occurs after st 27.

These results suggest that in avian embryos, spinal cord cells emigrate through the roof plate and the floor plate and contribute to the anlage of the developing pia, arachnoid and dura surrounding the spinal cord. Supported by the NIH.

614.12

Undifferentiated Neuroepithelial Cells In The Spinal Cord Retain The Potential To Develop Neural Crest Phenotypes. Z. Korade*, K. Sharma and E. Frank. Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh PA 15261.

A subpopulation of neuroepithelial cells in the spinal cord migrate into the periphery after neural crest emigration is complete to develop as DRG cells and melanocytes, formerly thought to be late neural crest derivatives. Could these spinal cells develop other crest-type phenotypes if they encountered the environment that earlier crest cells find? This was tested by placing the neuroepithelial cells into crest migratory pathways at various developmental stages.

Cells from dorsal or ventral halves of st 26 quail spinal cords were dissociated and placed into chicken hosts. Approximately 100 - 200 cells were injected under the skin at the dorsolateral margin of the neural tube in st 16-19 or st 22-24 embryos. Injected embryos were allowed to develop for 2 - 10 days and quail cells were identified using a quail-specific Mab. Cells isolated from ventral spinal cord failed to migrate from the site of injection whereas dorsal cord cells did migrate. This difference may result from the fact that more cells in the dorsal cord are undifferentiated and still dividing at st 26. The final location of the dorsal cells was dependent on the developmental stage of the host. When injected into st 16-19 hosts, dorsal spinal cells migrated into sympathetic ganglia and peripheral nerves, but in st 22-24 embryos these cells migrated to DRG, peripheral nerve and skin. The migration of spinal cells to different locations at different stages parallels the sequential developmental changes of crest phenotypes.

These results show that at least some undifferentiated neuroepithelial cells in the dorsal spinal cord retain the ability to develop a variety of neural crest-like phenotypes. Their differentiation as neural crest-like cells is dependent on the temporal environment they encounter during development. Supported by the NIH and the McKnight Foundation.

614.13

PHENOTYPE PLASTICITY AND IMMUNOCYTOCHEMICAL EVIDENCE FOR CHAT AND DβH CO-LOCALIZATION IN FETAL PIG SUPERIOR CERVICAL GANGLIA CELLS. P. Partoens[1], J.M. Wang[1], N. Fraeyman[2], J.M. Godfraind[3]* and W.P. De Potter[1] [1]Lab. neuropharmacol., Univ. Antwerp (UIA), Antwerp, [2]Heymans Inst. Pharmacol., Ghent and [3]Fac. Méd., UCL, Woluwé, Belgium

The early expression of the cholinergic phenotype in sympathetic neurons appears to be species dependent. Moreover a dual phenotype status during the noradrenergic/cholinergic transition in rodent has been electrophysiologically evaluated and recent immunohistochemical evidence indicates co-expression of tyrosine-hydroxylase and choline-acetyltransferase (CHAT) in avian sumpathetic neurons. We investigated dissociated neurons from the superior cervical ganglia (SCG) of fetal piglets for the presence of dopamine-β-hydroxylase (DβH) and CHAT using immunocyto-chemical methods, in control conditions, continuous stimulation with high K^+ or in co-culture with splenocytes. Four types of cells were distinguished: negative cells (non-neuronal cells), $D\beta H^+$ or $CHAT^+$ positive cells and double positive cells ($D\beta H^+$ & $CHAT^+$). After 2 days in culture the ratio of $CHAT^+/D\beta H^+$ single positive cells was 0.15 ± 0.01 indicating the predominance of the adrenergic phenotype. After 8 days in control medium the ratio increased to 0.30 ± 0.02 indicating a shift to the cholinergic phenotype. After 8 days in high K^+ medium, the ratio was 0.15 ± 0.03, while coculture of the neurons with splenocytes induced a shift in the ratio to 0.41 ± 0.03. For the double positive cells, after 8 days in culture, the percentage of the $D\beta H^+$ & $CHAT^+$ cells in total neuronal cells was 8.4%, 7.4% and 17.5% in control, high K+, and co-culture with splenocytes conditions respectively. We conclude that the fetal pig neurons are predominantly adrenergic and that during a certain culture period, catecholamines and acetylcholine biosynthesis enzymes are present in the same mammal sympathetic neurons.

614.14

CHARACTERIZATION OF A ECTOPIC CELL TYPE FROM THE HEARTS OF α-MHC-NGF TRANSGENIC MICE. A. F. Andrade-Rozental*, R. Rozental, A. Hassankhani, D.C. Spray and H.J. Federoff. Departments of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx NY 10461.

Previously, we have demonstrated that cardiac selective expression of NGF in a transgenic mouse produced sympathetic hyperinnervation and hyperplasia of an unknown cell population within the base of the heart (Hassankhani, et al, Soc Neurosci Abst **18**:1289, 1992). To further analyze the ectopic cell population, we have undertaken morphological, immunocytochemical and physiological studies. Ectopic cells prepared from the base of hearts of 3-4 week old transgenic mice were dissociated and cultured in HBSS containing 10% FCS. None of these cells showed spontaneous contractile activity and did not stain with an antibody against sarcomeric actin, ruling out a myogenic phenotype. At two days *in vitro* (DIV) a subpopulation of the cells displayed a spindle-shaped morphology. Immunocytochemical analysis indicated that this subpopulation stained positively for GFAP and vimentin. Physiological studies revealed that they were not electrically excitable that they were poorly coupled. Unitary junctional conductance of these cells were 60 pS and junctional conductances were 0.4-2 nS. After 7 DIV, a second subpopulation of cells that adopt a fiber-like morphology were detected. These cells immunostained for $gp140^{trkA}$ and peripherin. Overall, these results indicate that these ectopic cells are likely derived from neural crest. Experiments are being carried out to evaluate whether these cells arise by abnormal migration into the developing heart or abnormal mitogenic expansion of a normal cardiac neural crest-derived cell type.

614.15

MICROENVIRONMENTAL FACTORS IN THE DIFFERENTIATION OF ENTERIC NEURONS FROM NEURAL CREST-DERIVED PRECURSORS T.P. Rothman*, J. Chen and M D. Gershon, Columbia Univ. P&S New York, NY 10032

The enteric nervous system (ENS) develops from precursors that migrate to the bowel from the neural crest. Experiments involving back-transplantation of gut into younger host embryos have indicated that crest-derived cells are not committed to differentiate along enteric lineages at the time they colonize the bowel. Differentiation of these cells thus depends on signals they receive from the enteric microenvironment. Previous studies have revealed that enteric neuronal and glial development are specifically promoted by laminin, an effect that is blocked by a soluble peptide (IKVAV). This sequence is present on the Ae chain of laminin. In order to determine if laminin Ae might be one of the enteric factors that normally promotes ENS development, we examined its expression in the developing nervous system of control and congenitally aganglionic mice (*ls/ls*). mRNA encoding laminin Ae was detected in the fetal bowel (using RT-PCR) as early as day E10, was expressed through E17, but declined to trace amounts at later ages. Laminin Ae expression is therefore developmentally regulated and coincides with the period of enteric ganglion formation. *In situ* hybridization suggests that mRNA encoding laminin Ae is expressed first in the epithelium and then the mesenchyme of the bowel. Another factor that may also affect enteric neuronal development, directly or indirectly, is CNTF. Amplimers based on rat CNTF cDNA were used to clone a cDNA fragment encoding mouse CNTF, which was 94% homologous to that of rat. The mouse sequence was used to design primers (for RT-PCR) and a riboprobe (for *in situ* hybridization) to investigate CNTF expression. CNTF was expressed in the mesenchyme of the fetal mouse gut, but was not developmentally regulated. In contrast, mRNA encoding $CNTF_{\alpha}$, the component of the receptor that conveys CNTF specificity, was abundant in fetal bowel (E10-E17), but rare in adult gut. These data suggest that laminin Ae and CNTF are potentially factors that play roles in the development of the ENS. (Supported by grant HD21032).

614.16

TRANSIENTLY CATECHOLAMINERGIC CELLS IN THE BOWEL OF THE FETAL RAT EXPRESS mRNA FOR THE NMDAR1 RECEPTOR. M.S. Cumming*, G.A. Burns, C. Ulibarri, and K.E. Stephens. Department of VCAPP, Washington State University, Pullman, WA 99164

Tyrosine hydroxylase-like immunoreactivity (TH-li) has been shown to be a transient marker for precursors of enteric neurons (Baetge, *Dev Biol* 141:353 '90). In this study, we sought to ascertain whether neural crest cells, destined to innervate the fetal gut, express mRNA for the NMDAR1 subtype of the glutamate receptor. Serial sections of embryonic day 12 rat tissues were initially reacted with a polyclonal TH primary antiserum (Peninsula Labs), using a standard avidin-biotin immunocytochemical protocol with DAB as the chromagen. The same sections were then hybridized with a 1.4 kB NMDAR1 riboprobe, which had been transcribed in the presence of ^{35}S-UTP. The immuno-reacted\hybridized tissues were coated with radiosensitive emulsion and incubated for 5 weeks at -70° C. Clusters of silver grains were observed over cells exhibiting TH-li in the primitive gut. The expression of NMDAR1 mRNA by TH-reactive cells suggests a possible developmental role for this receptor, such as neuronal migration.

614.17

DEVELOPMENTAL EXPRESSION OF THE *MICROPHTHALMIA* GENE IN EYE AND EAR OF WILD TYPE AND MUTANT MICE . C. Chen*, A. Nakayama, M.Tachibana, H. Arnheiter. National Institutes of Health, NINDS-LVMP, Bethesda, MD 20892.

The *microphthalmia* (*mi*) gene encodes a novel member of the developmentally important family of basic-helix-loop-helix-zipper transcription factors. Mice with mutations in this gene may be completely white, deaf and virtually blind. These abnormalities are due to deficiencies in neural crest-derived melanocytes that populate the skin and the stria vascularis of the ear, and deficiencies in the pigment epithelium (PE) of the retina that lead to microphthalmia.

To better define the pathogenetic mechanisms leading to these abnormalities, we now study the expression of this gene during development of normal and *mi* mutant embryos via *in situ* hybridization. In wild type embryos, *mi* expression was first found at E9.5 in the PE and at E 11.5 in cells surrounding the otic vesicle (OV). TRP2, a marker for melanoblasts, was expressed almost as early as *mi* and apparently in the same cells. While *mi* expression started to decrease at E12.5 in the PE and E14.5 in the OV and was barely detectable at birth, TRP2 expression remained detectable even after birth both in the PE and in the stria vascularis. In embryos homozygous for a transgenic insertion at *mi*, expression of *mi* was low in the PE and essentially non detectable in the OV. TRP2, however, could be detected in the PE, but not the OV, consistent with the observation that PE cells seem to remain intact, while neural crest-derived melanoblasts of the OV apparently do not. In embryos homozygous for a single codon deletion in *mi* that does not impair the gene's expression, both *mi* and TRP2 gave strong signals in the PE. However, in the OV, the expression of either gene remained low and became undetectable after E13.5, again suggesting absence of melanocytes. Thus, *mi* may play a role in melanoblast proliferation and differentiation but not the maintenance of the differentiated melanocyte phenotype.

CELL MIGRATION AND DIFFERENTIATION IV

615.1

DENDRITIC DIFFERENTIATION OF RETINAL GANGLION CELLS IN CHICK. R.L. SNOW, D.J. STELZNER* AND J.A. ROBSON. Dept. of Anatomy and Cell Biology, SUNY HSC, Syracuse, NY 13210.

Development of dendritic morphology of chick retinal ganglion cells was investigated using retrograde transport of DiI. Label was applied to the optic nerves of embryos fixed between E6 and E14. The embryos were stored in fixative for 6-8 weeks at 37°C. Retinal wholemounts were examined using epifluoresence and confocal microscopy. Selected retinas were photoconverted and drawings of labeled cells were made using a computerized microscope.

At E6 many ganglion cells are still migrating. These cells are bipolar in shape and lack dendrites. They have axons in the optic nerve and trailing processes attached to the scleral margin. By E8 rapid dendritic growth is prevalent and trailing processes retract. This early dendritic growth is characterized by the appearance of many short spiny projections from all sides of the soma. As the cells mature most of these spiny processes appear to retract. However, a few elongate and branch profusely giving the cells a mossy appearance. Later, many of these branches disappear. As dendrites continue to mature, distinct ganglion cell morphologies become evident. Ganglion cells with large dendritic fields differentiate first, followed by several other distinct classes. [Supported by NIH grant EY03490]

615.2

POSTNATAL MATURATION OF THE MOUSE OPTIC PATHWAY. Zhi Ping Mi, and C. Lagenaur* Dept. Neurobiol., Univ. Pittsburgh, Pittsburgh, PA 15261.

Although mouse optic axons reach their targets during embryonic periods, postnatal maturation of the optic pathway is critical for normal function. In the present study we describe changes in cell surface antigens that reveal the progress of maturation in both neuronal and glial compartments. Following birth, M6 and L1 antigens delineate the path of optic axons; these are lost from axonal regions that are to be myelinated during the first week. M2, Thy-1 and P84 expression rise in the optic fiber layer during the first week. P84 is ultimately lost by the end of the second week, while M2 and Thy-1 are retained. P84 does not stain the fibers that are to be myelinated, while M2 and Thy-1 become strongly expressed in the optic nerve, apparently on distinct non-neuronal membranes. O11 is a late marker for myelination. This antigen is first detectable in the myelin forming proximal to the retina at P14, and staining extends just past the chiasm by P30. O11 staining appears to delineate this optic nerve-optic tract boundary in older animals as well. One striking feature of postnatal development of the optic nerve is the series of distal to proximal maturational events occurring in both neuronal and glial components. It is not clear if these events represent a cascade of responses to a signal originating in a single cell type or if they are instead intrinsic programs of the various cells of the optic pathway.

Supported by NIH grant EY05308

615.3

NEUROBLAST MIGRATION IN THE DEVELOPING CHICK OPTIC TECTUM VIA PERIKARYAL TRANSLOCATION. J.A. Robson* and R.L. Snow. Dept. of Anatomy and Cell Biology, SUNY HSC, Syracuse, NY 13210.

Migration of neuroblasts in the optic tectum was studied using immunocytochemistry and retrograde transport of DiI. The results demonstrate that many early neuroblasts do not migrate along radial glial processes. Instead, they retain connections with both the ventricular and pial surfaces while growing axons and translocating their nuclei. Immunocytochemical studies using a monoclonal antibody specific for postmitotic neurons (TUJ1) reveal a cohort of bipolar cells spanning the neuroepithelium during the early period of cell migration (E4-E7). In older embryos the immunocytochemical label is limited to postmigratory neurons with no trailing processes extending to the ventricular surface. Migrating neuroblasts were retrogradely labeled by applying DiI to the lateral margin of the tectum in embryos fixed between E4-E10. 6-8 weeks later the tissue was sectioned using a vibratome and examined using fluorescent and confocal microscopy. Many retrogradely labeled cells have a bipolar shape with leading and trailing precesses contacting the pial and ventricular surfaces. Axons grow from the leading processes before somata reach their final locations and prior to retraction of trailing processes. Several sections were photoconverted, mounted in Epon and serially sectioned at 2um. Reconstructions of these cells show that labeling is not transcellular. These results support the hypothesis that many neuroblasts in thin tissues migrate by translocating their somata while retaining connections with the pial and/or ventricular surfaces.

615.4

DEGENERATION OF LEECH NEPHRIDIA AFTER DENERVATION AND REMOVAL OF THE URINARY BLADDER DURING EMBRYOGENESIS. A. Wenning* and B. Janisch. Fak. f. Biologie, Univ.Konstanz, 78464 Konstanz, Germany.

During formation of the leech excretory system, the ectodermal bladder contacts the mesodermal nephridium. Sensory and neurosecretory innervation of each excretory complex occurs by a single peripheral neuron (NNC). The NNCs contain, and presumably release, FMRFamide at (1) the urine-forming cells, (2) the urethral sphincter muscles and (3) central neurons (Wenning et al, J Exp Biol 182, '93). Removal of the prospective bladder cells between embryonic days 8 and 10 (E8-10) causes the nephridium to degenerate. Until E16, however, its differentiation into two types of urine-forming cells is undisturbed, whether or not the NNC has been removed as well. As seen using immunoreactivity, the NNC contacts only differentiated parts of the excretory system (Wenning et al. Roux's Arch Dev Biol 202, '93). At E16, normal nephridia contact their bladders and become functional, and the NNCs sprout extensively. The nephridia lacking bladders stop growing and degenerate. The NNC projections lose their immunoreactivity in the nephridium but not at the sphincter. Degeneration of the nephridium is not due to a missing outlet since the ectodermal urethra with its mesodermal musculature develop normally and persist. Thus, to form a functional excretory system, the nephridium must contact the bladder. Whether a special cell or subset of bladder cells (as described for *Helobdella* in Martindale & Shankland, Dev Biol 125, '88) is required is not yet known.

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615.5

INNERVATION IS REQUIRED FOR MAINTENANCE OF TWO LARVAL MUSCLES DURING METAMORPHOSIS of THE MOTH, *MANDUCA SEXTA*. R. J. Bayline*, A. Khoo, and R. Booker. Sect. of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

During metamorphosis of the moth, the musculature of the abdomen is reorganized. While most of the larval muscles either die or are respecified during metamorphosis, several larval intersegmental muscles are maintained in a segment-specific manner; 0 in abdominal segments 1 and 2 (A1-A2) and A7-A8, 2 in A3, and 5 in A4-A6. These muscles are used to carry out pupal-specific behaviors. In segments A4-A6, denervation triggers degeneration of two maintained muscles, the ventral internal medial muscle (VIM) and the ventral internal oblique muscle (VIO). Following denervation, both the VIO and the VIM are reduced to 33% of the normal size by the end of metamorphosis. Other maintained muscles are unaffected by denervation. We compared aspects of the induced degeneration in the denervated VIO with normal degeneration in respecified muscles . Following denervation of the VIO, dying nuclei are observed between the second and the sixth day of pupal life (P+2 through P+6). In respecified muscles, dying nuclei are present from P+2 through P+4. We used the thymidine analog 5-bromodeoxyuridine (5-BrdU) to label nuclei undergoing DNA replication. No nuclei label in the innervated VIO, while some nuclei in the denervated VIO label beginning on P+4. 5-BrdU labelling also begins on P+4 in respecified muscles. The external fibers in the denervated VIO degenerate, while the internal fibers remain intact. It is the degeneration of the external fibers which leads to the reduced size of the denervated muscles. Unlike respecified muscles, no new adult fibers develop.

615.6

DIFFERENTIAL EFFECTS OF CORTICOSTEROIDS ON NEURONAL AND GLIAL CELLS IN THE DENTATE GYRUS OF ADULT RAT HIPPOCAMPUS. B. Liao* and E.C. Azmitia, Dept. of Biology, New York Uni., Wash. SQ, New York, NY 10003.

Corticosteroids can enter the brain and regulate cell proliferation and differentiation. The hippocampus, as a central component in the limbic system, is a major target of corticosteroids. Long-term (2 mo.) adrenalectomy (LT-ADX) induces a loss of Nissl staining and 5-HT_{1A} mRNA in the hippocampal dentate gyrus. Short-term dexamethasone (ST-DEX) treatment to the LT-ADX rats for 24-72 hr produces a rapid recovery of 5-HT_{1A} mRNA, followed by a reappearance of Nissl staining. In the present study, we further explored the neuronal and glial response to the change of corticosteroid level.

Female Sprague-Dawley rats weighing 150 g were adrenalectomized and given saline to drink. Sham operated rats were given water drink. After 2 months, some of the ADX rats were given DEX (10 mg/L) in saline drink for 72 hr. Bromo-deoxyuridine (Brdu) (50 mg/kg B.W., i.p.) was injected into all three groups of rats at 24 and 48 hr later after the start of giving DEX to one group of ADX rats.

LT-ADX produced a loss of calbindin immunoreactive (CaB-IR) granule cells, which included a decrease in cell number, a swelling and pale-stained cytoplasm, a decrease in the density of dendrites, and a narrower dentate molecular layer. Double immunostaining for histone showed that small nuclei without CaB-IR were sparsely distributed within the granule cell layer. ST-DEX to LT-ADX rats reversed partly all of above consequences. No massive neurogenesis after LT-ADX or ST-DEX were observed. After LT-ADX, numerous small vimentin (Vim)-IR cells emerged in the dentate area, which corresponded to Brdu-IR area. ST-DEX did not increase the Vim-IR cell number, but promoted the elongation of the Vim-IR cell processes.

The evidences suggest a neuronal dedifferentiation and astroglial proliferation after the loss of corticosteroid support (Supported by NIA P01 AG10208 to ECA).

615.7

CHICK EMBRYO PURKINJE CELL DENDRITES ARE REDUCED IN SIZE FOLLOWING CHRONIC TREATMENT WITH A NMDA RECEPTOR ANTAGONIST, NPC 12626. B. White* and M. W. Vogel. MD Psych. Res. Center, UMAB, Baltimore, MD 21228.

The development of Purkinje cell dendrites is dependent on interactions with afferents. The role of neuronal activity in this interaction is investigated by chronically treating chick embryos with a NMDA receptor antagonist. The NMDA receptor was chosen as the target for pharmacological blockade because of its importance in synaptic stabilization, and because granule and Purkinje cells are more sensitive to NMDA early in development, suggesting that NMDA receptor activation is important for cerebellar development.

Chick embryos were given daily injections from E14 to E17 of the competitive NMDA receptor antagonist, NPC 12626 (Guilford Pharmaceuticals). The dosage was increased daily, with a low dose group starting at 25 mg/kg (end 75 mg/kg) and a high dose group starting at 50 mg/kg (end 100 mg/kg). Drug-treated (n=21) and control (n=19) embryos were killed on E18 (HH St 43 to 44) and their brains processed for Golgi-Cox staining. Purkinje cell dendritic arbors were measured using MacMeasureII (54 Purkinje cells/cerebellum).

The NPC 12626 treatment significantly reduced embryo motility after the first injections, but motility increased to control levels during the following days. The morphology of Purkinje cell dendrites in drug-treated embryos appeared to be within the range of normal variation. Morphometric analysis showed, however, that there was a 20% reduction in the average size of Purkinje cell dendritic arbors. The results suggest that NMDA receptor activation is involved in Purkinje cell dendritic growth. Research support provided by NIH grant NS29277.

615.8

LOCALIZATION OF ANTI-CALBINDIN IMMUNOREACTIVE CELLS AND PROCESSES IN THE FROG CEREBELLUM DURING THYROXINE-INDUCED METAMORPHOSIS. **N.J. Uray[a*], A.G. Gona[b] and P.S. Sexton*. Depts. of Anatomy, Kirksville Coll. of Osteo. Med., Kirksville, MO 63501[a] and Univ. of Med. and Dent. of N.J., New Jersey Med. Sch., Newark, N.J. 07103[b].**

To study the expansion of the Purkinje cell layer in the frog cerebellum during metamorphosis, tadpoles of the bullfrog, *Rana catesbeiana*, were immersed in a thyroxine solution (1 part per 100 million) to accelerate the metamorphic process, and batches of tadpoles along with control animals were anesthetized and killed at 2 day intervals up to 30 days of thyroxine exposure. The brains were fixed in Bouin's fluid for 3 hours, rinsed in buffer and embedded in paraplast. Immunocytochemistry was done on Bouin's fluid fixed, paraplast embedded sections using anti-calbindin D-28k, rabbit antiserum from SWant (Switzerland), biotin conjugated secondary antibody, and visualized with a peroxidase chromogen.

Purkinje cells were immunoreactive at all stages of development, although the pattern of distribution was not uniform. We also noted a distinct gap in the Purkinje cell layer at a site where a cone of cells forms in the external granular layer (egl). Since the gap is present prior to the formation of the egl, the cone may not be responsible for forming the gap, rather, the cone may form at the site where a gap already exists. In addition to Purkinje cells, a population of small cells in the auricular lobes and the dorsal part of the corpus cerebelli were also immunoreactive. Because these small cells remain immunoreactive past metamorphosis, our earlier contention that these may represent immature Purkinje cells may not be totally accurate. While some small cells which are immunoreactive during development may indeed be immature Purkinje cells, others appear to be cells of the interauricular granular band. Immunoreactive commissural fibers in the dorsal part of the molecular layer of the cerebellum may be processes of these cells. **Supported by NIAA Grant AA97537.**

615.9

MORPHODIFFERENTIATION OF SUPRAEPENDYMAL CELLS: THEIR INTERACTIONS AND DEVELOPMENT OF THE SUBCOMMISSURAL ORGAN.

I. Herrera-Vázquez*,G.Espinosa,A.Zepeda. Depto. Anatomía, Facultad de Medicina. UNAM.

The subcommissural organ (SCO) differentiates between days 10 and 16 gestational development in the rat, it is probable that supraependymal cells (SEC) participate importantly in this differentiation. In this moment the SCO's susceptibility increases to diverse factors wich could produce displasy and induced hidrocephalus. After learning, the SEC's ontogeny with a scanning electron microscope (SEM) we identify the neural type SEC (Kolmer I) and fagocytes (Kolmer II) during their apparition in the ventricular lumen, migration and exit through the ventricles; now we are observing their interactions and superficial changes (probably laminin,fibronectin and adhesins) using SEM. The fagocytic SEC appear with vesicles and filaments in the ventricular surface, in adjacent zones to the proliferative neuroectoderm (PNE), and in the infundibular region. In the dorsal ventricular zones there appears to be less vesiculation; part of their prolongations in the (K.I) seem to be plattened other cells present an irregular discoidal shape in ventricular surface.. Fibrilary elements interconnect with the PNE near the blood vessels. Interependymal cell unions with a central cilia may be prominent or in a groove like form depending on the region analized. SEC and ependimocytes fuse together in some cases in others, the presence of pores suggest other types of SEC communication between intra and extraventricular spaces. We will continue with immunocytochemistry identifying this interaction. Acknowledgement for review and comment to: L. Cintra and A. Carabez (supported by DEP. Anatomía and Div. Investgación y Posgrado Fac. de Medicina UNAM.)

615.10

SUBEPENDYMAL LAYER IN ADULT RATS: CHANGES IN CELL NUMBER AND ADHESION MOLECULES AFTER CORTICAL LESIONS. F.G. Szele*, M.F. Chesselet Dept. of Pharmacology, U. of Penn., Philadelphia, PA 19104

The subependymal layer (SL) is derived from the fetal subventricular zone and in the adult rat retains developmentaly regulated adhesion molecules as well as immature dividing cells. This study examined the hypothesis that mitosis, growth factors, cell phenotype, and adhesion molecules in the SL change after brain injury in the adult rat. The number of Nissl-stained cells in the SL increased 2-fold after unilateral cortical lesions. However, the number of BrdU-labelled cells in the SL did not change significantly and very few BrdU-labelled cells were observed outside of the SL suggesting that mitosis and migration remained constant after lesion. Therefore the increased cell number in the SL may be due to decreases in the rate of cell death after cortical injury. The growth promoting factors fibroblast growth factor (bFGF) and epidermal growth factor (EGF) were also examined after lesion. Whereas a robust decrease in the expression of bFGF was observed in the SL following cortical lesions, EGF was undetected in the subependymal layer before and after lesion. Cells isolated from adult subependymal layer can express neuronal or glial phenotypes *in vitro*. *In vivo*, however, cells in the subependymal layer did not express mature neuronal (synaptophysin, neuron-specific enolase, MAP-2) or astrocytic (GFAP) phenotypes before or after lesion. Vimentin- (immature astrocyte protein) positive radial glia in the medial striatum also expressed GFAP after lesion. Immunoreactivity to polysialylated neural cell adhesion molecule (PSA-NCAM) increased 3-fold in the SL and medial striatum after cortical lesion. In contrast, immunoreactivity to tenascin decreased transiently in the SL and medial striatum after cortical lesions. This study demonstrates that in response to cortical lesion, the adult SL is a highly plastic area with regards to cell number and adhesion molecules. Supported by PHS grant NS29230.

615.11

FIRST TRIMESTER DEVELOPMENT OF THE NIGROSTRIATAL DOPAMINERGIC SYSTEM. P. Almqvist*, E. Jacobsson[1] H. Pschera[2], Å. Seiger[1] and E. Sundström[1]. Dept. of Clinical Neuroscience, Sections for Neurosurgery and Geriatric Medicine[1] and Dept. of Obstetrics & Gynecology[2], Karolinska Institute, S-171 77 Stockholm, Sweden.

The present study emphasize to characterize the morphological and neurochemical differentiation of mesencephalic dopaminergic neurons from human embryos, derived from elective first trimester abortions. Embryonic brain tissue was taken for analyses of tyrosine hydroxylase (TH) by immunohistochemistry and Western blot, and for analyses of endogenous dopamine (DA) content using HPLC-ED. TH was first detected immunohistochemically at 4.5 weeks of gestational age. In parasagital sections of embryonic brainstem at this developmental stage, a small, distinct population of rounded, densly packed, TH-immunoreactive perikarya was seen in the mesencephalic flexure. These cells were located in the centre of the mantle layer of the ventral basal plate, parallell to the ventricular zone. During the sixth to tenth gestational week, the number of TH-positive cells increased exponentially as did the intensity of the TH-immunoreactivity. TH-positive cells migrated ventrally and somewhat rostrally, away from the ventricular germinal layer. During this period, short primary processes developed into long axonal trajectories giving rise to the medial forebrain bundle, projecting to the neostriatum. At the tenth gestational week, varicose fibers were detected in most areas of the striatal anlage.

To confirm the identity of TH in the embryonic tissue, as recognized by the antisera used for immunohistochemistry (Pel-Freeze, USA), mesencephalic tissue of 5-10 weeks of gestation was analysed by Western blot technique. A single band with the molecular weight of 60 kDa was detected already at 5 weeks of age. The amount of TH/mg protein increased approximately ten-fold during these developmental stages. Mesencephalic tissue and forebrain/basal ganglia were taken for analyses of dopamine (DA) content using HPLC-ED. DA could be detected at 5.5 weeks of gestational age in both areas and was found to increase exponentially from 7-7.5 weeks of age to reach 4 - 5.5 ng DA/mesencephalon and 50-75 ng DA/g caudate nucleus-putamen at the end of the first trimester.

615.12

S103L NEURONAL PROTEOGLYCAN IN DEVELOPMENT: ASSEMBLY AND FUNCTION. M. Domowicz, D. Mangoura, H. Li, N. Sakellaridis* and N. B. Schwartz. Depts of Peds & BMB, U. of Chicago, Chicago, IL, 60637.

We have identified two distinct chondroitin sulfate proteoglycans (CSPG) in chick brain which can be differentiated by their immunoreactivity with S103L mAb, which recognizes an epitope in the aggrecan core protein, and HNK-1 (Krueger et al., 1992). In comparative studies using cell type specific tissue culture systems, we have provided evidence that the S103L CSPG is specific to neurons and developmentally regulated, so that its transient expression coincides with late migration and establishment of cortical nuclei. Immunocytochemical studies revealed that the in ovo pattern of expression of S103L CSPG is reproduced in primary neuronal cultures (Hennig et al., 1993). The HNK-1 CSPG is synthesized at high levels and constitutively in neuronal cultures as compared to glial cultures. In addition, astrocytes produce an abundance of CSPG with a larger core protein (>500 kDa), probably related to versican. Our lab has recently identified a mutation in the aggrecan gene in the nanomelic chick. The mutation introduces a stop codon in the translated sequence, and as a consequence a truncated core protein is synthesized which is not glycosylated or secreted in chondrocytes (Li et al., 1993). No expression of the complete S103L CSPG was found in nanomelic brain but the expression of the other brain CSPGs was not affected. Synthesis and processing of the S103L CSPG core protein as well as the functional consequences were studied in nanomelic neuronal culture. The regular order of aggregates was dramatically interrupted, with neurons often traveling in parallel. These data further support our hypothesis that the S103L CSPG functions to halt neuronal migration and together with sequence data confirm that the neuronal S103L CSPG and the cartilage S103L CSPG, aggrecan, are products of the same gene. (HD 09402)

615.13

CLINICAL CORRELATES OF ABNORMAL COLUMNAR CORTICAL ORGANIZATION IN CHILDREN WITH CHRONIC FOCAL EPILEPSY. R. Goldstein, A. S. Harvey, M. Duchowny*, J. H. Bruce, P. Jayakar, T. J. Resnick, N. Altman, L. Alvarez. Miami Children's Hospital, Department of Neuroscience, Miami, FL 33155.

Developmental pathology is a well recognized cause of chronic epilepsy and mental retardation, yet there are few studies linking specific histopathologic abnormalities with postnatal outcome. We report the clinical findings of 18 children (12 females, 6 males) aged 3 months to 15 years (mean 7.2, SD 4.7) with abnormalities of cortical neurogenesis, migration, and organization. All had undergone focal cortical resection (hemispherectomy or multilobar 9, frontal 5, temporal 4) for medically resistant partial seizures. Tissue analysis revealed immaturity of cortical cytoarchitecture characterized by longitudinal rows of neurons arranged perpendicular to the cortical surface rather than the usual horizontal lamination. Ectopic white matter neurons (N=17), white matter gliosis (N=13), morphologically abnormal neurons (large ill-defined occasionally binucleated or multinucleated cells) (N=8), and variable cortical width (N=6) were also identified. Mean age at seizure onset was 1.8 years (1 month to 7 years, SD 2.0) and mean seizure duration was 5.4 years (3 months to 13 years, SD 3.6). Fourteen children experienced daily secondarily generalized motor convulsions. Eleven children were developmentally delayed and 5 functioned in the low average range of intelligence.

These findings indicate that abnormal columnar cortical organization occurs in association with other abnormalities of neurogenesis, gliosis, and neuronal ectopia in children with chronic focal epilepsy. This histopathology correlates with early onset malignant seizures, widespread epileptogenecity, and a high frequency of developmental delay.

615.14

DEVELOPMENT OF THE NEOCORTEX OF THE MOUSE USING BrdU IMMUNOCYTOCHEMISTRY. Dr. P. Humphreys, Dr. W. Hendelman* and E. Leung. Depts. of Pediatrics (Neurology) and Anatomy & Neurobiology, University of Ottawa, Faculty of Medicine, Ottawa, ON, Canada, K1H 8M5.

Previous studies attempting to define the time of birth of neurons destined for layers II-VI of the mouse neocortex have given conflicting results, possibly due to differences in definition of embryonic day numbers, relatively long time periods (up to 12 hours) during which coitus is permitted to occur, and to inter-strain variabilities. We studied the time of generation of neocortical neurons in the Paris R-3 mouse. Animals were mated over periods varying from 1.5 to 5 hrs (average 2.5 hrs); the day of coitus was counted as embryonic day 0 (E0). Pregnant mothers were given an intraperitoneal injection of 5-bromo-deoxy-uridine (BrdU, 100 mg/kg) at one of six precise times: E14.0, E14.5, E15.0, E15.5, E16.0, E16.5. Offspring were divided into three groups and sacrificed on postnatal day 1 (P1), P9 and P22. Brains were fixed in 70% ethanol, coronally sectioned at the anterior hippocampal level, and reacted with antibody against BrdU by the peroxidase method (deFazio et al, 1987). BrdU-labeled nuclei in the dorsolateral cortical plate (P1) or neocortex (P9, P22) were counted using an image analyzer. Analysis of data from P9 and P22 animals revealed that neurons born at the 6 injection times were concentrated predominantly in specific layers as follows: E14.0-III-VI; E14.5-III-V; E15.0-III>II; E15.5-II>III; E16.0-II; E16.5-II.
(Supported by grants from the Children's Hospital of Eastern Ontario Research Foundation and the Bickell Foundation.)

616.1

MICROGLIAL REACTIVITY TO *N*-METHYL-D-ASPARTATE (NMDA) INDUCED EXCITOTOXICITY IN THE EARLY POSTNATAL BRAIN. L.Acarin, B.González, B.Castellano and A.J.Castro*. Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University Chicago, Maywood, IL 60130; and Dept. of Cell Biology and Physiology, Autonomous University of Barcelona, Barcelona, Spain.

Injections of NMDA into the early postnatal brain causes severe and rapid neuronal loss due to the high sensitivity of the developing brain to this excitotoxin. This excitotoxic process has been implicated in the neuronal damage occurring after hypoxia-ischemia. In the present study the time course of microglial reaction was examined in cortical areas receiving NMDA injections as well as in cortical projection areas such as the pontine gray. Under ether anesthesia, 6 day old rats received an injection of 50 nmols of NMDA (0.2µl) in the right sensorimotor cortex. At various survival times ranging from 10 hours to 28 days post lesion, microglial reactivity was studied using tomato lectin histochemistry.

Microglial reaction was seen as early as 10 hours after NMDA injections. Reactive cells in the neocortex, the hippocampus and the rostral thalamus showed an ameboid and pseudopodic morphology. At 1 and 3 days after injection, microglial morphology changed to a more ramified type, and a marked increase in the number of microglial cells was seen at day 3. From day 7, the lesion became nonprogressive and microglial cells tended to accumulate in the border between neuron depleted areas and undamaged surrounding regions. A mild type of glial scar was seen from 14 days. Additionally, a microglial response was observed in association with anterogradely degenerating cortical efferents coursing through the pons. This reaction was already seen at 10 hours post injection, it peaked at day 1 and was nearly absent by day 7.

In summary, after NMDA injection in the earlypostnatal brain microglial reaction was restricted to areas showing apparent neuronal or axonal degeneration. This reaction was characterized by a rapid onset and followed a specific time course.

(Supported by funds from the Bane Estate, CIRIT fellowship AIRE93-33 and DGICYT project PB92-0598)

616.2

SURAMIN INHIBITS THE GLIOTIC RESPONSE FOLLOWING BRAIN INJURY X.-R. Zhou, S. Meiners, R. Nowakowski*, and H.M. Geller, Dept. of Pharmacology, Robert Wood Johnson Medical School, Piscataway, NJ 08854.

These experiments were designed to examine the hypothesis that certain cytokines and growth factors can provide the cellular signals to initiate the process of reactive gliosis in pathological conditions such as brain trauma caused by a penetrating stab wound injury. Suramin, a polysulfanated napthylurea, has been shown to inhibit the binding of various growth factors to their cell surface receptors. We therefore investigated the effect of suramin in a stab wound model of neural trauma in rats. A stab wound injury was made through the rat's left cortex and striatum using a 22 ga. hypodermic needle. In some animals, suramin (5 µl, 75 µM) was injected directly through the needle at the time of lesioning. In control animals there was an increase in GFAP and tenascin immunoreactivity in the vicinity of the lesion at all time points tested (1, 3, 7, and 30 days post lesion). In suramin-treated animals there was an obvious reduction in GFAP and tenascin immunoreactivity as compared to control animals at 1 and 3 days after injury. There was no difference in GFAP expression in suramin-treated and control animals at 7 and 30 days after injury. The increased levels of GFAP after lesion and the reduction in GFAP levels by suramin at 1 and 3 days after lesion was confirmed by Western blot analysis in brain tissue homogenates from the lesioned area as compared to homogenates from the unlesioned contralateral side of the brain. These results demonstrate that suramin inhibits the gliotic response as reflected by upregulated GFAP and tenascin levels at early time points following injury and suggest that growth factors and cytokines may be involved in initiating reactive gliosis. Supported by N.I.H R01 NS 24168 and N.R.S.A. NS 09281.

616.3

GLIAL ACTIVITY IN WILSON'S DISEASE. S.C. Page, A.N. Kalehua, A. Czlonkowska, G. Raivich* and G.W. Kreutzberg. Max Planck Institute for Psychiatry, Am Klopferspitz 18a, 82152 Martinsried, Germany, and Institute of Psychiatry and Neurology, 1/9 Sobiesko Str, 02-957 Warsaw, Poland.

Wilson's Disease (WD) is an autosomal recessive disorder characterised by the toxic accumulation of copper in a number of organs, particularly the liver and brain. The disease prevalence is approximately 3 in 100,000 live births. Recently the gene for WD has been mapped to chromosome 13q14.3. It has been suggested that the gene product is a copper transporting P-type ATPase expressed in the liver. However, little is currently known of the mechanism by which neuronal damage occurs in the brain tissue, notably in the basal ganglia, although histological techniques have shown that reactive gliosis is a feature of the disease.

This study uses *post mortem* tissue from 3 patients exhibiting neurological symptomatology. Since no data are available concerning immunocytochemical studies in WD brain tissue, screening with a variety of antibodies was performed. GFAP staining was carried out on both frozen and formalin-fixed free-floating sections as a marker for astrocytic activity. Microglial cells were visualised using either EBM11 or peroxidase-conjugated RCA for frozen and free-floating sections respectively. An antibody against the MHC-II (HLA-DR) antigen was also employed.

GFAP staining revealed a large number of reactive astrocytes in the basal ganglia tisue, notably the putamen, in addition to the thalamus and cortex. RCA labelling was also intense in the putamen. Intense EBM11 immunoreactivity was also observed in the basal ganglia, cortex and dentate nucleus. HLA-DR immunoreactivity was localised to glial cells, shown to be GFAP-postive and therefore astrocytic, in double-labelling experiments utilising DAB as the initial chromogen and BDHC as the second. HLA-DR immunoreactive cells were present with high incidence in many areas of the brain, including the basal ganglia, corpus callosum, internal capsule and the cortex. Within these tissues, HLA-DR immunoreactivity appears to be primarily, although not exclusively, located in the white matter.

To further characterise the role of reactive glial cells in neurodegeneration in WD, current work centres around potentially cytotoxic substances that may be secreted by such cells and which subsequently lead to tissue degeneration.

616.4

TENASCIN/CSPG-RICH ASTROCYTES INHIBIT NEURONAL GROWTH: AN *IN VITRO* MODEL FOR GLIOTIC SCARRING S. Meiners, E. M. Powell, and H.M. Geller*, Dept. of Pharmacology, Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Astrocytes provide an optimal surface for attachment, survival, and neuritic outgrowth of dissociated embryonic neurons. However, not all astrocytes are alike: previous work from this laboratory has demonstrated a heterogeneity in the ability of a monolayer of cultured astrocytes to support neuronal adhesion and neurite outgrowth. Areas of the monolayer with a fibrous, uneven surface (termed "rocky" astrocytes) were shown to be poor substrates for neuronal growth, whereas surrounding, flat areas of the monolayer ("flat" astrocytes) were permissive substrates. The current study was initiated to investigate whether the rocky and flat astrocytes represent different subtypes of cells, as opposed to, for example, different stages of astrocyte maturity. Cultures of purified astrocytes were established from the cerebral cortex of neonatal rats. As cultures with particularly pronounced, and distinct, rocky and flat areas were identified, these areas were carefully removed from the flask with a sterile spatula, trypsinized, and replated. The flat astrocytes remained flat after reaching confluency, and the rocky astrocytes remained rocky. In addition, rocky astrocytes supported reduced neuronal adhesion, neurite generation, and neurite outgrowth in comparison to flat astrocytes. Immunofluorescence analysis and Western blotting showed that the fibrous astrocytes were notedly high in the production of the extracellular matrix proteins tenascin and chondroitin-6-sulfate proteoglycan. No differences were detected between rocky and flat astrocytes in the levels of laminin or fibronectin. It is our hypothesis that rocky astrocytes may represent a subtype of cells which form barriers to neuronal growth during the development of the cortex. Due to their inhibitory properties, we suggest that rocky astrocytes may also provide a cell culture model for reactive gliosis.

616.5

TOPICAL GLUCOCORTICOIDS MODULATE RESPONSE TO CEREBRAL CORTICAL STAB WOUNDS. M.S. Li*, S. David Centre for Research in Neuroscience, Montreal General Hospital Research Institute, Montreal, Quebec, H3G 1A4

Penetrating injury to the CNS results in the formation of an interface (glia limitans and fibrous scar) across which axons fail to regenerate. Its maturation is associated with the deposition of a basal lamina. We have examined the effects of topical steroids on the formation of this interface. Stab wounds (2 mm long and 1.5 mm deep) were made in the parietal cortex of adult rats. Immediately after lesioning animals received either 1) a topical application of halcinonide 0.1% ointment 2) placebo ointment or 3) no treatment. Animals were perfused 3 weeks later. Cryostat sections were labelled with anti-GFAP and anti-laminin antibodies and visualised by immunoflourescence. The length of laminin immunoreactivity along the lesion interface, and the number of reactive astrocytes 300 µm lateral to the lesion were quantified. Laminin deposition at the interface was diminished in the steroid treated animals by 24% compared to controls. The placebo's effect was negligible at 2%. Astrocyte reactivity was decreased in both the steroid and placebo groups by 36% and 26% respectively. This reduction in astrocyte reactivity may in part be from the barrier properties of the ointments, preventing migration of foreign material into the CNS. These results suggest that steroids could alter the formation of the interface and may have implications for axon growth across CNS lesions.

616.6

GLIAL REACTION IN THE RAT SEPTAL COMPLEX FOLLOWING BILATERAL FIMBRIA-FORNIX TRANSECTION. E. Hollerbach, M. Frotscher, and T. Naumann*. Institute of Anatomy, University of Freiburg, P.O. Box 111,Germany.

Transection of the fimbria-fornix leads to degenerative changes in the septal complex as well as in the hippocampal formation. In particular, mainly retrograde neuronal reactions take place in the medial septal nucleus (MS), whereas the lateral septal nucleus (LS) only contains anterogradely degenerating fibers.

Glial reaction was investigated in adult Sprague-Dawley rats 1, 3, 6 weeks and 6 months following bilateral fimbria-fornix transection (ff-t) using the following markers: GSA-I-B_4 for microglial cells, anti-GFAP for astrocytes, RIP for oligodendrocytes and anti-Fluoro-Gold (a-FG). Immunocytochemistry was carried out on vibratome sections from perfusion-fixed brains. In some animals MS neurons were prelabeled by intrahippocampal FG injections prior to axotomy in order to document both the fate of septohippocampal neurons and glial phagocytosis ultrastructurally.

At all postlesional stages only few microglial cells were detectable immunocytochemically by using a-FG. Thus, there is only limited neuronal death and glial phagocytosis following ff-t in the MS (Naumann et al., 1992). In contrast, a strong increase in GFAP and GSA-I-B_4 staining in the LS and hippocampus was observed already 1 week post lesion. Moreover, increase in glial staining was not restricted to areas affected by ff-t. The intensity of all glial markers returned to that of controls at about 6 weeks post lesion. Thus, glial reaction after ff-t is a widespread and long-lasting process.

Naumann et al. (1992) J. Comp. Neurol. 325:219-242.

(Supported by the DFG: Leibniz Program and Fr 620/4-1)

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616.7

GFAP IMMUNOREACTIVITY IN TRIGEMINAL GANGLION SATELLITE CELLS AFTER TOOTH INJURY IN RATS. J.L. Stephenson and M.R. Byers* Dept. of Anesthesiology, University of Washington, Seattle, Washington, 98195. USA

An increase in glial fibrillary acidic protein (GFAP) immunoreactivity (IR) is often seen in astrocytes of the central nervous system in response to a wide variety of injuries. We have investigated injury induced GFAP-IR in ganglion satellite cells, in response to graded tooth injuries. We compared GFAP-IR of trigeminal ganglia (TG) after maxillary or mandibular molar injury at 1,3,and 7 days after the injury, and for different degrees of injury. Fixed ganglia (4% formaldehyde) were cut in the horizontal plane at 50 microns, and were analyzed as a 1 in 3 series to avoid double counting of neurons encircled by reactive satellite cells. The number of neurons with satellite cell reaction in each experimental category was compared to the uninjured control TG, as well as to zones within each section that did not correspond to the site of injury. We found significant increases in the total number of GFAP-IR satellite cell profiles surrounding tooth related neuronal cell bodies in the 7 day group ($p=0.016$). The GFAP-IR at 3 days was dramatically increased, but with a larger variance in the total number of reactive cells than at seven days. The site of GFAP reaction in the TG, shifted depending on the injury site (max or mand). Satellite cell GFAP-IR corresponded to the zone labeled by retrograde labeling of neuronal cell bodies by DiI from the molars. EM confirmed the identity of the GFAP-IR cells to be satellite cells. We conclude that a GFAP-IR satellite cell reaction is induced in trigeminal ganglia by molar inflammation in a site specific manner at 3-7 days. Supported by NIH DE05159.

616.8

FGF RECEPTOR EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS. J. F. Reilly and V. K. Menon* Department of Cell Biology & Human Anatomy, School of Medicine, University of California, Davis, CA 95616.

Basic fibroblast growth factor (bFGF) is thought to play a role in astrogliosis following traumatic injury to the CNS. In the present study, trauma-induced changes in the spatial and temporal expression of FGF receptor 1 (FGFR-1, *flg*) were examined. A stereotaxic lesion through the hippocampus was placed in adult, male Fisher 344 rats. After survival times ranging from 1 to 30 days, brains were removed and processed for immunohistochemistry using a monoclonal antibody to FGFR-1. Quantitative image analysis was carried out to evaluate the magnitude of the changes. Cortical expression of FGFR-1 was increased adjacent to the wound cavity by day 2 post-lesion. Levels peaked at day 4 and decreased to control levels by day 10, except for reactive astrocytes immediately adjacent to the wound cavity. In the hippocampus, FGFR-1 immunoreactivity was increased on day 4, peaked at day 7, and remained elevated beyond day 10. By day 30, staining had returned to control levels except for reactive astrocytes immediately adjacent to the wound cavity. Double immunohistochemistry for FGFR-1 and GFAP demonstrated that astrocytes are expressing FGFR-1. bFGF colocalized with FGFR-1, suggesting that FGFR-1-expressing astrocytes are also expressing bFGF. These data demonstrate a time course for astrocyte expression of FGFR-1 which precedes and parallels the established time course for astrocyte hypertrophy. This suggests that endogenous bFGF may act directly on astrocytes to induce astrogliosis.

616.9

TYROSINE PHOSPHORYLATION IN RAT SPINAL CORD AFTER SCIATIC NERVE TRANSECTION. W.A. Eckert,[1] J.G. Valtschanoff,[2] C.A. Otey,[3] A. Rustioni*[2] and R.J. Weinberg.[2] Depts. of [1]Physiology, and [2]Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599, and [3]Dept. of Anatomy & Cell Biology, University of Virginia, Charlottesville, VA 22908.

Nerve injury produces central changes reflecting both degeneration and regeneration of nerve fibers. Release of a growth factor, likely to act via receptor tyrosine kinases, may be an important signal associated with peripheral injury. Immunocytochemistry using antibodies for phosphotyrosine was employed to identify changes in tyrosine phosphorylation in the rat spinal cord consequent to sciatic nerve injury. Increased immunostaining in the spinal gray matter, dorsal columns and gracile nucleus on the side of the lesion became evident after three days and was more pronounced with longer survival times up to three weeks (the longest survival tested). This increase was most prominent in the fourth lumbar segment (the focus of termination of sciatic nerve afferents). Immunostaining was in astroglial cells and their processes in the dorsal horn; stained microglia was also seen. Immunopositivity also increased in glial cells surrounding motoneurons at the same levels.

These results in combination with other work from our laboratory suggest that some factor released by primary afferent fibers over a sustained period results in sustained kinase activation, either directly or via a cascade involving sustained synthesis and release of other factors. This kinase activation is likely to play an important role in the full development of the glial response to nerve damage.

616.10

EXPRESSION OF A NOVEL NON-ANGIOTENSIN II [^{125}I]CGP 42112 BINDING SITE IN HEALING WOUNDS OF THE RAT BRAIN. M. Viswanathan*, A.M. de Oliveira, F.M.A. Correa, and J.M. Saavedra, Sec. on Pharmacology, LCS, Bldg. 10; Room 2D-45, National Institute of Mental Health, NIH, Bethesda, MD 20892

Angiotensin II (Ang II) receptor subtypes AT_1 and AT_2 seem to be involved in growth and repair processes during wound healing. Our studies using the AT_2 ligand [^{125}I]CGP 42112 unexpectedly revealed, in healing brain wounds of adult rats, a binding site that is recognized by CGP 42112 and not by angiotensin II. Using quantitative autoradiography, we localized and characterized this site. [^{125}I]CGP 42112 binding was restricted to the wound edge and the immediate periphery. Immunocytochemical localization using ED-1 monoclonal antibody raised against rat macrophages showed good correlation between the distribution of ED-1(+) cells and the binding which was saturable, reversible, and stable. Saturation studies and Scatchard analysis of the data revealed a single class of binding sites with a K_d $3.8X10^{-10}$ M and binding capacity (B_{max}) of 109 fmol/mg protein. The time course of injury-induced expression of the binding in the brain lesion revealed highest levels at 3 days after injury which became undetectable after 10 days. Our results suggest that activated microglia surrounding the penetrating wound express the novel binding site and that this site may have a role in mechanisms of tissue repair in the brain.

616.11

INTERLEUKIN-1β INDUCTION OF PROSTAGLANDIN G/H SYNTHASE-2 (PGHS-2) AND INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) IN CULTURED MURINE GLIAL CELLS. **M. K. O'Banion*, J. C. Dusel, J. W. Chang and P. D. Coleman. Departments of Neurology and of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642**

Brain injury results in activation of glial cells, including both astrocytes and microglia, and in an increase in the expression of several endogenous pro-inflammatory cytokines such as IL-1β and TNF-α. Increased expression of these factors has also been observed in the brains of individuals with Alzheimer's disease and AIDS. Previous studies indicate that astrocytes make both prostaglandins (PG) and nitric oxide (NO) in response to IL-1β. To determine whether cytokines influence the expression of genes responsible for the generation of PG and NO, we performed northern blot analyses of RNA from purified primary cultures of astrocytes and microglia which had been treated with various factors. Our results indicate that PGHS-2, but not PGHS-1, mRNA is strongly induced in astrocytes exposed to recombinant IL-1β in a dose-dependent fashion. Induction is highest at 2 h, and PGHS-2 mRNA levels return to control values by 24 h. Macrophage/inducible NOS mRNA levels are also increased in astrocytes treated with IL-1β but appear to peak at a later time point (4 to 8 h of exposure). TNF-α, LPS, and basic FGF also induce PGHS-2 in astrocytes, whereas dexamethasone pretreatment diminishes the level of PGHS-2 induced in response to IL-1β. Interestingly, PGHS-2 mRNA was not detected in cultured microglia treated with IL-1β or GM-CSF; however, PGHS-1 mRNA was present in these cells under all conditions tested. In summary, our results indicate that brain glial cells transiently increase their expression of synthetic enzymes for PG and NO in response to cytokines and suggest the elaboration of a cascade of mediators for cytokine action in the brain. [Supported by LEAD award AG09016 to P.D.C. and M.K.O.]

616.12

EFFECTS OF PLATINUM-RELATED CHEMICALS ON MS1 MOUSE SCHWANN CELL LINE. Y. Ogawa* and S.U. Kim. Div. of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, BC, Canada, V6T 2B5.

Specific toxic effects of platinum-related chemotherapeutic agents such as cisplatin and carboplatin on neurons or Schwann cells have not yet been thoroughly evaluated.

The MS1 mouse Schwann cell line (Watabe et al., J Neuropathol Exp Neurol, 49:455, 1990) was exposed to cisplatin and carboplatin, and the survival rate was measured by MTT assay. Blocking effects of NGF, $ACTH_{4-9}$, and α-MSH against platinum toxicity were also examined. Cisplatin displayed a marked cytotoxicity at concentrations greater than 1 μg/ml and carboplatin at concentrations greater than 20 μg/ml. NGF protected cell death from cytotoxic effects of cisplatin and carboplatin at the concentration of 50 ng/ml.

These results suggest that cisplatin and carboplatin can induce Schwann cell damage during platinum-induced neuropathy and that cisplatin is far more toxic to Schwann cells than carboplatin.

616.13

NEUROPROTECTION AGAINST CISPLATIN TOXICITY BY βNGF AND SCHWANN CELL CONDITIONED MEDIUM IN VITRO. E.M. Hol, D.A. Houweling, E.A.J. Joosten*, W.H. Gispen and P.R. Bär. Res. Lab. Neurology, Rudolf Magnus Inst. for Neurosciences, 3584 CX Utrecht, The Netherlands.

Treatment with the anti-tumor drug cisplatin (CPt) leads to sensory neuropathy in patients, indicating that CPt affects the dorsal root ganglia (DRGs). In vitro CPt 1) inhibits neurite outgrowth from DRGs, 2) decreases the migration of DRG glia along the neurites and 3) is toxic to Schwann cells. As glial cells seem to be very sensitive to CPt treatment, we studied the role of these cells in neuroprotection. Therefore we cultured sensory neurons or satellite cells obtained from E15 rat DRGs and Schwann cells from P4 rat sciatic nerves. The sensory neurons were treated with 1μg/ml CPt, which reduced neurite outgrowth with 37% (measured with neurofilament ELISA). After 24h the medium was replaced with conditioned medium (CM) from glial cells or medium containing 15 ng/ml βNGF. Schwann cell CM and βNGF diminished the toxic effect on outgrowth with 60%, but satellite cell CM was not effective. These results suggest that Schwann cells and not satellite cells produce a soluble factor that protects against CPt-induced neurotoxicity. βNGF also had a potent protective effect in the pure sensory neuron culture. As Schwann cells are known to produce NGF, the Schwann cell derived protective factor may be NGF.

616.14

LEAD INDUCED DEVELOPMENTAL ALTERATIONS IN GLIAL GENE EXPRESSION N.H. Zawia* and G.J. Harry, Environmental Immunology & Neurobiology, Laboratory of Biochemical Risk Assessment, NIEHS, P.O. Box 12233, RTP, NC 27709.

Lead is a neurotoxicant that is known to produce behavioral, biochemical and structural abnormalities in brain development. We have previously demonstrated that lead selectively disrupts developmental gene expression. The goal of this study is to examine whether these alterations in gene expression are related to regional timetables of development. To accomplish this, two brain regions with distinct prenatal (brainstem) and postnatal (cerebellum) schedules of development were chosen. Long Evans hooded rats were lactationally exposed to low levels of lead acetate (0.2 % in the drinking water of the dam begun after parturition). Tissue was then obtained from the cerebellum and brainstem on postnatal days (PND): 3, 6, 9, 12, 15, 20, 25, 30, 40, and 50. No changes in either brain structure and weight or animal body weights were observed in the pups following such exposure to lead. Total RNA was isolated and probed for myelin basic protein (MBP), glial fibrillary acidic protein (GFAP) and actin gene expression by Northern analysis. In the cerebellum, lead produced long term elevations in both MBP and GFAP gene expression that began on PND 20 and were sustained into adulthood; actin gene expression remained unchanged. No such shifts in the expression of these genes were observed in the brainstem. These results suggest that glial gene expression is more susceptible to the effects of lead in actively developing regions and that the state of gene expression may be permanently altered by lead.

616.15

A PRONOUNCED ASTROCYTIC AND MACROPHAGE RESPONSE TO PRENATAL METHYLAZOXYMETHANOL TREATMENT ACCOMPANIES THE ABNORMAL DEVELOPMENT OF THALAMOCORTICAL PROJECTIONS. J.R. Hoffman*, M.F. Barbe, P. Levitt, Dept. of Anat. & Neurobiol, Med. Coll. of Penn., Philadelphia, PA 19129, Dept. of Physical Therapy, Temple Univ., Philadelphia, PA 19140, Dept.of Neurosci. & Cell Biol., Robert Wood Johnson Med.School/UMDNJ, Piscataway, NJ 08854.

The development of axonal projections requires a complex series of spatially and temporally regulated cell interactions. The thalamocortical pathway is established prenatally, but grows most extensively in target cortical regions postnatally. This delay may be dependent upon target maturity, as well as nonneuronal cells. Exposure to methylazoxymethanol (MAM) kills mitotically active cells and when administered late prenatally, appears to alter thalamocortical fiber organization. We investigated the effect of MAM treatment (20mg/kg) when administered on either embryonic day 19, 20 or 21. Analysis of astroglia (anti-vimentin and GFAP), brain macrophages (anti-ED-1), and fiber connections (DiI labeling of thalamus) was performed at birth, postnatal day (P) 5 and P10. GFAP staining was more pronounced in reactive astrocyte-like cells in subcortical white matter and internal capsule at P10. Moreover, ED-1 stained macrophages accumulated in white matter at the earliest time point examined and were still evident even at P10, almost 2 weeks after the single injection of MAM. The degree of nonneuronal cell response appeared to correlate with the disruption of the DiI-labeled thalamocortical fibers. In some brains, disrupted projections, anomalous growth cone and fiber varicosities were seen at birth. The results suggest that the MAM treatment produced an abnormal gliotic and phagocyte response in the fetal brain, one which can have longlasting effects on cellular organization and developing fiber projections. Supported by NRSA fellowship 09519, NIMH grant 45507 and NIA grant AG10560.

616.16

AXONAL DEGENERATION CAUSES A SLIGHT INCREASE IN ASTROCYTE PROLIFERATION *IN VITRO*. V. Guénard*, G. Frisch, L.A. Gwynn, E. Cuervo, and P.M. Wood. The Miami Project and Department of Neurological Surgery, University of Miami School of Medicine, Miami, FL.

Mechanisms inducing gliosis in the CNS after injury are not well understood. Astrocytes (AS) proliferate and become hypertrophied under stimuli not yet identified. In this study, the influence of axonal injury on astrocyte proliferation was evaluated at various times post-injury and related to axonal degeneration and regrowth. Since the transplantation of Schwann cells (SC) into gliotic areas of the injured CNS is of therapeutic interest, we also assayed whether factors released in injured N-AS cultures affected SC proliferation on axons. Cultures of purified embryonic rat dorsal root ganglion neurons (N) were transplanted onto AS or SC monolayers. After the neurons extended axons on the AS or SC, a large fascicle was cut 1.5 mm from the neuronal cell bodies. Non-injured cultures were used as controls. To assess proliferation, the cultures were exposed to [^{3}H]-thymidine 0, 1, 3 and 7 days post-injury (DPI) and processed for autoradiography. On 1 and 2 DPI AS proliferation was slightly increased and SC proliferation in sibling cultures was increased to approximately the same extent. Neurofilament staining distal to the lesion disappeared more rapidly in N-AS than N-SC cultures. Eight DPI, axonal regrowth was observed, but neither AS or SC proliferation changed. In two experiments conditioned media collected from injured and non-injured N-AS cultures decreased neurite-induced SC proliferation when compared to media from sibling N-SC cultures, as assessed by autoradiography. However, no difference was observed between media obtained from injured and non-injured N-AS cultures. Our data demonstrate that AS proliferation in gliosis may be caused partially by factors released after axonal injury. Supported by NIH grant NS28059, NMSS grant 1RG 2210-A-2 and The Miami Project.

616.17

β_2-ADRENERGIC RECEPTORS ARE EXPRESSED BY GLIA *IN VIVO* IN THE NORMAL AND INJURED RAT, RABBIT AND HUMAN CNS. C.A. Hodges-Savola*, S.D. Rogers, M.D. Catton, J.R. Ghilardi, C.J. Allen, S.R. Vigna, J.E. Maggio, L.A. Levin, P.W. Mantyh. Mol. Neurobio. Lab (151), Mpls., MN 55417; Psychiatry Dept, Univ of Minn., Mpls., MN 55455; Dept. of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115; Dept. Cell Biology, Duke Univ. Med. Ctr., Durham, N.C. 27710; Dept. Opthamol., Univ. of Wisc., Mad. WI, 53792

Glial cells in culture express several subtypes of functional adrenergic receptors. To determine if similar receptors are expressed by glia *in vivo* we examined normal, crushed, and transected optic nerves of the rabbit and rat using quantitative adrenergic receptor autoradiography. We also obtained preliminary data regarding the expression of adrenergic receptors in normal and damaged human optic nerve. High levels of α_1, α_2, β_1, and β_2 adrenergic receptors were identified in the rabbit and rat forebrain but only α_1 and β_2 receptors were observed in the normal rat and rabbit optic nerve, and these were present in low to moderate densities. Normal, as well as damaged, human optic nerves also exhibited adrenergic receptor binding limited to the α_1 and β_2 species. After unilateral optic nerve crush or transection, only β_2 adrenergic receptors were significantly increased. This increase in β_2 receptors was first significant at day 7 and 28 post-transection in the rabbit and rat, respectively. The expression of β_2 receptors in the transected optic nerve continued to increase with time so that by 90 days post-transection, the density of β_2 receptors in both the rabbit and rat optic nerve was among the highest in any area of the forebrain. Taken together with previous studies, these results suggest that *in vivo*, glial β_2 adrenergic receptors may provide a therapeutic target for regulation of human astrocyte functions including glycogen metabolism, cytokine release, and the hypertrophy and proliferation that occurs in response to neuronal injury. (Supported by the VA and NIH)

616.18

DENERVATION-INDUCED CHANGES IN ASTROCYTIC GENE EXPRESSION ARE ALTERED IN AGING MICE. P. A. Trimmer* University of Virginia, Departments of Neurology and Neuroscience, Charlottesville, VA 22908.

This study is designed to evaluate if the time course of changes in astrocytic gene expression associated with Wallerian degeneration is altered by normal aging. Denervation was induced in the dentate gyrus of adult (40-60 days) and aging (1-2 years) C57BL/6 mice by a unilateral lesion of the entorhinal cortex. In situ hybridization with a radiolabeled probe for glial fibrillary acidic protein (GFAP) was used to detect changes in levels of GFAP mRNA. In adult mice, there was a peak in GFAP mRNA levels at 2 days post-lesion (DPL). GFAP mRNA levels declined at 4 DPL but did not return to control levels until about 3 weeks post-lesion. In aging mice, the peak in GFAP mRNA levels was reduced in size and was delayed until 4 DPL. GFAP mRNA levels returned to near control in lesioned aging mice by 8-10 DPL. These results indicate that up-regulation of gene expression in reactive astrocytes is compromised in the CNS of aging mice. Compromised astrocyte function as a consequence of normal aging could limit the ability of astrocytes to provide the metabolic and functional support neurons in the CNS need to cope with the degeneration that characterizes pathological conditions such as Alzheimer's disease. Supported by the Alzheimer's and Related Diseases Research Award Fund.

617.1

CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IN YOUNG MOTONEURONS AFTER NERVE INJURY.

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Changes in calcitonin gene-related peptide immunoreactivity (CGRP-IR) were examined in motoneurons of young rats after nerve injury to determine when during postnatal development, CGRP-IR typical of adult moto-neurons was elicited by axonal injury. Hypoglossal nerve crush or transection was performed in rats at 10, 14 or 21 days postnatal (dpn). Rats were killed by aldehyde perfusion at 1, 3, 7, 14, 20 days postoperative (dpo). Results from the 21 dpn injury cases resembled those in the adult rat. Increased CGRP-IR continued after nerve crush until regeneration was complete. After transection, a biphasic response of CGRP-IR consisted of an early increase (1-3 dpo), a decrease to basal levels (7 dpo) and a second elevation (14-20 dpo) that persisted until regeneration occurred. No consistent increase in CGRP-IR was seen after either injury at 10 dpn and regeneration was meager and nerve cell death was substantial. The same injuries in 14 dpn rats produced intermediate changes in CGRP-IR, regeneration and cell death compared to those obtained in the 10 and 21 dpn rats. The results strengthen the idea that persistent up-regulation or down-regulation of CGRP-IR can be used to predict successful regeneration or apoptosis of injured motoneurons.

617.2

INCREASED EXPRESSION OF NITRIC OXIDE SYNTHASE (NOS) AND GROWTH ASSOCIATED PROTEIN (GAP-43) IN VISCERAL NEURONS AFTER NERVE INJURY.

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NADPH-diaphorase (NADPH-d), a marker for NOS, is present in a large percentage of L6-S1 afferent neurons innervating the pelvic viscera in the rat; however, NOS-IR has not been detected in these neurons. The present experiments were undertaken to determine if NOS could be expressed in these neurons following axotomy. GAP-43, a substance commonly expressed in neurons after injury, was also examined. In male Wistar rats, the right major pelvic ganglion (MPG) was removed to produce axotomy. In some rats, 1 week prior to ganglionectomy, Fluorogold was injected into the left and right MPG to retrogradely label afferent neurons in the DRG. Two-four weeks later, the animals were perfused. A differential distribution of NOS-IR was detected in DRG cells at different levels of the spinal cord in control animals. Rostral lumbar (L1-L2) DRGs exhibited significantly ($p<.05$) greater numbers of NOS-IR cells compared to caudal lumbar and sacral DRGs (L5-S1). L1 and L2 DRGs exhibited 15 NOS-IR cells/section (c/s) and 3 NOS-IR c/s, respectively, whereas L5-S1 DRGs had only a few cells (0.2 -0.7 c/s). After axotomy, a significant increase ($p<.01$; 11 NOS-IR c/s in the L6 and the S1 DRGs) was observed ipsilateral to nerve injury. After axotomy, NOS-IR significantly increased ($p<.05$) in visceral (FG labeled) afferent neurons in the L6 DRG: 87% of visceral afferents in the L6 DRG and 91% in the S1 DRG were NOS-IR compared to 11% (L6) and 24% (S1) contralateral to nerve injury. Increased NOS-IR and GAP-43-IR were also detected in the lateral dorsal horn and in the SPN ipsilateral to nerve injury. A significant increase ($p<.01$) in NOS-IR and NADPH-d positive cells was observed in the sacral parasympathetic nucleus in L6 ipsilateral to nerve injury. The results indicate that NOS-IR and NADPH-d activity in visceral afferent and efferent neurons are plastic and can be upregulated by peripheral nerve injury. The unmasking of NOS gene expression by axotomy in some visceral afferent neurons (L6-S1) may be due to removal of tonic inhibitory influences arising in the peripheral target organ. [Supported by NIH grants DK 37241, DK 422369, NRSA 1 F32 DK 08916-01].

617.3

ANDROGEN RECEPTOR mRNA EXPRESSION IN ADULT HAMSTER FACIAL MOTOR NEURONS (HFMN).

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We have previously demonstrated that systemic administration of exogenous testosterone propionate (TP) to adult hamsters enhances the rate of facial nerve regeneration following injury in both genders, with the effects significantly greater in males as compared to females. Further studies also indicate that these effects of TP occur via a mechanism involving androgen receptors (AR), which are present in both male and female HFMN. In this study, we tested the hypothesis that the differential effects of androgens on regeneration rate in male/female HFMN are related to gender specific differences in the levels and/or regulation of AR mRNA in these motoneurons. Four groups of hamsters were used: intact and gonadectomized males/females. *In situ* hybridization was performed using an ^{35}S-labeled antisense AR cRNA probe encoding 350 NTDs of the ligand binding region. Slides were processed for routine autoradiography and quantitative analysis was performed using a computerized image analysis system. Preliminary results indicate that AR mRNA levels are increased in male HFMN when compared to females. Gender specific regulation was also noted; gonadectomy decreases AR mRNA levels in males, but not in females. Studies are in progress to evaluate the interactive effects of hormonal/injury states on AR mRNA expression in HFMN. Supported by NIH grant NS28238.

617.4

CHANGES IN RETINOBLASTOMA SUSCEPTIBILITY PROTEIN (RB)- AND C-JUN-LIKE IMMUNOREACTIVITY (IR) IN HYPOGLOSSAL NEURONS FOLLOWING HYPOGLOSSAL NERVE CRUSH.

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RB plays an essential role in regulating cellular proliferation and differentiation by interacting with transcription factors (e.g. E2F, c-Myc) which regulate genes necessary for cell growth. We hypothesize that RB may likewise regulate growth-related molecules involved in neuronal responses to injury and/or neuronal plasticity. In the present study, we examined RB-IR in hypoglossal neurons at different times following hypoglossal nerve crush to determine if changes in RB expression occur following injury and during the course of axonal regeneration. Expression of the early response genes Fos and c-Jun were also assessed and compared with the expression of RB-IR.

Twenty-four adult, female Sprague Dawley rats received either a unilateral hypoglossal nerve crush (n=20) or sham surgery (n=4). Four animals were killed at each of 3h, 1 day, 3 days, 1 week, and 2 weeks following nerve crush. Twenty-micron thick coronal sections through the hypoglossal nucleus were cut and adjacent sections were processed for immunocytochemical detection of RB-, Fos-, or c-Jun-IR.

Intense nuclear RB-IR staining and less intense cytoplasmic staining was detected in hypoglossal neurons of sham-operated animals. Minimal c-Jun-IR and no Fos-IR was detected. Hypoglossal nerve crush resulted in a rapid induction of c-Jun-IR, but not Fos-IR, within hypoglossal neurons on the injured side. In contrast, nuclear RB-IR staining appeared to decrease 1-7 days following hypoglossal nerve crush. Optical density measurements confirmed that significantly less ($p<0.05$) nuclear (but not cytoplasmic) RB-IR staining was detected 1-7 days, but not 3h or 2 weeks, following nerve crush relative to the average density of staining on the uninjured side.

These data suggest that RB expression changes significantly following hypoglossal nerve crush with a time-course which correlates with the time-course for axonal regeneration. This is consistent with the hypothesis that, in neurons, RB may regulate molecules involved in neuronal response to injury and axonal regeneration.

617.5

EXPRESSION OF MICROTUBULE-ASSOCIATED PROTEINS (MAPs) IN NORMAL AND REGENERATING CAT TROCHLEAR MOTONEURONS.

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While previous studies have shown that the levels of actin, tubulin, and neurofilament proteins are altered in peripheral neurons following axotomy, much less is known about the role of MAPs in axonal regeneration. In this study, we examined the expression of a variety of MAPs, by immunocytochemical methods, in both normal and peripherally axotomized trochlear motoneurons (TMNs) of the cat. The left trochlear nerve of each animal (n = 4) was transected where it coursed between the cerebellum and inferior colliculus, and animals were allowed to survive 2 weeks. Three unoperated cats served as controls. Brain sections were incubated with antibodies recognizing a high molecular weight form of tau, termed HMW tau, MAP-2, MAP1B, and a phosphorylated form of MAP1B, termed MAP1B-P. In normal control TMNs, HMW tau and MAP1B were observed in cell bodies and processes. In contrast, MAP-2 was present specifically in dendrites while MAP1B-P was found only in axons. Following nerve transection, the levels of HMW tau appeared to decrease in axotomized TMNs, as compared to TMNs in the unaxotomized nucleus or in control animals. MAP 1B-P was increased in the axons of axotomized TMNs and was also observed in some cell bodies of axotomized TMNs. The levels of MAP-2 and MAP1B in axotomized TMNs appeared similar to controls. These results suggest a potential role for MAPs in the regeneration of peripheral nerve since their expression was specifically regulated following axotomy. (Supported by NIH Grant NS 24707).

617.6

LOCALIZATION OF INSULIN-LIKE GROWTH FACTOR II (IGF-II) PROTEIN AND mRNA DURING NERVE REGENERATION.

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Sensory and motor axon regeneration is dependent upon IGFs following sciatic nerve crush in rats. In the present study we examined the cellular distribution of IGF-II protein and mRNA in rat sciatic nerve and soleus muscle after nerve crush or transection by indirect immunocytochemistry and *in situ* hybridization. Immunostaining correlated with IGF-II mRNA levels, and was higher in postnatal liver and muscle than adult. Immunostaining as well as silver grain density in nerve were elevated distal to the site of crush, particularly in macrophages and Schwann cells. Immunoreactivity was also elevated on the surface of denervated muscle fibers. These results support the interrelated hypotheses that (i) IGF-dependent regeneration of axons in peripheral nerves is due to the elevation of both IGF-II protein and mRNA in nerves distal to the site of lesion, and (ii) that elevated IGF-II protein in muscle may support the sprouting of motor nerve terminals that precedes re-establishment of synapses. (Supported by grant PO1 NS28323)

617.7

GALANIN EXPRESSION IN SYMPATHETIC GANGLIA AFTER PARTIAL AXOTOMY. A. M. Shadiack*, R. P. Mohney, and R. E. Zigmond. Dept of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

One of the ways neurons of the sympathetic nervous system display plasticity after axotomy is by altered expression of certain neuropeptides, such as vasoactive intestinal peptide (VIP), substance P (SP), and galanin (GAL). The increase in GAL-like immunoreactivity (-IR) in the superior cervical ganglion is maximal at 3 days and declines slowly, though remaining above control levels even at 14 days (Sun et al., 1993). In the middle and inferior cervical gangion (MICG), the increase in GAL-IR occurs by 2 days, plateaus through 7 days, and then increases 2-3-fold further by 14 days, returning to control levels by 60 days. The further increase in GAL-IR in the MICG between days 7 and 14 may be related to the distance between the site of axonal transection and the ganglion in this preparation.

With the use of a retrograde fluorescent marker, fast blue, we have shown that GAL-IR is mainly found in axotomized neurons of the MICG. In order to determine whether changes in GAL mRNA are also restricted to axotomized neurons, we performed *in situ* hybridization for GAL. Using fast blue applied to the cut nerve trunk to identify axotomized neurons, we observed a high degree of coincidence between GAL mRNA- and fast blue-containing cells most of which appeared in the middle cervical ganglion region.

The functions of VIP, SP, and GAL after axotomy are yet unknown. Two possible sites of action for these peptides are within the ganglion and/or at the site of transection. We have found an increase in VIP and GAL, and perhaps a small increase in SP, in the proximal portion of the nerve trunk after transection and a further increase after ligation of that transected nerve. This suggests that VIP and GAL produced in the sympathetic neurons after axotomy are transported anterogradely within their axons.

617.8

CHANGES IN THE MACROPHAGE POPULATION IN THE RAT SUPERIOR CERVICAL GANGLION (SCG) AFTER AXOTOMY. R. C. Schreiber*, A. M. Shadiack, and R. E. Zigmond. Department of Neurosciences, Case Western Reserve University, Cleveland OH 44106

Macrophages play a role in the biochemical changes that occur in a peripheral nerve after transection. To determine whether these cells might also be involved in changes occurring in the region of axotomized sympathetic cell bodies, sections of rat SCG were examined immuno-histochemically using two monoclonal antibodies: OX6 (which recognizes macrophages and other cells expressing the Ia MHC class II antigen) and ED1 (which recognizes circulating monocytes and macrophages). SCG from normal and sham-operated animals and from animals 1, 2, 5, 8, and 14 days after nerve transection were studied, and the number of immunostained profiles, quantified by image analysis. Immunostained cells were found using both antibodies in ganglia from normal animals, and no changes were seen after sham-operation. After axotomy, OX6 staining was unchanged at 1 and 2 days, increased significantly at 5, and continued to rise at 8 and 14. ED1 immuno-fluorescence was unchanged at 1 day, increased significantly at 2, and was maintained at this level at 5, 8, and 14. The differences in the time courses of immunostaining with the two antibodies may indicate that there is differential regulation of the two antigens in a single cell population or that more than one cell population is involved. The time course of apparent invasion by circulating monocytes suggests that they do not participate in early biochemical changes after axotomy, such as the rapid increase in expression of leukemia inhibitory factor mRNA in the SCG. Whether resident macrophages participate in these early events remains to be determined. Studies using ED2 (which recognizes resident macrophages) will be reported on.

617.9

THE CLONING AND CHARACTERIZATION OF A cDNA ASSOCIATED WITH NEURONAL INJURY AND SCIATIC NERVE REGENERATION IN THE RAT. M. De León*, R.L. Nahin§, Yi Liu , A.A. Welcher¶, E. M. Shooter¶ and M. A. Ruda§. *Dept. Physiology and Pharmacology, Loma Linda Univ., CA; ¶Dept. of Neurobiol., Stanford Univ. Stanford, CA; §Neurobiol and Anesthesiol Branch, NIDR, NIH, Bethesda, MD.

We report the cloning and characterization of DA11, a full length cDNA clone isolated from a rat dorsal root ganglia (DRG) cDNA library. The sciatic nerve of Sprague Dawley rats was crushed and allowed to regenerate. At three days after the crush the ipsilateral DRG were pooled together, and the total RNA extracted. The RNA was purified through an oligo (dT) cellulose column, and 5g of mRNA was used to construct a cDNA library (DA). The DA library was screened twice by differential hybridization as described before (De León et al., J. Neurosci Res. 29:437-448, 1991) and the cDNAs found to be elevated after sciatic nerve crush were analyzed. The screening of the library resulted in the isolation of a 0.7 Kb DA11 cDNA clone whose expression was found to be induced after nerve crush. DNA sequencing and analysis of this clone revealed that the DA11 cDNA contained a single open reading frame that coded for a protein of a calculated molecular weight of about 15 kD. Northern blot analysis showed that the DA11 cDNA recognizes a single ~ 0.6kb mRNA species that showed a two-three fold induction in the DRG ipsilateral to the sciatic nerve crush, as compared to the contralateral DRG. The 0.6 Kb DA11 mRNA was detected in total RNA extracted from the lung, heart, brain, sciatic nerve and spinal cord tissue, but the highest level was found in RNA extracted from rat DRG. The relative levels of DA11 mRNA did not change in the sciatic nerve during Wallerian degeneration. The DA 11 mRNA was also higher in the cerebral cortex during the first two weeks after birth and it was significantly induced in PC12 cell lines after treatment with nerve growth factor. Our data support the hypothesis that the DA11 cDNA may play a role during sciatic nerve regeneration and cerebral cortex post-natal development.

617.10

SERINE PROTEASE:SERPIN BALANCE IN MOUSE SCIATIC NERVE AFTER INJURY. J. Ma, I.V. Smirnova, E.J. Gregory and B.W. Festoff*. Neurobiology Research Lab., VA Med. Ctr, Kansas City, MO 64128; Dept. of Neurology, Univ. of Kansas Med. Ctr, Kansas City, KS 66103.

Serine proteases, such as thrombin (TH), cathepsin G (CG), plasminogen activators (uPA, tPA), tissue kallikrein (tKK) and plasmin (PL) are necessary for extracellular proteolysis in various tissues. Proteolytic cascades involving these enzymes participate in the development and regeneration of nervous tissue (Festoff, 1990). Serine protease inhibitors (serpins) provide post-translational modulation of serine proteases by forming covalent complexes. Thus, the balance between serine protease and serpin may be critical in determining the tissue remodeling, particularly in synapse elimination and reinnervation. In this study we investigated the induction of a variety of serine proteases as well as the serpin protease nexin I (PNI), potent inhibitor of TH and uPA, after adult mouse sciatic nerve crush. Samples were taken from sciatic nerve distal to the crush site at day 1, 3, 6, 9 and 14. We measured the protease activities by their cleavage of several specific tripeptide chromogenic substrates. We found that TH-like activity at day 3 time point was dramatically increased, compared to other crush time points and controls, and was inhibited by addition of highly specific TH inhibitors such as PNI, hirudin and Phe-Pro-Arg-chloromethyl ketone. No significant change was observed for the other serine proteases following crush. Induction of active plasmin, either in crushed nerve or in the controls, was not detectable. Since the balance of serine proteases and their serpins in nerve was altered by nerve damage, we also estimated active PNI levels in these crush samples by means of complex formation with ^{125}I-labeled human uPA. The rate of active PNI in crush samples initially increased by sevenfold at day 6, peaked at tenfold by day 9 and then decreased at day 14. Thus, increase of TH after nerve crush is followed by generation of active PNI. These results suggest that shifting the balance between TH and PNI might underlie synapse retraction after injury with re-establishment of the balance facilitating repair of damage of peripheral nerve after injury. They also suggest that TH and its cognate serpin participate in axonal regeneration to a greater extent than other serine proteases studied. (Supported by the MMD Foundation/ SEP, the ADRDA and the Med. Res. Service of the Dept. of VA).

617.11

INDUCTION OF *c-FOS-LACZ* AND *c-JUN-LACZ* TRANSGENES FOLLOWING SCIATIC NERVE TRANSECTION IN NEONATAL AND ADULT MICE. H.D. Soares*, S.-C. Chen, T. Curran, and J.I. Morgan. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ.

The developmental stage of an animal greatly affects outcome following CNS injury. For example, peripheral nerve transection in neonates elicits profound neuronal loss whereas adult neurons survive peripheral axotomy and attempt regeneration. Paradoxically, functional recovery in neonatal animals usually surpasses recovery in adults despite severe muscular atrophy in both adults and neonates. Very little is known concerning the underlying molecular mechanisms governing recovery following peripheral nerve injury. The following study utilizes a transgenic approach to examine the transcription factors, *fos* and *jun*, in defined cell populations undergoing degeneration, regeneration, plastic reorganization, and transynaptic activation following sciatic nerve transection. Using either *c-fos-lacZ* or *c-jun-lacZ* mouse lines, the left tibial and peroneal branches were transected and a 2mm piece removed in either PO or adult subjects. Animals were allowed to recover for 2 hrs, 1, 2, 5, 14, or 28 days post-injury. Both *fos* and *jun* were transynaptically induced in spinal cord dorsal horn. In addition, *fos* and *jun* were both present in peripheral nerve stumps, surgically traumatized thigh and in PO denervated leg muscle. Interestingly, *fos*, but not *jun*, was present in the adult denervated leg muscle even though *jun* mRNA was substantially elevated. Axotomy elicited only a transient *fos* response in both PO and adult neurons. *Jun* was present for prolonged periods in axotomized neurons and also in adjacent neuronal cells whose axons had not been transected. These results suggest that *jun* is post-transcriptionally regulated in adult denervated leg muscle. Prolonged *jun* expression in neurons may be more associated with a sprouting phenomena rather than a pure regenerative response. Finally, our data demonstrate that developmental differences in response to peripheral nerve injury can occur on a transcriptional level.

617.12

INDUCTION OF THYMOSIN ß10-*LACZ* TRANSGENE IN REGENERATING MOTOR NEURONS FOLLOWING PERIPHERAL NERVE INJURY. S.-C. Chen, M. Butler, R. J. Wurzburger* and J. I. Morgan. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ.

In an attempt to identify molecular markers for neurogenesis, we found a protein named thymosin ß10 that is predominantly expressed in the embryonic nervous system. Its expression levels reach a maximum around the time of axonal outgrowth and decline sharply after birth. Biochemically, thymosin ß10 has been shown to be a G-actin binding protein. A rat genomic DNA of thymosin ß10 was cloned and used to generate transgenic mice that carry a thymosin ß10-*lacZ* transgene. The expression of thymosin ß10-*lacZ* is first detected at embryonic day 8.5 in the nervous system. During late neuroembryogenesis, its expression is widely distributed in the developing nervous system, including retina, cortex, cerebellum, spinal cord, cranial motor neurons and ganglia. In the adult, high levels of thymosin ß10-*lacZ* expression are restricted to neurons that have elevated synaptic remodeling. In addition to its cytoplasmic expression in the neuronal cell body, thymosin ß10-*lacZ* fusion protein is also detected in dendrites and axons.

The expression of thymosin ß10-*lacZ* was investigated following peripheral nerve injury. Five days after facial nerve transection, an induction of thymosin ß10-*lacZ* was observed in motor neurons in the ipsilateral facial nucleus. The expression remained elevated for several weeks during regeneration. Induction of thymosin ß10-*lacZ* in the spinal motor neurons of L4 & 5 was also detected following sciatic nerve transection. Increased blue staining in axons in the proximal stump of the injured nerve was observed and extended into dorsal root fibers supplying the secondary sensory neurons. These observations suggest that thymosin ß10 plays an active role in axonal growth during development, regeneration and in synaptic reorganization.

617.13

DIFFERENTIAL DISPLAY PCR IDENTIFIES CHANGES IN GENE EXPRESSION FOLLOWING RAT FEMORAL NERVE LESION

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The rat femoral nerve divides into a terminal sensory branch (saphenous nerve) and a terminal motor branch to the quadriceps muscle. Motor axons preferentially regenerate into the terminal motor branch following nerve transection and repair proximal to the terminal bifurcation, even when the distal nerve branches are completely isolated from their end organs (Brushart, J. Neurosci., 13 (6), 2730-2738 1993). To identify factors present in the denervated pathways which may underlie preferential motor reinnervation, we are starting to analyze lesion induced changes in mRNA expression using differential display PCR (Liang and Pardee, Science, 257, 967-971, 1992). Total RNA was extracted from terminal motor and sensory branches from control nerves or nerves which had been transected and prevented from regenerating for two weeks. Subsets of the expressed mRNAs were reverse transcribed with an anchored dTMN-primer, and amplified with PCR using end-labeled random 10-mers. PCR products were separated by PAGE. Most labeled bands representing expressed mRNAs were similar between lesioned and control motor branches or sensory branches. Several bands were present only in lesioned motor or lesioned sensory branches, representing potential mRNAs which are expressed only under denervated conditions. Differentially expressed bands will be sequenced to determine their identity. Initial results show the utility of using this approach to identify mRNAs that are up- or down-regulated following nerve transection. NS22404-09 (RDM) and VA Merit Review (RDM).

617.14

BDNF LEVELS INCREASE IN THE TERMINAL MOTOR BRANCH FOLLOWING LESIONS OF THE PARENT FEMORAL NERVE: A COMPETITIVE PCR STUDY

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A 273 bp fragment of mature BDNF (nt 2573-2846) was amplified from rat muscle RNA and cloned into the pCRII vector (InVitrogen). An unrelated Hae III digested fragment of pCRII (~100 nts) was ligated into the Ssp I site within the BDNF fragment (at position 2665). This enlarged BNDF construct was verified by DNA sequencing and used to make sense riboprobe. Increasing concentrations of riboprobe were then used to spike reverse transcriptase reactions of total RNA harvested from: muscle, sciatic nerve, lesioned and non-lesioned terminal motor branch of the rat femoral nerve. The amount of radioactive label present in each band was determined by exposing the gel to a PhosphorImager. Total counts were then used to construct a standard curve relating concentration of spiked cRNA to target RNA. In previous studies using ribonuclease protection assays (Funakoshi et. al., J.Cell Biol., 1993. 123: 455-465) levels of BDNF were barely detectable in normal sciatic nerve. In our competitive PCR assays BDNF is easily detectable in normal sciatic nerve or normal motor branch of the femoral nerve, and is present in approximately 10-fold lower concentrations compared to muscle. The amount of BDNF in two-week lesioned nerve increases 10-30 fold in the motor branch of the femoral nerve (terminal branch to the quadriceps muscle) as compared to uniesioned nerve. Experiments are currently underway to determine BDNF levels in the other terminal branch of the femoral nerve which is purely sensory. Supported by NS22404-09 (RDM) and VA Merit Review (RDM).

617.15

CONSTITUTIVE HEAT-SHOCK-70 mRNA IS INDUCED IN RESPONSE TO FACIAL NERVE AXOTOMY.

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We have shown previously that constitutive heat-shock-protein 70 (hsp70) is increased in the regenerating facial nucleus. In this study, we extended these findings and examined if hsp70 mRNA would be induced in facial nucleus following peripheral nerve lesion. Nerve transection was done in Wistar rats at the level of the stylomastoid foramen and animals were sacrificed 6h, 12h, 24h or 3d later. Facial nuclei from operated and sham-op controls were extracted and prepared for Northern blot analysis using a cDNA probe complementary to constitutive hsp70 mRNA. Autoradiograms revealed a single band (ca. 2.5 Kb) which appeared to be maximally induced in operated nuclei at 24 hours following axotomy, as compared to controls. Preliminary results at 3 days post-lesion indicate that relative levels declined but remain elevated over time. The time course of hsp70 mRNA induction is consistent to that previously reported for constitutive hsp70 protein, where maximal protein levels were observed at 3 days post-axotomy and were maintained elevated thereafter. These results indicate that peripheral nerve lesion induces hsp70 mRNA. Further, these data suggest for a role of hsp70 in the early phase and long-term maintenance of the regeneration program of motoneurons.

617.16

EXPRESSION OF NITRIC OXIDE SYNTHASE (NOS) IN MOTOR NEURONS AFTER AXOTOMY.

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NOS, an enzyme for the synthesis of nitric oxide, becomes detectable in anterior horn cells following ventral root avulsion, and in motor neurons of cranial nerves after nerve transection. Since anterior horn cells died after root avulsion, and inhibition of NOS reduced neuron death, it was postulated that NOS expression signals the impending death of injured cells. However, extensive neuron death occurred only when cranial nerves were cut at young age, and fewer cranial neurons expressed NOS after axotomy at young age when compared with axotomy at adults. We hypothesize that expression of NOS is one of cellular events involved in neuronal response to injury. In this study, we examined the time course of NOS expression in motor neurons of cranial nerves following axotomy. Adult, female rats received either unilateral crush or transection of the vagus and hypoglossal nerves. Animals were killed at 1 and 3 days, 1, 2, 4, and 6 wks post operation (PO). Thirty-micron thick coronal sections cut through the lower brain stem were processed for NADPH-diaphorase reaction for NOS. Changes of NADPH-diaphorase reaction were observed 3 days PO in the two motor nuclei ipsilateral to axotomy. In the vagus nucleus, increases in the number of NOS positive neurons and intensity of labelling persisted 6 wks PO. In the hypoglossal nucleus, the number of NOS neurons reached maximally 3 days and 2 wks, and declined 4 and 6 wks after crush and transection, respectively. These data suggest that NOS was expressed in cranial motor neurons in a time course which could be correlated with axon regeneration, consistent with the hypothesis that expression of NOS is involved in neuronal response to injury, possibly regulated by axon regeneration.

617.17

LONG-TERM CHANGES IN HEMOPEXIN AFTER PERIPHERAL NERVE INJURY.

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Analysis of molecular environments that allow nerve repair has revealed the presence of hemopexin inadult rat peripheral nerves and the dramatic increase in the level of this glycoprotein after nerve transection (Swerts et al., J. Biol. Chem., 267, 10596-10600). Although it is a soluble protein, hemopexin is colocalized in the nerve with extracellular matrix proteins. Moreover, hemopexin synthesis has been detected within the nerve itself; thus this plasma protein can no longer be considered as a liver-specific product. In the present study, we have documented long term changes in hemopexin levels in permanently degenerated (transected) and regenerating (crushed) sciatic nerves, using western-blotting and immunohistochemical techniques. In both transected and crushed nerves, there was a 6-fold increase in hemopexin content at day 2 post-injury and a 12-fold increase at day 7 in the distal part of the nerves, compared with contralateral intact nerves. Beyond the first week, the fate of hemopexin was very different in each type of lesioned nerves: in transected nerves the level of hemopexin kept increasing, reaching over 20 times the basal level three months after injury, whereas in crushed nerves, hemopexin level declined progressively in a proximo-distal direction, returning to basal values 2 months post-injury. These results suggest that hemopexin could be regulated negatively, directly or indirectly, by growing axons and support the idea that hemopexin could be involved in the process of Wallerian degeneration and/or in nerve repair.

617.18

EXPRESSION OF BETA-ACTIN mRNA IN MOTONEURONS AFTER AXOTOMY.

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The peripheral neuron responds to axotomy by switching from translation of proteins needed for normal function (e.g. neurotransmitter synthesizing enzymes) to proteins needed for axonal outgrowth (e.g. actin and tubulin). At 1, 4, or 14 days after unilateral sciatic nerve crush (with contralateral sham crush), the L4/5 spinal cord was excised and hemisected. Northern blots of mRNA showed an increase in beta-actin mRNA at one day on the crush side compared to the sham side. The increase peaked at 4 days, and was returning to constitutive levels by 14 days. We have used *in situ* hybridization to further investigate beta-actin mRNA expression after axotomy. Riboprobes were synthesized using SP6 and digoxigenin labelled nucleotides and detected immunochemically. Motoneurons were retrogradely labelled with Fluoro-Ruby. Preliminary *in situ* data at 4 days showed larger, darker-stained motoneurons on the axotomized side. Increases in beta-actin mRNA after axotomy correlate with known increases in beta-actin synthesis and axonal transport.

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617.19

AXOTOMY OF MOTOR NEURONS ELEVATES c-JUN AND JUN D LEVELS. L.M. Lund, L.A. Campbell and I.G. McQuarrie*, VA Medical Center and Case Western Reserve University, Cleveland, OH

Stimulation of quiescent cells has been found to cause an early increase in mRNAs encoding regulatory proteins. Jun proteins regulate transcription and mediate mitogenic signal transduction. One member of this family, Jun D, is refractory to many signals that induce c-Jun. We have found that axotomizing lesions of sciatic motor neurons increase c-Jun mRNA expression in a manner similar to that described for beta-actin. Jun D also was constitutively expressed and increased during axon outgrowth. Using the L4/L5 spinal cord region, mRNA was extracted for Northern blot analysis from rats receiving bilateral axotomies (vs. bilateral sham-lesions) or unilateral axotomy (vs. contralateral sham-lesion). The cord was hemisected after unilateral lesions. Lesions were made either 20-30mm or 60-70mm from the spinal cord. Jun D and c-Jun mRNA levels increased by 24 hours after bilateral sciatic nerve crush and peaked by 4 days. Unilateral axotomy caused similar increases. By 14 days, these mRNAs had returned to near-control levels. Immunostaining reflected these changes. Jun D can mediate differentiation through homodimers or heterodimers with c-Jun, c-Fos and CREB, is nontransforming, and is a negative regulator of fibroblast growth. We are interested in Jun D as a potential negative regulator of gene expression during axonal regeneration. Supported by grants to I.G.M. from the DVA.

617.20

Trk B IN RAT SPINAL MOTONEURONS AFTER SCIATIC NERVE LESION. L.A. Campbell* and I.G. McQuarrie, VA Medical Center and Case Western Reserve Univ., Cleveland, OH 44106.

Neurotrophins act as trophic factors for specific cell groups in the nervous system during development and regeneration. Trk proto-oncogene receptors mediate these effects. Studies that localize trk B mRNAs in specific groups of neurons have been done using in situ hybridization. We have used a trk B polyclonal antibody for immunohistochemistry to localize trk B receptor protein in cerebellum, cerebral cortex, and spinal cord. To study effects of axotomy, we transected the L4/5 spinal nerves (with contralateral sham lesions), and retrogradely labeled motoneurons with Fluoro-Ruby. Immunohistochemistry was performed with a biotinylated secondary antibody conjugated with horseradish peroxidase, using a methyl green counterstain. Preliminary data at 4 days post-axotomy showed an increase in the number of trk B immunoreactive motor neurons on the axotomized side, consistent with the localization of fluorescence. This increase in trk B receptor protein above basal levels corresponds to known increases of its ligand, BDNF, in target tissues. (Supported by grants from the DVA).

NEUROGLIA AND MYELIN IV

618.1

TAURINE PRODUCTION FROM CYSTEINE IN CEREBRAL ASTROCYTE CULTURES EXPOSED TO HYPEROSMOLALITY. J.W. Beetsch* and J.E. Olson. Dept. Emerg. Med., Wright State Univ. Sch. of Med., Dayton, OH 45435.

Hyperosmotic conditions lead to elevated astrocyte taurine content as an adaptive mechanism to altered cell volume. To investigate the mechanisms underlying this increase in taurine content, ^{35}S-taurine production from ^{35}S-cysteine was measured in rat cerebral astrocytes grown in isoosmotic and hyperosmotic conditions.

Primary astrocyte cultures were prepared from 2-4 day-old rats. Culture medium was changed twice weekly. After 2 weeks, cultures were exposed to control medium (300 mOsm) or hyperosmotic medium (450 mOsm). After 1, 8, 24, or 48 hr, culture medium was removed and replaced with PBS containing ^{35}S-cysteine together with 1 mM unlabeled cysteine and sufficient NaCl to match the osmolality of the growth medium. 1 hr later, ^{35}S-taurine was extracted from the cells, isolated by HPLC, and quantified by radioactivity counting. Intracellular taurine content also was determined by HPLC.

Taurine production from cysteine decreased 38% after 1 hr exposure to hyperosmotic medium compared to control cultures. ^{35}S-taurine production recovered to a stable level within 24 hrs and remained constant at 48 hrs; however, ^{35}S-taurine production in hyperosmotically treated cultures was less than the production in control cultures at all time points measured.

Intracellular taurine concentration decreased in both control and hyperosmotic cultures 1 and 8 hrs after the medium change when compared to untreated cells. After 24 and 48 hrs in hyperosmotic medium cellular taurine concentrations were increased 70% and 24%, respectively compared to cultures grown in 300 mOsm medium.

Thus, hyperosmotic treatment of cerebral astrocyte cultures reduces the conversion of ^{35}S-cysteine to ^{35}S-taurine despite the elevated taurine content in these culutures. These data suggest that, while taurine content of the cultures is increased by prolonged exposure to hyperosmotic medium, exogenous cysteine does not contribute to the elevated intracellular levels of taurine. Supported by NIH (NS 23218).

618.2

DIFFERENTIAL EFFECTS OF BETA AND GAMMA INTERFERONS ON EXPRESSION OF MHC ANTIGENS AND ICAM-1 IN HUMAN ASTROCYTES IN CULTURE. J.-I. Satoh*, D.W. Paty and S.U. Kim. Div. of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, BC, Canada V6T 2B5.

Recent clinical trials have shown that recombinant human interferon beta (IFN-β) was effective in reducing exacerbations in relapsing-remitting multiple sclerosis (MS), while interferon gamma (IFN-γ) increased the frequency of relapses (Neurology 37:1097, 1987 & 43:655, 1993). To investigate mechanisms underlying the immunomodulatory effects of IFN-β and IFN-γ in MS, interferon-induced expression of class I/II major histocompatibility complex (MHC) antigens and intercellular adhesion molecule-1 (ICAM-1) was investigated in cultured fetal human astrocytes by flow cytometry and immunocytochemistry.

Under the basal condition, class I antigen and ICAM-1 were expressed in moderate numbers (23-76%) of astrocytes, whereas class II MHC antigen was expressed only in small numbers (0.3-8%). Following a 72-hour treatment with IFN-γ (10-100 U/ml), expression of all three antigens was increased greatly. However, IFN-β (10-100 IU/ml) did not induce significantly expression of class II MHC antigen or ICAM-1, while expression of class I MHC antigen was elevated. Furthermore, IFN-β significantly reduced IFN-γ-induced expression of class II MHC antigen but not of class I MHC antigen or of ICAM-1. The differential effects of IFN-β and IFN-γ on expression of class I/II MHC antigen and ICAM-1 in human astrocytes suggest that interferons act as modulators of astrocyte function in inflammatory demyelinating lesions of MS.

618.3

SYNERGISTIC ACTIVATION OF AP-1 COMPLEXES BY ATP AND bFGF IN ASTROCYTES. J.T. Neary, Q. Zhu, J.H. Bruce*, A.N. Moore[1], and P.K. Dash[1]. Lab. Neuropathol., Research Service, VA Med. Ctr., [1]Dept. Neurobiol. & Anat., Univ. Texas, Houston, TX, and Dept. Pathol., Univ. Miami Sch. Med., Miami, FL 33125.

Reactive gliosis that occurs following CNS injury is characterized by hypertrophy and proliferation of astrocytes. ATP and bFGF, agents that are increased after brain injury, can act synergistically to stimulate gliotic-like responses in astrocytes. c-Jun and c-Fos are also increased in response to brain injury. Therefore, we hypothesized that ATP and bFGF may enhance the expression of these transcription factors. To test this hypothesis, we examined the effect of ATP and/or bFGF on the level of AP-1 complexes, heterodimers consisting of Fos and Jun families of transcription factors. Primary rat astrocyte cultures were treated with ATP (100 uM), bFGF (25 ng/ml), or ATP + bFGF, nuclear extracts were prepared, and DNA binding activity was measured by a gel mobility-shift assay using a ^{32}P-labeled AP-1 consensus oligonucleotide. Binding to AP-1 was negligible in untreated cultures. After 1 hr of treatment with ATP or 3 hr treatment with bFGF, AP-1 binding was increased. Interestingly, a synergistic effect between ATP and bFGF was observed at 3 hr. The DNA-protein interaction was sequence-specific because the shifted bands were markedly reduced when excess unlabeled AP-1 oligonucleotides were included in the binding assay. These results indicate that ATP and bFGF induce AP-1 complexes and suggest that c-Jun and c-Fos stimulate changes in gene expression that mediate the gliotic-like responses elicited by ATP and bFGF. (Supported by the Department of Veterans Affairs.)

618.4

CYTOKINE PRODUCTION BY HUMAN ASTROCYTES. J. N. Stewart, Nalbantoglu, H. Durham* and J.P. Antel. McGill University and Montreal Neurological Insitute, Montreal, PQ, Canada.

Pro- and anti-inflammatory cytokine production by astrocytes and microglia in the central nervous system (CNS) may modulate the immune response in this compartment in pathological conditions. Tumor necrosis factor (TNF) has been detected by immunocytochemical techniques in inflammatory CNS disease. TNF and IL-6 mRNA have been variably observed by Northern blotting and in situ hybridization in primary cultures of fetal human astrocytes maintained under basal conditions or following stimulation with IL-1, LPS or IFN-γ (Lee et al, 1993; Sebire et al, 1993; Aloisi et al, 1993). In situ hybridization is particularly applicable to the study of adult human astrocytes which can only be obtained in mixed cultures containing microglia. Using ^{35}S-labelled riboprobes we detected TNF and IL-6 mRNA in fetal bovine serum-containing cultures of fetal (15-20 weeks of gestation) astrocytes under basal conditions and after stimulation with LPS and IFN-γ. Signal was also detected in smears of immediately ex vivo fetal CNS tissue. In contrast to cultured fetal astrocytes, IL-6 and TNF mRNA could only be detected in a proportion of adult astrocytes under basal conditions. This proportion increased after stimulation with lipopolysaccharide and IFN-γ. Both cytokines were detected in all of the microglia in these cultures under both basal and activating conditions.

618.5

NOVEL INHIBITORS OF ASTROCYTE GLUTAMINE SYNTHETASE. K.J. Farrell,* D.K. Willis,[1] and R.A. Swanson. Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94121, and [1]USDA/ARS, Dept of Plant Pathology, Univ of Wisconsin Madison

Glutamine synthetase is found in glia but not neurons. L-methionine-d,l-sulfoximine (MSO) has proven useful in the study of glial functions as an inhibitior of glutamine synthetase. However, MSO has many other effects that may be unrelated to this action. This study identified alternative compounds that can inhibit glial glutamine synthetase. Inhibition of free enzyme prepared from sheep brain (Sigma) was determined by the method of Meister. Inhibition in primary rat cortical astrocyte cultures was also assessed using a 3 hour incubation of intact cultures with these compounds, followed by washing and lysing of the cells.

Compound	IC_{50} (free enzyme)	IC_{50} (astrocyte cultures)
methionine sulfoximine	91 ± 6.1μM	13.1 ± 18.1 μM
glufosinate	10 ± 2.9	0.007 ± 0.003
phosalacine	> 2000	7.0 ± 0.88
tabtoxin lactame	400 ± 65	0.3 ± 0.032
4-N-diaminobutyric acid	212 ± 2	> 2000
oxetin	1500 ± 250	> 2000

Inhibitor concentrations of up to 2mM did not cause astrocyte death, as assessed by lactate dehydrogenase activity of the cultures 24 hours post-incubation. Previous work with MSO has suggested a link between astrocyte glutamine and glycogen metabolism. The effect of the novel inhibitors on astrocyte glycogen accumulation was assessed by the fluorometric method of Passonneau and Lowry. Each of the agents that inhibit astrocyte glutamine synthetase were also found to increase glycogen accumulation, suggesting a causal relationship. These agents may be useful alternatives to MSO for the study of glial functions.

618.6

INHIBITION OF PROTEIN SYNTHESIS PREVENTS INTERLEUKIN 1 α (IL1)-INDUCED INCREASES IN PROSTAGLANDIN (PG) PRODUCTION IN OVINE ASTROGLIA. C. Thore*, M. Nam, and D. Busija. Dept. of Physiology & Pharmacology, Bowman Gray Sch. of Med., Winston-Salem, NC 27157-1083.

Little is known concerning actions and mechanisms of cytokines on prostaglandin production in astroglia. The purpose of this study was to examine short term (1-4 hour) effects of IL1 on PG production in astroglia cultured from fetal sheep. We tested the hypothesis that increased PG production is dependent upon continued or enhanced protein synthesis. Immuno-identified astroglia from second passage fetal ovine cortex were grown to confluence in 12-well (22mm) plates. $PGF_{2\alpha}$ levels in medium were determined using enzyme immunoassay. Cells were exposed to 10 ng/ml IL1 in medium or to medium alone. Application of IL did not increase $PGF_{2\alpha}$ levels at 1 hour but augmented the production of $PGF_{2\alpha}$ from 1586±303 to 2868±393 pg/ml at 2 hours (n=29; $p<0.05$) and from 1356±369 to 5426±1571 pg/ml at 4 hours (n=20; $p<0.05$) Coapplication of an inhibitor of prostaglandin H synthase (indomethacin, 10 μg/ml; n=8) or of phospholipase A_2 (quinacrine, 10^{-6}M; n=8) prevented increases in $PGF_{2\alpha}$ production, as did H-7 (10^{-4}M; n=5), an inhibitor of protein kinase C. Further, coincubation of IL1 with actinomycin D (1 μg/ml; n=9), a RNA synthesis inhibitor or cycloheximide (10 μg/ml; n=9), an inhibitor of protein synthesis, completely blocked the increase in $PGF_{2\alpha}$. We conclude that IL1 increases $PGF_{2\alpha}$ production in ovine astroglia via a mechanism involving several steps, including activation of protein kinase C and phospholipase A_2, and continued or enhanced protein synthesis. Supported by HL-30260 and HL-46558.

618.7

IDENTIFICATION OF VOLUME-SENSITIVE ORGANIC OSMOLYTE CHANNELS IN HUMAN GLIAL CELLS. P.S. Jackson,* K. Strange, and J. R. Madsen. Depts. Neurosurgery and Medicine, Children's Hospital; Program in Neuroscience, Harvard Medical School, Boston, MA, 02115.

Regulation of cellular volume is a fundamental process critical to the survival of all cells, particularly in the central nervous system where even minor volume changes can cause severe neurological damage. Over the past several years much has been learned about the basic biology of cell volume control. It has become clear that organic osmolytes such as taurine and *myo*-inositol play a central role in brain volume homeostasis. During periods of cellular swelling these solutes are lost from cells through a shared transport pathway which is now known to be a relatively non-selective Volume Sensitive Organic osmolyte / Anion Channel (VSOAC; Jackson and Strange, *Am. J. Physiol.*, 465:C1489-1500, 1993). This channel requires non-hydrolytic binding of cytoplasmic ATP in order to activate in response to cell swelling. VSOAC has not been previously demonstrated in human glial cells. We report here that the channel is present in primary cultures of human cortical astrocytes and astrocytic tumors. VSOAC was activated by swelling in cells from four different patients suggesting that this channel is a consistent feature of human cortical astrocytes. The channel is outwardly rectified and inactivated at membrane potentials more positive than +60mV. Single channel measurements demonstrated a ~40 pS channel with inactivation kinetics analogous to those seen in whole cell data. These characteristics are effectively identical to those observed in model systems such as rat C6 glioma cells. Clinically available drugs (e.g., ketoconazole, clotrimazole) are capable of blocking VSOAC. Development of drugs that open or enhance the activity of this channel may provide new strategies to reduce cerebral edema and cellular swelling during brain injury and ischemia.

618.8

CHARACTERIZATION OF VIP-INDUCED PROTEINS AND mRNA IN MOUSE ASTROCYTES BY 2-D GEL ELECTROPHORESIS AND BY mRNA DIFFERENTIAL DISPLAY (DD). P.J. Magistretti[1], G. Pellegri[1], C.L. Bolis[2]* and J-R. Cardinaux[1]. [1]Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland. [2]Department of Biology, University of Milan, Italy.

In primary cultures of mouse cerebral cortical astrocytes, a rapid glycogenolysis followed by a massive glycogen resynthesis (six- to ten-fold over basal levels after 9 hr) are induced by vasoactive intestinal peptide (VIP) or noradrenaline (NA). Both actions of the neurotransmitters are mediated by cAMP. Since the induction of glycogen resynthesis triggered by VIP or NA is abolished by inhibition of transcription and translation, we applied the 2-D gel electrophoresis and the mRNA DD techniques to search for newly synthetized astrocytic gene products induced by VIP or NA. The comparison of ^{35}S-labeled proteins from primary astrocyte cultures treated or not with VIP 1 μM or NA 100 μM reveals 8 proteins in which labeling is increased after the treatment. We focused our attention on a 23 kDa protein (pI≈5.8) which seemed abundant enough for purification and microsequencing. The effect of NA is mimicked by isoproterenol, whereas methoxamine, an α1-agonist, is inactive, thus suggesting that the induction of this protein is mediated by adrenergic receptors of the β-subtype. As a complementary approach, the identification of VIP- and NA-modulated genes has been carried out by the technique of DD (Liang and Pardee 1992, Science 257:967). Using this technique we have observed several differences between the patterns of amplified mRNAs from untreated cultures and from cultures exposed to VIP 1 μM or NA 100 μM. cDNA fragments differentially expressed in astrocytes can be isolated from the gel and cloned into vectors. We are presently verifying by Northern blot that astrocytes treated with VIP or NA express higher levels of the mRNAs identified by DD.

618.9

EXPRESSION AND CHARACTERIZATION OF ATP- AND 5'-NUCLEOTIDE-RECEPTOR(S) IN *XENOPUS* OOCYTES INJECTED WITH poly(A^+) mRNA FROM RAT CORTICAL ASTROCYTES. B.F. King, [a]J.T. Neary*, [a]Q. Zhu, [a]M.D. Norenberg, S. Wang and G. Burnstock. Dept. Anatomy & Devel. Biol., University College London, WC1E 6BT, UK, and [a]VA Medical Center, Dept. Pathology, Univ. Miami, Fl. 33125, USA.

Burnstock, Barnard and colleagues (*FEBs Lett.*, **324**, 219-225, 1993; *TIPs*, **15**, 67-70, 1994) have reported cloning a G-protein coupled ATP-receptor from central neurons that has been designated a P_{2y1}-purinoceptor (with 2-methylthio-ATP>ATP>ADP, but UTP inactive). As a corollary to the study of neuronal purinoceptors, we have investigated ATP receptors on astroglial cells. Astrocytes were harvested from rat neonatal cortices and grown to confluence on culture dishes for 21-28 days. Total RNA was extracted from primary cultures using the Guanidinium method and poly(A^+) mRNA purified with biotinylated oligo(dT) and streptavidin-coated magnetic particles. *Xenopus* oocytes (stages V/VI) were injected with mRNA (50ng) and incubated in Barth's solution for 48h at 18°C. Under voltage clamp conditions, injected oocytes responded to ATP (10^{-8} to 10^{-3}M) with biphasic inward currents that reversed at -20mV, *i.e.* close to E_{CL}. Evoked inward currents were unaffected by the P_1-receptor antagonist, sulfophenyltheophylline (10^{-5}M), but were reduced (by 80%) by the P_2-receptor antagonist, suramin (10^{-4}M). Expressed P_2-purinoceptors were activated equally by 2-methylthioATP and 2-chloro-ATP which were slightly more potent than ATP and ADP; α,β-methyleneATP was weakly active and adenosine inactive. However, UTP also evoked biphasic inward currents and was as potent as ATP and 2-methylthioATP; both CTP and ITP were weakly active. The results suggested either i) expressed P_{2y}-purinoceptors were pharmacologically atypical or ii) heterologous mRNA expressed P_{2y1} and P_{2u}-purinoceptors equally. The possible combination of one or two metabotropic P_2-purinoceptors on astrocytes pharmacologically distinct from the neuronal P_{2y1}-subtype indicates important differences in purinergic signalling at glial cells and central neurons.
Supported by The Wellcome Trust and Department of Veterans Affairs.

618.10

AMMONIA ENHANCES INHIBITORY EFFECTS OF BENZODIAZEPINES AND NEUROSTEROIDS ON K^+ INFLUX IN ASTROCYTES. A.S. Bender* and M.D. Norenberg. Lab. of Neuropathology, Univ. of Miami Sch. of Med. & Vet. Adm. Med. Ctr., Miami, FL 33101.

Levels of ammonia and endogenous benzodiazepines (BZDs) (eg. DBI) are increased in hepatic encephalopathy (HE). Since astrocytes are involved in HE, and since K^+ uptake in astrocytes plays a major role in CNS K^+ homeostasis, we examined the effects of BZDs, as well as various neurosteroids (products of peripheral-type BZD receptor activation) on K^+ uptake. Rat astrocytes in primary culture were incubated with 2 μCi/ml of ^{86}RbCl (analog of K^+) for 1 hr in the presence of BZDs and neurosteroids with or without 5 mM NH_4Cl. Ammonia alone inhibited K^+ uptake by 26%. Agents that interact with the peripheral-type BZD receptor (eg., Ro5-4864 and PK 11195 at 30 μM) inhibited K^+ uptake by ~25%, whereas agents that interact with central-type BZD receptor (clonazepan and flumazenil at 30 μM) did not affect K^+ uptake. The effect of Ro5-4864 on K^+ uptake was significantly enhanced in the presence of 5 mM ammonia (55% inhibition). Pregnenolone sulfate (50 μM) and 5α-pregnane-3α,21-diol-20-one (5α-THDOC; 30 μM) inhibited K^+ uptake by 30 and 20%, respectively. The effects of neurosteroids were enhanced in the presence of ammonia (41-50% inhibition). Thus, agents involved in HE are able to affect astrocyte K^+ homeostasis which may result in altered CNS excitability. (Supported by the Veterans Administration and USPHS grants AM-38153 and NS-30291, and GRECC)

618.11

EFFECTS OF AMMONIA AND BENZODIAZEPINES ON ADENOSINE UPTAKE IN ASTROCYTES. R.S. Dombro*, A.S. Bender, and M.D. Norenberg. Lab. of Neuropathology, Univ. of Miami Sch. of Med. & Vet. Adm. Med. Ctr., Miami, FL 33101.

The mechanism of neuroinhibition in hepatic encephalopathy (HE) is not known. Benzodiazepines (BZDs) are elevated in HE and are known to reduce the uptake of adenosine, an inhibitory neuromodulator. This study was undertaken to determine whether agents acting on peripheral and central BZD receptors, products of peripheral BZD receptor activation (neurosteroids), as well as ammonia (elevated in HE), affect adenosine uptake. Rat astrocytes in primary culture were incubated with 1 μM [^{3}H]-adenosine (0.2 μCi/ ml) for 30 min in the presence or absence of the ammonia, BZDs and neurosteroids. Adenosine uptake was inhibited by 9% in the presence of 5 mM NH_4Cl; however, 2-3 days of prior treatment with ammonia resulted in a 20-25% decrease in adenosine uptake. Agents that interact with the peripheral (Ro 5-4864 and PK 11195) and central (clonazepan and flumazenil) BZD receptors at 10 μM, reduced adenosine uptake by ~50%. Endogenous BZDs, DBI (40 μM) and ODN (10 μM), inhibited adenosine uptake by ~14%. Pregnenolone sulfate (10 μM) and 5α-pregnane-3α,21-diol-20-one (5α-THDOC; 50 μM) also decreased adenosine uptake by 20 and 60%, respectively. Suppression of adenosine uptake by ammonia, BZDs and neurosteroids may contribute to the neuroinhibition in HE. (Supported by the Veterans Administration and USPHS grants AM-38153 and NS-30291, and GRECC)

618.12

REVERSIBLE, IN VIVO, CHANGES IN ASTROCYTE MORPHOLOGY. J.B. Bobak* and A.K. Salm. Department of Anatomy, West Virginia University, Morgantown, WV, 26505

Rearrangement of astrocyte processes has been suggested to participate in CNS plasticity, for example, by influencing synapse formation or neuronal subgroupings. However, little direct evidence for such morphological changes in vivo has been reported. The supraoptic nucleus, which displays much plasticity, was stimulated in adult male rats by replacing drinking water with 2% saline for 2 (n=5) or 9 (n=5) days. Control (n=4) animals received free access to tap water. A rehydrated group (n=5) was given 2% saline for 9 days, then returned to tap water for 9 days. Animals were sacrificed and their brain tissue was processed for electron microscopy. Electron micrograph montages of astrocytes (3,887X) in the ventral glial limitans (VGL) and dendritic zone subjacent to the supraoptic nucleus were constructed and judged, without knowledge of grouping, as to the orientation of perinuclear cytoplasm and/or thick processes. Astrocytes in control animals were found to exhibit cytoplasm oriented mostly vertical and perpendicular to the pial surface. With dehydration, a significant reorientation ($p<.02$) of astrocyte cytoplasm to a horizontal direction, parallel to the pial surface, occurred. The changes in the vertically oriented processes were only observed in those directed ventrally towards the pial surface ($P<.01$). Within individual animals, a highly significant inverse correlation ($r=.93$, $p<.001$) was found between the proportion of cells displaying vertical vs. horizontal orientations. Upon rehydration the astrocytes in the VGL returned to a vertical orientation. From these data we conclude that astrocytes in vivo are capable of reversibly changing their morphology. Supported by BNS-9109827.

618.13

EVIDENCE OF A LOCALIZED ABLATION OF ASTROCYTES BY INTRACEREBRAL INJECTION OF L-α-AMINOADIPATE. M. Khurgel* and G.O. Ivy. Dept. Anatomy & Cell Biology, Univ. of Toronto, Scarborough Campus, West Hill, Ont., M1C 1A4, Canada.

L-α-aminoadipate (LAA) has been proposed to be an astrocyte-specific toxin. However, the *in situ* effectiveness of this compound has not been conclusively demonstrated for adult animals. We have studied the consequences of intracerebral injections of LAA as part of our on-going inquiry into neurono-glial interactions. Adult (7 months old, 570-610 g) Long Evans hooded rats were stereotaxically injected with 5 μl of artificial cerebrospinal fluid (ACSF) or LAA (Sigma, 20 μg/μl in ACSF) into basomedial amygdala/piriform cortex area. The animals were sacrificed by intracardial perfusion 48 hr following the injections. Frozen brain sections were processed for immunohistochemistry with anti-GFAP and anti-vimentin to evaluate the response of astrocytes, as well as lectin histochemistry to visualize microglia. Alternate sections were counter-stained with cresyl violet. The levels of GFAP and vimentin immunoreactivity, the morphology and distribution of astrocytes and microglia, and the density of neurons in the area of injection were compared between the LAA and ACSF rats, and surgical controls. Injections of ACSF resulted in stab wound-like pattern of morphological changes, with neuronal loss and reactive microglia confined to the needle track, and activated astrocytes within 100 μm on either side of it. In contrast, injections of LAA resulted in a 400-800 μm wide space devoid of GFAP-positive astrocytes, but filled with vimentin-positive cellular debris. The selective nature of the lesion was supported by the presence of activated microglia and a normal density of neurons. We conclude that LAA is an effective astroglial toxin and that this compound may be useful for addressing the functional significance of astrocytes in mammalian brain.
Supported by NSERC

618.14

ASTROCYTE-SPECIFIC EXPRESSION OF CD44 IN RAT BRAIN CULTURES. N.R. Bhat*, P.S. Zhang and E.L. Hogan. Neurology, Medical University of SC, Charleston, SC 29425

CD44 is a transmembrane glycoprotein that serves as an adhesion molecule in cell interactions, migration and metastasis. Although originally identified in lymphocytes as a homing receptor, CD44 variants have been found in a variety of cell types and tissues including brain. This study examines the expression of CD44 in primary cultures of developing rat brain. Immunocytochemical studies using anti-CD44 antibodies indicated the paucity of expression of this antigen in neuron-enriched cultures. Type-1 astrocytes in primary glial cultures on the other hand displayed a rather high level of expression of CD44, the immunoreactive material being present on an extensive array of thin processes. Oligodendrocytes developing in the same cultures however did not express detectable level of the antigen. In order to examine the expression of the molecule in O-2A lineage cells, the glial progenitors developing in mixed glial cultures were isolated and grown either in the presence of 10% FCS which allows type-2 astrocytic differentiation or under serum-free conditions to promote oligodendrocytic differentiation. Neither O-2A progenitors nor oligodendrocytes contained appreciable level of CD44. Type-2 astrocytes in contrast stained intensely; the staining was more extensive than that of GFAP. The differential cellular distribution of CD44 was confirmed by immunoblot analysis of the cell extracts. The results of this study indicate an astrocyte specific expression of a CD44 variant in the developing brain. Supported by NMS Society RG-2474 and RG-2481.

618.15

ELECTROPHYSIOLOGICAL AND FUNCTIONAL PROPERTIES OF *IN VITRO* BLOOD-BRAIN BARRIER CELLS. K.A. Stanness, H.R. Winn and D. Janigro* Dept. of Neurosurgery, University of Washington, Seattle, WA 98104

Brain microvascular endothelial cells are the site of the anatomical blood-brain barrier (BBB), but astrocytes (AC) interacting with non-brain endothelium (EC) can induce BBB properties. We have established a dynamic cell culture system that allows co-culturing of AC and rat brain microvascular EC (RBMEC) in a hollow fiber artificial capillary system. Bovine aortic (BAEC) or RBMEC loaded together with either C6 rat glioma or rat astrocytes formed a permeability barrier to macromolecules and membrane-impermeant dyes (HRP and Evans blue) after two weeks in culture. In addition, after replating under two dimensional tissue culture conditions, BAEC cell displayed electrophysiological properties normally found in RBMEC. In fact, ATP (1-10 μM) caused activation of K currents in native BAEC, while after exposure to either C6 or AC, BAEC responded to ATP with activation of an inward current carried in part by Ca^{+2}, a property characteristic of RBMEC. During patch clamp experiments, cells were identified based on their uptake of Ac-LDL (EC) or for GFAP positivity (AC and C6). The latter was determined on living cells permeabilized with streptolysin-O and incubated with anti-GFAP sera. GFAP-negative cells were positive for the microglial marker OX-42. We conclude that 1) co-culturing of EC and glial cells in the artificial capillary system leads to the development of barrier properties in EC 2) the cells harvested from the cartridge retain their BBB properties and can be replated in a two dimensional culture amenable for patch clamping 3) the electrophysiological properties of BAEC are altered after co-culture with AC, providing additional evidence that BBB properties can be induced by exposing non-brain EC to glial cells. Supported by NS 51614, NS30305, NS21076 and NS07144.

618.16

ACTIVATION OF POTASSIUM CHANNELS BY GLUCOSE WITHDRAWAL IN CULTURED MICROGLIA. W. Walz* and K. Datta. Dept. of Physiology, Coll. Med., Univ. of Saskatchewan, Saskatoon, S7N 0W0, Canada.

The reaction of microglia to ischemic insults is neither well studied nor well understood. First reports show that microglia are an extremely sensitive indicator of subtle and morphological nonapparent neuronal damage during early stages of ischemic injury. We investigated the question if energy deprivation has a direct effect on physiological properties of cultured mouse microglia. We used the patch clamp technique to record the whole-cell current and the membrane potential. The cells were MAC-1 positive and exhibited the inward rectifier K^+ channel in addition to a K^+ outward current. Glucose withdrawal resulted in the activation of a transient K^+ inward current (130 pA), which is barium-sensitive and has a reversal potential of -65 mV. Within min the transient current disappeared and there was a decrease in the resting conductance to 44%. The membrane depolarized from -76 to -38 mV. If glucose was withdrawn in the presence of antimycin a (a blocker of oxidative metabolism) there was no transient current activation, but a decrease of the conductance to 16%. The membrane depolarized from -90 to -38 mV. It appears that glucose withdrawal alone activates an inward rectifying K^+ channel, whereas total block of ATP production leads to a general shutdown of ion channels, with both situations leading to a large depolarization. The results suggest that microglial ion channels are directly affected by ischemic conditions and might play a role in the cell's sensitivity in early stages of ischemia.

618.17

DIFFERENTIAL INDUCTION OF NITRIC OXIDE SYNTHETASE IN PRIMARY RAT MICROGLIAL VERSUS ASTROCYTE CULTURES. L.A. Radov, T. Ryan, D. Reif and J. Blosser.* Dept. of Biology, Fisons Pharmaceuticals, Rochester, N.Y. 14623.

In culture,microglia and astrocytes can express the inducible Type II form of nitric oxide synthase (NOS). It has been suggested that glia may contribute to neuronal damage associated with cerebral ischemia and/or other neurodegenerative diseases by generating nitric oxide (NO)(Proc. Natl. Acad. Sci. 98:10945-10949;1992) via this enzyme. Since microglia and astrocytes represent two distinct populations in the brain, we investigated conditions under which these cell types maximally expressed Type II NOS and produced NO. Primary cultures of neonatal rat microglia and astrocytes were prepared (J. Neurosci.;6(8):2263-2178; 1986) and harvested after 7 days in culture. The purity of each cell population was determined using specific fluorescent probes; acetylated low-density lipoprotein for microglia and anti-GFAP for astrocytes. Microglia and astrocyte cultures were exposed to a variety of stimuli, including bacterial lipopolysaccharide (LPS), gamma interferon, tumor necrosis factor α, IL-1B or a combination of these factors. The cultures were assayed for NOS activity by measuring nitrite levels and the production of L-[^{3}H]citrulline from L[^{3}H]arginine. Increases in Type II NOS mRNA and not other mRNA from other NOS isozymes showed that the observed increases in NO upon treatment were do to transcriptional upregulation of the Type II NOS gene. Quantitative differences in NOS activity was noted when both cell types responded to identical stimuli. However, microglial cultures did not respond to concentrations of LPS alone that were stimulatory for the astrocytes. These results suggest that *in vivo* microglia and astroglia NOS activation may be regulated differentially under different physiological or pathologic conditions.

618.18

REGULATION OF EXPRESSION OF MHC CLASS II ANTIGENS IN RAT ASTROCYTES AND MICROGLIA. R. P. Hellendall* and J. Ting. Lineberger Comp. Cancer Ctr., Univ. North Carolina, Chapel Hill, NC 27599.

We are studying factors regulating the expression of major histocompatability complex (MHC) class II (Ia) antigens on rat astrocytes and microglia to better understand the role of these immune-related molecules in various non-neuronal cells in the CNS. The function of MHC class II expression in the brain is poorly understood; the induction of these molecules during a number of neurodegenerative diseases indicates a possible role in the progression of pathology. Astrocytes and microglia were isolated from neonatal rat pups and stained for surface expression of Ia or transfected with a plasmid construct containing the human MHC class II promoter (HLA-DRα) driving a chloramphenicol acetyltransferase (CAT) gene (astrocytes only) to examine promoter function. Cells were treated with interferon-γ (IFN-γ), a known inducer of class II expression, in combination with a number of effectors of intracellular signaling pathways. Preliminary data indicate induction of surface expression on astrocytes by IFN-γ can be inhibited by blocking protein kinase C activity. PKC inhibition also resulted in a reduction in microglial class II surface signal although expression remained above background. Our findings also show that selective inhibition of protein tyrosine kinase fails to inhibit IFN-γ driven class II expression in either cell phenotype. Parallel experiments with transfected cells produced similar patterns of expression from the human promoter. This work suggests MHC class II expression on astrocytes and microglia may be under similar regulatory control through PKC and some tyrosine kinases but may differ in their sensitivity to external stimuli.

618.19

EVIDENCE FOR THE PROLIFERATION OF MICROGLIA AFTER EXCITOTOXIC INJURY OF ADULT BRAIN. E. Welter*, S. Collins, J.R. Fredieu, and D.M.D. Landis. Dept. Neurology, Case Western Reserve University, Cleveland, OH 44106.

When adult mammalian brain is injured, there is a complex response of non-neuronal cells which includes cell proliferation generating astrocytes in the affected region. We are using the technique of retrovirus-mediated gene transfer to identify the lineage and characteristics of cells proliferating after injury.

Kainic acid was injected into the rat caudatoputamen to kill neurons, and 48 hours later LZ1 BAG virus was injected at the same site, to infect proliferating cells and transfer the reporter gene β-galactosidase. We have used immunocytochemical detection of β-galactosidase immunoreactivity to detect proliferating cells in the tissue at various intervals after the injection of virus. This approach circumvents the technical problem that cytochemical demonstration of ß-galactosidase activity may obscure immunoreactivity detected with immunofluorescence methods.

Forty eight hours after viral injection, most of the β-galactosidase-immunoreactive cells lacked GFAP immunoreactivity. In many instances, the shape of cells with β-galactosidase immunoreactivity was similar to that of cells expressing OX-42 immunoreactivity, a marker of microglia. We are now examining the ß-galactosidase-immununoreactive cells for the presence of microglial and macrophage cell markers such as OX42 and ED1 at several survival intervals after injection of the retrovirus.

618.20

ION CHANNELS AND OXYGEN RADICAL PRODUCTION IN ACTIVATED MICROGLIA. B.A. Ballyk*, D.J. Phipps, P.S. Pennefather and L.C. Schlichter. Playfair Neurosciences Unit, Toronto Western Hospital, Toronto, Canada, M5T 2S8.

The objective of the present study was to characterize ion channel expression in activated microglia and their ability to release oxygen radicals.

Primary mixed cultures from the brains of newborn Wistar rats were starved to eliminate neurons and then shaken to isolate microglia, which were plated either in 96-well plates for functional assays or on glass coverslips for patch-clamp recording. Cultures which received conditioned media containing colony stimulating factor 1 (CSF-1) showed marked proliferation, as measured by ^{3}H-thymidine incorporation. Microglial cultures were judged to be >98% pure by staining with isolectin B_4.

Whole cell recordings showed that activated microglia expressed at least three ionic currents: an inward-rectifier K^+ current blocked by external Ba^{2+}, a delayed-rectifier K^+ current with several features of the cloned $K_V1.3$ channel, including block by charybdotoxin and margatoxin, and a Cl^- current blocked by flufenamic acid.

In functional assays, microglia activated with opsonized zymosan produced superoxide and nitric oxide, as measured by reduction of cytochrome C and Griess reagent, respectively.

We are now using ion channel blockers in functional assays to determine what roles these channels play in the physiology of microglia.

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GENE STRUCTURE AND FUNCTION V

619.1

DIFFERENTIAL EXPRESSION OF DNA CLONES CODING FOR INHERITED "HIGH" OR "LOW" HUMAN CATECHOL-O-METHYLTRANSFERASE (COMT). MH Grossman*, R Weinstein and JB Littrell. Dept. of Pediatrics, Temple Univ. Schl. of Med., St. Christopher's Hosp. for Children, Phila., PA 19134.

Inherited variations of human catechol-O-methyltransferase (COMT) activity levels are controlled at a two allele, autosomal locus. The "low" activity enzyme is also relatively thermolabile, compared to the "high" activity form. A single nucleotide change at position #472 resulting in an adenosine in *L/L* individuals and a guanosine in most *H/H* individuals has been identified. PCR-amplified coding regions from *L/L* and *H/H* specimens were subcloned into the pGEM-3Z vector and protein was expressed using the TnT lysate coupled transcription/translation system (Promega). ^{35}S-methionine-labelled expressed proteins were analyzed by SDS-PAGE followed by autoradiography. Two major protein bands of 25.5 and 29 kd, observed in each lane corresponded to soluble and membrane-bound forms of the COMT enzyme. SDS-PAGE analysis of lysate proteins, following immunoprecipitation with antisera to soluble COMT enzyme, revealed that the relative intensities of radioactive bands from the *H/H* specimen were greater than those from the *L/L* specimen. Scanning densitometry indicated that the relative amount of radioactive immunoprecipitable protein in the 25.5 kd band from the *H/H* specimen was 2.3 times greater than the corresponding band from the *L/L* specimen. This is in excellent agreement with measured liver COMT activity levels in these two specimens. These findings are supported by results on Western blots of human liver homogenates from individuals with inherited *L/L* and *H/H* genotypes. These studies provide good evidence that the single base change in the COMT coding region influences regulation of expression of the human COMT gene either at the level of transcription, translation or degradation of protein.

619.2

MAPPING THE ACHIASMATIC MUTATION IN THE DOG: A PROGRESS REPORT. Qing Tang*, Robert. W. Williams, Dan Goldowitz. Department of Anatomy & Neurobiololgy U.T. Memphis, 875 Monroe Ave., Memphis, TN 38163.

We have characterized a mutation in Belgian sheepdog that disrupts the formation of the optic chiasm (Williams et al, 1994). The pattern of inheritance over 6 generations indicates that the mutant allele is autosomal recessive (symbol NOX, no optic chiasm). To identify NOX we have taken complementary approaches. Candidate gene approach: Several mutations at the tyrosinase gene locus (TYR) are known to perturb ganglion cell projections at the chiasm. We have used primers derived from conserved sequences of human and mouse TYR to clone exon 1 of dog homolog through use of the polymerase chain reaction. The relationship between TYR and NOX will be determined through sequence comparison and linkage analysis. Chromosome mapping: We have begun to map canine genes that are linked on mouse chromosome 7 over a 25 cM interval. This interval, located between the *p* locus and the calcitonin gene, contains several loci that play key roles in brain development and pigmentation, including the TYR locus. To define the linkage group(s) in the dog, we outcrossed a mutant Belgian to a Siberian Husky, having first determined that the two breeds are genetically heterogeneous by using random amplified polymophic DNA (RAPD) markers (Goldowitz et al, in progress). The F1 progeny were backcrossed to mutant Belgians. DNAs from the backcross offspring are being typed using several forms of markers, including restriction fragment length polymorphism, polymorphic microsatellites, and RAPD. This will allow us to begin constructing a molecular linkage map of the dog genome. We are also screening a dog genomic library to obtain probes that will be used to assign chromosomal position amongst linked loci via fluorescence *in situ* hybridization.

Support: NEI RO1-9586

619.3

SOMATIC GENE TRANSFER IN THE ADULT CENTRAL NERVOUS SYSTEM: PRACTICAL AND THEORETICAL ISSUES. N.H. Liou, L.J. Fisher, J. Ray, S. T. Suhr, S. Thode, A. L. Miller* and F. H. Gage. Department of Neurosciences, University of California-San Diego, La Jolla, CA 92093-0627.

Somatic gene transfer has been extensively investigated using genetically modified primary fibroblasts as grafts for intracerebral transplantation in our laboratory. We use defined retroviral vector to investigate gene expression and regulation in the adult central nervous system (CNS) on a molecular and cellular basis. Our data suggest that expression from retroviral promoters/enhancers decreases with the onset of quiescence *in vitro* and in grafted fibroblasts *in vivo*. When genetically modified fibroblasts are implanted into the host CNS, the lack of tumor formation indicates that the cells reside in a quiescent status within the grafting area. As a consequence, it is necessary to enhance the capacity of engineered fibroblasts to express transgene products in a quiescent state, and the objective of this project is to examine strategies of achieving long-term stable and/or regulatable transgene expression in primary fibroblasts *in vitro* and following implantation into the adult rat CNS. To achieve this goal we use several strategies: 1) The effect of different viral, tissue-specific and housekeeping promoter/enhancer configurations in retroviral constructs is analyzed in growing and confluent cells *in vitro*. Fibroblast cultures in different serum levels have been established that may mimic conditions in the *in vivo* grafts. The effect of the size and composition of different transgenes on their expression is analyzed under the same conditions. 2) We also analyze whether potentially negative regulatory elements can be deleted from viral promoters/enhancers to direct transgene expression constitutively, and 3) The influence of exogenous factors on transgene expression is analyzed for full-length as well as truncated promoters/enhancers. In addition to *in vitro* experiments long-term stable and/or regulatable expression from various promoter/enhancer combinations is also monitored *in vivo* after grafting. Our results suggest that retroviral gene expression can be regulated by dexamethasone and retinoic acid *in vitro* and *in vivo*.

619.4

DEFECTIVE HSV VECTORS FOR THE STUDY OF GLUTAMATE DECARBOXYLASE EXPRESSION IN CELLS OF THE CNS

K. C. New[1*], R. L. Martuza[2], K. Gale[3], S. D. Rabkin[1,2]. Departments of Microbiology[1], Pharmacology[3] and Neurosurgery[2], Georgetown University Medical Center, Washington, DC 20007

The plasmid-based defective herpes simplex virus (HSV) vector system offers an efficient means to deliver and express genes in cells of the central nervous system (CNS). Glutamic acid decarboxylase (GAD) is the single enzyme in the mammalian brain that converts glutamate to γ-aminobutyric acid (GABA). Mammalian GAD exists as two isoforms of 65 and 67 kD, encoded by different genes.

GAD_{65} or GAD_{67} cDNAs under control of the CMV immediate-early promoter were inserted into HSV amplicon plasmids. Double-cassette defective HSV vectors, containing separate transcription units for both GAD and E. coli lacZ, were generated with a conditional-lethal helper HSV. Because GAD is expressed throughout the brain, the presence of the lacZ marker enables us to detect and quantify vector infection *in vivo*.

These defective vectors were used to infect Vero cells and primary cultures of cerebellar granule cells (primarily glutamatergic) and cortical astrocytes. Infection resulted in the expression of both ß-galactosidase and GAD in each of the cell types, as determined by immunocytochemistry. The expressed GAD protein was active and capable of GABA synthesis.

These vectors should permit us to determine the effects of GABA synthesis on glutamatergic neurons and glia *in vitro*, as well as modulate local concentrations of glutamate and GABA *in vivo*.

619.5

DIFFERENTIAL DISPLAY OF PCR-AMPLIFIED mRNA AS A SCREENING TECHNIQUE IN THE BRAIN. J.M. Babity*, B.J. Chiasson, and H.A. Robertson. Dept. of Pharmacology, Dalhousie Univ., Halifax, N.S., Canada, B3H 4H7.

We have adopted a technique of differential display of PCR-amplified mRNA to examine the nature and extent of changes in gene expression in mammalian brain. This technique utilizes a set of oligonucleotide primers, one anchored to the poly A^+ tail of the message and the other located upstream of the poly A^+ tail. While this technique has previously been used to study changes in cultured cells, little information is available on the application of this technique to heterogeneous tissues such as the brain. Currently, we are using this technique to examine changes in gene expression associated with seizure activity and focal cerebral ischemia. In the first example, we have isolated mRNA from animals that have been kindled to stage 5 seizures and left seizure free for at least 2 weeks or from animals acutely treated with kainic acid. Electrode implanted or vehicle injected animals served as a sources of control mRNA. In the second example, we are studying changes in gene expression associated with a model of focal cerebral ischemia based on the photochemical induction of thrombotic stroke using the dye Rose Bengal. We have identified a number of differentially displayed messages in the brains of animals that have been kindled or have experienced kainic-acid-induced seizures. Differentially displayed messages have been isolated, PCR amplified, and subcloned into plasmid vectors. We are attempting to use both PCR-amplified material directly and subcloned material in Northern blotting experiments to further characterize gene expression associated with these differentially displayed messages in control and experimental animals. It appears that this technique is useful for studying changes in gene expression in the brain.

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619.6

WITHDRAWN

619.7

FACTORS AFFECTING AVAILABILITY OF ANTISENSE OLIGONUCLEOTIDES IN BRAIN. Y. Yaida, W. A. Pulsinelli* and T. S. Nowak, Jr. Dept. of Neurology, Univ. Tennessee, Memphis, TN 38163.

The use of antisense oligonucleotides (oligos) to interfere with the expression of specific proteins is of demonstrated utility in vitro, and several reports have indicated that this approach may also be applicable to the intact brain. Few investigations have explicitly evaluated the localization of administered oligos within brain. In the present studies we have examined the distribution and stability of oligos after intraventricular (i.c.v.) and intrahippocampal (i.h.) injection in the rat.

These studies used synthetic oligos (19-30mers) antisense to various regions of a rat hsp70 mRNA sequence. Oligos were 3' end-labeled with [^{35}S]thio-dATP, and 50 nCi (1 pmol, 2-5 µl) injected to examine distribution 1-24 h after administration. Frozen sections (15 µm) were cut and subjected to film autoradiography. Localization and stability were evaluated 6-48 h after injection of unlabeled oligos (2 nmol) by in situ hybridization with ^{35}S-labeled sense sequences.

After i.c.v. injection labeled oligo was largely periventricular. A more propagated signal was seen at 6 h or later, especially in white matter tracts. This likely does not represent intact oligo, since in situ hybridization detected only periventricular accumulation. With direct i.h. administration labeled oligo accumulated along the hippocampal fissure, probably reflecting injectate bulk flow. There was occasional prominent labeling of dentate granule cells, and limited CA1 labeling was also seen along the track of the injection. Hybridization at 6-48 h detected neuronal uptake of oligo with a similar distribution, including mossy fibers remote from the injection site. Unmodified oligos can therefore accumulate in neurons after local administration.

619.8

DEVELOPMENT OF PCREB AND FOS-LI IN SUPRAOPTIC NUCLEUS AFTER SALT LOADING. P. Shiromani*, M. Magner, S. Winston, M. E. Charness. *Depts Psychiatry, Neurology, Harvard Medical School, VA Medical Center, West Roxbury, MA 02132.*

Phosphorylation of the cAMP response element binding protein (CREB) precedes the induction of immediate early gene expression. Using antibodies that distinguish CREB from phosphorylated CREB (PCREB), we studied the appearance of PCREB-like immunoreactivity (PCREB-LI) and Fos-LI in the hypothalamic supraoptic nucleus (SON) in response to hyperosmolar stress in rats. Increased numbers of PCREB-LI cells were present at 15, 45, and 90 min after injection with normal or hypertonic saline. The number of PCREB-LI cells did not differ significantly between the two groups and returned to normal after 6 hrs. The SON of hypertonic saline-treated rats showed higher levels of *c-fos* mRNA than that of normal saline-treated rats, and only minimal signal was detected in the SON of uninjected rats. The number of Fos-LI cells increased dramatically at 45 and 90 min after injection of hypertonic saline and did not change at 15, 45, or 90 minutes after injection of normal saline. The discrepancy between levels of PCREB-LI and *c-fos* mRNA suggests that hyperosmolar stress may activate additional transcriptional factors besides CREB. The lack of Fos-LI in the presence of modest increases in *c-fos* mRNA in normal saline-treated rats implies that levels of *c-fos* mRNA must exceed a threshold before increases in Fos-LI cells are detectable in the SON. Such a threshold might permit neuronal cells to activate diverse cAMP or calcium-responsive genes, through phosphorylation of CREB, without inducing the constellation of Fos-responsive genes.

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619.9

SOMATOSTATIN DECREASES SOMATOSTATIN MESSENGER RIBONUCLEIC ACID LEVELS IN THE RAT PERIVENTRICULAR NUCLEUS. M.C. Aguila*. Department of Physiology, UT Southwestern Med. Ctr., Dallas, TX 75235-8873.

Somatostatin (SRIF), the inhibitory hypothalamic peptide of pituitary growth hormone secretion was reported to inhibit its own release by a negative feedback mechanism. However, it is not known whether this negative regulation is exerted at the molecular level. Therefore, this study was conducted to examine the hypothesis that hypothalamic SRIF mRNA levels might be regulated by its own protein. Periventricular nucleus (PeN) from adult male rats were incubated in Waymouth's medium with SRIF or the SRIF analog RC 160 (10^{-9} to 10^{-7}M) for 6 hrs. Levels of SRIF mRNA were determined by a S_1 nuclease protection assay using a ^{32}[P] labelled rat SRIF riboprobe. SRIF (10^{-7}M) significantly ($P \leq 0.01$) decreased SRIF mRNA levels. Likewise, RC 160 (10^{-9} to 10^{-7}M) significantly ($p \leq 0.05$, $p \leq 0.01$ respectively) diminished SRIF mRNA levels. These results suggest that SRIF can regulate its own gene expression by a negative loop feedback. SRIF secreted from these neurons may be down-regulating a preceding stimulatory input. Supported by NIH grant NS26821.

619.10

THE TRANSCRIPTION FACTOR C/EBPδ IS INDUCED BY VIP, PACAP AND NORADRENALINE IN MOUSE CORTICAL ASTROCYTES.
J-R. Cardinaux and P.J. Magistretti*. Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland.

The CCAAT/enhancer binding protein (C/EBP) family of transcription factors belongs to a class of proteins, whose DNA-binding domain involves a basic region and a leucine zipper. This bZIP domain was first described for C/EBP and then for the Fos/Jun and ATF/CREB families. High C/EBP concentrations were found in the nuclei of fully differentiated hepatocytes and adipocytes. Therefore, it has been postulated that the regulation of energy balance may be channeled at least in part through C/EBP. Since we have previously described transcription dependent metabolic effects of VIP and noradrenaline (NA) in astrocytes (Sorg and Magistretti, 1992, J. Neurosci. 12:4923), we have examined the effects of these neurotransmitters on C/EBP protein levels. By Western blot analysis, we observed that C/EBPδ is rapidly induced by VIP 10 nM, by the VIP-related peptide PACAP at 10 nM or by NA 1 μM in mouse astrocytes prepared from the cerebral cortex. The effect of NA can be mimicked by the β-agonist isoproterenol, whereas methoxamine an α1-agonist is devoid of any effect. Moreover, the NA-induction can be antagonized by atenolol and not by prazosine, thus suggesting that the induction of C/EBPδ is mediated by β-subtype adrenergic receptors. VIP, PACAP and NA therefore probably increase the C/EBPδ expression via the cAMP second-messenger pathways. To our knowledge this is the first demonstration of the induction by neurotransmitters of a member of the C/EBP family in a mammalian brain cell type. We are currently searching putative target genes whose expression is modified in response to the C/EBPδ induction.

619.11

REGULATION OF Na AND K CHANNEL mRNA IN CULTURED NEUROBLASTOMA CELLS. F.N. Quandt*, J.K. Hirsh, and D. Sievert. Mol. Biophys. & Physiol. & M.S. Center., Rush Univ., Chicago, IL 60612

Factors which regulate transcription of mRNA for voltage-dependent ion channels were identified using cultured N1E-115 neuroblastoma cells as a model system. Na and K channel-specific mRNA was measured using a competitive-polymerase chain reaction technique following reverse-transcription of isolated total RNA. Amplified cDNA was sequenced to confirm identity of the transcript, and the absence of genomic DNA was verified. Cell division can be arrested resulting in an enhanced differentiated phenotype during incubation of cells in medium having reduced serum and 1.5% dimethylsulphoxide. K channel (a *shaw* homolog) mRNA abundance was found to increase by approximately 100% under this condition, but there was little change in Na channel (either type II or III) mRNA. Incubation of differentiated neurons with 1 μM A23187 or ionomycin for two days decreased Na channel mRNA to 50%, without significant reduction in K channel mRNA. Therefore, Na and K content of these neurons is differentially regulated. Mechanisms responsible for the alteration in Na channel mRNA by Ca loading were studied. Although growth of differentiated cells in high external K did not alter mRNA level by itself, it blocked the decrease in mRNA in the presence of A23187. Na channel mRNA was reduced when cells were incubated in 250 μM chlorophenylthio-cyclic AMP (cAMP). A reduction by cAMP was not observed in cells grown in A23187. These observations suggest that the effect of Ca on transcription is mediated by cAMP, and perhaps coupled to changes in membrane potential. Supported by the National Multiple Sclerosis Society.

619.12

CONSTITUTIVE EXPRESSION OF HEAT SHOCK PROTEIN 70 (HSP70) IN THE RABBIT NERVOUS SYSTEM. P. Manzerra*, S.J. Rush, I.R. Brown, Dept.of Zoology, Univ. of Toronto, West Hill, Ont., Canada, M1C 1A4

Western blot analysis utilizing the ECL detection system has revealed the constitutive expression of stress-inducible hsp70 protein in unstressed (control) rabbit brain. This observation is not unique to rabbit brain as both rat and mouse cerebellum also constitutively express stress-inducible hsp70 protein. Two-dimensional Western blotting has identified a number of inducible hsp70 and constitutive hsc70 isoforms. A range of antibodies which specifically recognize either stress-inducible hsp70, constitutive hsc70 or both members was utilized to verify that the hsp70 family member detected in control tissue was indeed the stress-inducible hsp70 protein. Highest levels of constitutive expression of stress-inducible hsp70 were observed in control retina, brain core and cerebellum with lesser levels observed in cerebral hemispheres, spinal cord and brain stem. Constitutive expression of hsp70 was also detected in non-neural tissue, however these levels were lower than that observed in neural tissue. In response to hyperthermia, immunocytochemical studies reveal a robust induction of stress-inducible hsp70 in a pattern consistent with a strong glial response. Despite the ability of the antibodies to detect stress-inducible hsp70 by Western blotting in both control and heat shocked tissue, no hsp70 positive cells were detected by immunocytochemistry in control brain, suggesting that the immunoreactive epitope may be masked in unstressed animals. Our Western data clearly indicate that stress-inducible hsp70 is present in unstressed neural tissue. What role this protein plays in the unstressed animal has yet to be determined. Recently, we have shown that a correlation exits between levels of constitutive hsc70 protein and the magnitude of hsp70 induction, that is tissues/cells with high levels of hsc70 show a dampening in the level of hsp70 induction following hyperthermia. Constitutive levels of stress-inducible hsp70 may also exert a dampening effect on hsp70 induction.

619.13

LOCALIZATION OF HEAT SHOCK mRNAs ENCODING HSC70 AND HSP70 IN THE RABBIT BRAIN. J.A. Foster*, S.J. Rush, and I.R. Brown. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

In response to stresses, such as elevated temperature, cells respond by increasing synthesis of a group of highly conserved proteins known as heat shock proteins (hsps). Our laboratory studies the expression of constitutive (hsc) and hyperthermia-inducible (hsp) members of the hsp70 multigene family in the New Zealand white rabbit. Using radioactive and non-radioactive (DIG) *in situ* hybridization protocols, both hsc70 mRNA and hsp70 mRNA were localized to hippocampal neurons in control animals. The role of the stress-inducible hsp70 mRNA species in neurons of the unstressed animal is yet to be determined. Following 1 hour of hyperthermia, glial cell types (including astrocytes, oligodendrocytes, and microglia) within the hippocampal region, the overlying corpus callosum and the fimbria showed an induction of hsp70 mRNA. We performed lectin cytochemistry using GSA I-B_4 from *Griffonia simplicifolia* to identify microglia and immunocytochemistry with anti-GFAP antibody to identify astrocytes. Having previously shown that hsc70 protein is found constitutively within dendritic processes of Purkinje neurons of the cerebellum, we now analyze transport of hsc70 mRNA in neuronal processes. In addition, a time course analysis of hsp70 mRNA induction in glial cell types investigates transport of hsp70 mRNA in glial processes following hyperthermia.

619.14

HIPPOCAMPAL ANATOMY AND ULTRASTRUCTURAL MORPHOLOGY OF α-CALCIUM CALMODULIN KINASE II DELETION MICE. T.A. Comery*, J.A. Kleim, A. Silva and W.T. Greenough. Depts. of Psych., Biochem., Cell and Struct. Bio., Neurosc. Prog., and Beckman Inst., Univ. Illinois, Urbana, IL 61801 and Cold Spring Harbor, NY 11724.

Calcium Calmodulin Kinase II (CAMKII) regulates both pre- and post-synaptic functions. CAMKII is highly abundant in the postsynaptic densities of excitatory synapses. Transgenic mice homozygous for a null mutation for the α-subunit of CAMKII show impaired formation of hippocampal long-term potentiation, long-term depression, and abnormal short-lived plasticity. Behavioral studies reveal profound deficits in both spatial and contextual learning (Silva et al., 1992; Silva et al., unpublished results). The present study used stereological techniques to examine the ultrastructural characteristics of the CA1 region of the hippocampus in the homozygous mutant mice. Pyramidal cell density, in CA1 stratum pyramidale, was obtained using the physical disector on serial 1.5 μm sections. Synaptic density was obtained from serial electron micrographs of stratum radiatum in CA1 using the physical disector. Preliminary results demonstrate similar neuronal and synaptic densities in the homozygous mutant (N=2) and wild-type animals (N=3). No differences were observed in the number of synapses per neuron. Initial analysis of the size of post-synaptic densities reveals no differences between the two groups. However, one mutant mouse appeared to display spontaneous seizure activity and examination of the hippocampal anatomy revealed cell loss is all CA regions of the hippocampus.
Supported by NIMH 35321, NSERC, Kiwanis Nerv. Sys. Res. Fnd., Whitehall Foundation and Klingenstein Foundation.

619.15

EXPRESSION OF CALMODULIN mRNA IN THE DEVELOPING RAT BRAIN. F. Berry and I.R. Brown*. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

In the rat, three genes have been identified which encode calmodulin (CaM). Previously in our laboratory, two cDNAs have been cloned and characterized which correspond to mRNAs of 1.8 and 4.0 kb derived from the rat calmodulin gene I. Both messages are preferentially expressed in neurons in the rat brain. We have extended our analysis to examine expression of these CaM I mRNAs during neural development in the Wistar rat. Northern blot analysis revealed that the 4.0 kb transcript increases in abundance from postnatal day 1 (P1) in the cerebral hemispheres (CH) and thalamus. In the superior colliculus (SC), the 4.0 kb mRNA demonstrates a transient increase from P1 to P10 and then declines in later ages (P15 and adult). Levels of the 1.8 kb message remain relatively constant over development, while the 4.0 kb message shows greater change. Using *in situ* hybridization, we observe that CaM I mRNA is localized to a discrete layer in the developing SC. By P5 and P10, expression is focused to neurons between the superficial gray and optic layers. In the developing CH, CaM I mRNA is localized in a punctate pattern at P1, while from P5 to P15, message is distributed in a columnar fashion in cortical layers II-V. Closer examination of cells in these layers reveals that CaM mRNA is present in the cell body and dendritic processes. The timing of CaM I mRNA expression in the developing SC coincides with the period of removal of mistargeted axons from retinal ganglion cells, while the expression pattern in the developing CH correlates to a period of synaptogenesis in the cortical layers. This work was supported by grants to I.R.B. from NSERC, Canada.

619.16

Concerted regulation of GAD and $GABA_A$ receptor family transcripts as a model system in the molecular physiology of CNS development.

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GABA (γ-aminobutyric acid) acts as a neuortrophic signaling molecule in embryonic development of the CNS, distinct from the well-established function of GABA as a fast-acting inhibitory transmitter in the adult. Evidence for this role includes the early embryonic detection of GABA, GABA-evoked pharmacological responses involving Cl^- and Ca^{2+} ions, and promotion of neurite outgrowth. The molecular basis for the embryonic role of GABA lies in 1) its synthesis from glutamate by glutamic acid decarboxylase (GAD), and 2) the presence of GABA receptors. We have implemented a quantitative PCR strategy to enumerate GAD transcripts during development of the rat cervical spinal cord. Three GAD mRNAs, GAD65, GAD67 and EP10 were detected at E11 and steeply increased during an early embryonic period of 4 days. GAD65 and GAD67 reached maximum expression at the end of embryogenesis, at levels 1000x higher than initially detected. During the postnatal period, they gradually declined to their adult level at one third of maximum. The alternatively spliced transcript of the GAD67 gene, EP10, reached a maximum before GAD65&67 and then steeply dropped in late embryonic development. EP10 expression correlated with the transition of spinal cord tissue from proliferative to differentiating. RTPCR of 13 $GABA_A$ receptor subunits revealed overall parallel expression to GAD. As a working model, we propose that production of GAD transcripts may involve a regenerative feedback loop, linking the product of GAD, i.e. GABA, to the induction of GAD and $GABA_A$ receptor subunit mRNAs. We are currently testing this hypothesis using embryonic spinal cord cells grown in a serum free tissue culture on an extracellular matrix substrate; these conditions enable the continuing differentiation of cells.

POSTSYNAPTIC MECHANISMS III

620.1

THE *EFFICIENCY* OF CHANNEL OPENING SUMMATION AT $GABA_A$ AND NMDA SYNAPSES: RELEVANCE TO MODULATION OF SYNAPTIC CURRENTS AND INTERPRETATION OF QUANTAL ANALYSIS. Y. De Koninck*, D.N. Lieberman and I. Mody. Depts. of Anesthesiology/Pain Mgmt. & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

Stochastic simulations (Faber *et al.*, *Science* **258**:1494, 1992; De Koninck & Mody, *J. Neurophysiol.* **71**:1318, 1994) have demonstrated the existence of intrinsic quantal variance (σ_q^2) at the peak of miniature postsynaptic currents (mPSCs) in spite of the postulated pharmacological saturation of receptors at central synapses. The σ_q^2 depends on the relationship between activation kinetics and bursting properties of subsynaptic receptor channels. The occurrence of gaps during bursts or clusters of channel openings may cause poor or *inefficient* summation of channel activity.

We examined how σ_q^2 and $Po_{(peak)}$, the probability that ligand-bound channels will be open at the peak of mPSCs, are affected by modulators of channel kinetics such as intracellular calcium, phosphatases and kinases. We simulated mPSCs based on cell-attached single channel data obtained in acutely isolated adult neurons. Clusters of channel openings were aligned by exponentially distributed random latencies based on the time to first opening of channels following brief agonist applications (Edmonds & Colquhoun, *Proc.Roy.Soc.Lond.B* **250**:279, 1992).

For unitary currents with burst and onset kinetics such as those underlying $GABA_A$ receptor mediated mIPSCs, the $Po_{(peak)}$ is high, and modulation of the intraburst kinetics has little effect on the peak mPSC amplitude. In contrast, as previously reported, (Jahr, *Science* **255**:470, 1992; Rosemund *et al.*, *Science* **262**:754, 1993), $Po_{(peak)}$ is much lower for NMDA receptor mediated mEPSCs. Thus, modulation of the intraburst kinetics will dramatically alter peak amplitude. The alteration in $Po_{(peak)}$ will lead to changes in σ_q^2. As the coefficient of variation (CV) used for quantal analysis assumes a fixed σ_q^2, variations in σ_q^2 by modulators of channel kinetics may produce erroneous interpretations of changes in CV at certain synapses.

Supported by NS-27528, NS-30549, and by the Sid W. Richardson Foundation. Y. De K. is a Fellow of the Canadian MRC. D.N.L. is a Hughes Predoctoral Fellow.

620.2

MONOSYNAPTIC GABAERGIC INPUTS TO RAT RUBRAL NEURONS IN SLICES G.-F. TSENG* and Y.-S. FU Department of Anatomy, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Mammalian red nucleus(RN) displayed a deafferentation induced sprouting of excitatory and inhibitory terminals, but the locations of inhibitory neurons remain enigmatic. Here we used intracellular recording and anatomical tracing methods to study the inhibitory connections of normal RN neurons in slices. In some cases, biocytin was used to reveal the morphology of recorded cells. They resembled Golgi-stained RN neurons. Recorded caudal cells were similar to identified rubrospinal neurons revealed by fixed tissue intracellular dye injection.

Stimulating electrode was placed in the mesencephalic reticular formation dorsolateral to RN(*dl*MRF), substantia nigra(SN), or ventral tegmental decussation (VTD). Stimuli in VTD evoked no postsynaptic potential(PSP) but antidromic spikes suggests the lack of a recurrent inhibition. Stimuli in *dl*MRF(11/11 caudal and 8/10 rostral neurons) and SN (16/19 caudal and 19/20 rostral neurons) evoked a short latency hyperpolarizing PSP. This PSP could temporarily delay the repetitive spiking evoked by depolarizing current injection and it was reversibly blocked by bath perfusion of 20 μM bicuculline. Blockage of this IPSP revealed no underlying depolarizing PSP indicating a direct activation of inhibitory neurons or their axons. Latencies of IPSPs evoked from both stimulus sites were around 1.1 ms consistent with the hypothesis of being monosynaptic PSPs.

Anatomically, anterograde tracer-Dextran applied in the *dl*MRF or SN of live slices labeled neurons with boutons-bearing axons extending into the RN. They were often found to oppose somata in the RN. Immunochemical staining shows that *dl*MRF and SN neurons contain high level of the calcium-binding protein-parvalbumin, known to present in high concentration in GABAergic neurons. Thus anatomical and physiological data suggest that *dl*MRF and SN neurons mediate a $GABA_A$-IPSP on rat RN neurons.(supported by NSC of Taiwan, R.O.C.)

620.3

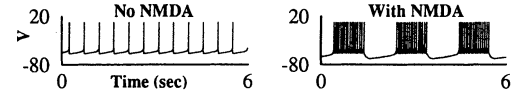
FUNCTIONAL CHARACTERIZATION OF THE CORTICO-NUCLEAR PROJECTION IN RAT CEREBELLUM *IN VITRO* D. Mouginot and B. H. Gähwiler* Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

Neurons of the deep cerebellar nuclei (DCN) constitute the major output of the cerebellum. Intracellular recordings were obtained to characterize the inhibitory synapses formed by Purkinje cells (PCs) on neurones in the DCN using organotypic cerebellar cultures. These preparations exhibit the unique property that PCs grow axons *de novo*, forming an impressive *in vitro* analog of the cortico-nuclear projection. In the presence of excitatory amino acid receptor antagonists (CNQX, 20 μM; D-APV, 40 μM) and bicuculline (40 μM), DCN neurons spontaneously fired action potentials whose frequency depended linearly on membrane potential. Thus, DCN cells displayed a beating-type pacemaker activity under conditions of synaptic isolation. DCN neurons possess $GABA_A$ and $GABA_B$ receptors, as shown with bath application of muscimol (5 μM) and baclofen (10, 100 μM). In the presence of CNQX and D-APV, field stimulation of PCs evoked graded IPSPs which were completely and reversibly abolished by bicuculline. IPSP amplitudes were not markedly reduced during fast repetitive stimulation, and the resulting hyperpolarization was not affected by the $GABA_B$ receptor antagonist CGP-35348. Trains of IPSPs (5 to 10 Hz) were able to transiently disrupt the pacemaker activity of DCN cells. In addition, experiments with bicuculline show that PCs exert a tonic hyperpolarizing tone on DCN neurones.

620.4

MODELING NMDA-INDUCED BURSTING IN DOPAMINE NEURONS Y.-X. Li*, R. Bertram and J. Rinzel. Math. Res. Branch, NIDDK, NIH, BSA Bldg., Suite 350, Bethesda, MD 20892

Burst firing of dopamine neurons *in vitro* is induced by the glutamate agonist N-methyl-D aspartate (NMDA). The hyperpolarization between bursts is believed due to Na^+ extrusion by a ouabain-sensitive pump (Johnson *et al.*, *Science*, 258:665). We formulate and explore a theoretical model for this novel mechanism of burst generation. We show that interaction between the regenerative, inward NMDA-mediated current and the outward Na^+-pump current is sufficient to generate the slow oscillation ($0.5Hz$) underlying the burst. The region of negative slope in the $I-V$ relation of the NMDA channel in the presence of Mg^{2+} is indispensable for the occurrence of this slow rhythm. As suggested by Seutin *et al.* (*Neuroscience*, 58:201), we find that at least 2 spatial compartments are required: a soma where action potentials (APs) are produced and a dendrite where the slow rhythm is generated. The time scale of Na^+-handling in the dendrite determines the burst period. In the absence of NMDA, the model shows tonic spiking ($\sim 2Hz$) which is insensitive to Na^+ pump inhibitors. When NMDA is present, tonic spiking is replaced by repetitive bursting. Tetrodotoxin blocks the APs but leaves the slow rhythm unchanged. Na^+ pump inhibitors tranform bursting back into tonic firing. When the soma is voltage-clamped, slow oscillations in current, which are generated in the dendritic compartment, are still present. These results are in good agreement with experimental observations. Insights obtained with our model may apply to other neuron types where bursting appears to involve NMDA channel activity.



620.5

NMDA CURRENT IS NEGATIVELY REGULATED BY AMPA/KAINATE AND METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS. M. Yuzaki*, L. M. Verselis, S. C. Sun, D. Forrest., T. Curran and J. A. Connor. Roche Inst. of Molecular Biol. Nutley, NJ 07110.

The major excitatory transmitter glutamate activates several subtypes of glutamate receptors. In this study, we investigated the interaction of these subtypes in single neurons. We carried out voltage-clamp recordings using the conventional whole-cell patch-clamp technique from hippocampal neurons, prepared from B6 mouse embryos (E16-E18) and cultured for 8-13 days. Glutamate (100 μM), applied by rapid pressure ejection, induced a large inward current with prominent desensitization in Mg-free, glycine- and TTX-containing solution at -60mV. The peak current induced by glutamate was paradoxically increased to 122 ± 5% (mean ± sem, n=9) of control by an AMPA/kainate receptor (A/K-R) antagonist, CNQX (10 μM). The enhanced current was almost completely blocked by an NMDA receptor (NMDA-R) antagonist, D,L-APV (200 μM). In addition, in mutant mice lacking functional NMDA-Rs, CNQX reduced the glutamate-induced current to 33 ± 5% (n=5) of control, indicating that the NMDA current was increased by about 90% in the wild-type animals by blocking A/K-Rs. The ratio of glutamate current at 3 sec to its peak amplitude was increased by CNQX, indicating that the NMDA current had been desensitized by A/K-R activation. In Ca-free solution, CNQX reduced the glutamate-induced current to 76 ± 2% (n=3) of control. In Na-free solution, with Li or Cs substitution, CNQX reduced the glutamate current to 79 ± 4% (n=3). Thus AMPA/kainate current may affect NMDA current via Na influx, which eventually increases Ca levels by the Na/Ca exchanger. Similarly, the NMDA-component of glutamate current was also increased to 120 ± 5% (n=6) by a metabotropic glutamate receptor (mGluR) antagonist, (+)-MCPG (200 μM). These results suggest that the NMDA component of glutamate current is inhibited by A/K-R and mGluR activities.

620.6

WHOLE CELL RECORDINGS FROM RAT HIPPOCAMPAL CA3 NEURONS DURING STIMULATION OF DENTATE GRANULE CELLS. A. C. Greenwood*[1] and T.H. Brown[1,2]. Depts. of Cellular & Molecular Physiology[1] and Psychology[2], Yale Univ., New Haven, CT 06520.

The mossy-fiber (MF) synapse onto CA3 pyramids offers some advantages for electrophysiological study of quantal synaptic transmission and long-term potentiation (LTP). For example, the synaptic input from a MF axon onto a CA3 cell is not widely dispersed over the dendrites and is relatively proximal to the soma.

We developed a method of analyzing populations of excitatory postsynaptic currents (EPSCs) to test the applicability of different models of transmission, estimate model parameters and test hypothetical mechanisms of plasticity, with estimates of uncertainty obtained for each synapse from asymptotic theory or many simulations.

The application of these analytical methods to data collected during minimal stimulation of MF axons and to simulated data suggest that 200+ unitary EPSCs, as may be obtained by simultaneously patching CA3 pyramids and dentate granule cells, could elucidate the quantal mechanism of a MF synapse with satisfactory confidence.

Brain slices (300-400 μm) from male Sprague Dawley rats (14-25 days) were placed in a submerged chamber on a fixed-stage upright microscope (Zeiss Axioskop) with a water immersion lens (40X, 0.75 NA) and video-DIC optics. Whole-cell electrodes were placed (under visual guidance) on "postsynaptic" pyramids in region CA3 or the hilus and on "presynaptic" dentate granule cells to obtain simultaneous recordings. During the selection of granule cells for patching, K^+ from the presynaptic pipette in or above the dentate sometimes evoked EPSCs recorded in CA3 cells. Similarly, K^+ from the descending postsynaptic pipette in region CA3 or the hilus sometimes evoked antidromic action potentials recorded in dentate granule cells.

Once simultaneous recordings were established (n>50), granule cells were current-clamped to fire pairs of spikes separated by ~45 ms every 5 sec. Postsynaptic cells were voltage-clamped (R_S < 10MΩ; peak-to-peak noise < 5pA; 1 kHz filtered). Spontaneous PSCs < 10pA have been observed. Work is still in progress to record unitary MF EPSCs evoked by controlled firing of a single granule cell before and after the induction of LTP. *Supported by NIH & ONR.*

620.7

NOVEL TIME COURSE OF TRANSMISSION AT A GIANT GLUTAMATE SYNAPSE IN RAT VESTIBULAR CEREBELLUM. D.J. Rossi*, E. Mugnaini and N.T. Slater, Department of Physiology, Northwestern University Medical School, Chicago, IL, 60611 U.S.A.

Unipolar brush cells (UBCs) of mammalian vestibulocerebellum receive innervation from a single mossy fiber in the form of an extraordinarily extensive synaptic contact (12-40 μm^2). UBCs are immunonegative for GABA and glycine (Mugnaini et al., *Synapse* 16:284, 1994), but immunopositive for glutamate. In the present study we have examined the properties of transmission at this synapse using whole-cell recording methods in thin cerebellar slices maintained *in vitro*. UBCs in the GCL of rat cerebellar nodulus and uvula were patch-clamped with Lucifer Yellow (LY)-filled pipettes, and stimulating electrodes were placed in the white matter to activate mossy fiber afferents. Confocal fluorescence imaging of their characteristic morphology was used to verify cell identity. LY filled the soma, dendritic brush, and revealed a branching axon that gave rise to 2-4 mossy-like rosettes in the GCL. In whole-cell recordings a biphasic excitatory postsynaptic current (EPSC) was observed in UBCs voltage-clamped at -80 mV in Mg-free saline. The fast initial inward current (τ_{on}=0.7 ms, τ_{off}=3.6ms) was followed by an unusually slow inward current (τ_{on}=220 ms, τ_{off}=1.4s), both of which were evoked in an all-or-none fashion at identical stimulus thresholds. At all postnatal ages the fast EPSC was blocked by the AMPA/KA receptor antagonist CNQX (10 μM). In Mg^{2+}-free saline both fast and slow EPSCs reversed near 0mV with linear *I-V* relations between -80 and +50 mV. The pharmacology of the slow EPSC changed with development. In young animals (6-20 days) it was preferentially blocked by the NMDA receptor antagonists D-AP5 (50 μM) and 7-chloro-kynurenate (100 μM) and displayed non-linear *I-V* relations in the presence of external Mg^{2+}. In contrast, it was blocked by CNQX (10 μM) and displayed no Mg^{2+} channel block in older animals (>20 days). It is proposed that the ultrastructural design of this synapse represents a specialization to prolong the lifetime of glutamate in the cleft following release, thus resulting in rebinding of glutamate to produce a long-lasting EPSC whose time course is independent of receptor type.

620.8

NMDA RECEPTOR-MEDIATED SYNAPTIC TRANSMISSION IN THE RAT MEDIAL VESTIBULAR NUCLEUS *IN VITRO*. G.A. Kinney*, B.W. Peterson and N.T. Slater Dept. of Physiology, Northwestern Univ. Med. School, 303 E. Chicago Ave., Chicago, IL 60611.

While synaptic activation of NMDA receptors within the intrinsic circuitry of the brainstem vestibular nuclei has been proposed to contribute to plasticity of vestibular function, to date there has been no demonstration using intracellular recording methods of an NMDA receptor-mediated component of synaptic potentials within the mammalian vestibular nuclear complex. In the present study we have re-examined this issue using whole-cell recording methods in 300-450 μm thick slices of rat medial vestibular nucleus (MVN) maintained *in vitro*. Slices were cut at the level of the vestibular nerve (nVIII), and stimulating electrodes were placed in the nVIII to evoke EPSPs. In normal saline, the nVIII-evoked EPSP was blocked by the AMPA/KA receptor antagonist DNQX (10 μM). However, in the presence of the $GABA_A$ receptor antagonist bicuculline (10 μM) a late, slow component of the EPSP was observed which was blocked by the NMDA receptor antagonist D-AP5 (50 μM). This monosynaptic NMDA-mediated component could be isolated in the presence of DNQX, and displayed the non-linear voltage relations charactersitic of NMDA-mediated responses in the presence of external Mg. In some cells, DNQX also blocked a large (>50%) proportion of the D-AP5-sensitive component. Polysynaptic AMPA/KA-mediated EPSPs were observed in the presence of D-AP5. Prolonged bath application (10-20 min) of the metabotropic glutamate receptor (mGluR) agonist 1S,3R-ACPD (10-100 μM) produced a moderate, slowly desensitizing depolarization (2-8 mV) and an acute depression of the EPSP in all cells. A long-term potentiation of the NMDA-mediated EPSP was observed in 9/13 cells following the washout of 1S,3R-ACPD. These results demonstrate that NMDA receptors contribute both to the monosynaptic nVIII-evoked EPSP and to transmission within the intrinsic circuitry of the rat MVN, and are modulated by activation of mGluRs.

620.9

SATURATION OF AMPA RECEPTORS FOLLOWING QUANTAL TRANSMITTER RELEASE. C-M. Tang*, M. Margulis, Dept of Neurology, Univ. of Maryland Sch. Med.

While it is generally believed that post-synaptic NMDA receptors reach saturation following the release of glutamate from a single transmitter vesicle, it remains unclear whether AMPA receptors reaches saturation. Using brief focal application of Ba^{+2} and K^+ mEPSCs were elicited from small numbers of release sites on proximal dendrites of cultured hippocampal neurons. Closely timed mEPSCs did not follow Poisson behavior nor did they summate linearly. A minority but statistically significant number of closely timed mEPSCs were also closely matched in terms of amplitude. Addition of cyclothiazide (100 uM) increased the non-linearity of summation and the stastical significance of "amplitude pairing". Receptor saturation best explains these observations. AMPA receptor saturation following quantal transmitter release has implications for synaptic transmission in the CNS.

620.10

DOES GLUTAMATE RELEASE SATURATE NMDA RECEPTORS AT A SYNAPSE? W.R. Holmes*. Neurobiology Program, Dept. of Biological Sciences, Ohio University COM, Athens, OH 45701.

The fact that few NMDA receptor channels are ever open at a synapse and that NMDA receptors have a high affinity for glutamate suggests that a single vesicle of glutamate might saturate all NMDA receptors at a synapse. In our previous model of LTP (Holmes and Levy 1990), the first few pulses of a 400 Hz input caused the peak number of NMDA receptors bound to glutamate to increase. However, if glutamate saturates NMDA receptors, this peak number should be the same for all pulses in the train. Whether or not the peak number of bound NMDA receptors increases during a tetanus affects model predictions of the amount of calcium entry through NMDA receptor channels.

A diffusion model similar to that reported for nicotinic synapses by Wathey et al. (1979) was constructed. A single vesicle of glutamate was assumed to be released either once or 8 times at 400 Hz. The numbers of bound and open NMDA and non-NMDA receptor channels were computed. Conditions when NMDA receptors would or would not be saturated were determined.

For saturation of NMDA receptors to occur with the first pulse of a tetanus, i) the number of glutamate molecules released into the synaptic cleft must be much larger than the number of NMDA receptors and ii) the first binding constant (k_{on}) must be large. Under these conditions, a tetanus allows many more receptor channels to enter the open state even though the peak number of bound receptors never rises above that of a single input.

The first binding constant has been estimated to be 5 $\mu M^{-1}s^{-1}$ (Clements and Westbrook 1991). With this value, the number of NMDA receptors bound with glutamate increases with successive pulses of a tetanus, indicating a lack of saturation. This occurs even when the number of released glutamate molecules is 10 times the number of NMDA receptors.

620.11

SATURATING CONCENTRATIONS OF GLYCINE REVEAL UBIQUITOUS PRESENCE OF NMDA RECEPTORS AT EXCITATORY SYNAPSES IN CULTURED HIPPOCAMPAL NEURONS. [1]K.S. Wilcox*, [2]R. Maki, [1,2,3]M.A. Dichter. Depts of [1]Neurology and [2]Pharmacology, and the [3]David Mahoney Institute of Neurological Sciences, University of Pennsylvania School of Medicine and the Graduate Hospital, Phila., PA 19104.

In the mammalian hippocampus, excitatory postsynaptic currents (EPSCs) are mediated by activation of both non-NMDA and NMDA receptors colocalized at the synapse. Evidence for colocalization comes from many studies in which miniature EPSCs (mEPSCs) have been shown to consist of both non-NMDA and NMDA components. An early report (Bekkers & Stevens, 1989) suggested that approximately 70% of mEPSCs recorded in cultured hippocampal neurons consisted of both components, whereas the remaining 30% could be evenly divided between synapses containing either non-NMDA or NMDA receptors exclusively. In the present study, we examined the contribution each glutamate receptor subtype makes at individual postsynaptic boutons in hippocampal neurons maintained in low density culture. Using the whole cell patch clamp technique to simultaneously record the pre- and postsynaptic neuron, it was possible to record mEPSCs arising from the same terminals that were activated during evoked responses. We found that as the concentration of glycine was increased from 0 to 10 μM, the NMDA component of the evoked EPSC became significantly greater in both amplitude and duration. Furthermore, in 10 μM glycine, we saw no evidence of mEPSCs comprised solely of the non-NMDA component. Therefore, in our hippocampal culture preparation, there are no postsynaptic sites comprised exclusively of non-NMDA receptors. In addition, although there were mEPSCs comprised of only the NMDA component, it is not possible to conclude that non-NMDA receptors were not present, as desensitization of those receptors, as well as a weaker affinity than the NMDA receptor for the endogenous ligand, could mask their presence. NS24260 (MAD) & AG1200301 (RM).

620.12

DIFFERENCES IN INHIBITORY SYNAPTIC INPUT INFLUENCE EXCITABILITY OF CAT CORTICAL NEURONS. J.F.M. van Brederode* and W. J. Spain. VA Medial Center, Seattle, WA 98108 and Depts. of Biological Structure, Neurology, and Physiology. & Biophysics, Univ. of Washington, Seattle, WA 98195.

We compared intracellularly recorded post-synaptic potentials (PSPs) evoked in regular spiking layer 2/3 (L2/3) and layer 5 (L5) neurons by stimulation of the gray matter of cat area 4γ in vitro. Trains of stimuli (9 pulses; 100 Hz) caused depression and hyperpolarization in L2/3 cells, but facilitation and depolarization in L5 cells. The mechanism of this difference was investigated. **1)** Single shocks evoked a stereotyped tri-phasic PSP consisting of a fast excitatory PSP (fEPSP), and a fast and slow inhibitory PSP (fIPSP and sIPSP, res.) in L2/3 cells. PSPs in L5 cells, in contrast, were more variable, and entirely depolarizing in 85% of neurons at rest. **2)** In response to single shocks isolated γ -aminobutyric acid (GABA)-A mediated fIPSPs were found in all L5 cells after blockade of glutamate receptors with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonovaleric acid (AP5). They were smaller and their duration shorter compared to fIPSPs in L2/3 cells and overlapped completely in time with fEPSPs. **3)** Large (5-15 mV) GABA-B mediated sIPSPs were always evoked in combination with fIPSPs in L2/3 cells but only in 30% of L5 cells in which they were also much smaller (< 1mV). Isolated IPSPs were able to sum during trains of shocks and caused long-lasting hyperpolarization in L2/3, but not in L5 cells due to a lack of sIPSPs. **4)** After GABA-release was enhanced by 4-aminopyridine sIPSPs were evoked together with fIPSPs in all layer V cells. **5)** Blockade of the inward rectifier current I_h with extracellular Cs^+ lengthened and amplified isolated fIPSPs in L5 cells, but not in L2/3 cells in which I_h was absent or small. **6)** N-methyl-D-aspartate-(NMDA) mediated responses were apparent in L2/3 cells only after GABA-A blockade, in contrast to L5 cells in which AP5-sensitive slow EPSPs were demonstrable in control perfusate. We conclude that the differences in temporal summation of PSPs in cells from deep and superficial layers is due to attenuation of GABA-A responses by I_h and weaker GABA-B mediated responses in L5 compared to L2/3 cells. Supported by a VA Merit Review and NIH EY 01208.

620.13

PATCH-CLAMP ANALYSIS OF SPONTANEOUS SYNAPTIC CURRENTS IN THE RAT BASOLATERAL AMYGDALA. B.N. Smith* and F.E. Dudek Dept. of Anat. and Neurobiol., Colorado State Univ., Fort Collins, CO 80523.

Alterations of local excitatory and inhibitory amino acid transmission in the CNS can lead to epileptiform activity. Amino acid transmission in the basolateral amygdala (BLA) has been demonstrated, but not extensively studied with whole-cell recordings in the slice. Because of the potential for certain substances (e.g, corticotropin-releasing hormone) to contribute to seizure activity in the amygdala, we examined spontaneous synaptic activity in the rat BLA using the whole-cell voltage-clamp technique in coronal slices (400-500 μm) from young rats (postnatal day 6-14). Biocytin (0.2%) was used to verify the location of the recorded neurons.

Resting membrane potential for BLA neurons was -65 ± 6.3 mV (mean ± SEM); input resistance was 293 ±71 MΩ. At holding potentials near rest, most neurons exhibited 10 to 60 pA spontaneous inward, but usually not outward, post-synaptic currents (PSCs). At more negative potentials (-80 to -100 mV), inward PSCs of up to 300 pA were observed. Both inward and outward PSCs could usually be discerned at potentials 10 to 20 mV depolarized from rest. In some cells, Cs^+ was used in the electrode to block voltage-activated K^+-currents. These cells usually exhibited outward PSCs of between 10 and 300 pA at depolarized potentials (-20 to 0 mV). Three Cs^+-loaded cells also showed bursts (up to 20 Hz, 0.5 sec) of spontaneous inward and outward PSCs, suggesting that the somata of some of the cells contacting the recorded neuron were contained within the slice.

These results indicate that BLA neurons have spontaneous synaptic currents that are similar to amino acid-mediated EPSCs and IPSCs. Resolving synaptic mechanisms in the BLA may help identify how extrinsic perturbations exert epileptogenic effects. Supported by postdoctoral fellowship NS09289 (BNS) and NIH grants HD05958 and NS16683 (FED).

620.14

THE TIME COURSE OF QUANTAL SYNAPTIC CURRENTS IN RAT SUBMANDIBULAR GANGLION CELLS. R.J. Callister* & B.Walmsley. The Neuroscience Group, Discipline of Medical Biochemistry, Faculty of Medicine and Health Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia.

Neurons in the submandibular ganglion innervate the salivary glands. In rats, these simple spherical cells lie in a very thin connective tissue sheet and, after about 5 weeks of age, 75% of the cells receive innervation from only a single parasympathetic preganglionic fiber (Lichtman, *J. Physiol. (Lond.)* 273:155-177, 1977). We are using this preparation to study quantal synaptic transmission in order to avoid the usual problems associated with multi-fiber inputs and dendritic attenuation of synaptic potentials and currents. Ganglia from 5-10 week old rats were pinned out in a small perfusion chamber and viewed under an inverted microscope. Afferent fibers in the chorda tympani nerve were electrically stimulated using a suction electrode. Intracellular recordings of excitatory postsynaptic potentials (EPSPs) were obtained in normal Krebs' solution and in solutions containing 100 μM cadmium chloride. Under conditions of reduced transmitter release, a large proportion of failures of release and single quantal events were evident in amplitude histograms of the evoked EPSP. Two-electrode voltage clamp recordings of excitatory postsynaptic currents (EPSCs) were then obtained to investigate previous evidence (Rang, *J. Physiol. (Lond.)* 311:23-55, 1981) that the time-course of decay of the evoked EPSC and the single quantal (spontaneous) EPSC are markedly different. In agreement with Rang, the EPSC in normal Krebs' solution exhibited a decay which could be fitted by the sum of two exponentials (with time constants of approximately 30 ms and 7 ms). However, in contrast to previous evidence, this two phase decay persisted when evoked release was reduced to the level of single quanta.

620.15

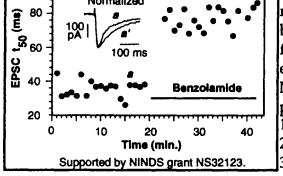
A PROMINENT ROLE FOR NMDA RECEPTORS IN FREQUENCY CODING E. D'Angelo*, P. Rossi, G. DeFilippi, V. Taglietti. Inst. General Physiol., Univ. Pavia, I-27100, Pavia, Italy.

We have investigated synaptic currents (EPSCs) and potentials (EPSPs) at the mossy fibre - granule cell synapse of the rat cerebellum using whole-cell recording techniques in a slice preparation. As in other central synapses, EPSCs and EPSPs were brought about by NMDA and non-NMDA receptors. The NMDA component, both in EPSCs and EPSPs, showed a remarkable slowing down at depolarized membrane potentials. This allowed the NMDA component to control EPSP time-course in spite of the slowing down of membrane discharge due to inward rectification, and determined a dramatic enhancement of EPSP temporal summation extending the input-output relationship of granule cells toward the low frequency range (<=50 Hz). Moreover, the NMDA component determined 30% of EPSP amplitude already at potentials as low as -80 mV. This result indicates a remarkable contribution of NMDA receptors to synaptic depolarization during low-frequency transmission and near rest, and in the process of signal coding. This result extends the functional role of NMDA receptors, which are already known to participate to the induction of LTP and LTD during high-frequency transmission.

620.16

NMDA RECEPTOR-MEDIATED EPSCs MODULATED BY ENDOGENOUS pH TRANSIENTS IN RAT HIPPOCAMPUS. **J.A. Gottfried* and M. Chesler.** Depts. of Physiol. & Neurosurgery, NYU Med. Center, New York, NY 10016.

The occurrence of extracellular alkaline shifts during excitatory synaptic transmission (1) suggests that the NMDA-receptor H^+ modulatory site (2,3) may play a functional role. We amplified these pH shifts with benzolamide (BZ), a carbonic anhydrase inhibitor, and studied the EPSCs of whole-cell clamped CA1 neurons in rat hippocampal slices. In Mg^{2+} free, CO_2/HCO_3^- media, 1 μM BZ caused an immediate increase in EPSC 50% decay time (t_{50}) at holding potentials of -80 or -40 mV (see Fig.)(mean increase 78 ± 14% at -80 mV, $p<0.01$, n=10). This was time-locked to an amplification of the extracellular alkaline shift (154 ± 14%). In CO_2/HCO_3^- plus APV (20-75 μM), the EPSC t_{50} was unaltered by BZ, while the alkaline shifts were still increased (111 ± 23%, n=8). In HEPES, where buffering is independent of carbonic anhydrase, neither the EPSC t_{50} nor the alkaline shift was affected by BZ (n=9). These data demonstrate that kinetics of the NMDA receptor-mediated EPSCs depend on the buffering capacity of the extracellular fluid. The results indicate that endogenous pH shifts can modulate NMDA receptor function in a physiologically relevant time frame.



1) Chesler & Kaila, *TINS* 15:396
2) Tang et al. PNAS 87:6445.
3) Traynelis & CullCandy *Nature* 345:347

Supported by NINDS grant NS32123.

621.1

POTENT NAPHTHALENIC AGONISTS FOR MELATONIN RECEPTORS IN BRAIN. L.P. Niles*, L. Smith, J. Wang, J.J. Chen[1] and G. Firnau[1]. Departments of Biomedical Sciences and [1]Nuclear Medicine, McMaster University, Hamilton, Ontario, Canada, L8N-3Z5.

In studies aimed at the identification of selective ligands for melatonin receptors in the CNS, we have assessed the binding and functional activities of a series of naphthalenic compounds bearing variable halogen substitutions on the N-acylamino group. Binding experiments were carried out with the melatonin receptor radioligand, 2-[^{125}I]iodomelatonin ([^{125}I]MEL), in either whole brain homogenates or synaptosomal (P_2) fractions. Competition experiments performed on ice, in 50 mM Tris-HCl buffer (pH 7.4 at 4^{0}C), revealed that these compounds recognize both high (picomolar) and low (nanomolar) affinity binding sites for melatonin. In assays conducted at room temperature in the presence of Mg Cl_2 (4 mM), both binding components were also evident. More importantly, under these conditions, some naphthalenic drugs exhibited a marked increase in their competitive potency at the high-affinity sites labelled by [^{125}I]MEL, as indicated by affinities in the sub-picomolar range.

Preliminary functional assays, in chick brain and rat hypothalamic membranes, show that these ligands inhibit forskolin-stimulated adenylate cyclase activity. These findings indicate that compounds characterized by a naphthalene nucleus, a 7-methoxy group and halogen substituents on the N-acetyl side chain are high-affinity agonists at CNS receptors for melatonin. (Supported by a NSERC Strategic grant and SERVIER).

621.2

ENDOGENOUS GLUTAMATE INDUCES DEPHOSPHORYLATION OF THE NEURONAL CYTOSKELETAL PROTEIN MAP2 E.M. Quinlan and S. Halpain* Department of Neuroscience, University of Virginia Health Sciences Center, Charlottesville, VA 22908

Cytoskeletal proteins are likely targets of glutamate transmission in the nervous system. To examine transmembrane modulation of the neuronal cytoskeleton, we have focused on the the microtubule-associated phosphoprotein MAP2 in rat hippocampal slices. Changes in MAP2 phosphorylation are known to regulate its interaction with microtubules and actin. Accordingly, multiple phosphorylation sites along the primary amino acid sequence represent potential targets for synaptic regulation of MAP2 function. Previous studies suggested that NMDA receptor activation is potently coupled to MAP2 dephosphorylation. Additionally, depolarization of slices with 40 mM KCl induced a rapid dephosphory-lation of MAP2. Such dephosphorylation was blocked by the NMDA receptor antagonist MK-801, suggesting that depolarization activates NMDA receptor-induced dephosphorylation by stimulating the release of endogenous glutamate. It is well established that glia possess efficient glutamate uptake mechanisms, and that endogenous glutamate is released from slices by spontaneous neuronal activity. Application of 1 mM dihydrokainate, an inhibitor of glutamate uptake by glial cells, induced a rapid dephosphorylation of MAP2, suggesting that endogenous glutamate is a potent signal for MAP2 dephosphorylation. NMDA-induced dephosphorylation is dependent on extracellular calcium concentration. These data support a model in which glutamate transmission activates the calcium-dependent protein phosphatase 2B (PP2B or calcineurin), which targets specific proteins, such as MAP2, for dephosphorylation.

621.3

SUBSTRATES FOR MAP KINASE ASSOCIATED WITH SYNAPSES IN THE RAT CENTRAL NERVOUS SYSTEM. T. Suzuki[1]*, K. Okumura-Noji[1], E. Nishida[2], [1]Dept. of Biochemistry , Nagoya City Univ. Med. Sch., Nagoya 467 and [2]Dept. Genetics & Mol. Biol., Inst. for Virus Res., Kyoto Univ. 606, Japan.

Roles for MAP kinase in synaptic function have been suggested by (1) its occurrence in the brain, especially in the neuronal dendrites, and (2) excitatory neurotransmitter, glutamic acid, and electric stimulation induce MAP kinase activation. In the present study, to clarify the function of the enzyme in the synapse, we surveyed MAP kinase substrates that occur in synaptic fractions. Supernatant and synaptic fractions (P_2, synaptosome, synaptic plasma membrane [SPM], and postsynaptic density [PSD] fractions) were prepared from Wistar male rats. MAP kinases were purified from Xenopus oocyte or obtained from UBI (sea star p44mpk). Protein kinase activities in each fraction were heat-inactivated before phosphorylation by the MAP kinases. Western blotting showed the occurrence of both MAP kinase and MAP kinase kinase in all the fractions mentioned above. Fractions other than PSD contained two bands immunoreactive to anti-MAP kinase antiserum, these possibly corresponding to ERK1 and ERK2, respectively. ERK2 was greater than ERK1 in content in both synaptosome and SPM fractions, and only ERK2 was detected in the PSD fraction. All the fractions contained substrates for both enzymes, but each fraction showed a different substrate profile. Interestingly, sea star MAP kinase strongly phosphorylated the substrates in the supernatant and P_1 fractions, while Xenopus MAP kinase (ERK2 type) acted more on the substrates in the synaptic fractions, especially those in the PSD fraction. Phosphorylation of the PSD proteins by Xenopus MAP kinase was increased after maturation of synapses. These results indicate that part of ERK2 occurs in the synapse, especially in the postsynaptic side, and that it may play some role(s) in the synaptic function via phosphorylation of synapse-specific substrates.

621.4

ACTIVATION OF NFkB UPON GLUTAMATE STIMULATION IN CULTURED MOUSE CEREBELLAR GRANULE CELLS. L. Guerrini, F. Blasi and S. Denis-Donini* Genetics and Biology Departments and Center of Cytopharmacology, Milan, Italy.

NFkB (the relA/p50 heterodimer) is a pleiotropic transcription factor. It is present in an active form in the nucleus of a restricted member of cell types (lymphocytes); in most other cell types it is present in a covert cytoplasmic form and it can be induced to move to the nucleus in response to a wide variety of extracellular stimuli. The localization of relA has been studied through immunohistochemistry in brain sections and in cultures of cerebellar granule cells. In brain sections, the pattern of staining suggests a synaptic localization, as already described in the rat (Kaltschmidt et al. Mec.Dev.'93). In neuronal cells in vitro, relA is mostly concentrated at the tip of processes and at contact sites. This type of spatial distribution prompted us to investigate whether physiological stimuli, such as nanomolar concentrations of neurotransmitters, could activate NFkB in such cells. Glutamate pulses induce the nuclear translocation of NFkB, as observed through band shift experiments and this induction is specifically blocked by the NMDA receptor antagonist APV. With specific antibodies, we have assessed that the activated transcription factor is the heterodimer relA/p50. We are currently investigating the potential target genes of glutamate activated NFkB.

621.5

STRONGLY ANISOSMOTIC SOLUTIONS SUPPRESS $GABA_A$ CURRENTS IN FRESHLY DISSOCIATED HIPPOCAMPAL NEURONS. G.G.Somjen*[1], M.Vreugdenhil, W.J.Wadman. Dept. Exper. Zool., Univ. Amsterdam, the Netherlands and [1]Dept. Cell Biol. Duke Univ. Durham, NC 27710.

We have reported that sudden exposure of dissociated hippocampal neurons to strongly anisosmotic or glucose deficient solutions suppressed voltage gated Na^+, K^+ and Ca^{2+} currents (Brain Res., 632: 180-194, 1993). We now investigated whether $GABA_A$-induced ion currents were similarly shut down by exposure to anisosmotic solutions. Freshly dissociated rat CA1 pyramidal neurons were patch-clamped in whole-cell configuration. Voltage gated Na^+ and K^+ currents were pharmacologically blocked. The effect of hypo-osmotic, NaCl deficient (mannitol-substituted), or hyper-osmotic test solutions delivered from a micropipette was tested on membrane currents evoked by brief puffs of muscimol and on voltage gated Ca^{2+} currents. Hyper-osmotic solution caused cells to shrink, but hypo-osmotic solution had no effect on cell size or shape. All three test solutions depressed the muscimol-induced current and usually also the muscimol-induced increase of membrane conductance. V-gated Ca^{2+} currents were depressed by anisosmotic, but not by NaCl deficient isosmotic solution. We conclude $GABA_A$ receptor controlled membrane channels are partially shut down in a strongly anisomotic or NaCl deficient environment.

621.6

HYPOTONIC CELL SWELLING, TISSUE ELECTRICAL RESISTANCE AND SYNAPTIC TRANSMISSION IN CA1 ST. RADIATUM OF HIPPOCAMPAL TISSUE SLICES. S.R. Chebabo[1], M.A. Hester, J. Jing, P.G. Aitken*, G.G. Somjen. Dept Cell Biol., Duke Univ. Durham, NC

In order to determine the degree of cell swelling in brain tissue caused by lowering of osmotic pressure, hippocampal slices were exposed to bathing fluid from which varying amounts of NaCl was deleted. Interstitial volume (ISV) change was determined from the volume of dilution of marker ions TMA^+ or TEA^+ administered by iontophoresis. Tissue electric resistance was measured as the voltage drop generated by subliminal constant current pulses. The decrease of ISV determined from the dilution of probe ions was dependent on the degree of hypotonicity used. In the most severe condition ISV shrank to 3.3% of the total tissue volume. Prolonged hypotonic exposure revealed regulatory volume decrease (RVD). After restoring normal osmotic pressure, the cells in the tissue underwent post-exposure shrinkage. The electric resistance of the tissue increased in part due to lowered ionic strength and in part due to the effect of restricted interstitial space. Isosmotic NaCl deficient (mannitol or fructose-substituted) solutions had either no effect or caused erratic changes of TMA^+ space. The corrected electric resistance did not increase in low NaCl solution. Extracellular EPSPs increased much more in hypotonic than in NaCl-deficient solution, and in both cases more than could be accounted for by the increased tissue resistance, confirming increased synaptic efficacy. ([1]On leave from Dept. Pharmacol., Feder. Univ. Rio de Janeiro, Brazil) (Support by CNPq (Brazil), the Hughes Med.Inst., & NIH grant NS17771)

621.7

The Serotonergic Inhibitory Postsynaptic Potential in Prepositus Hypoglossi is Mediated by Two Potassium Currents. D. H. Bobker* and J. T. Williams. Dept of Neurology and Vollum Institute, L226, Oregon Health Sciences University, Portland, OR 97201

Synaptic inhibition mediated by the activation of potassium channels has been reported from several types of neurons. In each case, the K^+ conductance underlying the synaptic potential is activated by a G protein and inwardly rectifies. We report here a second K^+ current that contributes to synaptic inhibition. Intracellular recordings were made from guinea-pig nucleus prepositus hypoglossi *in vitro*, where we have described a 5-hydroxytryptamine (5-HT)-mediated inhibitory postsynaptic potential (IPSP). Voltage-clamp analysis of the current induced by applied 5-HT revealed two separate conductances: an inwardly rectifying, rapidly-activating K^+ current (I_{IR}) and an outwardly rectifying, slowly-activating K^+ current (I_{OR}). I_{IR} was blocked by extracellular Ba^{2+} (200 μM) and TEA^+ (126 mM). I_{OR} was insensitive to this concentration of Ba^{2+} and TEA^+, but was inhibited by Cd^{2+} and intracellular BAPTA, indicating Ca-dependence.

Single focal electrical stimuli evoked a 5-HT-mediated IPSP, or under voltage-clamp, an inhibitory postsynaptic current (IPSC). Ba^{2+} blocked only a component of this IPSC, which corresponded to the current caused by I_{IR}. When multiple stimuli were applied (to prolong the release of transmitter), the time-dependent current I_{OR} was more fully activated, resulting in an augmentation of the IPSC. We conclude that the IPSC is caused by both currents and that its amplitude can be modulated by the degree to which I_{OR} is activated. This represents a mechanism by which synaptic responses can be potentiated.

621.8

$GABA_B$ INHIBITOR BLOCKS THE EXPRESSION OF ANTIDROMICALLY-CONDITIONED EPSP-TO-SPIKE POTENTIATION. Lee W. Campbell* and Terrence J. Sejnowski. Howard Hughes Medical Institute, Salk Institute, PO Box 85800, San Diego, CA 92186.

EPSP-to-spike or E-S potentiation is a component of LTP which results from a shift in the excitation/inhibition balance. In the presence of bicuculline, a $GABA_A$ antagonist, most of the E-S potentiation component of LTP is eliminated.

Pairing antidromic theta-burst stimulation in phase with 5 Hz orthodromic stimulation produces an E-S potentiation without affecting EPSP slope (Soc Neurosci Abstr 17:533.15). This associative form of E-S potentiation is not blocked by $GABA_A$ antagonists and is mechanistically distinct from the E-S potentiation of LTP (Soc Neuro Abstr 19:547.15). Is $GABA_B$ involved in the induction or expression of antidromically-conditioned associative E-S potentiation?

The EPSP and population spike were recorded from the CA1 layer of rat hippocampal slices. The antidromic conditioning stimulus was 50 bursts of 5 pulses at 100 Hz with an interburst interval of 200 ms delivered to the alveus. The Schaffer collaterals were stimulated at 5 Hz. The compound 5-aminovaleric acid (5AV) was used in the bath to block $GABA_B$ receptors.

Several results suggest $GABA_B$ receptors are involved in the expression, but not the induction, of associative E-S potentiation. **1)** In slices showing E-S potentiation following paired stimulation, adding 1 mM 5AV 10 min after pairing reduced the potentiation of the population spike to baseline; washing in normal bathing medium restored the potentiation to its former value. **2)** In slices failing to potentiate following paired stimulation and in slices given the antidromic conditioning separate from the orthodromic stimulation, 5AV had no effect on the population spike. **3)** Delivering paired stimulation in 5AV medium resulted* in no E-S potentiation; washing out 5AV 10 min after pairing unmasked the potentiation.

621.9

METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED INCREASE OF PHRENIC MOTONEURON (PMN) EXCITABILITY *IN VITRO*. D. Morin, X-W. Dong & J.L. Feldman*. Systems Neurobiology Laboratory, Dept. of Physiological Science, UCLA, Los Angeles, CA, 90024-1527.

The role of metabotropic glutamate receptor (mGluR) in modulation of phrenic motoneuron excitability was studied in the *in vitro* neonatal rat brainstem/spinal cord preparation. Spontaneous PMN activity was recorded from cervical ventral root (C4) or by whole-cell patch-clamp techniques. In order to activate mGluR, trans-(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid agonist (ACPD) was applied locally via pressure ejection over the ventral spinal cord surface at the level of the phrenic nucleus. To block mGluR, (R,S)-α-methyl-4-carboxyphenylglycine (MCPG) was added to the bathing solution. Local application of ACPD (0.2 - 0.7 mM) induced up to a 30% increase of the inspiratory activity, in a dose-dependent manner. At higher concentrations (0.5, 0.7 mM), tonic activity was superimposed on the inspiratory activity. MCPG (0.6-1 mM) effectively blocked the actions of ACPD. Local application of ACPD depolarized PMN resting membrane potential (10-15 mV) and produced a concurrent 9% decrease in the inspiratory drive potential. Current pulse injection which induced one spike under control condition induced several spikes after ACPD application. Under voltage-clamp conditions, ACPD caused a tonic inward current (-150 pA) and a 32% drop in amplitude of the inspiratory synaptic current. The I-V curve exhibited a 10% increase of input membrane resistance. The ACPD-induced inward current remained after TTX was added to the bathing solution and was partially blocked by MCPG (0.4 mM).

These results show a postsynaptic action of ACPD and suggest that mGluR can modulate the excitability of PMNs. Supported by NIH Grant NS24742 and the Conseil Régional PACA.

LONG-TERM POTENTIATION: PHARMACOLOGY I

622.1

A CENTRALLY ACTIVE DRUG THAT MODULATES AMPA RECEPTOR GATED CURRENTS. A. Arai, M. Kessler*, J. Ambros-Ingerson, P. Xiao, G. Rogers and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717-3800.

Systemic administration of the drug 1-(1,3-benzodioxol-5-ylcarbonyl)-piperidine (BDP) has been reported to enhance monosynaptic responses in the hippocampus in vivo and to improve spatial and olfactory memory in rats. The mechanisms of action of BDP and recently developed, more potent analogs were investigated using a receptor binding assay, ligand-gated currents in patches excised from cultured hippocampal slices, and the field EPSP in acute hippocampal slices. The results were compared with those obtained with cyclothiazide, a structurally dissimilar diuretic compound known to affect the desensitization kinetics of the AMPA receptor. BDP and some but not all of its analogs caused an increase in ^{3}H-AMPA binding whereas cyclothiazide produced a decrease in the affinity of the AMPA receptor. BDP and analogs increased the amplitude and decay time of AMPA receptor currents in excised patches with the greater effect being on the latter variable. Cyclothiazide enhanced the steady state current mediated by the receptor, as reported by other investigators. Detailed comparisons and modelling studies indicate that BDP and analogs have substantially different effects on the kinetics of AMPA receptor currents than does cyclothiazide. Finally, the BDP drugs markedly and reversibly facilitate the field EPSP in a manner predicted from their actions on excised patches. Significant differences in potency and effects on waveform were evident across the analogs tested. In summary, the available evidence indicates that the BDP drugs— for which the name "Ampakine" has been proposed — provide tools for testing the functional consequences of pharmacologically enhancing AMPA receptor mediated transmission in behaving animals. Supported by AFOSR 92-J-0307.

622.2

AMPLIFICATION OF MONO- AND POLY-SYNAPTIC TRANSMISSION IN HIPPOCAMPUS BY A NOVEL MODULATOR OF AMPA RECEPTORS. J. Sirvio*[1,2], G. Rogers[3], G. Lynch[1], & J. Larson[1]. [1]CNLM, Univ. Calif., Irvine, CA 92717, [2]Univ. Kuopio, Finland, [3]Neurosci. Inst., Univ. Calif., Santa Barbara, CA.

Electrophysiological studies of drug actions typically assess direct membrane effects or effects on monosynaptic responses in order to characterize cellular mechanisms of action. However the ultimate effects of drugs on behavior may depend on the compounding of their actions across serial brain circuits. Drugs that modulate the function of AMPA-type glutamate receptors are of interest since AMPA receptors mediate responses at most excitatory synapses in the brain. The present study was undertaken to compare the effects of a novel AMPA receptor modulator, CX-516, on mono- and poly-synaptic responses in hippocampus.

Hippocampal slices were prepared from adult male rats using conventional methods. Stimulation electrodes were positioned to activate perforant path (PP) fibers in the molecular layer of the dentate gyrus and Schaffer-commissural (SC) fibers in CA1. A recording electrode was placed in the CA1 apical dendritic field.

PP stimulation evoked small (0.1-0.3 mV) negative field potentials in CA1 with latency and waveform characteristics consistent with them being mediated by the trisynaptic hippocampal circuit. SC stimulation was set to elicit monosynaptic responses of the same amplitude as the trisynaptic response. CX-516 (250 μ M, n=6) reversibly increased the amplitude of the monosynaptic SC-evoked field EPSP by 52% (±18, S.D.) and that of the trisynaptic response by 188% (±65).

These results indicate that it is feasible to test drug effects on a serial, polysynaptic circuit in vitro. The AMPA receptor modulator had much larger effects on trisynaptic than on monosynaptic responses, suggesting that its effects are amplified across serial connections. These findings should be of use in correlating physiological and behavioral effects of drugs in vivo.

(Supported by AFOSR, ONR, NIH, and the Academy of Finland).

622.3

CENTRALLY ACTIVE MODULATORS OF AMPA RECEPTORS FACILITATE THE INDUCTION OF LTP *IN VIVO*. U. Staubli*, Y. Perez, F. Xu, G. Rogers[1], M. Ingvar[2], S. Stone-Elander[2] and G. Lynch[3]. Ctr Neural Sci, New York University, New York, NY 10003; [1]Neuroscience Research Inst., Univ. of Calif., Santa Barbara, CA 93106; [2]Karolinska Hospital, Clinical Neurophysiol., Stockholm, Sweden S-1040; [3] Ctr Neurobiol. Learning & Memory, Univ. Calif., Irvine, CA 92717.

Previous work has shown that an experimental drug (BDP: 1-(1,3-benzodioxol-5-ylcarbonyl)piperidine) increases the amplitude and duration of EPSPs recorded in slices of hippocampus, while having little effect on the slope of responses. Studies with outside-out patches indicate that BDP modulates AMPA receptor gated currents. After intraperitoneal (ip) injections and for 2-3 hours, BDP influences monosynaptic responses in the dentate gyrus and area CA1 *in vivo* in a manner similar to that observed in slices. Here we tested the effects of BDP on LTP in field CA1 of freely moving rats. LTP was induced using a minimal stimulation paradigm involving pairs of short high-frequency bursts (paired theta bursts) repeated 5 times at 30 seconds; this pattern produces a small and transient (24hrs) potentiation effect. Each of nine animals was tested both in absence and presence of the drug in a counterbalanced fashion. Injection of the drug prior to theta burst stimulation greatly facilitated the subsequent induction of LTP; i.e., the increase in slope and amplitude was larger in magnitude and more persistent (several days) following drug than vehicle injections. Biodistribution and pharmacokinetics were examined with positron emission tomography (PET) using BDP radiolabelled with carbon-11; the results confirmed that the drug rapidly migrates from the injection site and crosses the blood-brain barrier; time activity curves for brain, heart and liver showed that the drug reaches a maximum in the brain in less than 4 minutes. The facilitatory effects on LTP induction in freely moving animals were replicated with an analogue of BDP (BDP-5), which was also found to improve retention in a radial maze and in an odor matching problem. These results define the first tool for enhancing LTP *in vivo* and confirm an important prediction from the hypothesis that LTP is a substrate of memory.

622.4

FACILITATION OF OLFACTORY LEARNING BY A NOVEL MODULATOR OF AMPA RECEPTORS. J. Larson*[1], T. Lieu[1], V. Petchpradub[1], G. Rogers[2], & G. Lynch[1]. [1]CNLM, Univ. Calif., Irvine, CA 92717, [2]Neurosci. Inst., Univ. Calif., Santa Barbara, CA 93106.

Recent studies indicate that drugs that enhance excitatory currents mediated by AMPA receptors facilitate the induction of hippocampal long-term potentiation. The present experiment tested the effects of a novel AMPA receptor modulator, CX-516, on acquisition, retention, and performance in an olfactory discrimination paradigm.

Adult rats (n=12) were trained on a series of simultaneous cue, two-odor discriminations until new problems were acquired in one session. They were then given a series of novel problems in which only 3-5 training trials were used. Prior to each problem, half the rats were given CX-516 (30 mg/kg) and the other half vehicle injections; positive and negative cues were counter-balanced across rats. Each rat was trained on 2-3 novel problems under drug and vehicle conditions. Retention was assessed 1-3 days post-training by giving 5 unrewarded probe trials.

The rats trained on odor problems after vehicle injections showed chance responding on subsequent retention trials (51.1 $\pm$ 6.6% correct); the same rats trained after CX-516 injections showed significantly better retention (73.4 $\pm$ 7.0% correct; $p<.01$). The animals also made more correct responses on training trials 2-5 on drug vs. vehicle days (79.3 + 6.3% vs. 45.8 + 12.4%; $p<.05$). There were no evident drug effects when the rats were tested on already-learned odors.

These results suggest that facilitation of AMPA receptors can enhance acquisition of novel information without causing substantial changes in performance variables (e.g., cue processing, motor responses, motivation) involved in the training paradigm. Accordingly, drugs such as CX-516 may be of use in the treatment of memory disorders.

(Supported by AFOSR, ONR, NIA, and NIH).

622.5

ENHANCEMENT OF SYNAPTIC TRANSMISSION IN HIPPOCAMPUS OF AGED RATS BY NOVEL MODULATORS OF AMPA RECEPTORS. M. Coogan[1], J. Larson[1], G. Rogers*[2], & G. Lynch[1]. [1]CNLM, Univ. Calif., Irvine, CA 92717, [2]Neurosci. Inst., Univ. Calif., Santa Barbara, CA 93106.

Recent studies have identified a novel class of drugs (Ampakines) that facilitates excitatory synaptic currents mediated by AMPA receptors. Since aging is accompanied by decreases in excitatory communication, it has been suggested that such drugs might offset certain types of age-related deficits. This idea rests on two unexamined assumptions about AMPA receptors in the aged brain: i) that the binding site(s) for the drugs is unchanged from that found in young brains and ii) that the channel kinetic properties affected by the drugs are also comparable in young vs. aged brains. These assumptions were tested by comparing drug effects on synaptic responses in young (1-4 months) and aged (24-28 months) rats.

Hippocampal slices were prepared using conventional methods and field EPSPs recorded in the CA1 region. Two Ampakines (CX-516 and CX-517) found to produce potent effects in pilot studies were used. Both drugs produced qualitatively similar, dose-dependent, reversible enhancements of field EPSP amplitude and prolongations of decay time in slices from young and aged rats. CX-516 had more potent effects on response amplitude whereas CX-517 was more potent in modulating the decay time. Quantitative comparisons involving several response parameters and patterns of synaptic activation will be described and shown to reinforce the conclusion that the drugs have essentially the same effects on synaptic responses in the young and aged hippocampus. Based on these findings, it appears that Ampakines can be used to test the hypothesis that facilitation of fast, excitatory transmission will have positive effects with regard to age-related changes in brain functioning.

(Supported by AFOSR, ONR, NIA, and NIH).

622.6

QUANTITATIVE LOCALIZATION OF AMPA RECEPTOR mRNAs - EVIDENCE FOR REGIONALLY SPECIFIC SUBUNIT STOICHIOMETRIES S. J. Gold*[2], M. Hennegriff[2], G. Lynch[2], and C. M. Gall[1]. [1]Dept. of Anatomy and Neurobiology, and [2]Psychobiology, University of California, Irvine, CA 92717

Quantitative in situ hybridization and reverse-transcriptase PCR were used to determine the relative levels of AMPA receptor subunit (GluR1-3) mRNAs in rat forebrain. In all regions sampled, GluR2 cRNA hybridization was dense, being greater than or equal to the other two subtypes. Levels of GluR1 and GluR3 mRNA, however, varied markedly between hippocampus, neocortex and piriform cortex. In hippocampal neuronal layers, GluR1 cRNA hybridization density was equal to GluR2 but was 3-4 fold higher than GluR3. In contrast, parietal cortex (layers II-III) had relatively low levels of GluR1 mRNA (40% of GluR2), but high levels of GluR3 (60% of GluR2). In piriform cortex (layer II), GluR1 and GluR3 mRNA levels were both low (33% of GluR2). If relative levels of mRNA are predictive of relative levels of subunit protein, then it is likely that the stoichiometry of the AMPA receptor varies substantially between synaptic systems in cortical zones of the telencephalon. Attempts to test this using immunoprecipitation with antibodies directed at the subunits of the receptor are in progress. It will also be of interest to determine if the regional variations described here are associated with differences in plasticity of the LTP type which is reported to be dependent upon the AMPA receptor subunit composition as well as with the effects of recently developed drugs ("Ampakines") that facilitate AMPA receptor mediated responses in the hippocampus of behaving animals. Supported by AG00538.

622.7

TEA PRODUCES A PERSISTENT ENHANCEMENT OF SYNAPTIC TRANSMISSION IN RAT NEOCORTEX. Marc R. Pelletier* and John J. Hablitz. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

We investigated the ability of tetraethylammonium (TEA) to induce long lasting changes in neocortical synaptic activity. Intracellular recordings were obtained from layer II/III pyramidal neurons in *in vitro* brain slices. Bath application of 25 mM TEA for 7 min produced transient effects on passive membrane properties. The resting membrane potential depolarized by 4.1 $\pm$ 0.4 mV ($\pm$ SEM; n=9) and the input resistance increased by 16 $\pm$ 4.2%. The duration of action potentials, evoked by depolarizing current, was increased dramatically; an effect consistent with the blockade by TEA of K^+ channels. Action potential duration was not different from control after 30-40 min of wash; TEA was then considered to have been washed out. TEA produced a persistent (45 min of wash) increase of 158.7 $\pm$ 7.6% in the amplitude of EPSPs evoked by weak orthodromic stimulation (layer IV/V; 0.05 Hz). The amplitude of chloride-dependent IPSPs evoked by strong orthodromic stimulation was increased by 180 $\pm$ 10.0 %. These effects on synaptic transmission were APV (20μM)-resistant (n=4). Orthodromic stimulation during TEA application was not required for enhancement (n=5). The enhancement of EPSPs, but not IPSPs, was blocked by recording with pipets containing 200 mM BAPTA (n=4). TEA-induced enhancement of EPSPs in the neocortex is similar to that described in hippocampus. Additionally, we report an enhancement of IPSPs, which might be mediated presynaptically via an increase in GABA release.

622.8

INDUCTION OF LTP VIA VOLTAGE-DEPENDENT CALCIUM CHANNELS (VDCCs) IS NOT SYNAPSE SPECIFIC. K.M. Huber*, M.D. Mauk, and P.T. Kelly, Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School-Houston, TX 77225.

Tetraethylammonium (TEA) induced potentiation (LTP_K) was used to examine the properties of LTP induced by Ca^{2+} influx through VDCCs in area CA1 of hippocampal slices. We previously showed that LTP_K in isolated CA1 slices was strictly dependent on NMDA receptor activity (Huber et al., SN abstract # 547.10, 1993). We found that delivering 80 msec pulses of 25 Hz stimulation once every 5 sec to Schaffer collaterals during TEA applications induced LTP_K (26 $\pm$ 4%, n = 12) in the presence of APV (100-200 μM), but was blocked by APV plus nifedipine (10-50 μM; 5 $\pm$ 3%, n = 6). We utilized this protocol in two pathway experiments to determine the synapse specificity of VDCC-dependent LTP_K. Stimulation was turned off to one pathway during TEA applications, while the other independent pathway was stimulated using the 25 Hz stimulation protocol (described above). Significant potentiation was observed in the stimulation off pathway (27 $\pm$ 3%, n = 12), indicating that induction of VDCC dependent LTP_K is not synapse specific. This finding is supported by experiments in which CA1 neurons were stimulated antidromically (alveus) using the 25 Hz stimulation protocol and all synaptic (Schaffer collateral) stimulation was ceased during TEA applications. The antidromic stimulation protocol in APV (100- 200 μM) resulted in potentiation of EPSPs (20 $\pm$ 5%, n = 5) when synaptic stimulation was resumed 30 min after TEA application. This property contrasts with NMDA receptor-dependent LTP which has been shown to be specific to synapses that are active during induction and suggests that the route of Ca^{2+} entry can control the synapse specificity of LTP. These data support the hypothesis that a function of dendritic spines is to localize NMDA receptor mediated increases in postsynaptic Ca^{2+} and are responsible for synapse specificity. In contrast, VDCCs have been shown to be localized on proximal dendrites and may primarily mediate Ca^{2+} increases in dendritic shafts (Westenbrock, et al. Nature, 1990; Müller and Connor, Nature, 1991) and therefore do not contribute to the mechanisms underlying synapse specificity.

622.9

THE NMDA RECEPTOR REDOX SITE: DTNB BLOCKS INDUCTION AND EXPRESSION OF TETANIC NMDA-LTP BUT NOT AMPA-LTP

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In physiological conditions, the induction of LTP is mediated by NMDA receptors, whereas the expression is mediated primarily by AMPA receptors (AMPA-LTP). LTP of NMDA receptors (NMDA-LTP) can be induced however, in several conditions, notably when AMPA receptors are blocked partially or completely and (or) when (Mg++) is reduced to boost NMDA responses (Asztely et al., Europ. J. Neurosci.,1992, 4: 681). Since DTNB and DTT have no effect on AMPA receptor-mediated responses in rat hippocampal slices and since a redox site sensitive to these drugs has been shown to modulate NMDA receptor responses (Tang and Aizenman, J. Physiol. 1993, 465: 303), we have investigated the effect of DTNB (200μM) on the two types of tetanic LTP using extracellular recording techniques. The following results have been obtained: 1) DTNB does not affect the induction or expression of AMPA-LTP. 2) DTNB irreversibly reduced (maximally) 35-45% of control NMDA receptor- mediated responses. 3) DTNB prevented the induction of NMDA-LTP. 4) The expression of NMDA-LTP was completely reversed by DTNB.These results indicate that at least one disulfide bridge exists in NMDA but not in AMPA receptors. This S-S group is probably reduced during the tetanic stimulation and the SH groups generated in the NMDA receptor are necessary not only for the induction but also for the expression of NMDA-LTP (see Gozlan et al. this meeting). Interestingly, the induction of AMPA-LTP by NMDA receptors apparently does not require these SH groups. These observations provide the first direct evidence of a major difference between the expression of NMDA-LTP and AMPA-LTP. They cannot be readily reconcilied with a presynaptic hypothesis for the maintainance of LTP.

622.10

THE NMDA RECEPTOR REDOX SITE: INDUCTION AND EXPRESSION OF ANOXIC LTP ARE BLOCKED BY DTNB.

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A novel form of LTP induced by a brief anoxic-aglycemic episode has been recently described in this laboratory (Crepel et al., J. Neurophysiol.,1993, 70: 2045).This anoxic LTP is mediated exclusively by NMDA receptors, in contrast to tetanic LTP which is primarily mediated by AMPA receptors. Since NMDA receptor responses are modulated by redox conditions (Tang and Aizenman, J. Physiol.,1993, 465: 303), and since an anoxic episode induces modifications of redox equilibriums, we have studied the effects of the thiol specific oxidizing reagent DTNB on the induction and the expression of anoxic LTP. In control slices anoxia generated a LTP, and this process was fully prevented by DTNB. Once potentiated, the NMDA response returned to control levels in the presence of DTNB and application of disulfide reducing agents potentiated NMDA responses reversing the inhibition induced by DTNB. In the presence of DTNB (200μM), 35-45% of control NMDA field epsp was irreversibly reduced and the anoxic LTP could not be generated. These results indicate that anoxia induces a modification of the redox site of the NMDA receptor, probably a reduction of at least a disulfide bond and the free SH groups generated by this process are required for the induction and expression of anoxic LTP. This effect is specific to NMDA receptors: tetanic LTP of NMDA but not AMPA receptors requires a redox modification (Chinestra et al., this meeting). If such a process could be demonstrated to occur in vivo, then disulfide drugs would have some interest in the treatment of the consequenses of ischemia.

622.11

(RS)-α-METHYL-4-CARBOXYPHENYLGLYCINE AND (RS)-4-CARBOXY-3-HYDROXYPHENYLGLYCINE BLOCK TETANUS-INDUCED LTP, BUT NOT METABOTROPIC GLUTAMATE AGONIST-INDUCED INCREASES IN ADENYLYL CYCLASE ACTIVITY.

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Induction of long-term potentiation (LTP) in the CA_1 region of hippocampus is dependent on N-methyl-D-aspartate (NMDA) receptor activation, however, stimulation of this receptor alone is not sufficient to maintain LTP. In our previous work, we have demonstrated that coactivation of a metabotropic glutamate receptor (mGluR) coupled to postsynaptic increases in adenylyl cyclase activity and NMDA receptor is required for LTP induction (Musgrave, Ballyk and Goh, Neuroreport, 4, 171-174, 1993; Musgrave, Madigan, Bennett and Goh, J. Neurochem, in press, 1994). In these studies we examined the ability of novel mGluR antagonists, analogues of phenylglycine, to block induction of LTP as well as the increase in cAMP induced by mGluR agonists. For LTP studies, extracellular EPSPs were recorded at the CA_1 dendritic zone of rat hippocampal slices. Bath application of either 4-carboxy-3-hydroxyphenylglycine (500 μM) or (RS)-α-methyl-4-carboxyphenylglycine (500 μM) both blocked tetanus-induced (100 Hz, 1s) LTP in a reversible manner. For assessment of adenylyl cyclase antagonism, rat hippocampal synaptoneurosomes were incubated with each phenylglycine compound alone, or in combination with the mGluR agonists 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R ACPD; 100 μM) or quisqualate (1mM) or L-amino-4-phosphonobutyric acid (L-AP_4; 1 mM). Neither 4-carboxy-3-hydroxyphenylglycine nor (RS)-α-methyl-4-carboxyphenylglycine had any effect on either basal levels or mGluR agonist-induced increases in adenylyl cyclase activity at any concentrations of antagonist tested (100 μM, 500 μM, 1 mM, 10 mM). These studies suggest that while these compounds are effective in blocking tetanus induced LTP they probably do not do so via inhibition of the mGluRs coupled to the cAMP system. (Supported by the Medical Research Council of Canada).

622.12

1S-3R ACPD can cause long-term depression and depotentiation of synaptic transmission in the dentate gyrus of rat hippocampal slices.

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The effect of the metabotropic glutamate agonist 1S-3R ACPD (200 nM - 100 μM) on synaptic transmission was examined in both naive (non-potentiated) and potentiated submerged rat hippocampal slices (400 μm). A bipolar stimulating electrode and a blunt aCSF-filled recording pipette were lowered into the middle third of the perforant path of the dentate gyrus under visual control; field excitatory postsynaptic potentials (fEPSP's) were evoked at .05 Hz (stimuli were 3-15 volts, 100 μsec). Application of 1S-3R ACPD (20 min) depressed fEPSP amplitude in a dose-dependent fashion in the dentate gyrus of 8 of 8 naive hippocampal slices. At 200 nM ACPD the average EPSP amplitude was 88% ± 1% of baseline; at 100 μM EPSP amplitude was 29% ± 2% of baseline. Enhancement of synaptic transmission was observed in one slice (1 μM, 20 mins) of 23 tested; EPSP amplitude increased by 25% ± .05% and subsequent tetanic LTP was occluded. Long-term potentiation (LTP) was induced using 8 trains of 8 pulses at 200 Hz, inter-train interval of 2s; the average increase in fEPSP amplitude at 20 mins post-high frequency stimulation was 53% ±5%. When applied for 2 mins, 1S-3R ACPD (10 μM) transiently reduced fEPSP amplitude in naive slices (94% ± 3% of baseline) and potentiated slices (91% ± 1% of potentiated baseline); all slices returned to baseline in 120 secs post-washoff. ACPD (100 μM) applied for 20 minutes reduced the magnitude of potentiated EPSP's; the average decrease in EPSP amplitude 20 min post-washoff was 75% ±12% of baseline in 9 of 9 slices. When homosynaptic long-term depression was induced (average decrease after 20 min was to 62% ±.5% of baseline) using low frequency stimulation (1 Hz, 10 mins), 100 μM 1S-3R ACPD induced a further long-term depression (20 mins, decrease to 46% ±.4% of baseline) in fEPSP amplitude which was reversible with high frequency stimulation.

We conclude that sustained activation of metabotropic glutamate receptors with 1S-3R ACPD can induce both long-term depression and depotentiation of synaptic transmission in the dentate gyrus of the hippocampal slice.

622.13

INOSITOL HEXAKISPHOSPHATE CAUSES DEPRESSION OF POPULATION SPIKE AMPLITUDE IN SCHAFFER COLLATERAL-CA1 SYNAPSES.

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Hydrolysis of phosphatidylinositols generates various inositol phosphates including inositol hexakisphosphate (IP_6) which can reach a concentration of 25-100μM in mammalian cells. While the neurophysiological roles of IP_6 are not well defined, IP_6 application has been reported to cause Ca^{2+} influx and to release neurotransmitters such as aspartate and catecholamines as measured biochemically. Interestingly, IP_6 can act extracellularly by binding to AP-2, a clathrin-associated protein involved in endocytosis and synaptic vesicular recycling processes. Thus, it appears that IP_6 could play a role in the modulation of synaptic transmission. To assess this hypothesis, IP_6 was bath-applied to guinea pig hippocampal slices and the population responses evoked by Schaffer collateral stimulation were recorded in area CA1. Following 60 min of baseline recording, IP_6 (25μM) was applied for 30 min and synaptic transmission was monitored for another 90 min. A significant depression of the population spike amplitude (recorded in s. pyramidale) was observed 60 min after the onset of IP_6 treatment (27% decrease) and had reached a plateau after 120 min (79% decrease, n=7). However, no significant change in the population EPSP (recorded in s. radiatum) was observed over the same monitoring period (n=4). A preliminary finding indicates that pretreatment with the GABAergic antagonist bicuculline (1μM; n=2) blocked the depression of the population spike amplitude produced by IP_6, suggesting that this compound may exert its electrophysiological effects by potentiating GABAergic feed-forward inhibition. Supported by FRSQ Québec, PHS MH51106, the Sloan Foundation and the Klingenstein Foundation.

622.14

IMPAIRED LTD AND PAIRED PULSE FACILITATION IN THE CA1 REGION OF THE HIPPOCAMPUS OF COGNITIVE DEFICIENT MICROENCEPHALIC RATS.

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Recently we have reported that in the CA1 region of the hippocampus of cognitive deficient microencephalic rats LTP is disturbed, and that this defect could be restored by D-Serine, an agonist for the glycine-site of the NMDA receptor (Ramakers et al., Neuroscience 54, 49-61, 1993). In the present study we have investigated long-term depression (LTD) and paired pulse facilitation (PPF) in slices from these rats. Microencephaly was induced by injection of methylazoxy methanol (MAM, 25 mg/kg) to pregnant rats on day 15 of gestation (G15). Field EPSPs were recorded in the radiate layer of the CA1 field by stimulating (at 0.1 Hz and 1/2 maximum intensity) orthodromically. Glass-electrodes filled with artificial cerebrospinal fluid (ACSF) were used for the recording. In slices from control rats (injected with saline on G15) 1 Hz stimulation for 15 minutes elicited LTD which lasted for at least one hour (-52.5 ± 3.3%, 60 minutes after induction). In slices from microencephalic rats identical stimulation failed to induce LTD (+3.6 ± 2.7%). D-Serine (2 mM) added to the ACSF before and during the 1 Hz stimulation did not correct the defect in induction of LTD. In addition, paired pulse stimulation with interstimulus interval (ISI) of 50, 100 and 200 ms failed to induce PPF in slices from microencephalic rats, instead a depression of the second respons was observed. This depression depended on the stimulus strength, as weak stimulation showed no depression (but still no potentiation). At present the effect of D-Serine on this is investigated. The fact that D-Serine restores LTP and fails to correct LTD in slices from MAM-treated rats suggests that both forms of plasticity may require a different NMDA receptor activation. The absence of PPF and the finding of PPD suggests that the defects in plasticity of glutamatergic synapses in MAM-treated rats involve both pre- and postsynaptic mechanisms.

622.15

ENHANCED LTP PRODUCED BY EXPOSURE TO THE REDUCING AGENT DITHIOTHREITOL (DTT) IS REVERSED BY THE REDOX AGENT PYRROLOQUINOLINE QUINONE (PQQ).

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PQQ is a putative essential nutrient shown *in vitro* to diminish NMDA ionic currents and neurotoxicity by direct oxidation of the NMDA receptor redox site (*J. Neurosci.* 1992;12). Administration of PQQ prior to hypoxia/ischemia in a rodent stroke model significantly reduces infarct size (*Neuroscience*, 1994; in press). Reduction of the NMDA redox site by DTT enhances LTP (*Br. Res.* 1990; 519). In order to establish whether PQQ modulates NMDA activity via the redox site, we compared the effect of DTT and PQQ on LTP in hippocampal slices from adult rats. Extracellular synaptic potentials were recorded from s. rad. of area CA1 by stimulating CA3 Schaffer collaterals. LTP was induced by tetanic stimulation (100Hz for 1mscc, repeated once after 20 sec) at an intensity evoking 1/2 of the maximal response. In slices perfused with control medium, the tetanus increased the slope of the EPSP 52 $\pm$ 8.5% (mean $\pm$ SE, n=12 slices, n=12 rats). DTT exposure (100μM for 30 min, followed by 30 min wash) significantly increased the magnitude of LTP (88.9 $\pm$ 16.6% (n=8 slices, n=8 rats, $p<0.04$). In contrast, following exposure to DTT, PQQ (100μM) significantly reduced LTP (10.5 $\pm$ 7.4%, n=7 slices, n=7 rats, $p<0.005$). These results are consistent with *in vitro* observations from cultured cortical neurons and demonstrate that, in intact pathways in hippocampal slice, PQQ reverses the action of DTT on NMDA receptor mediated events. (NS31718,EFA, AHA).

LONG-TERM POTENTIATION: PHARMACOLOGY II

623.1

THE INFLUENCE OF $GABA_A$- AND $GABA_B$-ANTAGONISTS ON THE MAINTENANCE OF HIPPOCAMPAL LONG-TERM POTENTIATION IN RATS.

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Hippocampal long-term potentiation (LTP) is thought to serve as an elementary model for the investigation of processes underlying learning and memory formation.

Since a possible involvement of GABAergic transmission on the prolonged maintenance of LTP (> 4 hours) has not yet been shown, we examined the influence of GABAergic antagonists on late stages of hippocampal LTP in the CA1 region *in vitro*. The method used has been described previously by Frey et. al (*Brain Res:*. **452**, 57 - 65, 1988).

Here we present some evidence, that bath application of the $GABA_A$-antagonist picrotoxin (10 µM) and of the $GABA_B$-antagonist 5-aminovaleric acid (50 µM) did not block the induction and maintenance of LTP for at least 8 hours. Even the application of 50 µM picrotoxin and 50 µM 5-aminovaleric acid together had no influence on the induction or maintenance of LTP. Since application of 10 µM picrotoxin (as used in the first set of experiments) in combination with 50 µM 5-aminovaleric acid did not cause complete inhibition of the GABAergic transmission, 50 µM picrotoxin was used in experiments, where both substances were applied simultaneously.

Our results indicate, that the induction and prolonged maintenance of hippocampal CA1-LTP is independent of GABAergic transmission.

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623.2

Inhibitory Synaptic Transmission Modulates the Induction of Long Term Depression in CA1 Region of Hippocampus.

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The goal of this study was to examine the role of GABAergic input in the induction of long-term depression (LTD) at the Schaffer collateral/commissural synapses in area CA1 of hippocampus slices. The induction of LTD in the hippocampus (Yang et al., *Soc. Neurosci. Abstracts*, #183.3, 1993) and possibly in the cerebellum (Ekerot and Kano, *Brain Research*, 1985) is regulated by GABA. Therefore, we hypothesize that GABAergic input is required for LTD induction in the hippocampus. To test this hypothesis we attempted to induce LTD in the presence of 50 µM picrotoxin ($GABA_A$ antagonist) and again in the same slice one hour after picrotoxin washout. We find that low frequency synaptic stimulation (600 pulses at 1 or 3 Hz) in the presence of picrotoxin produces only a transient decrease of EPSP slope. This decrease returned after 25 minutes to 98% $\pm$ 1 of baseline after 1 Hz stimulation and 95%$\pm$ 2 after 3 Hz (n=5). In the same slice LTD could be reliably induced after picrotoxin washout; EPSPs were reduced to 86% $\pm$ 2 and 80% $\pm$ 1 after 1 and 3 Hz stimulation respectively . These results suggest that GABAergic synaptic transmission can influence LTD induction.

623.3

GABAergic AND DEVELOPMENTAL MODULATION OF HOMOSYNAPTIC LTD AND DEPOTENTIATION IN THE HIPPOCAMPUS.

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To test the hypothesis that GABA may regulate the induction of homosynaptic LTD and depotentiation (DPT), we compared the effects of GABA antagonists on hippocampal slices from young (16-22 days) and mature (5-10 weeks) rats. The slopes of extracellular CA1 field EPSPs were used to monitor synaptic transmission in s. radiatum. Low-frequency stimulation (LFS, 1 Hz/900 pulses) elicited LTD with respect to baseline synaptic transmission, or DPT from a potentiated level following LTP induction by HFS (100 Hz/1 sec). In young animals, LTD was inhibited by 1 mM CGP 35348 (a $GABA_B$ antagonist), -12 $\pm$ 2%, n=10 vs a -27 $\pm$ 1%, n=9 decrease in EPSPs from control slices. In contrast, homosynaptic DPT was unaffected by CGP 35348. In mature animals, LFS did not induce significant LTD (-4 $\pm$ 2%, n=38, from baseline), although significant DPT could be consistently elicited by an LFS given 30 minutes after LTP induction (24 $\pm$ 5%, of potentiation remaining, n=15). Bicuculline (10 uM, a $GABA_A$ antagonist) had no significant effect on LTD magnitude in young animals, but significantly enhanced LTD expression in slices from mature animals. In addition, after HFS, LTD (relative to the initial baseline) was expressed in mature slices following 2-3 LFS episodes. Our results suggest that the influences of both age, and of prior synaptic activity (i.e. HFS) on LTD induction can be explained by changes in GABAergic systems in young vs mature, and naive vs tetanized slices.

623.4

NITRIC OXIDE REGULATES THE THRESHOLD OF FREQUENCY DEPENDENT PLASTICITY IN AREA CA1 OF RAT HIPPOCAMPUS.

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Inhibition of nitric oxide synthase (NOS) blocks the induction of certain forms of long-term potentiation (LTP) and disrupts the acquisition of several different forms of learning. We examined the effects of both nitric oxide (NO) donors and NOS inhibitors on plasticity in area CA1 of the rat hippocampal slice.

Standard techniques were used for hippocampal slice preparation. Slices were obtained from Sprague-Dawley rats (14-35 days old) and experiments were performed in a submersion-type recording chamber at 31.6° C. Stimulating and recording electrodes were placed in stratum radiatum and extracellular field potentials were elicited every 15 seconds. Drugs were washed in via a continuous perfusion system.

In the presence of an NO donor, stable LTP could be induced by a variety of tetanic stimuli that were normally below threshold for LTP induction. We were able to induce LTP at tetanus parameters as weak as 25 pulses delivered at 10 Hz in the presence of the NO donors hydroxylamine (200 µM) or S-nitroso-N-acetylpenicillamine (200 µM). In the presence of the NMDA receptor antagonist AP5, a subthreshold tetanus of 25 pulses delivered at 50 Hz in combination with hydroxylamine induced a slowly developing potentiation.

The frequency of synaptic activation is a critical factor in determining the resulting plasticity. While holding the absolute number of stimuli constant (900), varying the frequency of stimulation from 1 Hz to 30 Hz will shift the resulting plasticity along a curve from depression to potentiation. Inhibition of NOS at frequencies that normally induce long-term depression (1-3 Hz) does not alter the resulting plasticity. Our preliminary results indicate that although NO does appear to lower the threshold for LTP induction, it is apparently without effect on the induction of long-term depression.

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623.5

PLATELET ACTIVATING FACTOR (PAF) RELEASED DURING HIPPOCAMPAL LTP: ROLE AS A RETROGRADE MESSENGER.

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Platelet activating factor (PAF) is an alkyl-ether phospholipid with significant neuroregulatory and neuropathological actions (Science, 240:1792,1988), including the induction of LTP in the hippocampus that could be blocked by PAF receptor antagonists, as well as by the NMDA receptor antagonists, APV and MK801 (Neuron, 10: 553, 1993). Here we have used radioimmunoassays (RIA) to measure PAF release from hippocampal slices during LTP induced by high frequency stimulation (HFS). During basal activity, before HFS, PAF levels in the medium were 0.533±0.033 ng/ml. 2 min after HFS this level did not change significantly (0.605±0.058 ng/ml PAF, $p = 0.37$). 15 min after HFS a significant increase to 1.33 ±0.20 ng/ml PAF was detected ($p < 0.05$). Upon establishment of stable LTP (60 min after HFS) PAF levels increased 9 fold above basal (to 4.57±0.27 ng/ml, $p < 0.001$), and remained the same up to 2 hours post stimulation (4.60±0.10 ng/ml PAF). We propose that postsynaptic calcium influx stimulated by NMDA receptors increases PAF biosynthesis. The PAF released from postsynaptic membranes interacts with presynaptic receptors to increase glutamate release. Each turn of this cycle amplifies subsequent responses. These biochemical reverberations in the Hebbian synapse would create a permanent increase in synaptic efficiency.

623.6

ACTIVATION OF ADENOSINE A_2 RECEPTORS POTENTIATES SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS.

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Adenosine is released into synapses in proportion to neuronal firing rate. Adenosine agonists have been shown to decrease synaptic transmission via activation of an A_1 receptor subtype. We have previously shown that non-A_1 receptor activation significantly potentiates P-type Ca current in acutely isolated hippocampal pyramidal neurons from the CA3 region (Mogul et al., *Neuron*, **10**: 1993). This effect appears to occur via activation of the A_{2b} receptor since the effect is observed with the non-selective A_2 agonist DPMA but is not seen with selective A_{2a} activation. We tested the effect of A_2 activation on synaptic transmission in young adult rat transverse hippocampal slices. Stimulation was applied to Schaeffer pathway and extracellular potentials were recorded in stratum radiatum of CA1. The EPSP was potentiated in the presence of the A_1 antagonist CPT (5 μM), presumably by the release of endogenous adenosine activating a non-A_1 receptor. In the presence of the A_2 antagonist DMPX (10 μM), the EPSP responded to a submaximal tetanic stimulation (T_s; 100 Hz, 1sec) with a post-tetanic potentiation (PTP) that returned to baseline within 10 min. LTP induced by T_s in control solution was not affected by subsequent DMPX exposure, however no additional LTP could be induced (although PTP was observed). These results suggest that A_2 receptors may be involved in the induction of some component of LTP.

623.7

DEVELOPMENTAL CHANGES IN CELLULAR MECHANISMS OF LONG-TERM POTENTIATION IN THE HIPPOCAMPUS : MATURATION OF A1 ADENOSINE RECEPTORS.

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Memory processes are generally thought to change during maturation of the brain. Long-term potentiation (LTP) and post-tetanic depression (PTD) in hippocampus have been proposed as important processes underlying memory. We previously reported that endogenous adenosine is released during tetanic stimulation and plays an essential role in the induction of LTP and PTD in rat hippocampal slices (Sekino,Y., et.al. B.B.R.C. 181, 1010-1014, 1991; Sekino,Y., Soc. Neurosci. Abstr. 18, 640, 1992). Here we examine how the role of adenosine in synaptic plasticity changes during brain development. We evaluated the pharmacological effects of an A1 receptor antagonist, 8-cyclopentyltheophylline (8-CPT), on synaptic transmission in slices of guinea pig hippocampus. Synaptic responses were measured in CA1 neurons in response to single stimuli and trains of stimuli applied to Schaffer/commissural fibers. In slices from adult animals, 8-CPT (1 μM) increased synaptic responses produced by single stimuli and had no effect on LTP elicited by trains of stimuli. However, in slices from juvenile animals (3 and 4 weeks), the same treatment had quite different effects : 8-CPT had no effect on responses to single stimuli but inhibited the induction of LTP by trains of stimuli. To determine whether this change in adenosine responsiveness was associated with changes in A1 receptors, we measured adenosine binding activity in hippocampal membrane preparations obtained from rats of different ages. A1 receptor activity increased gradually during the first 2 postnatal weeks and reached a plateau by the third week. Thus, the age-dependent changes in synaptic transmission may depend upon the maturation of adenosine receptors. We speculate that such developmental changes in adenosine receptors may contribute to the cellular mechanisms underlying the development of LTP and that such changes may produce some of the alterations in memory that occur in the developing brain.

623.8

INDUCTION OF LTP IN A SYMPATHETIC GANGLION NEEDS ACTIVATION OF 5-HT_3 RECEPTORS.

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In the superior cervical ganglion (SCG) of rat, brief preganglionic supramaximal tetanic pulse induces LTP manifested as long-lasting enhancement of the compound action potential (population spike) of the nicotinic pathway. LTP in the SCG, is independent of activation of cholinergic or adrenergic receptors during tetanic stimulation. Serotonin has been reported to be present in some of the ganglionic "interneurons"; the small intensely fluorescent (SIF) cells. We examined the role of the serotonin 5-HT_3 receptor subtype in LTP because it is widely distributed throughout the peripheral nervous system and exists at high density in peripheral neurons. Pretreatment of ganglia with the 5-HT_3 receptor antagonist MDL72222 (0.5 μM) 1 hr before the train had no significant effect on the basal ganglionic transmission but prevented the expression of LTP. To confirm the role of endogenous serotonin in the generation of LTP we used ganglia from rats treated with reserpine (3 mg/kg) 24 hrs prior to removal of ganglia. In contrast to ganglia from untreated rats, in those from reserpinized animals tetanic stimulation resulted only in post-tetanic potentiation (PTP) with no LTP or short-term potentiation (STP). In these experiments, when the 5-HT_3 receptor agonist 1-(m- chlorophenyl) biguanide (PBG) was applied 3 min (during PTP) or one hour after tetanic stimulation no LTP was seen. However, when PBG was superfused on reserpinized ganglia 30 min before tetanic stimulation, the LTP consistently appeared. These results present evidence that activation of serotonin 5-HT_3 receptor subtype is necessary for the induction of LTP in the SCG.

623.9

HISTAMINE AND LONG-TERM POTENTIATION.

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Long-term potentiation (LTP) of glutamatergic synaptic transmission in the hippocampus, a candidate mechanism for learning and memory formation, is modulated by a number of other neurotransmitters. Since histamine can increase calcium influx through the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, which is the initial trigger for LTP, we wanted to see if histamine could also modulate the induction of LTP.

We tested this possibility in the CA1 region of rat hippocampal slices, prepared according to standard procedures. A weak tetanus (0.25 s, 50 Hz) was delivered twice in the same slices with an interval of 140 min. In control experiments this tetanus led to a short-term potentiation (lasting less than one hour) of the field excitatory postsynaptic potentials on both occasions ($n = 6$, $p > 0.05$). However, when histamine (100 μM) was washed in 20 minutes prior to the second tetanus, a long-term potentiation was observed which remained significant for up to 2 hours following the tetanus ($n = 6$, $p < 0.01$). A lower concentration of histamine (10 μM) also gave significant potentiation at 60 min following the tetanus but by 90 min the potentiation was no longer significant ($n = 7$).

We tried to block this effect with the H1 antagonist, mepyramine (1 μM), and the H2 antagonist, cimetidine (50 μM), applied together before the first tetanus but were unable to do so ($n = 8$, $p < 0.01$). The antagonists did not affect the LTP produced by the combination of weak tetanus and histamine (100 μM) application. In additional experiments we confirmed that histamine could enhance NMDA-gated responses in slices and therefore, we conclude that histamine facilitated the induction of LTP by enhancing NMDA currents.

623.10

PROPRANOLOL SUPPRESSES LTP INDUCTION IN BOTH THE LATERAL AND MEDIAL PERFORANT PATH INPUTS TO THE DENTATE GYRUS.

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Recent evidence suggests that the lateral (LPP) and medial (MPP) perforant path inputs to the dentate gyrus can exhibit different forms of long-lasting synaptic plasticity. Norepinepherine application induces a lasting potentiation of MPP responses and a simultaneous depression of LPP responses, while LTP induction in the LPP, but not MPP, requires opioid receptor activation. β-Adrenergic receptor activation is also known to be important for LTP induction in the dentate gyrus. However, the role of β-receptors in LTP has not been assessed using selective stimulation of LPP and MPP fibers.

High-frequency stimulation (HFS; 100 Hz, 1 or 2s) applied to the dentate outer or middle molecular layer of rat hippocampal slices induced selective LTP of LPP or MPP responses, respectively, as assessed by field EPSP slope measurements obtained from the synaptic layers. The population spike was recorded by an electrode in the granule cell layer. In the MPP, the β-adrenergic receptor antagonist (-)-propranolol (1 μM) reduced in magnitude, but did not completely block, LTP of the field EPSP and population spike. In the LPP, LTP of the field EPSP appeared to be completely blocked by propranolol. The results suggest that β-adrenergic receptor activation is required for full LTP induction of both the lateral and medial perforant path inputs to the rat dentate gyrus. Supported by NIH NS23865

623.11

DELTA AND MU OPIOID RECEPTOR ACTIVATION IS REQUIRED TO INDUCE LTP IN THE LATERAL PERFORANT PATH OF NORMAL, BUT NOT DISINHIBITED, HIPPOCAMPAL SLICES. C.R. Bramham* and J.M. Sarvey
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Opioid receptor-dependent LTP has been demonstrated in the lateral perforant path (LPP) input to the dentate gyrus, a system which is thought to use glutamate and opioid peptides as co-transmitters. However, the receptor pharmacology and mechanism of LTP induction have not been characterized. High-frequency stimulation (HFS; 100 Hz,1s) applied to the dentate outer molecular layer of rat hippocampal slices induced selective LTP of LPP responses, as assessed by EPSP slope measurements obtained 40 min post-HFS. In slices maintained in standard ACSF, LTP induction was blocked or significantly reduced in magnitude when HFS was applied in the presence of the general opioid receptor antagonist, naloxone (5 μM), the delta receptor antagonist, naltrindole (50 nM), the mu receptor antagonist, CTAP (100 nM), or the NMDA receptor antagonist, AP5 (20 μM), while the kappa-1 opioid receptor antagonist nor-BNI (60 nM) did not affect LTP. Selective LTP of the LPP was also obtained in slices maintained in ACSF containing picrotoxin (50 μM) which attenuates GABA-A receptor mediated inhibition. However, in disinhibited slices, HFS applied in the presence of naloxone induced LTP equivalent to control values. The results suggest that delta and mu opioid receptors regulate LTP induction in the LPP by a mechanism which depends upon GABAergic inhibition. Supported by NIH NS23865

623.12

CORTICOTROPIN-LIKE INTERMEDIATE LOBE PEPTIDE [CLIP; ACTH 18-39] AND ITS´ N-TERMINAL FRAGMENT ACTH 18-24 BLOCK THE INDUCTION OF LONG-TERM POTENTIATION IN THE RAT DENTATE GYRUS. D. Balschun*, T. Seidenbecher, D. Vogel and K. G. Reymann. Federal Institute for Neurobiology, POB 1860, D-39008 Magdeburg, Germany.

Physiological functions of the POMC-derived peptide CLIP [ACTH 18-39] are scarcely documented. Recently, we demonstrated that the intracerebroventricular administration of CLIP caused a marked enhancement of neuronal excitability in the hippocampal CA1 region as well as a selective increase of paradoxical sleep. The sleep studies led us to suggest that the active sequence is located in the N-terminal part of CLIP.

The present study was conducted to test whether CLIP can modulate neuronal transmission in the dentate gyrus and whether the full CLIP sequence is essential for such actions. Experiments were performed on freely moving male Wistar rats (8-9 weeks old), housed individually with food and water ad libitum. A monopolar recording and a bipolar stimulation electrode were implanted stereotaxically in the granule cell layer of the dentate gyrus and in the perforant path, respectively, for recording of electrically evoked population spikes. Long-term potentiation (LTP) was induced by short tetanic bursts (6 bursts, each 15 stimuli at 200 Hz, at 10-second intervals) thirty minutes after intracerebroventricular administration of equimolar doses of either CLIP, ACTH, ACTH 1-24 or ACTH 18-24. Whereas none of the investigated peptides displayed significant effects on baseline recordings the infusion of 20 ng CLIP or an equimolar dose of the N-terminal CLIP-fragment ACTH 18-24 led to a blockade of the induction of LTP. In contrast, application of ACTH or ACTH 1-24 did not affect the induction of LTP. Therefore, the active sequence responsible for the blockade of LTP seems to be contained in the N-terminal segment of CLIP.

623.13

ENDOGENOUS SUBSTRATES FOR Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II DURING THE INDUCTION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. Kohji Fukunaga*[1], Dominique Muller[2] and Eishichi Miyamoto[1]. [1]Department of Pharmacology, Kumamoto University School of Medicine, Kumamoto 860, Japan and [2]Centre Médical Universitaire, Université de Genève, Faculte de Medicine, CH 1211 Genève 4, Switzerland.

Among the molecular mechanisms that have been proposed to contribute to long-term potentiation (LTP) in the hippocampus is the activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) following the stimulation of the NMDA receptor. Recently, we documented long-lasting increases in the Ca^{2+}-independent and total activities of the enzyme as well as an increase in the ratio of Ca^{2+}-independent to total activity following the induction of LTP (J. Biol. Chem., 268, 7863,1993). It could suggest that autophosphorylation of the enzyme is responsible for the change in CaM kinase II activity. Here we demonstrate with ^{32}P-labeled hippocampal slices that high, but not low frequency stimulation applied to two groups of CA1 afferents resulted in increases in autophosphorylation of both α and β subunits of CaM kinase II 1 hr after LTP induction. In addition, significant increases in phosphorylation of endogenous CaM kinase II substrates, synapsin I and microtubule-associated protein 2 (MAP2), were observed in the same slices. The stimulations of phosphorylation of CaM kinase II and its substrates could be blocked by preincubation of slices with an NMDA receptor antagonist, D-2-amino-5-phosphonopentanoate. These results suggest that LTP is associated with increases in phosphorylation of synapsin I and MAP2 through the activation of CaM kinase II.

623.14

TEMPORAL DIFFERENCES IN THE PHOSPHORYLATION STATE OF PRE- AND POSTSYNAPTIC PKC SUBSTRATES B-50/GAP-43 AND NEURO-GRANIN DURING LONG-TERM POTENTIATION. P.N.E. De Graan*, P. Pasinelli, G.M.J. Ramakers, I. Urban, J.J.H. Hens and W.H. Gispen. Rudolf Magnus Institute for Neurosciences, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands.

Pre- and postsynaptic PKC substrates have been implicated in the molecular mechanism underlying LTP. The major presynaptic PKC substrate implicated in LTP is B-50 (a.k.a. F1, GAP-43, neuromodulin). A potential postsynaptic target for PKC during LTP is neurogranin (a.k.a. BICKS, RC3), a protein which shares an 18 amino acid sequence with B-50, containing the unique PKC phosphorylation site and the calmodulin binding domain. In this study we monitored the time course of B-50 and neurogranin phosphorylation during LTP.

Rat hippocampal slices (450 μm) were labelled with $^{32}P_i$ and were either tetanized (100 Hz, 1 sec), received low frequency stimulation or were not stimulated. After field EPSPs recordings in the CA1 region, the *in situ* B-50 and neurogranin phosphorylation state was determined in individual slices after different times by immunoprecipitation and phosphoimaging. B-50 phosphorylation was increased from 10 to 90 min, neurogranin only at 60 min after the tetanus. A high degree of correlation was found between the degree of B-50 and neurogranin phosphorylation and the degree of potentiation. Slices treated with APV, or in which tetanic stimulation failed to induce LTP, did not show increased B-50 and neurogranin phosphorylation 60 min after the tetanus.

To investigate whether phosphatases may contribute to the increase in phosphorylation, we treated slices with okadaic acid (inhibitor of PP1 and PP2a). This resulted in a dose- and time-dependent increase in B-50 and neurogranin phosphorylation and this increase was additive to the effect of PKC stimulation by phorbol esters.

Our data show that the increase in presynaptic B-50 phosphorylation during LTP precedes the increase in postsynaptic neurogranin phosphorylation. This may be due to a differential activation of pre- and postsynaptic PKC, but the contribution of phosphatases should also be considered. (supported by an ENP grant of the ESF).

623.15

ACTIONS OF ALUMINUM ON SYNAPTIC TRANSMISSION AND LONG-TERM POTENTIATION OF CA1 NEURONS IN HIPPOCAMPAL SLICES. B. Platt[1], D. Büsselberg[1], Y. Lin*[2] and D.O. Carpenter[2]. [1]Univ. Düsseldorf, Physiology II, Moorenstr. 5, 40225 Düsseldorf, FRG and [2]Wadsworth Labs, NYS Dept. Health and School of Public Health, Albany, NY 12201.

Aluminum (Al) intoxication is known to induce cognitive dysfunctions and multiple forms of dementia. The precise mechanisms of the Al effects are not clear. In order to investigate the action of Al on synaptic transmission and long-term potentiation (LTP) in area CA1 of hippocampal slices, field potentials were evoked by stimulation of the Schaffer collaterals (0.2 Hz) and recorded in the stratum pyramidal of the CA1 region. After stable population spikes were recorded for 30 min in modified ringer solution, Al was applied for 30 min by bath perfusion. Low Al concentrations (0.68 μg/ml = 25 μM) increased the amplitude of the population spike slightly (121%) whereas 2.7 μg/ml (100 μM) decreased the amplitude (75%) and concentrations ≥ 4 μg/ml (150 μM) blocked the population spike completely. The actions on synaptic transmission were irreversible. Induction of LTP by high-frequency stimulation in the presence of 0.68 μg/ml Al lead to a reduced level of potentiation compared to control experiments. In the presence of 2.7 μg/ml Al potentiation was further reduced and declined to baseline level within 60 min. Subsequent washout did not lead to any recovery of the signal. For low Al concentrations (0.68 μg/ml), a rebound of the effect was found in cases of washout 15 min after tetanisation. The amplitude of the population spike increased to values above those in control experiments (> 250%).

Our data suggest multiple sites of actions of Al on synaptic transmission and LTP. Both stimulating and inhibitory effects were found in a concentration dependent manner. These interactions might contribute to the neurotoxic potency of Al.

623.16

MICE CARRYING A TARGETED DISRUPTION OF THE RIβ REGULATORY SUBUNIT OF PKA HAVE DEFECTS IN HIPPOCAMPAL SYNAPTIC PLASTICITY. [1]E.P. Brandon*, [2]Y-Y. Huang, [2]M. Zhuo, [1]M. Qi, [1]K.A. Gerhold, [2]E.R. Kandel, [1]G.S. McKnight, and [1]R.L. Idzerda. [1]Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195 and [2]HHMI, Center for Neurobiology & Behavior, Columbia Univ., New York, NY 10032

RIβ is the regulatory subunit of cyclic AMP-dependent protein kinase (PKA) that is expressed primarily in neurons and therefore might play a role in several types of synaptic plasticity. Mice carrying a null mutation in the gene encoding RIβ were produced via homologous recombination in embryonic stem cells. These mice show a compensatory increase in RIα protein in various regions of the brain. The hippocampal slice preparation was employed to measure multiple forms of synaptic plasticity. In the CA1 region, the mutants show normal field potentials in response to Schaffer collateral stimulation, normal paired-pulse facilitation, and normal long-term potentiation (LTP), even at three hours post-tetanus. In contrast, a protocol that reliably produced long-term depression (LTD, induced by 900 pulses @ 1 Hz) in slices from control mice produced only a transient depression in mutant slices. Depotentiation following LTP showed a similar defect. In the CA3 region, a protocol that normally produced NMDA receptor-independent LTP of the field potential evoked by mossy fiber stimulation (two 100 Hz tetani of 1 second duration each, separated by 10 seconds) produced no LTP in mutant mice. Thus, the RIβ isoform of PKA is necessary for at least two forms of hippocampal plasticity.

623.17

INDUCTION OF ANOXIC LTP IS PREVENTED BY PROTEIN KINASE C ANTAGONISTS. Y. BEN-ARI*, P. CONGAR, D. DIABIRA and C. HAMMOND. INSERM U29, 123 Bd Port Royal, 75014 Paris France.

We have recently described a novel form of LTP (anoxic LTP) induced in CA1 pyramidal neurons of the hippocampus by a brief anoxic-aglycemic episode in slices (Crepel et al., J. Neurophysiol., 1993, 70: 2045): i) its induction requires the activation of NMDA receptors and changes in membrane potential; ii) its expression is mediated by a persistent increase of the NMDA (but not AMPA) receptor-mediated component of the EPSP. It is due to a persistent upregulation of postsynaptic NMDA receptors and is associated with an apparent reduction of the voltage dependant Mg^{2+} block of NMDA receptor. We now report that the induction of anoxic LTP requires the activation of protein kinase C (PKC). The NMDA receptor-mediated field EPSP was recorded in isolated CA1 slices (in the presence of 10μM bicuculline, 10μM CNQX and 0.6mM Mg^{2+}). Sphingosine (10 μM), bath applied before, during and after the anoxic episode (2-3 min), prevented the induction of anoxic LTP in 8 out of 11 slices. At the same time, we checked that non treated slices obtained from the same rats exhibited anoxic LTP. Furthermore, intracellular recordings were performed in presence of selective PKC inhibitor (50μM PKCI 19-36 in 3M KCl and 50mM QX-314) in the pipette and 10μM bicuculline, 15μM CNQX and 1.3 mM Mg^{2+} in the bath. After the injection of PKCI (200 pA pulses for 15 min), anoxic LTP of the glutamatergic EPSC (V_H = -35 to -75 mV) was not observed (n= 5 cells out of 5). Interestingly, a LTD was observed instead in 4 cells out of 5. In comparison, in control experiments where injections were performed with no PKCI present in the pipette, LTP of the EPSP in 3 cells out of 5 and no LTD were observed. We conclude that the induction of anoxic LTP requires the activation of PKC.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION I

624.1

MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION BY DOPAMINE AND PSYCHOSTIMULANTS IN THE NUCLEUS ACCUMBENS *IN VITRO*. S.B. Kombian* and R.C. Malenka. Depts. of Psychiatry and Physiology, University of California, San Francisco, CA. 94143.

The nucleus accumbens (NA) is an important limbic-motor interface that is involved in many complex behaviors. *In vivo* evidence suggests that dopamine (DA), cocaine and amphetamine modulate these behaviors. However, the cellular actions of these substances and their underlying mechanisms are poorly defined. Using *in vitro* slices from rats and the whole cell recording technique, we examined the actions of these substances on excitatory synaptic responses recorded in cells of the "core" region of the NA evoked by stimulation of prelimbic cortical afferents in the presence of picrotoxin (25 μM). DA consistently produced a reversible, dose-dependent decrease in EPSP/EPSC slope/amplitude with an estimated EC50 of 75 μM. DA (50 μM) produced an inhibition of -34 ± 4% (mean ± SE, n=4) which was blocked by a D1 receptor antagonist SCH23390 (1-5 μM; -2 ± 2%, n=6, $p<0.001$) but not the D2 receptor antagonist sulpiride (10 or 20 μM; -31 ± 3%, n=4, $p>0.5$) Further, this effect of DA was mimicked by the D1 receptor selective agonist SKF38393 (10 μM, -23 ± 3% n=10) but not the D2 receptor selective agonist quinpirole (10 or 20 μM; +1 ± 2%, n=9). The DA-induced inhibition was accompanied by an increase in paired pulse facilitation (+19 ± 4%, n=8) and appears to be independent of DA's postsynaptic membrane effects. Cocaine at non-anaesthetic concentrations produced a reversible dose-dependent inhibition of EPSPs (30 μM, producing -33 ± 3% inhibition, n=6) which was blocked by SCH23390 (1-5 μM; -6 ± 5%, n=7, $p<0.001$) but not sulpiride (10 μM; -29 ± 14% n=3, $p>0.5$). Amphetamine (10 μM) also induced a reversible inhibition of EPSPs (-61 ± 4%, n=3) which was blocked by SCH23390 (1-5 μM; -4 ± 4%, n=4, $p<0.001$) but not sulpiride (10 μM; -54 ± 15%, n=2, $p>0.5$). Manipulation of cAMP levels by forskolin (10 μM) or 8-Br-cAMP (1 mM) in the presence of 10 μM CPT (an adenosine A1 receptor antagonist) did not mimick (+28 ± 6%, n=4 and +34 ± 10%, n=8 respectively) but partially occluded (-13%, n=1 and -16 ± 3%, n=3, $p<0.02$ respectively) the actions of DA (50 μM). These results suggest that DA and the psychostimulants reduce excitatory synaptic transmission in NA by activating presynaptic D1-like receptors which may not be linked to the cAMP second messenger pathway. SBK is supported by HFSPO.

624.2

SUBCHRONIC GLUCOCORTICOID EXPOSURE ENHANCES AMPHETAMINE- INDUCED CHANGES IN [^{3}H]TCP INTACT CELL BINDING TO THE PRIMARY CULTURED NEURONAL CELLS. H. Yamamoto[1], X. Yang[1], T. Yamamoto[1,2], N. Sagi[1], A. Baba[1], E. Takamori[1], Y.L. Murashima[1]* and T. Moroji[1]. [1]Dept. of Psycho- pharmacol., Tokyo Inst. of Psychiatry, Tokyo 156, [2]Lab. of Mol. Recog., Grad. Sch. of Integrated Sci., Yokohama City Univ., Yokohama 236, Japan.

We have previously demonstrated that prolonged exposure of cultured cortical cells to amphetamine(AMP) increases [^{3}H]TCP intact cell binding in a dose- and time- dependent manner. To examine whether there are similar effects of glucocorticoids on cortical cells, the cells were exposed to corticosterone (CORT), dexamethazone (DEX) or deoxycorticosterone (DECORT) for at least 6 days. Glucocorticoids (CORT, DEX) significantly induced increases in [^{3}H]TCP intact cell binding in a dose-dependent manner, but mineralcorticoid (DECORT) did not. Scatchard analysis revieled that subchronic exposure of CORT induced reduction of both K_D and Bmax values of [^{3}H]TCP intact cell binding as well as subchronic exposure of AMP. In addition, AMP-induced increases in [^{3}H]TCP intact cell binding was enhanced by co-administration of CORT. Considering the findings that cross-sensitization occurs between stimulants and a variety of stresses, it is of interest that glucocorticoid (one of the biological indicators for stresses) affet the [^{3}H]TCP binding and suggested that NMDA receptor/ion channel complex is involeved in the cross-sensitization.

624.3

ELECTROPHYSIOLOGICAL "SENSITIZATION" OF DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS *IN VITRO* FOLLOWING CHRONIC COCAINE ADMINISTRATION *IN VIVO*. S. Shoji, D. Simms and J.P. Gallagher*. Dept. of Pharm. and Tox., Univ. of Texas Med. Br., Galveston, TX 77555.

We used intracellular electrophysiological recording techniques to investigate the actions of cocaine (3 μM) applied to brain slices which contained the DLSN and were obtained from drug naive rats (NR) or rats administered cocaine (CR, 15 mg/kg, IP, 2x daily) for periods of 7, 14 or 28 days. Although cocaine did not alter the configuration or threshold levels of sodium action potentials (APs) in any of these neurons, a significant proportion of neurons recorded from CR exhibited a decrease of input resistance and an increase in the appearance of slow depolarizing after potentials following cocaine. Only 50 % of NR or 7 day-CR responded to cocaine with a hyperpolarization and inhibition of spontaneous APs. The hyperpolarization was TTX-insensitive and persisted in zero calcium media; its reversal potential varied (-70 to -90 mV). On the other hand, 100 % of neurons from 14 & 28 day-CR were hyperpolarized by cocaine. Moreover, application of bicuculline (10 μM), which had no effect on resting membrane potential of NR, induced a TTX-insensitive depolarization of 6 mV and 10 mV in 14 day- and 28 day-CR, respectively. The same CR neurons were hyperpolarized following a combination of CNQX (10 μM) and AP5 (25 μM).

These results demonstrate an electrophysiological "sensitization" to cocaine in CR that involve the intrinsic cell properties of DLSN neurons and their responsivity to excitatory and inhibitory amino acids. Supported by DA-07190.

624.4

COCAINE INHIBITS SYNAPTIC RESPONSES IN RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS THROUGH PRE- AND POST-SYNAPTIC MECHANISMS. D. Simms*, S. Shoji and J.P. Gallagher. Dept. of Pharm. and Tox., Univ. of Texas Med. Br., Galveston, TX 77555.

Focal stimulation of fimbrial fibers from the hippocampus results in the appearance of an EPSP-IPSP-LHP sequence when recording from the DLSN slice preparation. GABA, acting at $GABA_A$ and $GABA_B$ receptors, mediates the inhibitory postsynaptic potential (IPSP) and the late hyperpolarizing potential (LHP), respectively. In addition to these inhibitory responses, excitatory postsynaptic potentials (EPSPs) mediated by excitatory amino acid receptor activation are also recorded from DLSN neurons. We now report that cocaine acts to inhibit synaptic transmission in the rat DLSN through pre- and postsynaptic mechanisms.

Standard intracellular current-clamp and voltage-clamp recordings were made from neurons in rat brain slices containing the DLSN. Orthodromically-induced synaptic responses were obtained before and during drug superfusion. Superfusion of cocaine (3 μM) produced a membrane hyperpolarization due to an outward current that gradually faded during continuous superfusion. The amplitude of the IPSP and LHP were reduced during the cocaine-induced hyperpolarization even after the membrane potential was restored to its pre-cocaine value. Following the hyperpolarization and during cocaine superfusion, the depression of the LHP and IPSP persisted. Voltage-clamp experiments revealed, moreover, that the amplitude of the late hyperpolarizing current (LHC) was decreased throughout cocaine superfusion. Cocaine did not reduce a hyperpolarization produced by bath application of baclofen. In addition, cocaine was able to reduce the EPSP and LHP without producing a membrane hyperpolarization. Our results suggest that cocaine suppresses synaptic responses in DLSN neurons, not only by changing the membrane potential, but also by producing a persistent increase in membrane conductance and by presynaptically affecting neurotransmission between the hippocampus and the DLSN. Supported by DA-07190.

624.5

PROPOFOL FACILITATES SUBSTANCE P-MEDIATED INHIBITION OF CALCIUM-ACTIVATED K-CONDUCTANCE IN A GUINEA-PIG SYMPATHETIC GANGLION. W.H. Stapelfeldt*, J.M. Oleszewski. Depts. of Anesthesiology & CCM, Univ. of Pittsburgh, Pittsburgh, PA 15261, and VA Medical Center, Pittsburgh, PA 15240.

The general anesthetic agent propofol (2,6-diisopropylphenol, DIPRIVAN®) specifically augments substance P (SP)- but not VIP-mediated postsynaptic responses of guinea-pig inferior mesenteric ganglion (IMG) neurons. Single-electrode current and voltage clamp recordings from isolated perfused IMGs demonstrated that propofol (1-100 μM) had no effect on the resting membrane potential or input resistance of principal ganglion cells, but augmented in a dose-dependent fashion the amplitude and duration of membrane depolarization and inward current responses evoked by exogenous SP applied via pressure microejection from nearby pipets (Picospritzer). The increase in SP-evoked inward current responses resulted from propofol-induced inhibition of an outward current component which was also inhibited by 20mM TEA, but not 1 mM TEA. SP-evoked inward current responses augmented by 20 mM TEA were not further altered by the additional administration of propofol. During the SP response in normal Krebs solution, intermittent depolarizing voltage step (20 mV, 5 s, 0.05 Hz)-evoked calcium-activated potassium ($G_{K(Ca)}$)-tail currents were transiently reduced in amplitude. Propofol had no effect on $G_{K(Ca)}$-tail currents in the absence of SP, but extended the time period during which $G_{K(Ca)}$-tail currents were reduced following SP application for the duration of the prolonged SP-evoked inward current response. These findings suggest that the increase in SP-evoked inward current response by propofol is the result of an increase in SP-mediated inhibition of a $G_{K(Ca)}$-conductance which limits the net inward current and membrane depolarization in response to SP. This action represents a novel mechanism of anesthetic action of propofol not attributable to the known facilitatory property of this compound on GABA-A receptor/anionophore complexes.

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624.6

URETHANE ANESTHESIA AFFECTS EVOKED POTENTIALS IN DENTATE GYRUS. Y.Shirasaka*, C.G.Wasterlain. Dept. of Neurology, BRI, UCLA Sch. of Med.,Los Angeles, CA90024 and Epi.Res.Lab. VAMC, Sepulveda, CA91343.

Urethane (ethyl carbamate) is a commonly used anesthetic in animal studies. Although its mechanisms of action is not known, urethane is widely used in physiological or pharmacological studies of the nervous system. In this study, we examined the effect of urethane on the evoked potentials recorded in dentate gyrus by stimulating the perforant path.

Male adult Wistar rats (n = 18) were used in this study. Evoked potentials were examined in the awake state and 30 min after the beginning of urethane anesthesia (i.v., loading dose, 1000 mg/kg, maintenance dose 50 mg/kg in saline).

Under urethane anesthesia, short interstimulus interval-dependent paired-pulse inhibition (20-60 msec) decreased significantly compared with that in the awake state. Population spike amplitude and excitatory postsynaptic potentials during input/output response examination were significantly smaller than in the awake state at low stimulus intensity. Voltage-dependent paired-pulse inhibition was weak at each stimulus intensity under urethane anesthesia, and the difference of inhibition between awake and anesthetized states increased with the stimulus intensity. Frequency-dependent paired-pulse inhibition was significantly weaker than in the awake state at 0.1 Hz and non-significantly weaker at 2 Hz.

These results clearly show that urethane affects neurotransmission in hippocampus, and indicate a possible relationship with excitatory neurotransmitter systems. Supported by EFA fellowship(YS), by the VA research service and by research grant NS13515 from NINDS.

624.7

EFFECTS OF THIOPENTAL AND PHORBOL ON CA1 FIELD POTENTIALS IN THE RAT HIPPOCAMPUS Y-C. Tsai, E. Narimatsu, T. Gerhold, S. Kamath and M. Sokoll and E. Wegrzynowicz* . Dept of Anesthesiology, Univ of Iowa College of Medicine, Iowa City IA. 52242

Thiopental (THIO) has been used primarily as an induction agent for the past 60 years. Because of its structural difference, it has been assumed that its site of action differs from that of the inhalational anesthetics in producing the anesthetic state. We studied the effects of thiopental on the field potential of the CA1 cell group of the rat hippocampal slice and its interaction with phorbol di-acetate (PDA). In previous studies we examined the interaction between halothane and PDA. Rats were anesthetized with ketamine, decapitated and 400μm thick slices of hippocampus prepared and mounted in a bath. The Schaffer pathway was stimulated using a platinum bipolar electrode and field potentials recorded with glass microelectrodes (resistance 3-8MΩ). The two negatively going potentials N1 and N2 were analyzed for amplitude, and latency. N2 was also analyzed for slope of the onset of the potential. Control observations were recorded following which THIO (400 or 800 μM) was applied for 30 minutes. Recordings were again made and then PDA 0.25, 0.5 or 1.0 μM were applied sequentially and potentials again recorded. THIO application resulted in approximately a 50% decrease in the amplitude of N2. Application of PDA 1.0μM resulted in approximately a 20% recovery of the amplitude of N2. This reversal is less than that which was seen with halothane. These results support the concept that the inhalation and intravenous anesthetics, at least to some extent, act at different sites.

624.8

HALOTHANE AND PROPOFOL INCREASE THE COFACTOR SENSITIVITY OF PURIFIED BRAIN PROTEIN KINASE C. HC Hemmings, Jr.* AIB Adamo, MM Hoffman. Departments of Anesthesiology and Pharmacology, Cornell University Medical College, NY, NY 10021.

Protein kinase C (PKC) has been implicated as a target for general anesthetics. The activation of purified brain PKC is stimulated by general anesthetics when assayed with a physiologically relevant lipid bilayer preparation *in vitro*. Here we report the further biochemical characterization of the stimulatory effects of halothane and propofol on PKC activation. PKC was purified to >80% homogeneity from rat forebrain and assayed with 0.2 mg/ml histone H1, 2 μM *sn*-1,2-dioctanoylglycerol (DG)/20 μM phosphatidylserine (PS)/80 μM phosphatidylcholine lipid vesicles, 50 mM HEPES (pH 7.4), 1 mM EGTA, 1.5 mM $CaCl_2$, 10 mM Mg-acetate, and 100 μM [γ-^{32}P]ATP at 30°C minus or plus 2.4 vol% halothane or 200 μM propofol.

Parameter	n	- Halothane	+	- Propofol	+
K_m (mg/ml histone H1)	2	0.14	0.14	0.11	0.10
V_{max} (μmol/min/mg)	2	0.46	0.73	0.27	0.48
PS EC_{50} (mol %)	3	18±2.5	11±0.6*	18±1.9	11±1.2**
DG EC_{50} (mol %)	3	1.6±0.3	0.87±0.2*	2.5±0.3	1.2±0.4**
Ca^{2+} EC_{50} (free Ca^{2+}, μM)	3	4.5±1.0	2.8±0.4*	2.8±0.7	1.9±0.2*

Values are expressed as mean±SD. *p<0.05, **p<0.01 (*versus* value without anesthetic).

Both halothane and propofol increased the sensitivity of PKC to activation by DG, PS, and Ca^{2+}, without affecting its apparent affinity for the artificial substrate histone H1. These data suggest that general anesthetics may stimulate PKC activity not by mimicking one of its regulators, but by stabilizing its active conformation. Efforts are underway to extend these observations made with purified PKC *in vitro* to endogenous neuronal PKC and PKC substrates in order to further assess the role of PKC mediated protein phosphorylation in general anesthetic action.

Supported by a FAER/BOC Anesthesiology Young Investigator Award and a Cornell Scholar Award in Biomedical Science.

624.9

INTERACTIONS OF APV, PICROTOXIN AND PHORBOL DI-ACETATE ON HALOTHANE DEPRESSED CA1 POTENTIALS IN THE HIPPPOCAMPUS. E. Narimatsu, Y.C. Tsai, S. Kamath, and M. Sokoll* Dept. of Anesthesia, Univ. of Iowa College of Medicine, Iowa City, IA 52242

The effects of the inhalation anesthetic halothane and its interactions with protein kinase C (PKC) have been partially investigated. In this study we examined the effects of halothane and phorbol di-acetate (PDA) following the administration of APV and picrotoxin on the field potential of the CA1 layer of the hippocampus. Rats were anesthetized with ketamine and hippocampal slices 400μm thick were cut and mounted in a bath. A platinum bipolar electrode was used to stimulate the Schaffer pathway supplying the CA1 cell group. AP5 and picrotoxin were applied to block the NMDA and GABA potentials. Halothane was then applied followed by PDA. Application of AP5 produced little alteration in the amplitude of the N2 potential. Addition of picrotoxin produced a small but statistically significant increase in the amplitude of N2. The subsequent application of halothane resulted in a 52% decrease in the amplitude compared to control. The addition of PDA resulted in an increase in the amplitude of N2 to 90% of control, a value not significantly different from the control. The addition of AP5 and picrotoxin resulted in a small increase in the N2 amplitude suggesting that, in this protocol, NMDA receptors account for only a small part of the potential. The ability of PDA to produce significant reversal may be related to its stimulatory effect on the non-NMDA system.

624.10

PHARMACOLOGY OF TRANSMITTER RELEASE IN THE DEVELOPING CHICK HEART. D. B. Gray* and C. Eielson. Department of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

Potassium-evoked secretion of acetylcholine (ACh) and norepinephrine (NE) from cardiac parasympathetic nerve endings and cultured sympathetic cell somas respectively, is mediated by Ca influx. In this report we have examined the calcium pharmacology of ACh release from cholinergic terminals in intact excised right atria of embryonic stage 40 and hatchling chick hearts. At embryonic day 14, evoked ACh release from cholinergic nerve terminals in atria can be inhibited by over 80% with 10 uM nifedipine. This sensitivity to nifedipine is gradually reduced to less than 30% at hatching. The remaining ACh release at hatching can be blocked by omega-conotoxin suggesting a switchover from L type calcium channels to N type channels involved in excitation-secretion coupling. Cultures of St. 40 sympathetic ganglia release labeled NE during a high potassium challenge (56 mM), and this evoked release is not sensitive to nifedipine. However, co-cultures of St 40 sympathetic neurons with live heart cell cultures do show sensitivity to nifedipine (>60%) suggesting that exposure to target may induce coupling of L type channels to transmitter release at least transiently, in these neurons. The effects of muscle target and developmental age resemble those in other autonomic ganglia (Gray et al., Neuron 8: 715, 1992). Additionally, embryonic sensitivity of vertebrate autonomic ganglia to nifedipine may have implications for hypertensive pregnant women. Supported by a grant to B. Gray from the Catherine and Patrick Weldon Donaghue Foundation for Medical Research.

624.11

RILUZOLE: FREQUENCY-DEPENDENT ACTIONS LINK INHIBITION OF SODIUM CHANNELS AND INHIBITION OF SYNAPTIC TRANSMISSION. G.A. Böhme*, S. Le Guern, Ph. Boudeau and J.C.R. Randle, Rhône-Poulenc Rorer S.A., Central Research, 94403 Vitry-sur-Seine, France.

Riluzole (6-trifluoromethoxy-2-aminobenzothiazole) is an anti-convulsant and neuroprotectant that gave promising results in a Phase II study in amyotrophic lateral sclerosis. In cultured cerebellar granule neurons and NG108-15 hybridoma cells, riluzole (0.3-30 μM) induced a 5-30 mV negative shift of Na^+ current steady-state inactivation, but had little effect on Na^+ current activation. Riluzole slowed the recovery of Na^+ current from inactivation ($t_{1/2}$: control = 8-20 ms, riluzole = 30-60 ms). Thus, inhibition of Na^+ currents was frequency-dependent only at high activation frequencies (≥ 20 Hz). Depolarizing current pulses evoked trains of action potentials at 20-50 Hz that were slowed by 1 and 3 μM riluzole and reduced to a single spike by 10 μM riluzole. Riluzole has previously been shown to inhibit the release of glutamate *in vitro* and *in vivo*. In hippocampal slices, riluzole (1-30 μM) increased the electrical activation threshold of the Schaffer collateral→CA1 pathway evaluated as the appearance of an evoked pre-synaptic fiber volley and a post-synaptic glutamate-mediated field potential, and increased the latencies of these responses, but did not reduce the maximal response amplitudes evoked at high stimulus intensities. When trains of 10 stimuli were applied at 20-100 Hz, marked "use-dependence" of the inhibitory effects was observed that was not overcome at increased stimulus intensities. We conclude that use-dependent Na^+ current inhibition underlies riluzole's selective inhibition of high frequency electrical activity. Reduced pre-synaptic excitability would contribute to the inhibition of synaptic glutamate release and excitatory neurotransmission. This selective action of riluzole may allow it to exert anticonvulsant and neuroprotective actions while respecting lower frequency "normal" synaptic transmission.

624.12

CHANGES IN SYNAPTIC TRANSMISSION PRODUCED BY PHOSPHOLIPASE A_2-NEUROTOXINS IN CHOLINERGIC PAIRS OF NEURONS IN *APLYSIA CALIFORNICA*. M.L. Winfree, J.P. Apland, T-M Shih* and M.G. Filbert. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

The site of action of three snake phospholipase A_2-neurotoxins (PLA_2), taipoxin, crotoxin, and corticotoxin I has been examined using synaptically coupled cholinergic neurons in the buccal ganglia of the marine mollusc *Aplysia californica*. Corticotoxin II was also examined because it produces neurotoxic effects similar to corticotoxin I but lacks PLA_2 enzyme activity. Toxins were either superfused over isolated ganglia or micropressure injected directly into the presynaptic neuron of an identified pair of cholinergic cells. Microinjection of toxins into presynaptic neurons produced changes consistent with inhibition of transmitter release as discerned by a decrease in the amplitude of postsynaptic responses to stimulation of the presynaptic cell. Superfusion of PLA_2-neurotoxins into the experimental chamber resulted in depolarization of both pre- and postsynaptic cell membranes and changes in the amplitudes and decay phase of action potentials evoked by stimulation of presynaptic cells. Chamber application of toxins also resulted in spontaneous excitatory activity in postsynaptic cells. This spontaneous activity was reduced by dizocilpine, a non-competitive inhibitor of NMDA receptors. The observations reported here suggest that the above PLA_2-neurotoxins have multiple sites of action, both external and internal.

POTASSIUM CHANNELS V

625.1

INTRACELLULAR Cl^- DIMINISHES G-PROTEIN-ACTIVATED K^+ CONDUCTANCES IN RAT HIPPOCAMPAL NEURONS. R.A. Lenz[1,2], T.A. Pitler[1], P.J. Yarowsky*[2], & B.E. Alger[1]. [1]Dept. Physiol., [2]Dept. Pharm. & Exp. Therapeut., Univ. MD Sch. Med., Baltimore, MD 21201.

We made whole-cell recordings from CA1 cells in rat hippocampal slices with various electrode recording solutions. We found that G-protein-mediated K^+-dependent responses are suppressed when 160 mM KCl is in the intracellular recording solution instead of KCH_3SO_3 or potassium gluconate (KGluc). With KCl, outward current responses to applications of the $GABA_B$ agonist, baclofen, were reduced ~60% relative to responses in cells recorded with KCH_3SO_3 or KGluc electrodes. Additionally, monosynaptic $GABA_B$ responses in the presence of glutamate antagonists and picrotoxin, which were reliably obtained when using KCH_3SO_3 electrodes, were small or nonexistent in cells loaded with KCl.

Membrane potential and resting input resistance did not differ among cells recorded with the different solutions. When the non-hydrolyzable GTP analog, GTPγS, was included in a KCH_3SO_3 electrode, cells were unusually hyperpolarized and input resistances were significantly reduced. However, cells recorded with KCl electrodes containing GTPγS had membrane potentials and input resistances that were not significantly different from control. These data suggest that high $[KCl]_i$ prevents activation of a K^+ conductance by GTPγS.

We conclude that internal Cl^- can interfere with the activation of G-protein-activated K^+ conductance. It will be important to determine if the interference occurs at the channel, the G protein or at some other step.

625.2

GDP IS REQUIRED FOR ACTIVATION OF A GLYBURIDE-, ATP- AND CROMAKALIM-SENSITIVE OUTWARD CURRENT IN RAT HIPPOCAMPAL NEURONS **G. Erdemli* and K. Krnjević, Anaesthesia Research Dept., McGill University, Montréal, Québec, H3G 1Y6, Canada.**

There is no agreement between different groups working on cromakalim (CROM) effects on hippocampal neurons. For example, according to Politi and Rogawski (Mol. Pharmacol 1991,40:308) CROM activates a glyburide (GLYB)-sensitive K current in cultured cells. However, we found that cromakalim *decreases* a voltage-dependent outward current - probably a delayed rectifier - in CA1 neurons in slices (Erdemli and Krnjević Soc. Neurosci. Abst. 1993, 19:709). The present data were obtained with whole-cell recording from CA1 pyramidal neurons in slices. In nine cells, held at -54 ± 3 mV (where membrane current was ≈ 0) and recorded with the "standard" internal solution ($KMeSO_4$, HEPES, EGTA, $CaCl_2$ and $MgCl_2$), CROM (100 μM) reduced both input conductance (G_N) (by 14 ± 4 %) and outward currents evoked over a wide range by brief depolarizing pulses: at -4 ± 3.0 mV, currents *diminished* by 30 ± 10 %. When the standard internal solution contained also 1 mM GDP, there was a significant outward current at ≈-54 mV; and CROM *increased* outward currents at -4 ± 2.6 mV by 99 ± 26.4 % (n=10). The enhanced outward currents were reduced by CROM washout (in 2 cells) and by 10 μM GLYB (in 4 cells). When six other cells were recorded with electrodes containing both ATP (5 mM) and GDP (1 mM), there was no net outward current at ≈-54 mV and CROM *reduced* outward currents (at ≈ 0 mV, by 38 ± 11%). We conclude that, like many muscle cells, CA1 neurons have outward current channels that are both ATP- and GLYB-sensitive, and are opened by CROM - and therefore resemble classical K_{ATP} channels - but can be activated only when cytosolic GDP is present.

Supported by the Medical Research Council of Canada.

625.3

PROPERTIES OF POTASSIUM M-CHANNELS IN INSIDE-OUT PATCHES EXCISED FROM RAT SYMPATHETIC NEURONS. A.A.Selyanko and D.A.Brown*. Pharmacology Dept., University College, London, WC1E6BT, U.K.

M-channels in rat sympathetic neurons have previously been studied using cell-attached and excised outside-out membrane patches (Selyanko *et al.*, 1992: *Proc.Roy.Soc.Lond.B.*,250,119; Stansfeld *et al.*,1993: *Neuron*,10,639). We have now succeeded in maintaining M-channel activity in excised inside-out patches. The most striking change on excision was a substantial and irreversible "run-up" in single channel P_o (Figure 1). This was accompanied by an increased opening frequency and decreased shut time, with a loss of the medium and long shut times observed in cell-attached patches (Selyanko & Brown, 1993: *J.Physiol.*,472,711). P_o retained its voltage-sensitivity, but with a shallower slope. Single channel conductances were unchanged. Run-up was not prevented by including 3 mM ATP + 1 mM GTP in the bath solution or by adjusting Ca to 50-100 nM. This suggests tonic inhibition of M-channels *in situ* by some other cytoplasmic component(s).

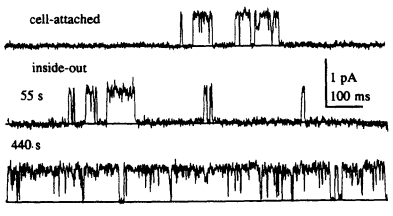

Fig.1. M-channel before and after patch-excision. Internal (bath) solution (mM): KCl, 175; HEPES, 5; BAPTA, 1. (pH 7.2). Pipette solution: Hepes-buffered Krebs (2.5 mM [K]). -30 mV. *c.*22°C.

625.4

MUSCARINE-INDUCED INCREASE OF AN INWARD RECTIFIER K^+ CURRENT IS MEDIATED BY GI2 IN ATT-20 CELLS. T. Kozasa, T. Ohtsuka, Y. Kaziro, S. Nakajima* and Y. Nakajima. Dept. of Anat. and Cell Biol., and Dept. of Pharmacol., Univ. of Illinois at Chicago, Chicago, IL 60612, and Institute of Med. Sci., Univ. of Tokyo, Tokyo 108.

Muscarine and somatostatin induce an inward rectifier K^+ current in a pituitary tumor cell line (AtT-20), and these effects are mediated by a pertussis toxin (PTX)-sensitive G protein, Gi or Go. To investigate which subtype(s) of Gi mediates these agonist effects, we constructed PTX-insensitive mutants of α-subunit cDNAs of Gi1, Gi2, and Gi3, and transfected them stably into AtT-20 cells. PTX ADP-ribosylates the cysteine residue at the 4th from the carboxyl terminal of Giα or Goα, and uncouples those subunits from receptors. We mutated this cysteine residue of Giα to serine. The function of the transfected Gi was examined after the endogenous Gi and Go of the transfected cells were inhibited by PTX. As controls, wild type Giα cDNAs (Gi1α, Gi2α, or Gi3α, PTX-sensitive) were transfected into AtT-20 cells. Muscarine (100μM) and somatostatin (500 nM) effects on cell lines were examined with the whole cell-clamp. Only in the cell lines into which the mutated (PTX-insensitive) Gi2α cDNA was transfected, did the muscarine response become PTX-insensitive, suggesting that Gi2 couples to the muscarinic receptors and enhances the activity of the inward rectifier K^+ channel. As for the somatostatin response, transfection of any of the mutated Giα cDNAs did not alter the PTX-sensitivity of the response. Supported by NSF grant IBN-9209785.

625.5

COMPARISON OF THE INCREASE IN POTASSIUM CONDUCTANCE BY GALANIN AND OXY-M (OXOTREMORINE M) IN MUDPUPPY PARASYMPATHETIC NEURONS. J.M. Mulvaney* and R.L. Parsons, Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Galanin and muscarinic agonists hyperpolarize mudpuppy parasympathetic neurons by activating a similar inwardly rectifying potassium conductance (G_K). The present experiments were done to quantitate and compare the increase in G_K produced by galanin and the muscarinic agonist Oxy-M. All experiments were done on isolated parasympathetic neurons dissociated from the cardiac ganglion of the mudpuppy, *Necturus maculosus*. Recordings were made using the perforated patch mode of the whole cell voltage clamp technique on cells bathed in physiological solution containing an elevated potassium concentration (in mM: 120 NMG, 12.5 KCl, 3.6 $CaCl_2$, 10 Hepes). Both agonists produced a concentration dependent increase in G_K. At low concentrations (10^{-9} uM Galanin and 10^{-8} uM Oxy-M) the increase in G_K was additive. In contrast, high concentrations of either agonist occluded the response to subsequent application of the other agonist. The time course of G_K activation was very different. The increase in G_K by Oxy-M occurred rapidly, remained relatively constant in the presence of agonist, and reversed quickly following removal of the agonist. The galanin-induced G_K developed slowly and then faded. The time course of the fade was consistent whether or not galanin was removed from the bath. Previous experiments demonstrated that arachidonic acid (AA) activates an inwardly rectifying G_K with a time course similar to the galanin-induced G_K. At maximum concentrations of Oxy-M or galanin, the AA-induced G_K was occluded. Our results indicate that galanin and Oxy-M activate the same inwardly rectifying G_K but that the mechanism of activation may be different. We suggest that the galanin-induced increase in G_K may be mediated through a second messenger, possibly AA.
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625.6

CESIUM AND BARIUM BLOCKADE OF DOPAMINE-MODULATED K^+ CHANNELS IN RAT STRIATAL NEURONS. Yong-Jian Lin*, Gabriela J. Greif and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

Inwardly-rectifying K^+ conductances are reported to have a prominent role in striatal (caudate-putamen) neuron firing properties, with heterogeneity at the whole-cell level in sensitivity to Cs^+ and Ba^{2+} blockade. Using cell-attached patch-clamp recording from freshly dissociated rat striatal neurons, we are studying blockade of an inwardly-rectifying 85 pS K^+ channel, which is activated by D_2-like dopamine receptors. Both Cs^+ (3 mM) and Ba^{2+} (0.5 mM) partially blocked this channel when applied to the external surface of the membrane via the patch pipette; block was rapid, flickery and voltage-sensitive. At this concentration, Ba^{2+} also produced intervening periods of stable blockade up to 1 sec. Cs^+, but not Ba^{2+}, slightly permeated the channel at voltages near the K^+ reversal potential. These cells also express other inwardly-rectifying K^+ channels with conductances ≤30 pS, which are dopamine-insensitive. These channels were blocked more completely by 3 mM Cs^+ than was the 85 pS channel. Our results extend to the single channel level the finding that striatal inwardly-rectifying K^+ channels are heterogeneous in their block by these ions, and indicate that the dopamine-modulated K^+ channel is both blocked and permeated by Cs^+. (Supported by MH-48545.)

625.7

INVESTIGATING THE SITE OF Zn^{2+} ACTION BY SINGLE AMINO ACID SUBSTITUTION IN A VOLTAGE-GATED K^+ CHANNEL. S.J. Gibbons[1]*, G. Talukder[1], M.M. Tamkun[2], H. Shear[2] and N.L. Harrison[1] [1]Anes and Crit Care & Pharm/Phys., U. of Chicago, Chicago Il 60637, [2]Mol. Phys. and Biophys., Vanderbilt U. Nashville TN.

Zn^{2+} modulates the gating of a human cloned voltage-dependent potassium channel (Kv1.5) by binding to a specific site on the extracellular surface of the protein (Harrison et al. (1993) Mol. Pharm., 43 482). We have employed the techniques of site-directed mutagenesis and transient expression of mutant channels in HEK-293 cells to establish the amino acids which are important for the integrity of the Zn^{2+} binding site. Single amino acids in the S5-S6 domain ("P region") were chosen for mutation by comparing the amino acid sequence of known Zn^{2+}-sensitive (Kv1.1, 1.4 and 1.5) and insensitive (Kv1.2 and 2.1) channels. Whole cell recordings were made at 25°C, using intracellular solutions based on K gluconate and continuous extracellular perfusion with HEPES-buffered saline containing 3mM K^+. hKv1.5 channels expressed in HEK 293 cells activated at a significantly more depolarized potential than the same channels expressed in mouse L cells. Modulation by Zn^{2+} was unaltered. Our initial approach has been to generate mutants of Kv1.5 with similar amino acids in the P region to Kv2.1, which is very weakly modulated by Zn^{2+}. Activation gating of Kv1.5 (A455K) is shifted to the right ($V_{0.5}$ =25mV) compared to the wild-type (w.t., $V_{0.5}$ =11mV) whereas activation of Kv1.5 (H461K) is not significantly changed ($V_{0.5}$ =19mV, n=4). Robust modulation of gating by 200μM Zn^{2+} is still observed for both channels although the right shift (Δ) is slightly reduced (w.t. Δ=25.3mV, A455K Δ=20mV, H461K Δ=17mV, n=3-9). We are presently examining the affinity of these channels for Zn^{2+}.
SJG is supported by DHHS training grant # DA07255.

625.8

CHEMICAL INHIBITION OF SDH IN VIVO ALTERS TIME COURSE OF ATP CONCENTRATION AND MEMBRANE POTENTIAL UPON IN VITRO INHIBITION OF SDH. M. Riepe[1,2], W.D. Niemi[1,3], D. Megow[2], and D.O. Carpenter*[1]. [1]Wadsworth Labs, NYS Dept. of Health, Albany, NY 12201, [2]Dept. of Neurology, Humboldt-Univ., Berlin, Germany and [3]Russell Sage College, Dept. of Biology, Troy, NY 12180.

Treatment with inhibitors of succinic dehydrogenase (SDH) can induce neurodegeneration similar to that seen in Huntington's Disease. Hippocampal brain slices were prepared from rats treated with a single in vivo injection of 3-nitropropionic acid (3-NPA), a suicide inhibitor of SDH which slowly alters the level of ATP. Rats were given a single injection (IP) of 20 mg/kg of 3-NPA and sacrificed by cervical dislocation at intervals of 1 to 8 h. The brains were quickly removed and placed in cold oxygenated Krebs, sliced, and then oxygenated in warm Krebs for 1.5 h. Relative to controls these slices showed a decrease in ATP and ADP concentrations within the first 2 h, an intermittent increase at 4 h, and then a second decline between 4 and 8 h, bringing the level down to that of the first 2 h. We assessed the amount of remaining SDH activity with 2,3,5-triphenyltetrazolium chloride (TTC) staining. We found that the intensity of staining paralleled the levels of ATP. Intracellular recordings from pyramidal neurons from control animals showed that acute perfusion of 3-NPA on isolated slices results in a hyperpolarization, while in slices taken from animals treated in vivo for 8 h this effect was not seen. Application of glibenclamide (10 μM), a selective antagonist at the KATP channel, reduced the hyperpolarization compared to controls. We conclude that brief periods of interruption of energy metabolism in vivo can be somewhat compensated, and this is reflected in such physiologic parameters as activation of ATP-regulated potassium channels. Supported by NS23807-09.

625.9

AN ATP-SENSITIVE K^+ CHANNEL IN ISOLATED RAT NEOCORTICAL NEURONS: ACTIVATION BY GANGLIOSIDES. Z.-Q.TONG* and X.-D.TANG Dept.of Physiol., First Military Medical Univ., Guangzhou 510515, P.R. China

Our previous studies have indicated that gangliosides (GM) protect cerebral ischemia by measuring infarcted area, contents of free radical and ECoG.The ionic mechanisms were further studied here with patch clamp technique.

Single neurons were acutely isolated from Sprague-Dawley rat neocortex at 18~23 °C. The pipette and bath were all filled with TTX (1μM) and CdCl2 (0.3 mM) contained but glucose-lacked solutions, with potassium concentrations of 5, 5 mM (cell-attached) or 135, 135 mM (inside-out).

In 7 cell-attached patches, 2~10 μM of monosialoganglioside-GM1(Sigma, approx. 95%) produced a class of single channel currents whose durations of the burst openings showed concentration-dependent. The values of the burst times resulted from 2, 5, 10 μM GM1 were 6± 2 s (n=3), 21± 4 s (n=3) and 42 s (n=1), respectively. The long-time burst-like openings would disappear after washing GM1 out. After formation of inside-out recording from the same cell-attached patch, the channel activities were depressed by either 2 mM of Na2-ATP (Sigma) or 1 μM of glibenclamide (Sigma). The reversal potential was ~ 0 mV (pipette). So the potassium channels were of ATP-sensitive type.

In 6 inside-out patches, after applications of 5~10 μM GM1, single channel recorded often became multichannel activities which then recovered to single channel openings after GM1 was washed out. The GM-evoked multichannel currents were inhibited by applications of 4 mM ATP or 2 μM glibenclamide, with a reversal potential of ~ 0 mV and a channel conductance of ~ 90 pS.

The results demonstrate that, under the conditions of metablism inhibition (bath without glucose and ATP), GM can activate ATP-sensitive K^+ channels in rat neocortex. The mechanism was suggested to involve protein kinase C.

625.10

PROSTAGLANDIN E_2 REGULATES VOLTAGE-GATED POTASSIUM CHANNELS IN NEUROHYOPOPHYSEAL NERVE TERMINALS. Lei ZHANG, Edward KARPINSKI and Christina BENISHIN*. Dept. of Physiology, Univ. of Alberta, Edmonton, AB Canada T6G 2H7.

A high concentration of prostaglandin E_2 (PGE_2) has been demonstrated in the neurohypophysis and may have an important role in hormone release from the posterior pituitary. In the present studies, the whole-cell version of the patch clamp technique was used to investigate the effect of PGE_2 on potassium (K^+) currents in nerve terminals which were acutely dissociated from the rat neurohypophysis. PGE_2 initially increased the delayed rectifier K^+ current (I_K). The effect of PGE_2 on I_K was transient with a return to control levels after 15 to 20 min. Additionally, 15 to 20 min after PGE_2 administration, the transient outward current (I_A) was increased. These effects were concentration dependent, within the concentration range of 50 to 500 μM PGE_2. Forskolin, 8-bromo cyclic-AMP and dibutyryl cyclic-AMP also significantly increased I_A and simultaneously decreased I_K. These results show that cyclic-AMP modulates K^+ channels in nerve terminals, and may mediate some of the effects of PGE_2 on K^+ channels and, hence, hormone secretion from the neurohypophysis.

625.11

5-HYDROXYTRYPTAMINE MODULATION OF DELAYED-RECTIFIER-TYPE POTASSIUM CHANNELS IN ANTENNAL-LOBE NEURONS OF THE MOTH *MANDUCA SEXTA*. P. Kloppenburg*[1], A.R. Mercer[1,2] and J.G. Hildebrand[1]. [1]ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721; [2]Dept. of Zool., Univ. of Otago, Dunedin, NZ.

A principal goal of our studies of the olfactory system of the sphinx moth *Manduca sexta* is to determine the role(s) played by 5-hydroxytryptamine (5HT or serotonin) in the antennal lobes (ALs, the primary olfactory centers) of the brain. In *Manduca*, each of the two ALs is innervated by a single 5HT-immunoreactive neuron (J. Neurobiol. 19:451-465, 1987). Synaptic contacts between AL interneurons and the 5HT-containing cell occur within the glomerular neuropil of the AL. The majority of these contacts are output synapses from the 5HT-containing cell (J. Comp. Neurol. 338:5-16, 1993). In the brain of the adult moth, exogenously applied 5HT (100 μM) leads to broadening of action potentials and increased excitability of AL interneurons (Soc. Neurosci. Abstr. 18:303, 1992). These effects can be mimicked by application of 5HT to AL neurons in primary-cell culture (Soc. Neurosci. Abstr. 19:126, 1993). Both *in vivo* and *in vitro*, treatment with 5HT leads to an increase in cell input resistance. Here we report that 5HT causes prolonged closure of delayed-rectifier-type K^+ channels in these cells. Applied to AL neurons *in vitro*, in brain slices, or in semi-intact brain preparations, 5HT (5-100 μM) decreases channel opening probability and causes reversible reduction of delayed-rectifier K^+ current. Single-channel recordings in cell-attached mode suggest that the effects of 5HT on K^+ channels of this type are mediated via a diffusible second messenger, the identity of which has yet to be established. Closure of delayed-rectifier-type K^+ channels could account for the increased duration of action potentials observed in many AL interneurons in response to 5HT. [Supported by a grant to ARM from the USA/NZ Cooperative Science Program and by NIH grant AI-23253 to JGH.]

625.12

BK CHANNEL: ITS WHOLE CELL OUTSIDEOUT RECORDING AND ITS ROLE IN DETERMINING THE RESTING MEMBRANE POTENTIAL IN BASILAR ARTERY CELLS. Y. Song, K. Tewari, L.K. Liem, S. Xu*, J.M. Simard. Division of Neurosurgery, Univ. of MD Sch. of Med. Baltimore, MD 21201.

We studied large-conductance, Ca^{2+}-activated K^+ (BK) channels in smooth muscle cells from the basilar artery of the guinea pig using the patch-clamp technique. In inside-out patches, the single channel slope conductance was 260 pS and in outside-out patches, the apparent dissociation constant for block by tetraethylammonium Cl (TEA) was 280 μM. Our principal goal was to assess the contribution of BK channels to conductance at negative potentials, near the resting membrane potential. We measured $N \cdot P_o$ (number of channels in the cell $\times$ open channel probability) at physiological potentials by recording single channel currents in a whole-cell configuration, a hybrid technique designated the "outside-out-whole-cell" configuration. With physiological solutions inside and outside, including 1 μ M Ca^{2+} internally, values (mean $\pm$ S.D.) of $N \cdot P_o$ were 0.17$\pm$0.05 and 0.49$\pm$0.39 at -40 and -30 mV, respectively. Our data on the single channel properties of this current indicate that, under conditions that simulate in part those expected with active myogenic tone, including a membrane potential of -40 mV and $[Ca^{2+}]_i$>0.1 μM, BK channels could contribute on the order of 10-20 mV to the membrane conductance, a value that compared favorably with the amount of depolarization observed under current clamp following external application of 1 mM TEA.

625.13

TETRANDRINE: EFFECTS ON BK_{CA} CHANNELS

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The alkaloid tetrandrine (TET) is used in traditional medicine in China because of its analgesic, antiphlogistic and for its vasodilatory and antihypertensive properties. Recently, TET has been used for differentiation of certain subtypes of Ca^{2+} dependent potassium channels of high conductance (BK_{Ca}) in rat neurohypophysis nerve terminals and pars intermedia cells (Wang and Lemos, 1992). In these systems, it has been shown to block only (charybdotoxin) ChTx insensitive type II currents, whereas ChTx sensitive type I BK_{Ca} currents remained unaffected.

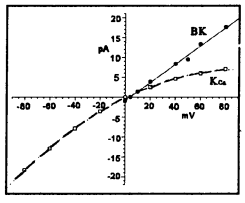
In the present study the effects of TET were characterized in detail in two different cell systems. In the rat glioma cell line C6-BU-1, ionomycin stimulated rubidium (Rb^+) efflux was found to be sensitive to charybdotoxin, iberiotoxin and tetraethylammonium (TEA), while apamin and 4-aminopyridine (4-AP) were ineffective. This pharmacological profile suggests the existence of BK_{Ca} currents in these cells. Since ionomycin stimulated Rb^+ efflux was not influenced by TET, BK_{Ca} channels in C6-BU-1 cells seem to belong to the class I BK_{Ca} channel subtype. In GH_3 (rat anterior pituitary tumor) cells BK_{Ca} currents were characterized using the patch clamp technique. In parallel with our results in the study above, external applied ChTx and TEA reversibly blocked single channel currents, whereas 4-AP had no effect. Surprisingly, also TET turned out to inhibit BK_{Ca} channel currents in GH_3 cells, suggesting the presence of a channel type not classified so far.

To get insight into the role of TET sensitive BK_{Ca} channels in the rat brain, TET effects were characterized in microdialysis experiments measuring dopamine release from the N. accumbens. Local administration of TET results in a profound and transient increase of dopamine release, while the metabolites DOPAC and HVA were hardly affected. The latter results indicate that tetrandrine sensitive processes are involved in the regulation of dopamine release in this brain area.

625.14

SINGLE CHANNEL STUDIES OF TWO "LARGE" CONDUCTANCE Ca-ACTIVATED K CHANNELS IN SYMPATHETIC NEURONS. T. Fagaly & D. Lipscombe*. *Dept. of Neuroscience, Brown University, Providence, RI 02912.*

It has long been recognized that multiple classes of Ca-activated K channels (K_{Ca}) are present in neurons with different pharmacological profiles. In comparison to the well studied *big* conductance (BK) channel, less is known of the single channel properties of other non-BK K_{Ca} channels. We have studied K_{Ca} currents in inside-out patches from sympathetic neurons of adult frogs. Using 140 mM KAsparate and 10 mM EGTA on both sides of the membrane and exposing the inside of the patch to 1-10 mM Ca, a K_{Ca} channel distinct from BK channels was observed in at least 30% of our recordings (see fig.). The single channel conductance γ_{sc} of this channel displayed strong inward rectification such that γ_{sc} increased from 80 pS to 220 pS over 120 mV (+60 mV to 0 mV and 0 mV to -60 mV, respectively). In contrast BK channel-like openings exhibited a linear conductance of about 220 pS over the same range. The inwardly rectifying K_{Ca} channel was also distinct from the BK channel in that it was only weakly voltage-dependent even at relatively low Ca concentrations (2 mM Ca/10 EGTA). We have observed this rectifying K_{Ca} channel in isolation from BK channels in at least 4 recordings and also in the presence of 500 nM Apamin in the patch pipette. Experiments are ongoing to determine whether this channel is charybdotoxin- or iberiotoxin-sensitive. Interestingly, the activity of this channel runs up in the absence of Ca with time after patch excision from the cell.



625.15

ETHANOL ACTIVATES LARGE CONDUCTANCE, Ca^{++}-ACTIVATED K^+ CHANNELS IN NEUROHYPOPHYSIAL TERMINALS. **A.M.Dopico, J.R. Lemos and S.N. Treistman*. Department of Pharmacology, University of Massachusetts Medical Center, and Worcester Foundation for Experimental Biology. Worcester, MA 01655.**

Large conductance, Ca^{++}-Activated K^+ (maxi CAK) channels are thought to underlie interburst intervals and, thus, control hormone release from neurohypophysial terminals. Since ethanol (EtOH) inhibits the release of vasopressin (AVP) and oxytocin (OT), we studied its effects on maxi CAK channels from these terminals using patch-clamp techniques. CAK channels show a reversal potential close to 0 mV in excised, inside-out patches in symmetric 145 mM $[K^+]$. They have a unitary conductance of 250 pS, and increase activity at more positive potentials and/or when $[Ca^{++}]$ is increased at the cytosolic side of the membrane. EtOH (25-100 mM) applied to the cytosolic surface of the patch reversibly increases CAK activity without changing the unitary conductance of the channel. This activation by EtOH may reflect a direct interaction with the channel, possibly altering the EC_{50} for Ca^{++}-activation of $I\text{-}K^+_{(Ca)}$, and in conjunction with the previously-reported inhibition of L-type Ca^{++} channels, can explain the reduced release of AVP and OT after EtOH ingestion. **Supported by N.I.H. grant AA-08003.**

625.16

POTASSIUM CHANNELS IN THE HYPERKALEMIC RESPONSE TO SUCCINYLCHOLINE FOLLOWING DENERVATION. J.A.J. Martyn, P. Yanez, D.B. Carr*, Dept. Anesthesia, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114

Denervation induces a proliferation of nicotinic acetylcholine receptors (nAChRs) at the muscle membrane. This upregulation of nAChRs is associated with supersensitivity to agonists such as succinylcholine (SCh) and is believed to be responsible for the potentially lethal hyperkalemic response to SCh observed in patients with denervation injuries. The present study uses K^+ channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) to characterize the contributory role of K^+ channels in this hyperkalemic response.

Rats underwent bilateral sciatic denervation. After 2 weeks, the plasma K^+ levels to SCh (3 mg/kg I.V.) was studied. The rise in plasma K^+ induced by SCh was significantly greater in denervated rats (2.9$\pm$0.3 MEq/L, n=6) than in controls (0.7$\pm$0.1, n=6). Pretreatment with either 4-AP (3 or 5 mg/kg) or TEA (20 or 40 mg/kg) failed to inhibit the rise in K^+. TEA (60 mg/kg) attenuated the hyperkalemia to SCh (1.2$\pm$0.3 MEq/L, n=6, $p < 0.5$). At this dose, however, twitch tension was decreased to 20% of control (80% muscle paralysis), suggesting that inhibition of AChR channel also occurred.

Thus, K^+ channels alone may not play a significant role in the hyperkalemia to SCh. K^+ channel blockers may not be clinically useful for the prevention of hyperkalemia to SCh.

625.17

ACTIN FILAMENTS REGULATE ION CHANNELS IN IDENTIFIED RETINAL NEURONS. Greg Maguire*@, Victoria Connaughton@, Adriana Pratt#, George R. Jackson, and Horacio Cantiello#, @Sensory Sciences Center, The University of Texas, Houston, TX 77030 and #Renal Unit, Massachusetts General Hospital, and Department of Medicine, Harvard Medical School, Charlestown, MA 02129.

The role of the actin-based cytoskelton was assessed on ion channel currents of identified retinal amacrine and bipolar neurons of the tiger salamander, Ambystoma tigrinum both in isolation and as a functional network. Neurons were identified by fluorescence labelling and whole-cell currrent patterns as previously described (Maguire et al., PNAS, 86:10144, 1989). Whole-cell voltage-gated K+ currents increased by addition of the actin filament depolymerizing agent cytochalasin D (1-5 uM) to the bathing medium. This regulation was time dependent with the peak current occurring 10-15 minutes after addition of the drug. Addition of phalloidin (1 uM), an actin filament-stabilizing agent, to the whole-cell recording pipette, completely prevented the stimulatory effect of cytochalasin D on the voltage-gated K+ currents. Voltage-gated Na+ channels were similarly affected by the changes in actin filament organization. The addition of cytochalasin D to the bathing medium activated single channnels under both cell-attached and inside-out conditions. Actin (1mg/ml) and/or filamin (10 nM) added to the cytosolic side of inside-out patches also increased the frquency of channel openings. The results are consistent with a dynamic role of actin filament organization on the regulation of voltage-gated ion channels in neurons.

625.18

FLUFENAMATE AND MEFENAMATE: EFFECTS ON NEURONAL CAN CHANNELS. L.D. Partridge*, M. Sandquist, T. Shaw. Physiol Dept, Univ of New Mexico, Albuquerque, NM 87131.

Calcium-activated non-selective (CAN) channels respond to cytoplasmic Ca^{++}, do not inactivate, and are capable of producing maintained depolarization; they thus play important roles in the physiology and pathology of neuron function. CAN channels have been studied in neurons following bursts and after Ca^{++} injection, and in patches isolated from neuronal membranes. Although CAN channels have been shown to be modulated by a PKA mechanism, no direct drug effects on neuronal I_{CAN} have been reported.

We studied I_{CAN} in bursting neurons of *Helix aspersa* by measuring the difference in tail currents at E_K following 50 and 500 ms depolarizing pulses to 0 mV. These current tails were identified as being I_{CAN} because they had decay time constants of at least 500 ms, could be measured at E_K, were eliminated by Ca^{++} current blockers, and were unaffected by removing extracellular Cl^-.

Flufenamate and mefenamate have been shown to block I_{CAN} in non-neuronal cells but there are no reported blockers of neuronal CAN channels. While lower concentrations had no effect on neuronal I_{CAN} tails, 500 μM of flufenamate or mefenamate had a biphasic effect, first increasing and then depressing I_{CAN} tails. This may represent an effect similar to that seen in non-neuronal cells where these drugs first release Ca^{++} from intracellular stores and then block CAN channels.

625.19

A NOVEL Na^+ GATED K^+ CURRENT OF FROG TECTAL NEURONES. A. Zaykin* and A. Nistri. Biophys. Lab., Int. Sch. Adv. Studies (SISSA), 34013 Trieste, Italy.

In the frog brain the optic tectum plays an essential role in processing visual signals from the retina. The basic properties of the layer VI cells, which receive monosynaptic inputs from the optic nerve, are still largely unclear. Using a slice preparation of the adult frog optic tectum we investigated with whole cell patch clamp recording voltage-activated currents of these neurones. The recording pipette contained a K gluconate solution plus ATP, EGTA and usually 5 mM Na^+. From holding potential of -70 mV depolarizing steps elicited a fast inward current (-50 mV threshold) peaking at 1.5-2 ms, followed by two outward currents. The first outward current was transient and invariably followed the fast inward current at all potential above inward current threshold. The second, slower current had a higher voltage threshold(-30 mV). The tail current of the fast inward current reversed at +75 mV, which was very close to E_{Na} while the tails of the outward currents reversed at values coincident with E_K. At test potentials more positive than E_{Na} the fast inward current reversed polarity but it was still followed by the transient inward current. Ca^{2+}-free extracellular solution did not change any of these currents. The transient outward current was also observed when intracellular Na^+ was changed from 0 to 15 mM. TTX (1 μM) simultaneously blocked the fast inward and outward currents leaving the slow outward component. Replacing extracellular Na^+ with Li^+ selectively blocked only the fast transient current; similar results were obtained in 0.2 mM 4-AP. It is suggested that a fast inward Na^+ current elicited a transient K^+ current. The novel properties of this latter current include its strong sensitivity to transmembrane Na^+ flux rather than intracellular Na^+, and its selective block by low doses of 4-AP or by Li^+. Supported by INFM.

625.20

INTRACELLULAR SODIUM EVOKES A SULFONYLUREA-SENSITIVE POTASSIUM CURRENT IN DOPAMINE NEURONS. S.W. Johnson*, Ke-Zhong Shen, R. Alan North and Vincent Seutin. Departments of Pharmacology and Neurology, Oregon Health Sciences University, Portland OR 97201, USA.

In Parkinson's disease, dopamine neurons may have reduced ability to synthesize the ATP needed to maintain intracellular Na^+ homeostasis. To study the electrophysiological effects of increased intracellular [Na^+], we used whole-cell patch pipettes to dialyze Na^+ (40 mM) into dopamine neurons in the rat midbrain slice. We found that Na^+-loading increased membrane conductance and evoked an outward current at -60 mV (205 $\pm$ 22 pA; n = 36). About 70% of current evoked by Na^+-loading was blocked by Ba^{2+} (300 μM), glipizide (IC_{50} = 4.4 nM), tolbutamide (IC_{50} = 20 μM), and glibenclamide (1 μM). This current was mediated by K^+ because it reversed direction at the expected reversal potential for K^+ (-98 $\pm$ 6 mV, n = 9; 100 mM K^+ in pipette, 2.5 mM in bath), and was also blocked by extracellular TEA (30 mM) and intracellular Cs^+ (100 mM in pipette). About 30% of current evoked by Na^+-loading was not blocked by Ba^{2+}; this current was generated by Na^+/K^+ ATPase because it was blocked by a reduced extracellular concentration of K^+ (0.5 mM), and by dihydroouabain (3 μM). We suggest that the sulfonylurea-sensitive K^+ current evoked by Na^+-loading may have a neuroprotective function in Parkinson's disease.

POTASSIUM CHANNELS VI

626.1

ELIMINATION OF DELAYED RECTIFIER POTASSIUM CHANNEL EXPRESSION BY ANTISENSE OLIGONUCLEOTIDES IN A RAT PITUITARY CELL LINE.
S. Chung*, D. Saal and L.K. Kaczmarek. Dept of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

A large number of different subfamilies of *Shaker* potassium channels have been cloned and expressed in heterologous expression systems, such as *Xenopus* oocytes. It has, however, been difficult to attribute any specific cloned K^+ channel to a specific K^+ current in real cells. We have now used antisense oligonucleotides against the Kv1.5 delayed rectifier K^+ channel to show that this channel contributes to a hormone sensitive K^+ current in the GH4C1 pituitary cell line.

Treatment with a glucocorticoid hormone, dexamethasone, rapidly increases Kv1.5 mRNA in GH4C1 cells by up to 3 fold in 4 hours. This is accompanied by an increase in the delayed rectifier K^+ current. Using an antibody against the Kv1.5 protein, we have now observed an increase in Kv1.5 protein on hormone treatment. Pre treatment of the cells with a 20-mer antisense phosphorothioate oligonucleotide, but not with a random nonsense oligonucleotide, blocked the increase in the expression of the protein induced by dexamethasone. The treatment of the cell with antisense oligonucleotide also specifically blocked the increase in the K^+ current. These results indicate that the Kv1.5 channel contributes to the glucocorticoid-sensitive component of K^+ current in $GH4C_1$ cells.

626.2

SELECTIVE EXPRESSION OF THREE DISTINCT POTASSIUM CHANNEL ACTIVITIES IN GIANT FIBER LOBE OF SQUID.
W.-S.D. Griggs, Y. Hanyu and G. Matsumoto*. Supermolecular Division, Electrotechnical Laboratory, Tsukuba, Ibaraki 305, Japan.

Cell bodies of the Giant Fiber Lobe (GFL) of squid *Loligo Bleekeri* are dissociated and cultured. Currents are recorded at 16~18°C using whole-cell patch clamp techniques. Within 24 hours of plating, the axonal bulbs (AB) and the bulb-like structures (BLS) exhibit sodium currents while the cell bodies without processes (CB) do not. Meanwhile, all CB have transient potassium currents that inactivate with time constants 50~100ms. This slow-inactivating current is totally blocked by external application of 1~2mM 4-aminopyridine (4-AP). Some CB have another fast-inactivating potassium current with time constants 10~13ms. This fast-inactivating current is less sensitive to 4-AP than the slow-inactivating one. The fast- and slow-inactivating currents reach half maximum at 4.5~8ms, depending slightly on the voltage. In some BLS, a third type of slightly inactivating current similar to the delayed-rectifier current in squid giant axon is recorded (I_K). It is tetramethylammonium-sensitive and insensitive to 4-AP. Depending on the voltage, it reaches half maximum at 9~25ms. In AB and some BLS, a slow-inactivating component similar to that in CB is present in addition to I_K. Other BLS have, in addition to I_K, a component similar to the fast-inactivating current in CB with respect to their inactivation time constants and 4-AP sensitivity. I_K and sodium current have been recorded in some CB after day 3 in culture. Our results show that each of CB, AB, and BLS in GFL expresses selective combinations of three potassium currents which are distinct in their kinetics and pharmacology. This selectivity can vary in time in culture.

626.3

A CALCIUM ACTIVATED POTASSIUM CURRENT IN X-ORGAN SOMATA. R. Godínez, B. Mendiola, R.F. Valdiosera and J. Hernández*. Depto. de Fisiología, CINVESTAV-IPN. Apartado Postal 14-740. México D.F. 07000 and Depto. de Ingeniería Biomédica, UAM-Iztapalapa. México, D.F.

Single electrode voltage clamp experiments were carried out in axotomized X-organ neurons somata of the crayfish (Procambarus clarkii). The outward currents recorded in response to depolarizing voltage pulses from a holding potential of -45 mV depended on $[Ca]_o$. These currents increased 80% when $[Ca]_o$ was increased from 13.5 mM to 23.5 mM. Decreased about 50% when $[Ca]_o$ was decreased to 2 mM from 13.5 mM and the same diminution occurred when the Ca^{++} current was blocked with 200 μM of Cd^{++}. A diminution of the same order was observed when 1 mM TEA or 20 nM charybdotoxin were added to the bath. Apamin however, had no effect. On the other hand, a 220 pS (in 227 mM KCl) K channel was observed in inside-out patch clamp recordings of these neurons. This channel is activated when the internal [Ca] is increased from 1 μM to 50 μM. The presence of this high conductance Ca^{++} activated K channel may be important as a burst terminating mechanism in these neurons. Supported by CONACYT 1245-N9203.

626.4

IDENTIFICATION OF A CALCIUM-ACTIVATED POTASSIUM CHANNEL IN PROLACTIN SECRETING CELLS OF THE TELEOST GILLICHTHYS MIRABILIS. Lisa A. Romano and Charles R. Fourtner* Dept. of Biological Sciences, State Univ. of NY at Buffalo, Buffalo, N.Y. 14260.

Prolactin is the main freshwater adapting hormone in teleost fish. Prolactin secreting cells are located exclusively in the teleost rostral pars distalis. We have identified using patch-clamp technique a calcium-activated potassium channel in dissociated cells of the rostral pars distalis of Gillichthys mirabilis. In excised patches the channel has a conductance of 170 pS and is voltage dependent over the physiological range of membrane potentials, with depolarization leading to an increase in channel open probability. The channel is activated by calcium on the cytoplasmic face of the membrane in a dose-dependent manner. Application of 1mM TEA, a known K channel blocker, to outside-out patches reversibly blocks the channel, while application to inside-out patches (up to 10mM) was without effect. This channel may function to modulate action potential duration and therefore prolactin secretion in these cells.

626.5

VOLTAGE ACTIVATED POTASSIUM CURRENTS IN RAT PRIMARY LACTOTROPHS AND PITUITARY CELL LINE GH3. S. Sankaranarayanan and S.M. Simasko*. Dept of VCAPP, School of Veterinary Medicine, Washington St. Univ., Pullman, WA 99164.

How voltage activated potassium currents (K_V) influence membrane potential behavior in lactotrophs and GH3 cells was addressed by characterizing their kinetic and pharmacological properties using whole cell patch clamp techniques. Exponential fits to the decay of K_V showed that both lactotrophs and GH3 cells have 3 components, a fast inactivating component (K_{V1}), a slowly inactivating component (K_{V2}), and an offset current. Voltage at 50% activation and rate of decay of K_{V1} was similar in lactotrophs (5 mV & 15/s) and GH3 cells (4 mV & 14/s). Voltage at 50% inactivation was -52 mV in lactotrophs and -41 mV in GH3 cells. Rate of recovery from inactivation of K_{V1} fit a single exponential (9/s) in lactotrophs, while in the GH3 cells the recovery from inactivation of K_{V1} fit a double exponential [0.12/s (70%) & 6/s (30%)]. In both lactotrophs and GH3 cells, 4-aminopyridine, 300 μM, blocks K_{V1} (~60%) and K_{V2} (~50%). Dendrotoxin (DTX), 100 nM, was found to block only the K_{V1} (41%) component in GH3 cells but had no effect on K_{V1} or K_{V2} in the lactotrophs even at a concentration of 300 nM.

These results show that although K_{V1} components in both lactotrophs and GH3 cells are similar, their differences are perhaps significant in terms of influencing membrane potential behavior. DTX will be a useful probe to investigate the role of K_{V1} in GH3 cell function.

626.6

POSTNATAL DEVELOPMENT OF HYPERPOLARIZATION-ACTIVATED INWARD CURRENTS IN RAT NEOCORTICAL NEURONS. J.J. HABLITZ* AND B. SUTOR, Neurobiology Research Center, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Neocortical neurons in vitro display inward rectification during hyperpolarizing current pulses. Voltage responses reach a peak and then "sag" back towards resting levels. We have used whole-cell patch-clamp techniques to study the postnatal development of this response in rat neocortical neurons. Slices of anterior frontal cortex were prepared from rat pups on postnatal day (PN) 2-30. Under current clamp conditions, hyperpolarizing inward rectification or "sag" was observed as early as PN2 and was prominent in neurons from PN2-10. "Sag" was observed upon hyperpolarizations to -70 mV from a rest potential of -50 mV. The difference between the peak and steady state voltages reached during long (500-800ms) current pulses increased with pulse amplitude, suggesting activation of a voltage-dependent current. Hyperpolarizations beyond approx. -130 mV were often associated with an abrupt depolarization during a sustained current pulse. Under voltage clamp conditions, using a holding potential of -50 mV, a non-inactivating, time and voltage-dependent inward current was observed during hyperpolarizing voltage steps to membrane potentials negative to -70 mV. The "sag" and inward current were sensitive to block by both Cs^+ and Ba^{2+}. PN 10-30 neurons showed less prominent sag although IV-curves showed inward rectification. These results indicate that hyperpolarization activated inward currents are present in neocortical neurons early in development.(Supported by NS22373)

626.7

BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF INWARDLY RECTIFYING K^+ CURRENTS IN RAT SPINAL CORD ASTROCYTES

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Department of Neurology, Yale, School of Med., New Haven CT 06510 and *Biology, Cal. Polytechnic State University, San Luis Obispo CA 93405.

Astrocytes are known to posses a large resting K^+ conductance. We characterized the resting conductance of astrocytes derived from rat spinal cord and found that this conductance was primarily mediated by inwardly rectifying K^+ (Kir) channels. We identified two types of astrocyte Kir channels with single channel conductances of ~28 pS and ~80 pS respectively. Channels displayed voltage-dependent block: open probabilities were ~0.8 at potentials near and negative to the cell's resting membrane potential, but were near zero at potentials positive of the resting potential. The conductance (gK) of inwardly rectifying K^+ currents (Kir) depended strongly on $[K^+]_o$, and gK was approximately proportional to the square-root of $[K^+]_o$. Kir currents inactivated in a time and voltage-dependent manner due to block by $[Na^+]_o$. Kir currents were also blocked in a dose-dependent manner by extracellular Cs^+ (Kd=72 μM at -160 mV), Ba^{2+} (Kd=8.5 nM) and TEA (90% block at 10 mM), but were insensitive to 4-AP (5 mM). Ba^{2+} and TEA inhibition of Kir currents caused a marked depolarization suggesting that Kir channel activity is essential to establish the negative resting potential typical for astrocytes. The biophysical features of astrocytic inwardly rectifying K^+ channels are compatible with properties required for their proposed involvement in $[K^+]_o$ clearance according to the "glial spatial buffer" hypothesis: i.) high open probability at the resting potential, ii.) increasing conductance with increasing $[K^+]_o$, iii.) and rectification, e.g. channel closure at positive potentials. It is proposed, therefore, that the dissipation of $[K^+]_o$ following neuronal activity is mediated by the activity of astrocytic Kir channels.

626.8

POTASSIUM CURRENTS UNDERLYING ACTION POTENTIALS OF HIPPOCAMPAL CA1 St. ORIENS-ALVEUS INHIBITORY NEURONS. L. Zhang* and C. J. McBain. Laboratory of Cellular and Molecular Neurophysiology, NICHD, NIH, Bethesda, MD 20892

Whole-cell recordings were made from 88 CA1 st. oriens-alveus interneurons in hippocampal slices from 13-20 day-old rats to determine the compliment of potassium currents underlying single action potentials (AP). AP repolarization was dependent on the transient D- and A-currents, since the spike width was broadened by 58% and 120% by 50 μM and 2 mM 4-AP respectively. Further, a prepulse to -80 mV to deinactivate the A current increased the rate of spike repolarization. A role for the Ca^{2+}-activated K current (I_C) in spike repolarization was also determined, the specific blocker iberiotoxin (IBTX, 100 nM), increased the duration of APs by 36%. Ca^{2+}-free Co^{2+}-containing solution produced similar effects (38% increase) which were non-additive with IBTX. In contrast, 4-AP (2 mM) further prolonged the APs in the Ca^{2+}-free medium by 130%. APs were followed by two prominent AHPs of fast (2 ms) and "medium" (270 ms) duration. IBTX (100 nM) or Ca-free solution completely eliminated this fast AHP, while 4-AP was without effect. The amplitude of the medium duration AHP was reduced by apamin (100 nM, 26%), Ca^{2+}-free Co^{2+} solution (63%), the muscarinic agonist carbachol (10 μM, 20%) and histamine (10 μM, 23%). A slow-inactivating and two transient outward currents could be elicited by membrane depolarizations in excised outside-out, or whole-cell patches. Subtraction of the currents elicited with or without conditioning prepulses to -90 or -40 mV allowed isolation of each current. The half-maximal activation of the total transient current was reached at -22 mV and the slow-inactivating at -4 mV. At -10 mV, 4-AP (2 mM) inhibited both the transient current by 66% and the slow-inactivating current by 11%. In conclusion, I_A, I_D and I_C are three of the major K currents involved in AP repolarization; while I_C, I_{AHP} and I_M mediate part of the AHP in O-A inhibitory neurons.

626.9

POTASSIUM CURRENTS IN PREVERTEBRAL AND PARAVERTEBRAL SYMPATHETIC NEURONS. Hong-Sheng Wang and David McKinnon*, Dept. of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794

Intracellular recordings were made from rat sympathetic neurons in isolated superior cervical ganglia (SCG), celiac ganglia (CG), and superior mesenteric ganglia (SMG). Based on their response to a maintained depolarizing current stimulus, neurons were classified as 'phasic' or 'tonic'. All neurons in the SCG were phasic, 85% of the neurons in the SMG and 58% of the neurons in the CG were tonic and the balance were phasic. The voltage response of phasic and tonic neurons around threshold to a constant current step was markedly different. The response of phasic neurons was biphasic with an initial depolarizing response followed by significant repolarization of the membrane potential. In contrast, tonic neurons became more depolarized during a prolonged current step. The underlying currents were studied using single-electrode voltage-clamp recording. The M-current was present in all phasic neurons, but was very weak or absent in tonic neurons. An A-current was apparent in both phasic and tonic neurons. The voltage-dependent activation, steady-state inactivation, and current density of the A-current were all similar in phasic and tonic cells. A low-threshold, slowly inactivating outward current (D2 current) was observed exclusively in tonic neurons. The slow inactivation of this current appeared to underlie the slow depolarizing ramp seen in response to a maintained depolarizing current step. Computer simulations, based on the voltage-clamp data, suggested that the different firing properties of phasic and tonic neurons could be accounted for by differential expression of the M-current and the D2-current.

626.10

A-TYPE POTASSIUM CURRENT (I_A) GATING IS ALTERED IN SYMPATHETIC NEURONS FROM HYPERTENSIVE RATS. Walter P. Robertson and Geoffrey G. Schofield*. Department of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

Increased sympathetic nerve activity has been implicated in the development and maintenance of hypertension. The cellular basis for the exaggerated sympathetic nerve activity has yet to be determined. I_A may mediate neuronal excitability via modulation of action potential duration, regulation of the interspike interval during repetative firing, and modulation of the time course of EPSP decay. We examined I_A in the spontaneously hypertensive rat (SHR) to investigate the possibility that alterations in I_A may be involved in the hyperexcitability of sympathetic neurons. Our preliminary data shows that half inactivation voltage of I_A from SHR (n=4) is ~13 mV hyperpolarized than control Wistar rats (n=6) (Fig.). In the SHR, inactivation at such hyperpolarized potentials could reduce the availability of I_A and thus mediate the increased sympathetic activity in hypertension.

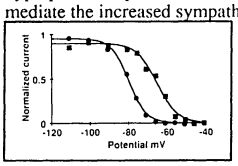


Figure. H infinity plots of peak A-current. Neurons were held at the plotted potentials for 1.0 second before stepping to +20 mV. Current is normalized to maximum. Mean V_h for SHR sympathetic neurons (circles) was -78 ± 1 mV and -66 ± 2 mV for Wistar rats (squares). Solid lines are least-squares fit of the data to a Boltzman function.

626.11

ROLE OF IA-DEPOL IN FREQUENCY-DEPENDENT SPIKE BROADENING OF R20 CELLS IN *APLYSIA CALIFORNICA*. M. Ma* and J. Koester. Center for Neurobiology and Behavior, Columbia University, N.Y., NY 10032.

We studied the mechanisms and consequences of frequency-dependent spike broadening in the 2 R20 neurons in the abdominal ganglion. These neurons, which activate the circuit that initiates respiratory pumping, contain the neuropeptide SCP. When fired at 4-8 Hz, the falling phase of the action potential increases 6-fold during a 6-10 sec train, from 5 to 30 msec.

Spike broadening recorded from the somata of the R20 cells was shown to affect transmitter release from nearby terminals. We tape-recorded a 7 Hz, 10 sec train of gradually broadening spikes. They were then replayed as command signals for a 2-electrode voltage clamp that controlled soma potential. The synaptic output of the R20 cell produced in this way in a postsynaptic RB cell or in the R25 cells was the same as that produced under current clamp. But when only the first (non-broadened) spike from a train was played back for 10 sec at the same (7 Hz) frequency, the synaptic output was reduced by 50 - 70 %. Because the release sites are some distance from the soma,the effect of broadening on release is underestimated by this protocol.

Using pharmacological methods and 2-electrode voltage clamp, we isolated voltage-gated I_{Na}, I_{Ca} (L- and N-type), $I_{K\text{-}V}$, I_{Adepol} and $I_{K\text{-}Ca}$ in the R20 cells. I_{Adepol} activates in a range more depolarized than that for traditional A currents (Pfaffinger et al.,1991). The contributions of different currents to spike broadening were determined by using the gradually broadening action potential train as the command for the clamp. We found that: (i) Although the peaks of I_{Ca} show 40% inactivation during the spike train, the time integral of I_{Ca} during individual spikes increases by 200%, indicating that there is more Ca^{2+} influx during broadened spikes than during the non-broadened ones. (ii) Surprisingly, I_{Adepol} is the major outward current in the non-broadened spikes. Moreover, it undergoes profound and rapid cumulative inactivation, which is the major cause of frequency-dependent spike broadening in the R20 cells.

626.12

IONIC CURRENTS INVOLVED WITH AUTOMATICITY IN A CLONAL CELL LINE. H. Bryant[1]*, V. Kowtha, K. Iwasa[2], D. Stenger. Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC 20375, Department of Physiology, [1]Uniformed Services University of the Health Sciences, Bethesda, MD 20814, [2]Section on Biophysics of Sensory Processes, LCB/NIDCD/NIH, Bethesda, MD 20892.

NG108-15 (neuroblastoma x glioma) cells are capable of exhibiting stable automaticity (the spontaneous occurrence of regenerative action potentials) following transient exposure to extracellular perfusates containing 20 mM NH_4Cl. The electrical activity of NG108-15 cells was monitored using single electrode intracellular recording and whole cell patch clamp techniques after culture in serum-containing medium (SCM) or serum-free media (SFM) containing N2 or B27 supplements. Cells cultured in SCM did not exhibit automaticity. Whole cell recordings revealed a pronounced outward current component in cells cultured in SFM which was insensitive to TEA and cesium. Action potential profiles of SFM cells were characterized by prolonged after hyperpolarization (AHP) which predisposed SFM cells to fire repetitively. Cadmium blocked the AHP. K^+ channel blockers Apamin and Charybdotoxin reduced the AHP component while CTX prolonged the action potential duration. SITS, a potent anion transport inhibitor, revealed another chloride sensitive outward component that slowed the firing rate. These results suggest that the AHP and the chloride outward currents present in cells cultured in SFM modulate automaticity in NG108-15 cells.

626.13

A DENDROTOXIN-I SENSITIVE POTASSIUM CONDUCTANCE IN AUDITORY NEURONS OF THE RAT MNTB. H. M. Brew and I. D. Forsythe. SPON: Brain Research Association. Dept. of Cell Physiology and Pharmacology, Univ. of Leicester, P.O. Box 138, Leicester, LE1 9HN, UK.

Neurons of the medial nucleus of the trapezoid body (MNTB) are part of brainstem auditory pathways that underly sound source localisation. MNTB neurons possess rapidly activating potassium currents (Forsythe & Barnes-Davies 1993, Proc. Roy. Soc. B, 251, p143-150) that are thought to contribute to their ability to follow accurately the action potentials in their single giant synaptic input. We used whole-cell patch techniques to characterize one of the conductances underlying these currents in MNTB neurons in 200μm slices of brainstem from Lister hooded rats (5-12 day old). Slices were perfused with a standard artificial CSF (aCSF) at 25°C. Drugs were applied in the perfusate. Patch pipettes contained a pseudo-intracellular solution. A modified aCSF containing 1μM tetrodotoxin, 10μM strychnine, 0.5mM calcium and 2.5mM magnesium was used for voltage clamp recordings.

One potassium conductance produced most of the outward current in MNTB neurons between -60mV and -30mV. This current, I_d, was blocked by 100nM dendrotoxin-I (DTX-I) and mostly blocked by 100μM 4-aminopyridine. I_d activated and deactivated rapidly, peaking at 5msec at -30mV, and closing with a mean time constant of 1.8msec at -100mV. I_d showed voltage-dependent inactivation, inactivating very slowly at -30mV (>10sec), and 500msec at a holding potential of -110mV gave near complete deinactivation. The mean reversal potential for Id tail currents was -72mV.

MNTB neurons respond to depolarizing current clamp steps (≥0.75nA) with a single action potential (AP). In the presence of 100nM DTX-I, current steps (≥0.25nA) produced a train of APs (140Hz in response to 1.5nA).

The I_d described here is similar to the current, I_d, described in A-type neurons of rat nodose ganglion (Stansfeld et al. 1986, Neurosci. Lett. 64, p299-304). We suggest that Id prevents MNTB neurons from firing multiple APs in response to one giant epsc, while the other high threshold potassium conductances facilitate high frequency following of presynaptic APs. Supported by the Wellcome Trust.

626.14

A COMPARATIVE PROFILE OF [125I]-α-DENDROTOXIN BINDING TO VOLTAGE-DEPENDENT POTASSIUM CHANNELS IN RODENT, NONHUMAN PRIMATE AND HUMAN BRAIN. L.E. Schechter*, D.M. Pearsall, S.P. Nawoschik, N.X. Barrezueta and K.J. Rhodes. American Cyanamid Co., Med. Res. Div., CNS Biological Research, Pearl River, NY 10965.

Alpha-Dendrotoxin (α-DTX) isolated from the venom of the African Green Mamba (*Dendroaspis angusticeps*) has previously been reported to be a neurotoxic peptide that selectively labels with high affinity voltage-dependent potassium (K+) channels. Previous investigators have extensively studied the binding profile of radiolabelled α-DTX in rodent and bovine brain. This study compared the radioligand binding profile of [125I]-α-DTX in hippocampal tissue of rat, cynomolgus monkey and human as defined by saturation analysis, competition experiments and quantitative receptor autoradiography. A rapid filtration binding assay was developed for [125I]-α-DTX using whole cell aggregates of brain tissue. The Kd and Bmax determined by saturation analysis from hippocampal tissue of rat, cynomolgus monkey and human was 63 ± 5 pM; 723 ± 31 fmol/mg prot., 81 ± 2 pM; 553 ± 63 fmol/mg prot. and 92 ± 4 pM; 570 ± 27 fmol/mg prot., respectively. Competition studies examining the displacement of [125I]-α-DTX by peptide toxins which are known to block voltage-dependent K+ channels indicated that the rank order of affinity constants was similar across species: α-DTX > charybdotoxin > mast cell degranulating peptide > β-bungarotoxin. This similarity of the pharmacological profile in the hippocampus among the different species was confirmed using a variety of structurally and pharmacologically diverse agents. Interestingly, the laminar distribution of α-DTX binding sites in hippocampal tissue as studied by quantitative receptor autoradiography indicated that species differences exist in localization. Taken together, these data suggest that there is significant conservation of the voltage-dependent K+ channel subunits that bind α-DTX across species but the neuroanatomical distribution appears to be different.

626.15

DISTRIBUTION OF [^{125}I]α-DENDROTOXIN BINDING SITES IN THE AMYGDALA, HIPPOCAMPUS AND ENTORHINAL CORTEX OF THE MONKEY. N.X. Barrezueta*, D. M. Pearsall, L. E. Schechter and K. J. Rhodes, CNS Biological Res., American Cyanamid Co., Pearl River, NY 10965.

Voltage-gated potassium (K^+) channels play a critical role in neuronal function by controlling the resting membrane potential. In the present study we used [^{125}I]α-dendrotoxin ([^{125}I]DTX) as a radioligand for quantitative autoradiographic localization of voltage-gated K^+ channels in cynomolgous (n=1) and fascicularis (n=2) monkey brain. Twenty micron thick coronal sections were incubated for 2 hr at 4 °C in 50 mM Tris buffer (pH 7.5) containing (in mM): NaCl (140), KCl (5), $MgSO_4$ (1.3) and 40 pM [^{125}I]DTX. Adjacent sections were incubated in the same buffer containing unlabeled DTX (100 nM) to define non-specific binding. Sections were then washed in buffer and dH_2O, rapidly dried, and apposed to Hyperfilm-^{3}H along with [^{125}I]-plastic standards (Amersham) for 48 hr.

Quantitative analysis of the autoradiograms indicated that specific [^{125}I]DTX binding was 80-85% of the total and that the distribution of [^{125}I]DTX binding sites was qualitatively similar in cynomolgous and fascicularis brain. Analysis of binding site density within the medial temporal lobe indicated that in the amygdala there was a high density of [^{125}I]DTX binding sites in the lateral and lateral basal nuclei, a moderate density in the medial basal nucleus, and a low density in accessory basal nucleus. In the entorhinal cortex (area 28), there was a high density of binding sites in layers II and V, and a moderate density in layer III. There was also a higher density of [^{125}I]DTX binding sites in area 28S than in other entorhinal subfields. In the hippocampal formation, there was a high density of [^{125}I]DTX binding sites in the molecular layer of the dentate gyrus, stratum pyramidale, radiatum and moleculare of CA3, and in the subiculum and presubiculum. These data suggest that DTX-sensitive voltage-gated K^+ channels may have important modulatory functions within medial temporal lobe circuits crucial for memory function.

626.16

Cd^{2+}-BLOCKADE OF HYPERPOLARIZATION-ACTIVATED CURRENT I_{AB} IN CRAYFISH MUSCLE. A. Araque, D. Cattaert and W. Buño*. Instituto Cajal, CSIC, Madrid 28002, Spain.

A novel hyperpolarization-activated current mediating inward rectification in opener crayfish muscle has been recently described (Araque and Buño, *J. Neurosci.*, 1994). This voltage- and time-dependent current, called I_{AB}, is selectively mediated by K^+, its activation curve depends on the Vm-E_K, and its underlying conductance is unaffected by $[K^+]_o$. It is insensitive to Cs^+, Rb^+ and Ba^{2+}, but is blocked by extracellular Zn^{2+} or Cd^{2+}.

The effects of Cd^{2+} on I_{AB} were studied under two-electrode voltage-clamp in opener muscle fibres. Cd^{2+} (10 mM) totally and reversibly reduced I_{AB}, without modifying the I_{AB} equilibrium potential. Lower Cd^{2+} concentration decreased the maximal conductance of I_{AB} and shifted its activation curve towards more hyperpolarized potentials without affecting the slope of the activation curve. While the I_{AB} activation time constant increased, the I_{AB} deactivation time constant was not modified by Cd^{2+}. The effects of Cd^{2+} on I_{AB} were independent on $[K^+]_o$. Cd^{2+} effects on I_{AB} were dose-dependent, obeying the Hill equation with $IC_{50} = 0.452 \pm 0.045$ mM and a Hill coefficient of 1.

We conclude that: *a*) the block of I_{AB} by Cd^{2+} was exerted in a dose-dependent manner, obeying the Hill equation and suggesting that one Cd^{2+} ion binds reversibly to one receptor with a dissociation constant of 0.452 mM; *b*) the selective permeability of I_{AB} channels was not modified by Cd^{2+}; *c*) the binding site for Cd^{2+} to block I_{AB} was different to the binding site for K^+ to permeate; *d*) the Cd^{2+}-blockade of I_{AB} was not voltage-dependent; *e*) Cd^{2+} blocked the I_{AB} channel gate interfering with its opening but not with its closing mechanism.

Supported by DGICYT (Spain) and DGXII CEC (Europe) grants to W.B. A.A. is an Areces Foundation postdoctoral fellow.

626.17

BLOCK OF THE Kv1.2 K^+ CHANNEL BY AMINOPYRIDINES AND QUININE. Taco R. Werkman* and Michael A. Rogawski. Neuronal Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Block of Kv1.2 voltage-dependent K^+ channel currents by aminopyridines and quinine was investigated in fibroblast cells stably transfected with Kv1.2 K^+ channel cDNA (Werkman *et al., Neuroscience* 50:935-946, 1992). Voltage-clamp experiments were conducted in the whole cell mode using the switch clamp technique, and also in the cell attached and excised patch modes using conventional patch recording methods. 4-Aminopyridine, 3,4-diaminopyridine and quinine all produced a concentration-dependent, reversible block of the Kv1.2 K^+ current elicited with voltage steps from -60 to +30 mV (K_D's = 230, 860 and 18 μM, respectively). Aminopyridine-induced block was voltage-dependent with the fractional block diminishing at depolarized potentials. Quinine-induced block was also voltage-dependent, but with block increasing at depolarized potentials. Aminopyridine block occurs by a mechanism that doesn't require open channels, although an initial depolarization is needed to allow the aminopyridine access to its binding site. In contrast, quinine appears to block by a simple open-channel mechanism. Using intracellular perfusion, it was possible to demonstrate that the aminopyridines block the K^+ channel by binding to an intracellular site, whereas the location of the quinine binding site could not be defined with confidence. The transfected fibroblast preparation provides a useful system to characterize in detail the mechanism underlying the K^+ channel block produced by aminopyridines and quinine, compounds that are well know to act as K^+ channel antagonists, but whose mechanisms of action are incompletely understood.

626.18

TETRAPENTYL AMMONIUM (TPA) BLOCKS THE SLOWLY-ACTIVATING, SLOWLY-INACTIVATING K^+ CURRENT (I_K(s)) OF RAT PITUITARY MELANOTROPHS. K. Wong, J.-L. Davidson and S.J. Kehl*, Dept. of Physiology, U.B.C., Vancouver, B.C., V6T 1Z3.

The I_K(s) of the melanotroph has been shown in this and other labs to be blocked by external TEA. The aim of the work outlined here was to investigate the effects of TPA, a structural analogue of TEA, on the I_K(s). Whole-cell currents were recorded from cultured melanotrophs by using conventional patch electrode techniques. The block of I_K(s) by TPA was manifest as a decrease of the steady-state current amplitude and an increased rate of current decay ("inactivation"). At a given voltage, the extent and rate of the TPA-induced inactivation was proportional to the TPA concentration; and, at a given concentration of TPA, the degree and rate of inactivation increased with membrane depolarization. The K_i at 0 and 50 mV was estimated to be 2.0 and 0.8 μM, respectively, and the Hill coefficient was near one. Tail currents recorded in TPA following a brief, intense depolarization that opened I_K(s) channels were smaller than control responses but "deactivated" at the same rate. When TEA and TPA were applied simultaneously there was no evidence for any competitive interaction. These results are consistent with an open channel blocking mechanism for TPA.

626.19

NOISE FLUCTUATION ANALYSIS OF PINEAL DELAYED RECTIFIER CHANNELS AND MODIFICATION BY THE BLOCKER, DOCOSAHEXAENOIC ACID. Richard E. Frye, Jon S. Poling, and Stefano Vicini*. Department of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D.C. 20007; Laboratory of Membrane Biochemistry and Biophysics, NIAAA, Rockville M.D. 20852.

Docosahexaenoic acid (22:6n3) potently inhibits the delayed rectifier current of rat pinealocytes. 22:6n3 reduces peak K^+ current amplitude and produces a dose-dependent "secondary inactivation" of macroscopic outward current. This effect is probably due to open-channel blocking ability of 22:6n3. To investigate this hypothesis we identified single channel properties through noise analysis and single channel recording techniques. A computer program to analyze current fluctuations due to ion channels was developed utilizing LabTalk™ and the user interface module of Origin™ for Windows™. The technique employs mathematical routines based on Fast Fourier Transformations (FFTs) similar to those described by Dempster (Computer Analysis of Electrophysiological Signals, Academic Press, 1993). The power spectra of whole-cell current fluctuations due to delayed rectifier K^+ channels are well described by a double Lorentian function. The slow component of the double Lorentian is shifted to a higher corner frequency when 22:6n3 is applied. To further investigate this effect, 22:6n3 was applied to outside-out patches excised from pinealocytes. In control conditions, records of single channel currents exhibit bursting behavior and a conductance of about 20 pS is observed when the membrane is depolarized from -40 mV. In the presence of 22:6n3 (6 μM), the channel burst length is shortened and longer closed intervals become apparent. Fluctuation analysis of outside-out recordings produce parameters which correspond to the kinetics of single channel currents.

626.20

EFFECTS OF EXTRACELLULAR PH ON MEMBRANE ELECTRICAL AND MECHANICAL PROPERTIES OF VASCULAR SMOOTH MUSCLE

Beixing Liu, P.C. Johnson and G.F. Koshland*. Department of Physiology, University of Arizona, Tucson, AZ85724

We have investigated the effect of changing extracellular pH (pH_o) at constant level of CO_2 on the membrane electrical and mechanical properties of vascular smooth muscle in isolated guinea-pig mesenteric and femoral arteries, using intracellular microelectrode recording and force measurement. Lowering pH_o depolarizes but reduces force development of vascular smooth muscle, whereas increasing pH_o hyperpolarizes and increases force development. From measurement of the decay time constant of excitatory junction potentials, we also found that lowering pH_o from 7.4 to 6.6 decreased the total membrane conductance of vascular smooth muscle whereas increasing pH_o had the opposite effect. The relationship between membrane potential and pH_o was unchanged as we increased the $[K^+]_o$ (in mM) from 4.7 to 25 at pH_o 6.6, 7.4, and 7.8. The value of membrane potential measured at $[K^+]_o$ of 25 mM was close to that predicted by Nernst equation. These data indicate that the resting potential of vascular smooth muscle is predominantly determined by the membrane conductance to K^+ and that the effect of pH_o on resting potential may be mediated through altering K conductance. Finally, application of cromakalim and glibenclamide did not significantly affect the resting potential at pH_o 6.6, 7.4, and 7.8, which suggests that changing pH_o did not affect ATP-regulated K channels. The maximum effect of pH on membrane potential occurs within about 5 min, after which the potential tends to return toward the control level. This secondary change is blocked by 1 mM TEA, suggesting that it is due to change of intracellular pH acting on Ca-regulated K channels. Supported by NIH grant HL 15390.

627.1

ANALOG VLSI MODEL NEURON: A MULTIPLE PURPOSE PROGRAMMABLE DEVICE. G. Le Masson*, S. Le Masson, Y. Deval, and M. Moulins. LNPC,Univ. Bordeaux I-CNRS Arcachon 33120 FRANCE and IXL-URA CNRS 846 Bordeaux FRANCE.

Using a BiCMOS full custom technology, we developed an analog VLSI circuit (ASIC) for modeling conductance-based neurons. Currents with voltage dependent, sigmoidally-shaped activation and inactivation are produced and summed through an external serial capacitance. The maximal gain and voltage dependence of each current are fully and easily programmable via analog inputs, as well as the slope of both activation and inactivation curves. Simple kinetics and calcium dependent currents are available, and a synaptic function allows the construction of simple networks. Assembling several circuits is possible, to model neurons with complex intrinsic properties such as burstiness, or to reproduce multicompartmental structure. We tested VLSI models in both static and dynamic range, and we successfully reproduced with the same circuit, different neuronal behavior. To further validate the models, we compared results with an identical numerical model using classical Hodgkin Huxley's conductances. Due to its analog structure, it require no computation time, and allows a fast screening of the parameter space. We recently reported a new technique for real time interaction between model neurons and biological neurons, trough artificial synapses. These VLSI models are ideally suited for such hybrid network interactions.

627.2

MULTIPLE CONDITIONS FOR SLOW OSCILLATIONS IN THE MEMBRANE OF A BURSTING NEURON O.E. Dick, K.-D. Kniffki, C. Vahle-Hinz* Physiologische Institute, Universität Würzburg, Röntgenring 9, D-97070 Würzburg and Universität Hamburg, Martinistr. 52, D-20251 Hamburg.

We have examined the possibility of different conditions for the existence of slow oscillations underlying the bursting mode (Epstein and Marder, Biol. Cybern. 63:25,1990; Destexhe et al., Biophys. J. 65:1538,1993). The problem has been solved by searching for several nonintersecting regions in the parameter space of the model of the stomatogastric bursting neuron (Guckenheimer et al., Phil. Trans. R. Soc. 341:345,1993). Since the considered system has two slow variables: intracellular calcium concentration (Ca) and activation variable for the Ca-current (Z_{Ca}), two systems have been chosen for the analysis of the conditions of slow oscillations. The first system contains the equations for the membrane potential V and Ca, the second system involves the equations for V and Z_{Ca}. Using the methods of bifurcation theory we have obtained for each system the explicit parametric forms of the boundaries limiting the regions of slow oscillations. Analyzing these forms we have found that over the range of physiological values of the model parameters there are two nonintersecting regions on (g_{Ca}, I), (g_{kCa}, I) and (g_a, I) planes, where g_{Ca}, g_{kCa}, g_a are the maximal calcium, calcium-dependent potassium and transient potassium conductances and I is the stimulus value. These results suggest that there are multiple conditions for slow oscillations in the membrane of the stomatogastric neuron. By means of numerical integration we have tested that the frequency of slow oscillations for the parameter values belonging to the regions obtained for the V, Ca system is lower than the frequency of oscillations obtained for the V, Z_{Ca} system. The neuronal membrane is likely to display the "slow bursting mode", which has a slow component determined by intracellular Ca oscillations and a fast component caused by oscillations of the variable Z_{Ca}.

Supported by the N.N.-Stiftung i.Gr., Würzburg.

627.3

RECONSTRUCTION OF HIPPOCAMPAL CA1 PYRAMIDAL CELL ELECTROPHYSIOLOGY BY COMPUTER SIMULATION D.M. Durand*, E.N. Warman, and G.L.F. Yuen, Depts of Biomedical Engineering and Neurosciences, Case Western Reserve University, Cleveland, OH. 44106.

Computer models of neural activity have been very successful in simulating the neurophysiological behavior of many neurons. In the hippocampus, the electrophysiology of neurons such as the CA1 cells has been studied in great details. A large number of ionic currents have been described but previous computer models were not able to reproduce several important features of these cells. We have developed a model based on the simplest combination of ionic channels that could best reproduce CA1 electrophysiology. The model was designed using seven active ionic conductances: g_{Na}, g_{Ca}, g_{DR}, g_{CT}, g_M and g_{AHP} whose kinetics have been inferred from the available voltage clamp data. Particular emphasis was placed on accommodation, slow depolarization potential and spike broadening during repetitive firing. The action potential was accurately simulated and the role of the three repolarizing currents I_A, I_{CT} and I_{DR} investigated. The model also reproduces all four after-potentials recorded following activation of the cell. The fast, medium and slow AHPs were generated by I_{CT}, I_M and I_{AHP} respectively. The model accurately reproduces features observed during the injection of long current pulses such as changes in firing frequency and a gradual broadening of action potential. The role of each current in mediating these responses has been investigated. The model also suggested that I_M may be the principal outward current regulating the CA1 resting potential. Supported by NSF grant # BNS 8809504.

627.4

A COMPUTATIONAL MODEL OF MYELINATED AXONS IN FROG DORSAL ROOT. A.L.Padjen* & T. Hashiguchi, Dept of Pharmacology & Therapeutics, McGill University, Montreal and Dept of Physiology, Tokyo Medical College, Tokyo.

We have used a mathematical model to simulate electrophysiological characteristics of large myelinated axons (long charging and discharging processes of the electrotonic potentials, ETPs) obtained by intracellular recordings from frog dorsal roots. The simulation program (modifed NEURON simulation program by M.Hines & J.W. Moore) contained parameters based on experimental data of all recognized conductances in myelinated axons based on Hodgkin-Huxley equations, including inward rectifier and fast and slow K+ conductances. It also incorporated all known sections of myelinated axons, such as nodal, internodal, myelin, unmyelinated sections, definitions for perinodal shunt, serial and parallel internodal resistances and their measured or estimated properties.

In order to simulate ETPs the myelin leakage conductance and the ionic conductance of internodal membrane were found to be the crucial factors. In contrast to the classical estimate of 1.5 $\mu S/cm^2$ a much larger value of 80 $\mu S/cm^2$ is required as specific myelin conductance. The likely pathways for this conductance are Schmidt-Lantermann incisures or paranodal opening. The conductance of the internodal axolemma requires values that are <1% of the measured conductance of the node of Ranvier.

The model supports the notion that not only the ion conductances of node of Ranvier but also those of the internodal axolemma contribute to electrophysiological properties and conductance mechanism in myelinated axons. (Supported in part by MRC of Canada)

627.5

BUSRSTING, SPIKING, CHAOS, FRACTALS, AND UNIVERSALITY IN BIOLOGICAL RHYTHMS. T.R. Chay*, Y.S. Lee and Y-S Fan. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Biological systems offer many interesting examples of oscillations, chaos, and bifurcations. Oscillations in biology arise because most cellular processes contain feedbacks that are appropriate for generating rhythms. These rhythms are essential for regulating cellular function. In this tutorial review, we treat two interesting nonlinear dynamical processes in biology that give rise to bursting, spiking, chaos, and fractals: endogenous electrical activity of *excitable cells* and Ca^{2+} releases from the Ca^{2+} stores in *non-excitable cells* induced by hormones and neurotransmitters. We will first show that each of these complex processes can be described by a simple, yet elegant mathematical model. We then show how to utilize bifurcation analyses to gain a deeper insight into the mechanisms involved in the neuronal and cellular oscillations. With the bifurcating diagrams, we explain how spiking can be transformed to bursting via a complex type of dynamic structure when the key parameter in the model varies. Understanding how this parameter would affect the bifurcating structure is important in predicting and controlling abnormal biological rhythms. Although we describe two very different dynamic processes in biological rhythms, we will show that there is universality in their bifurcation structures.

627.6

MEASUREMENT ERRORS FROM CLAMPING VOLTAGE-DEPENDENT CONDUCTANCES IN CELLS WITH ELONGATE PROCESSES Daniel K. Hartline* and Ann M. Castelfranco, Békésy Lab., Univ. of Hawaii, Honolulu, HI 96822

Voltage clamp data are often obtained from cells with attached processes. Space-clamp errors potentially invalidate such data. To assess these errors, clamp currents were simulated for somata with a single cylindrical process containing voltage-dependent Hodgkin-Huxley (HH) conductances. Leak-subtracted I(t) and I(V) data were fitted with HH parameters as if the cells were space clamped.

Time constants (τ's) for mh kinetics (as opposed to m^3h) were voltage-independent. Resulting clamp currents had an activation "delay" and voltage-dependent kinetics fittable over a broad range of parameters by an m^qh form. For an outward current mechanism uniformly distributed in a compact (0.5λ) process, activation τ's were lengthened up to several fold, with lesser effect on inactivation τ. Errors in activation τ and q increased, and in inactivation τ decreased, with increased conductance densities. Fitted kinetic parameters for both activation and inactivation displayed a spurious voltage dependence near threshold.

Parameters of the I(V) curve could be well fitted up to *ca* 10-100x leak conductance density. Measurement errors depended strongly on the time of the measurement, well after peak current being optimal. For a uniformly distributed conductance, measurement error was most severe in conductance and activation-width and less severe for activation midpoint and reversal potential. If enough is known about the distribution of channels in processes, it may be possible to correct clamp data for some of the measurement errors.

627.7

ENTORHINAL CORTEX LAYER III NEURONS: CHARACTERIZATION AND CHOLINERGIC EXCITATION. A. Mena* and A. Alonso. Montreal Neurological Institute and McGill University, Montreal, Qc, CANADA H3A 2B4 .

Layers II and III of the entorhinal cortex (EC), via the perforant path, project massively to the hippocampus, and receive input from the cholinergic neurons of the basal forebrain. By means of intracellular recordings in the *in vitro* slice preparation, we have already characterized the electrophysiology of neurons in layer II (Alonso and Klink, 1993) and studied the modulation of their excitability by carbachol (CCh) (Alonso and Klink, 1991 and this meeting). Here, we report on the extension of this study to layer III neurons. Layer III cells appear a rather homogeneous population both morphologically and electrophysiologically. Intracellular staining with biocytin revealed a typical pyramidal shape with a thick apical dendrite and a skirt of basal dendrites. Current clamp recordings showed prominent fast inward rectifcation in both the depolarizing and hyperpolarizing directions. Many neurons displayed rhythmic tonic firing at rest which appears primarily sustained by a persistent Na-current. In contrast to layer II neurons, no rhythmic subthreshold oscillations were observed upon DC depolarization. Pressure-pulse applications of CCh resulted in a dose-dependent depolarization which could be potentiated after eliciting firing by manual injection of current. Clamping back to the control level showed an increase in input resistance. With continued bath application (20-60 μM) of CCh, the response displayed pronounced desensitization. These data demonstrate clear-cut differences between EC layer II and layer III neurons both in their basic electrophysiological properties and in their modulation by basal forebrain cholinergic inputs.

627.8

WHY DO CORTICAL NEURONS SPIKE IRREGULARLY? M. Tsodyks, A. Bell, Z.F. Mainen and T.J. Sejnowski,* Howard Hughes Medical Institute, Salk Institute, La Jolla, CA 92037

The spike trains of many cortical neurons in vivo are highly irregular during both spontaneous and driven activity. Although this variability may simply reflect large correlated fluctations in synaptic input, an additional consideration is the balance between synaptic drive and the intrinsic conductances generating action potentials.

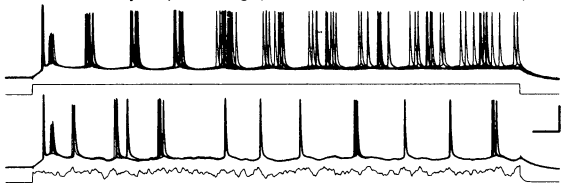
Using a single-compartment Hodgkin-Huxley model, we show that when excitation and inhibition are balanced to maintain the neuron in the region of optimal sensitivity, near firing threshold, then variable spike trains result even in the absence of large input fluctuations. Increased levels of balanced synaptic input increase the rate of input fluctuations, producing higher firing rates with maintained irregularity. Decreased spike repolarization (weaker K^+ rectification) increased the steepness of the input-firing curve, giving greater sensitivity to input fluctuations.

Very similar results were obtained with a simpler integrate-and-fire model, which was used to explore networks of interacting excitatory and inhibitory neurons. Despite positive feedback between the excitatory neurons, proper balance was achieved robustly through inhibitory feedback. With weak repolarization, the network was sensitive to small inputs and could be switched rapidly between stable states dominated by fluctuations. This regime is not consistent with the mean-field approximations commonly made in network models. We propose that neuromodulatory control of K^+ conductances (weakening or strengthening spike repolarization) may dynamically regulate the time-scale of information to which cortical networks are sensitive. (We are grateful to M. Shadlen for useful discussion. Supported by ONR. ZFM is an HHMI Predoctoral Fellow.)

627.9

RELIABILITY OF SPIKE INITIATION IN NEOCORTEX. Z.F. Mainen* and T.J. Sejnowski. Howard Hughes Medical Institute, Salk Institute, La Jolla, CA 92037.

Chemical and electrical signalling in the central nervous system appear remarkably variable, yet it is not known whether this variability carries meaningful information or simply reflects the intrinsic unreliability of underlying mechanisms. We have assessed the reliability of one process critical to neural signalling, the generation of action potentials. Whole-cell recordings were made from rat neocortical pyramidal cells *in vitro* and spikes were elicited by somatic current injection in the presence of synaptic transmission blockers (DNQX, AP5, BMI). Shown below are two examples of 10 consecutive responses (superimposed) to repetitions of identical stimuli (scale bars: 50 msec, 0.5 nA, 50 mV). A constant current step (0.2 nA) produced spike trains (f=11-12 Hz) with individual spike times that drifted from trial to trial (top). In contrast, repetition of a stimulus with transients resembling synaptic activity (filtered gaussian white noise, μ=0.2 nA, σ=0.05 nA) evoked trains (f=12 Hz) with highly consistent spike times (bottom). Our experiments suggest that spike generation is under some conditions an extremely reliable process able to faithfully translate an input signal into a sequence of nerve impulses, and that synaptic transients may enhance the fidelity of spike coding. (ZFM is an HHMI Predoctoral Fellow.)



627.10

Electropharmacological Characterization of Cryopreserved Rat Cortical Neurons T. Weiser, J. Sabourin°, M. Wienrich*, Boehringer Ingelheim KG, Ingelheim, Germany; °Battelle Europe, Geneva, Switzerland

Primary cultures from embryonic rodent brain are widely used for neurophysiological investigations. Unfortunately, these cultures have only a limited survival time of a few days to weeks, and longer storage so far has been impossible. We therefore set out to improve the cryopreservation technique of cortical neurons from embryonic rat brain.

Cryopreserved neurons were tested using the patch-clamp technique, and their properties regarding voltage- and transmitteractivated ion channels were compared with those from unpreserved control cells.

All preserved cells had resting membrane potentials in the physiological range (-55 ±2.8 mV S.E.M, n=14) and responded to voltage jump protocols with typical neuronal sodium- and potassium currents. The application of glutamate, NMDA or GABA induced currents indistinguishable from those of the control cells. The NMDA induced currents where suppressed by AP5; GABA-ergic responses where inhibited by bicuculline and amplified by the steroid 3-alpha-dihydroprogesterone.

We conclude that cryopreservation does not alter the properties of cortical rat neurons, and that these cells can be used for electrophysiological and neuropharmacological investigations.

Supported by the BMFT (Grant No. 0319520A)

627.11

THE HYPERPOLARIZATION-ACTIVATED CATION CURRENT (I_h) IN NEOCORTICAL NEURONS IS BLOCKED BY EXTERNAL PROTEOLYSIS AND INTERNAL TEA. T. Budde*, J.A. White and A.R. Kay. Dept. of Biological Sciences, Univ. of Iowa, Iowa City, IA 52242

After one day in culture neurons derived from the neonatal cerebral cortex exhibited a slowly activating current gated by hyperpolarizing voltage-clamp pulses. The current was blocked by extracellular cesium (2 mM) and unaffected by barium (1 mM), and was permeable to both Na and K (P_{Na}/P_K = 0.29). Its form and pharmacology are consistent with a current termed I_h in other preparations.

I_h was absent from cells acutely dissociated from both the neonatal and mature cerebral cortex, despite of the use of low enzyme concentrations. The sensitivity of I_h to extracellular proteolysis was demonstrated by superfusing the cells with trypsin (1 mg /ml) while monitoring the presence of I_h in the whole-cell mode of recording. I_h was rapidly abolished ($t_{1/2}$ ~ 5 min) by proteolysis and exhibited no shifts in its range of activation or changes in its activation kinetics during the course of the digestion, suggesting the abolition of the ability of the current to gate rather than a shift in its range of activation.

Intracellular TEA, at a concentration of <15 mM was shown to block I_h, while extracellular TEA and 4AP had no effect on the current. This suggests that I_h may be structurally related to potassium channels.

Supported by grants from NIH and ONR.

627.12

FUNCTIONAL ROLE OF AXON HILLOCK AND INITIAL SEGMENT IN CORTICAL SPIKE INITIATION. J.R. Huguenard*[#], J. Joerges, Z.F. Mainen and T.J. Sejnowski. Howard Hughes Medical Institute, Salk Inst., La Jolla, CA 92037 and [#]Dept. of Neurol., Stanford Univ. Sch. Med., Stanford, CA 94305.

The processes that lead from synaptic input to spike output are critical factors in information processing in cortical neurons, yet the precise mechanisms of spike initiation are not well understood. Although neocortical pyramidal cells have voltage-dependent somatic and dendritic Na^+ channels that could promote dendritic spikes, recent data [Stuart and Sakmann, Nature 367:69] support the long-standing hypothesis that spikes are initiated preferentially in the axon hillock or initial segment (AH-IS). This is consistent with our findings in acutely isolated neurons that only cells retaining a visually detectable axon segment can support robust spike generation. A biophysical model was constructed in order to explore the implications of these findings.

When reported somatic and dendritic Na^+ densities (40 pS/μm^2) are combined with AH-IS densities at least 200-fold higher, a density comparable to measurements at nodes of Ranvier, spikes are initiated in the initial segment and then invade the soma and dendritic tree antidromically, even when the site of depolarization is in the distal dendrite. In addition to high channel density, the geometry of the AH-IS was found to be critical to this behavior. The IS achieves a lower initiation threshold by a combination of high local current density and electrical isolation from the soma, while the conical shape of the AH promotes complete invasion of spikes into the soma. Simulations of axo-axonic inhibition, as believed to occur at chandelier cell synapses on the AH-IS, demonstrate that this type of inhibition is more effective in delaying spike onset than equivalent inhibition in the somatodendritic compartment. Voltage-clamp simulations indicate that caution should be exercised in the interpretation of somatic voltage-clamp data. For example, IS spike initiation can occur after distal dendritic synaptic input, even with apparent voltage clamp in the soma. These results underscore the capacity of the AH/IS to function independently of the soma, and indicate the importance of further studies of its physiology. Supported by NIH Grant NS12151 and Office of Naval Research. ZFM is an HHMI Predoctoral Fellow.

627.13

CAESIUM PREVENTS THE MAINTENANCE OF LONG TERM DEPRESSION IN RAT HIPPOCAMPAL CA1 NEURONS. D. DiFrancesco, D. Janigro#, E. Wanke* and G. Maccaferri. Dip. di Fisiologia e Biochimica Generali, Univ. di Milano, Italy and #Dept. of Neurosurgery, Univ. of Washington, Seattle, WA.

Hippocampal synaptic plasticity involves long-term modification of the efficiency of information processing at synaptic sites. We measured long-term depression (LTD) of field EPSP in the CA1 region of acutely isolated hippocampal slices by delivering a 15 minutes train of pulses at 1 Hz to the Schaffer-commissural-CA1 pathway. LTD was prevented by adding a NMDA receptor antagonist (AP-5, 50 μM) to the perfusing medium. Superfusion of the slices with Cs (2 mM) during or just after the 1 Hz stimulation period could both inhibit the maintenance phase of the depression itself and elicit spontaneous rhythmic activity. Since a major effect of Cs is a block of the hyperpolarization-activated current (I_h; *J. Neurophysiol.* **69**, 2129-2136), these results suggest the possible involvement of I_h in the maintenance of long-term depression and in the regulation of hippocampal excitability.

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627.14

TWO TYPES OF INTERNEURONS IDENTIFIED BY POTASSIUM CURRENTS IN ISOLATED HIPPOCAMPAL CELLS FROM ADULT GUINEA-PIG. Shouhui Fan* and Robert K. S. Wong. Department of Pharmacology, State University of New York Health Science Center, Brooklyn, NY 11203.

Interneurons were isolated from the CA1 lacunosum-moleculare region of adult guinea-pig hippocampal slices by a modified enzymatic and mechanical treatment. Isolated interneurons were multipolar with several dendritic processes and thereby could be distinguished from CA1 pyramidal neurons. Outward potassium currents were recorded under whole-cell voltage-clamp when the cell was depolarized following a hyperpolarizing prepulse. The holding potential was -50 mV. Since the transient potassium current (i.e. A-current) and delayed potassium currents have significantly different properties, it is possible to activate them separately and examine them independently.

It was observed that interneurons could be divided into two groups based on whether or not they had the A-current. Approximately twice as many isolated interneurons had an A-current (N=9) than those that did not (N=4). These results suggest that there are two distinctive subpopulations of interneurons which may play different inhibitory roles in this region.

627.15

MEMBRANE POTENTIAL OSCILLATIONS IN IMMATURE RAT HIPPOCAMPAL CA3 NEURONS IN VITRO. C. Psarropoulou*[1] and M. Avoli[2], [1]Dept of Physiology & Biophysics, Sch. of Med., Sherbrooke Univ., Sherbrooke, QC, J1H 5N4 and [2]Dept of Neurology & Neurosurgery, MNI, McGill Univ., Montreal QC H3A 2B4, Canada.

Sharp-electrode intracellular recordings were made from 43 CA3 pyramidal neurons *in vitro*, in hippocampal slices obtained from Sprague-Dawley rat pups taken 2-17 days postnatally (P2-P17). Membrane potential oscillations (MPOs) were recorded at resting membrane potential (RMP) or following depolarization to levels more positive than - 60mV, in 31/43 neurons (72%). The RMP of oscillating neurons (-64.8 ± 1.25 mV, n = 31) did not differ from that of non-oscillating (-63.9 ± 1.38 mV, n = 12). MPO amplitude (3 - 5 mV measured peak to peak at -50 mV) as well as MPO frequency (ranging from 3-15 Hz, measured at -50 mV) increased upon depolarization. MPOs induced action potential firing, whose frequency increased with depolarization. The percentage of oscillating neurons increased with maturation but not significantly (P2-P10: 13/20 neurons, 65%; P11-P17: 18/22 neurons, 82%). No developmental changes in MPO amplitude or frequency were seen. Tetrodotoxin (TTX, 1μM) blocked MPOs in 4/4 slices (P10-P16). Tetraethylammonium (TEA, 5mM), added after TTX, revealed slower, higher amplitude MPOs (2/3 slices) that were subsequently blocked by cobalt (5 mM). Following IPSPs or afterhyperpolarizations, MPOs were transiently inhibited, an effect outlasting the return to RMP and not mimicked by negative current injection. The non-NMDA receptor antagonist CNQX (10 μM, n = 2) reduced substantially intrinsic MPO amplitude; adenosine (50-100 μM, n = 2) did not depress, but increased transiently during washout MPO amplitude.

These experiments indicate that intrinsic MPOs, possibly facilitating synchronization, are present in the majority of CA3 hippocampal pyramidal neurons from the early postnatal life. These MPOs are generated by the activation of Na-conductance(s) and possibly Ca-conductance(s) and are depressed by the activation of K-conductance(s); in addition their features are modified by synaptic activity.

627.16

THE IONIC MECHANISM OF A NONSELECTIVE CATION CONDUCTANCE INDUCED BY NEUROTENSIN IN THE VENTRAL TEGMENTAL AREA. P. Y. Chien[1*], R. H. Farkas[1], S. Nakajima[2] and Y. Nakajima[1] Dept. of Anat. and Cell Biol.[1] and Dept. of Pharmacol.[2] Univ. of Illinois, College of Med. at Chicago, IL, 60612.

Neurotensin (NT) excites rat ventral tegmental area (VTA) dopaminergic neurons in culture by inducing a nonselective conductance. NT also decreases the inwardly rectifying K^+ conductance induced by the D_2 agonist quinpirole. The I-V relation of the NT-induced current had almost a zero slope between -60 mV and -140 mV. After decreasing the external Ca^{++} concentration, the NT-induced current increased by ≈4 times and had a positive slope-conductance. This result suggests that in the presence of $[Ca^{++}]_o$, the channel is blocked by Ca^{++}. When K^+_i and K^+_o are replaced by Cs^+, and E_{Cs}= -68 mV and E_{Na}= +13 mV, the nonselective conductance reverses at -7 ± 2 mV, between E_{Cs} and E_{Na}. When the internal Cl^- concentration was reduced, shifting E_{Cl} from near 0 mV to about -80 mV, the nonselective current still reversed at -7 ± 2 mV, suggesting that the nonselective conductance was impermeable to Cl^-, and that it was probably a nonselective cation conductance. Neurokinin B (NKB) and ACPD also induce inward currents in VTA neurons. The I-V relations of the NKB- and ACPD-induced currents had almost a zero slope between -60 mV and -140 mV, similar to the NT-induced current. Some of the NKB- and ACPD-responding cells were found to be dopaminergic based on tyrosine-hydroxylase immunoreactivity.

627.17

WHOLE-CELL AND SINGLE-CHANNEL PROPERTIES OF A LINEAR MEMBRANE CONDUCTANCE IN CAT RGCS. D.W. Robinson*, S-J. Huang, R.P. Scobey and L.M. Chalupa. Dept. of Neurology, Section of Neurobiology, Physiology and Behavior and Center for Neuroscience, University of California, Davis, CA95616.

Whole-cell recordings from isolated cat retinal ganglion cells (RGCs) revealed the presence of a previously unreported linear current, which increased with ontogeny to a mean conductance of 0.27nS/pF at postnatal ages (Robinson et al., 1993). Changing the external Cl^- concentration had no measureable effect on the reversal potential implicating K^+ as the major permeant ion. When the external K^+ concentration was increased from 5 to 35 mM the reversal potential changed from -54 to -18 mV. This change was close to the expected value of 34.5 mV calculated from the GHK voltage equation, assuming a 4% permeability to Na^+. We also used the cell-attached and ripped-off patch variations of the patch-clamp technique to examine the single-channel basis of this linear whole-cell conductance. From -100 to +100 mV little or no voltage-dependency was observed in the isolated single-channel conductance. Changing K^+ concentrations on both sides of the patch resulted in changes in reversal potential similar to those observed in whole-cell. This potassium channel could therefore play an important role in setting the resting membrane potential in these neurons.

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627.18

TTX-RESISTANT PERSISTENT NA^+ CURRENT UNDERLIES PACEMAKER POTENTIALS OF FISH GONADOTROPIN-RELEASING HORMONE (GNRH) NEURONS. **Y. Oka*. Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, Japan**

Gonadotropin-releasing hormone (GnRH)-immunoreactive terminal nerve (TN) cells show endogenous regular beating discharges, which may be related to their putative neuromodulator functions. The ionic mechanism underlying the pacemaker potentials (PPs) was studied using intracellular and patch-pipette current clamp recordings from whole brain *in vitro* preparation of a small fish brain. Addition of 1.5-3 μM TTX to the Krebs-Ringer solution blocked spontaneous action potentials, but small regular PPs remained. The PPs were not affected by 1 mM amiloride or 1 mM Ni^{2+} (blockers of low-voltage-activated Ca^{2+} currents), or 2 mM Co^{2+} or 0.5 mM Cd^{2+} (blockers of high-voltage-activated Ca^{2+} currents), or in Ca^{2+}-free solution. Thus, Ca^{2+} currents are not essential for the generation of PPs. On the contrary, the PPs were readily blocked by substituting tetramethylammonium (TMA) or choline for Na^+ in the perfusing solution, and the resting membrane potential became more hyperpolarized than the control level. This is probably because of the block of persistent inward current that is carried by Na^+ and supplies the depolarizing drive during the normal beating discharge mode. The present results suggest that the TTX-resistant persistent Na^+ current, $I_{Na(slow)}$, plays an important role in the generation of PPs in TN-GnRH cells.

628.1

EFFECT OF HYPOTHERMIA AND EXTRACELLULAR pH ON CORTICAL NMDA RECEPTOR ACTIVITY. A.T. Gray, L.T. Buck, J.R. Feiner, B. Hansen and P.E. Bickler*. Dept. of Anesthesia, University of California at San Francisco, S.F., CA 94143-0542

Extracellular acidity is known to inhibit N-methyl-D-aspartate (NMDA) receptor activity and may therefore protect neurons during cerebral ischemia. However, the importance of this effect during induced hypothermia is not clear. Cortical slices from rats age 10 to 11 days were loaded with fura-2 to estimate cytosolic free calcium. Baseline intracellular calcium was not different between the normothermic (37°C) and hypothermic (17°C) groups (142 ± 18 vs. 102 ± 12 nM, respectively; mean ± S.E.M., n=8 in each group). Baseline ATP levels were 61 ± 11 nmoles/mg at 37°C (n=4). Hypothermia reduced NMDA stimulated calcium influx (156 ± 25 vs. 41 ± 6 nM, respectively, n=8 in each group). Extracellular acidity at 37°C decreased NMDA stimulated calcium influx 86% over the in vivo pHe range of 6.9 to 7.4. However, no significant effect of extracellular pH on NMDA stimulated calcium influx was seen at 17°C (in vivo pHe range 7.3 to 7.8). We conclude that the effect of extracellular pH on NMDA receptor activity depends on temperature. Further studies are needed to estimate these effects over similar in vivo pH ranges.

Supported by the UCSF Anesthesia Research Foundation.

628.2

EFFECT OF ACIDIC pH ON EXCITATORY AMINO ACID-STIMULATED PHOSPHATIDYLINOSITOL METABOLISM IN NEURONS. D.L. Yourick* and M.A. DeCoster. Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, DC 20307-5100.

During hypoxic brain injury, extracellular pH may drop to as low as 6.4. Most studies to date evaluating neuroprotection do so at physiological pH, 7.4. Little is understood about the role of [H⁺] on promotion/reduction of neuronal death itself. The role of excitatory amino acid (EAA)-induced phosphatidylinositol (PI) metabolism in neuronal injury is yet to be established, but, at least in young animals, agonists that specifically stimulate the metabotropic receptor can cause neuronal injury. In the present study, we investigated the effect of reduced or acidic pH on basal and EAA-stimulated PI metabolism in neuronal cultures. Basal and glutamate -stimulated PI metabolism were decreased at all concentrations tested (10-100 μM), with maximal inhibition detected at 100 μM, when pH was reduced from 7.4 to 6.4. Considering the multiple EAA receptor types at which glutamate acts, we expanded these studies to include the EAA receptor agonists NMDA and trans-ACPD, as well as the non-EAA receptor agonists norepinephrine and carbachol to stimulate PI metabolism. The rank ordered potencies of agonists stimulating PI metabolism (pH 7.4) were: glutamate ≈ trans-ACPD > norepinephrine ≈ carbachol >> NMDA. While decreasing the pH to 6.4 reduced both 100 μM glutamate and trans-ACPD stimulation by approximately 25%, stimulation by NMDA was reduced by 77%. Preliminary results indicate that PI metabolism at pH 6.4 is slightly reduced for the non-EAA agonist norepinephrine while unaffected for carbachol. Our results indicate that PI metabolism, via direct (trans-ACPD) or indirect (NMDA) metabotropic receptor activation, is decreased by acidosis and are consistent with the known effects of pH on NMDA receptor function. Acidic pH effects on multiple EAA receptor types may be beneficial to neuronal outcome subsequent to CNS trauma.

628.3

VISUAL TOXICITY OF ETHAMBUTOL IS MEDIATED THROUGH THE NMDA RECEPTOR OF MAMMALIAN RETINAL GANGLION CELLS. J. E. Heng*, K. Moscaritolo, & E. B. Dreyer. Harvard Medical School, Dept. of Ophthalmology, MEEI, Dept. of Neurology, Children's Hospital, Boston, MA, 02115.

Ethambutol is a mainstay in the management of tuberculosis and other mycobacterial infections. Although its ability to cause optic neuropathy and visual loss is well known, a mechanism has not heretofore been established. We have established that ethambutol is directly toxic to retinal ganglion cells *in vitro*, and that this toxicity is mediated through the NMDA receptor. In cell culture studies we have shown that the addition of 100 μM ethambutol to retinal cultures results in the death of 50% of retinal ganglion cells within 24 hours. Ethambutol mediated retinal ganglion cell death is dose-dependent. Other cell types in these preparations are unaffected. This toxicity can be prevented in several ways: (1) the elimination of endogenous glutamate; (2) the addition of the NMDA antagonists APV (2-amino-5-phosphonovalerate), MK-801 (dizocilpine), or the calcium channel blocker nimodipine. In addition, the non-NMDA antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) did not reduce toxicity.

Taken together, these experiments suggest: (1) ethambutol is directly toxic to retinal ganglion cells in culture; (2) ethambutol increases the sensitivity of retinal ganglion cells to endogenous levels of glutamate; (3) ethambutol toxicity is mediated through the NMDA receptor. These findings suggest that visual loss due to prolonged ethambutol treatment for tuberculosis may be prevented by the use of selective NMDA antagonists.

628.4

Cyclic-AMP ANALOGUES PRODUCE EXCITOTOXICITY. J.W. McDonald* and D.D. Schoepp. Dept. Neurology, Washington Univ. Sch. Med., St. Louis, MO 63108 and Lilly Res. Labs, Indianapolis, IN 46285.

The pathologic role of cAMP in excitotoxic brain injury was assessed in a perinatal rat model. Stereotaxic intrastriatal injections of 8-bromo-cAMP or dibutryl-cAMP (600-1200 nmol, 0.5 ul), but not cGMP analogues or vehicle, produced dose-dependent seizures and brain injury in PND 7 rats. The severity of brain injury was assessed histologically and by hemispheric weight disparities 5 days following injection (McDonald et al., 1989). Co-treatment with the NMDA antagonist MK-801 (2.5 mg/kg i.p.) abolished cAMP induced seizures and injury. Intrastriatal injections of cAMP in adult rats (600 nmol) produced "wet dog shake" like limbic seizures and a pattern of local and distant injury that was prevented by MK-801 (2.5 mg/kg,i.p.). Co-injection of cAMP (600 nmol) selectively potentiated 1S,3R-ACPD (1000 nmol), but not NMDA (2 nmol) or AMPA (2 nmol) induced injury in PND 7 rats [%damage, mean±SEM (unpaired t-test, n=8/group), cAMP 0.7±0.4%, 1S,3R-ACPD 2.0±0.5% vs cAMP+1S,3R-ACPD 5.4±0.4% ($p<0.001$), NMDA 1.1±0.3% vs cAMP+NMDA 1.9±0.4% (ns), AMPA 0.8±0.4% vs cAMP+AMPA 2.0±0.3% (ns)]. The results suggest that activation of the cAMP cascade may selectively potentiate metabotropic mediated brain injury and that cAMP injury is blocked by NMDA antagonists.

628.5

SUBLETHAL NMDA RECEPTOR ACTIVATION RAPIDLY DISRUPTS MICROTUBULES IN CULTURED NEURONS. M.P. Goldberg*, J.K. Freeman, and A.M. Rosengarten. Center for the Study of Nervous System Injury, Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Brief deprivation of oxygen and glucose, or direct glutamate receptor activation, causes focal swelling in dendrites of cultured cortical neurons (Soc. Neurosci. Abstr. *18*: 1583, 1992, *19*: 26, 1993). We examined NMDA-induced alterations in the neuronal cytoskeleton, using immunofluorescence and laser scanning confocal microscopy. In control cultures, antibodies to the microtubule-associated protein, MAP2, revealed diffuse immunoreactivity within neuronal somata, and smooth dendritic contours. Exposure to 10-50 μM NMDA for 10 min resulted in little neuronal death, but was sufficient to cause extensive appearance of focal swelling, or varicosities, in MAP2-labeled dendrites of most neurons. Dendritic varicosities were apparent within 3 min of NMDA exposure, and MAP2 fluorescence was substantially diminished within 1 hr. At low NMDA concentrations (10-30 μM x 10 min), these changes were reversible within several hours. Confocal microscopy with monoclonal antibodies to α-tubulin revealed distinct microtubules arranged circumferentially in neuronal cell bodies, and in longitudinal bundles in axons and dendrites. Immediately after 10 min NMDA exposure, this pattern was replaced with diffuse immunofluorescence uniformly filling the dendritic and somatic cytoplasm. Glial microtubules were not altered. Loss of distinct microtubules was most apparent with antibodies specific for the tyrosine (dynamic) form of α-tubulin (YL1/2, Serotec; TUB1A2, Sigma). NMDA-induced varicosity formation, and loss of microtubule labeling, were blocked by pretreatment with the microtubule-stabilizing drug, taxol. Rapid disruption of dendritic microtubules may underlie early alterations in neuronal structure in cerebral ischemia, and may permit lasting changes in neuronal signaling after prolonged synaptic activation.

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628.6

EXCITOTOXIC INTERACTION BETWEEN ACPD AND NMDA AFTER INTRAHIPPOCAMPAL ADMINISTRATION. J.P. Kesslak*, A. Theis, C.W. Cotman. Departments of Neurology, Psychobiology and I.R.U. in Brain Aging, University of California, Irvine, CA 92717.

Glutamate activates two broad categories of receptor subtypes, ionotropic (i.e., selective agonists AMPA, Kainate, or NMDA) and metabotropic (mGluRs). Maintaining the balance between plasticity and pathology may involve receptor subtype interactions *Trans*-aminocyclopentane-1,3-dicarboxylic acid (ACPD), a selective mGluR agonist, can attenuate NMDA neurotoxicity (Koh et al., 1991) in cortical cultures. Although other neuroprotective actions of ACPD have been subsequently demonstrated both *in vitro* and *in vivo*, recent studies indicate neuropathological effects of mGluR activation with fairly high doses of ACPD. In the present study we examine the interaction between NMDA and ACPD to determine *in vivo* the neuroprotective or neuropathological effects of mGluR activation.

Fifteen adult male Sprague Dawley rats received intrahippocampal injections of 10 mM ACPD+100 mM NMDA, and in the contralateral side 10 mM ACPD or 100 mM NMDA. At 14 days after the injections, ACPD resulted in minor damage in the dorsal blade of the dentate gyrus, NMDA alone did not produce significant neural damage. The combination of ACPD and NMDA produced significant damage to CA3 and also involved the ventral blade of the dentate. Excitotoxic actions of the ACPD+NMDA may indirectly modify uptake or directly effect receptors. The potential neuroprotective and toxic activity of mGluRs may be an important factor in normal CNS development, disease related pathology and provide insights into the development of therapeutic interventions.

628.7

NMDA TOXICITY IN DEVELOPING CEREBELLAR GRANULE CELLS MEDIATED BY A MAGNESIUM-INSENSITIVE NMDA RECEPTOR. Y. Xia*, K.N. Kumar, R. Ragan, M. Michaelis, and E. Michaelis, Pharmacol. & Toxicol. Dept., Univ. of Kansas, Lawrence, KS.

NMDA receptors play an important role in glutamate-induced neurotoxicity and are related to neurodegenerative diseases. Cerebellar granule cells in culture were used as a model system to study the NMDA-mediated neurotoxicity. Rat cerebellar granule cells were grown under depolarizing conditions (25 mM KCl) and the medium was not changed except for the addition of dH_2O to compensate for evaporation. Cells were treated with NMDA that was directly added to the culture medium in the presence of 0.8 mM Mg^{++}. Cell death was monitored by both the LDH and the MTT assay. The dose response curve of NMDA-induced toxicity showed that the toxicity was increased through DIV 8 to DIV 14. This increased toxicity corresponded to the increased expression of the 63-70 kDa glutamate-binding protein as determined by Western blots. The NMDA (100 μM)-induced toxicity at DIV 14 was blocked by AP-5 (500 μM) and MK801 (10 μM) and it was not sensitive to CNQX (25 μM). This indicates that the NMDA-induced neurotoxicity is mediated by NMDA receptors rather than non-NMDA receptors which might have been activated as a result of NMDA-induced glutamate release. The NMDA-activated increase in $[Ca^{++}]_i$ measured by Fura-2 in the condition favoring the Mg^{++}-sensitive NMDA receptors (5 mM KCl, 10 μM glycine, and Mg^{++}-free) did not show the developmental increase through DIV 5 to DIV 14 and did not correspond to the developmental pattern of NMDA-induced toxicity. These results may suggest a distinct role of Mg^{++}-insensitive NMDA receptors in NMDA toxicity of cerebellar granule cells. [Supported by a Marion Merrell Dow Fellowship and a Grant].

628.8

ASSESSMENT OF MEMANTINE AS A NEUROPROTECTIVE DRUG IN RATS POISONED WITH SOMAN. S.S. Deshpande, C.D. Smith, J.S. Forster, S. Phann, R.E. Sheridan* and M.G. Filbert. Neurotoxicology Branch, Pathophysiology Division, USAMRICD, APG, MD 21010-5425.

Memantine a potent noncompetitive antagonist at N-methyl D-aspartate activated receptor channels(Eu. J. Pharmacol., **166**, 591, 1989) and has been shown to protect embryonic cortical neurones against glutamate-induced cell death (Eu. J. Pharmacol., **198**, 215, 1991). Pretreatment of rats with memantine also has been shown to prevent seizures after soman (an irreversible cholinesterase inhibitor) injection (Toxicol. Appl. Pharmacol., **112**, 95, 1992). The purpose of this study was to examine efficacy of memantine as a pretreatment drug in protecting rats from seizure-induced neuronal damage after soman. Adult male Sprague Dawley rats received pyridostigmine (0.13 mg/kg i.m.) and atropine methyl nitrate (5 mg/kg s.c.) 30 and 5 min respectively before a single injection of soman (0.1 mg/kg s.c. equivalent to a 0.9 LD_{50} dose). Memantine (18 mg/kg s.c.) was administered 60 min prior to soman. At 24 hr rats were anesthetized with sodium pentobarbital (65 mg/kg i.p) and transcardially perfused with buffered 10% formalin. Neuropathology of various areas of brain was examined in H&E stained coronal sections. Approximately 64% of rats receiving soman alone showed severe seizure activity and died within 24 hr. Pretreatment of rats with memantine reduced the severity of seizures and provided 100% protection from lethality. The brains of surviving rats in soman alone group showed necrotic lesions in frontal cortex, piriform cortex and hippocampus. Although memantine reduced the intensity of seizures in 66% of the pretreated rats, the drug could not protect the rats from seizure-induced neuronal degeneration in the brain areas described above. It is likely that repeated administration of memantine is needed to maintain the blockade of channels activated by excitatory amino acids in soman poisoning.

628.9

REDUCTION OF NMDA-INDUCED EXCITOTOXICITY IN CEREBELLAR GRANULE CELL CULTURES BY ANTISENSE OLIGONUCLEOTIDES FOR NMDAε SUBUNITS. M.Didier*, S.A. Berman and S.Bursztajn, Lab. for Molecular Neuroscience, McLean Hosp/Harvard Med. Sch. Belmont, MA 02178.

The recent molecular cloning of several NMDAε subunits demonstrated the heterogeneity of NMDA receptor localizations and properties in the central nervous system. Using antisense oligonucleotides (AONs), we investigated the involvement of the NMDAζ1 and the four NMDAε subunits in the formation of NMDA receptors which mediate excitotoxicity. Cerebellar granule cells displayed mRNAs for these subunits after 10 days in culture as revealed by RT-PCR. Preliminary experiments showed that a maximum amount of stable ONs was incorporated inside the cells 24-36 hr after their addition to the culture medium. Treatment of neuronal cultures with NMDAζ1 AONs decreased dramatically the level of NMDAζ1 protein and reduced by 60% NMDA-induced excitotoxicity. We found that AONs but not sense ONs, for NMDAε 1-2 and 3 protected also neurons from NMDA excitotoxicity when 40 to 60% of cells were damaged by NMDA. However, only AONs for NMDAε 1 and 3 displayed some protective effect when the NMDA-dependent neuronal death exceeded 70%. AONs against NMDAε 4 were much less efficient in rescuing neuronal cells whatever the level of excitotoxicity. The protective effects obtained with AONs for NMDA receptor subunits were not additive. The reduction of neuronal cell death after AON treatments was correlated with a decrease in the NMDA-stimulated calcium influx. Finally, NMDA AON treatment did not affect the toxicity induced either by activation of AMPA glutamate receptors or by MPP+. These results suggest that AONs for NMDAε subunits can be used to knockout the pathological roles of specific NMDA receptor subpopulations in neuronal cells.

628.10

MITOCHONDRIA SEQUESTER NMDA RECEPTOR-MEDIATED CA^{2+} LOADS. G. J. Wang*, S. A. Thayer, Dept. of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455.

Glutamate induces an intracellular acidification in cultured hippocampal neurons that results from sequestration of NMDA receptor-mediated Ca^{2+} loads by mitochondria with subsequent acceleration of cellular metabolism. Consistent with this hypothesis, pretreatment of hippocampal neurons with the mitochondrial uncoupling agent FCCP (1 μM, 5 min), significantly increased the amplitude of glutamate-induced $[Ca^{2+}]_i$ transients as measured by indo-1 based microfluorimetry. Removal of extracellular Na^+ (Na^+_o) for 20 min prior to exposure to NMDA (200 μM, 3 min), presumably reducing intracellular Na^+(Na^+_i), revealed a prolonged plateau phase in the recovery of the $[Ca^{2+}]_i$ transient which resembled the mitochondrion-mediated $[Ca^{2+}]_i$ plateau previously observed in sensory neurons. Mitochondria release Ca^{2+} via Na^+/Ca^{2+} exchange; thus, a reduction in Na^+_i will slow Ca^{2+} extrusion across the inner membrane consequently increasing the retention of $[Ca^{2+}]_i$ within mitochondria. Readdition of Na^+_o during recovery of NMDA-induced $[Ca^{2+}]_i$ transients increased the height and shortened the duration of the plateau phase. Application of FCCP during the plateau elicited a large $[Ca^{2+}]_i$ increase in the absence of extracellular Ca^{2+}. When Na^+ was maintained throughout the experiment the recovery lacked a plateau phase and FCCP failed to increase $[Ca^{2+}]_i$. These data indicate that mitochondria sequester NMDA receptor-mediated Ca^{2+} loads and that an accompanying Na^+ load will have profound effects on the accumulation and retention of Ca^{2+} by mitochondria.

628.11

TOXIC DOPAMINE-GLUTAMATE INTERACTIONS IN CULTURED NEURONS: EVIDENCE FOR OXIDATIVE STRESS. K.R. Hoyt*, I.J. Reynolds and T. G. Hastings. Depts. of Pharmacology and Neuroscience, Univ. of Pittsburgh , Pittsburgh, PA 15261.

Dopamine (DA) and glutamate (Glu) are neurotransmitters important for normal brain function. However, these neurotransmitters can cause neuronal damage when their extracellular concentrations are elevated, in pathological conditions such as cerebral ischemia and methamphetamine toxicity, and possibly in neurodegenerative conditions such as Parkinson's disease. We have found, using the fluorescent dyes CMFDA and monochlorobimane to measure intracellular glutathione, and dichlorofluorescin to measure intracellular oxidation, evidence for DA and Glu-induced oxidative stress. We also investigated the interaction between DA and Glu on cell death in cortical neurons cultured for 14-17 days. We used concentrations and exposure times of DA and Glu that were themselves not toxic, and we found in that in combination they caused a significant loss of cell viability measured by retention of calcein-AM. Cells treated with 250 uM DA for 2 h or cells treated with 100 uM Glu/1uM glycine for 5 min showed 4% loss of viability compared to untreated controls, when measured 6 h after treatment. When these two treatments were combined, there was a 30% loss of viability ($p < 0.05$). In another experiment, 100 uM DA for 1 h or 100 uM Glu/1uM glycine for 2 min resulted in a 4% loss of viability when measured 20 h after treatment. Combining these two treatments resulted in a 36% loss of viability ($p < 0.05$). We are currently investigating the mechanisms underlying these toxic interactions. Supported by MH18273 and MH45156.

628.12

STUDIES ON THE ROLE OF MITOCHONDRIA IN NMDA RECEPTOR-MEDIATED EXCITOTOXICITY. I.J. Reynolds*, T.G. Hastings and K.R. Hoyt Depts Pharmacol. and Neurosci., U. Pittsburgh, Pittsburgh PA 15261.

The intracellular events that link intense NMDA receptor activation to neuronal death are not clear, although Ca^{2+} influx is essential. This series of studies in neurons cultured from fetal rat forebrain sought to identify cellular events that are correlated with neuronal death in order to characterize the lethal stimulus.

We recently reported that the fluorescent dye, 2',7'-dichlorodihydrofluorescein can be used to monitor the production of reactive oxygen species (ROS) induced by glutamate (Soc. Neuro. 19:1350, 1993). This process requires NMDA receptor activation and Ca^{2+} entry. As mitochondria are an important source of ROS we investigated a possible role for this organelle using the mitochondrial proton ionophore FCCP. FCCP (5μM) completely blocked glutamate (100μM)-induced ROS generation. As FCCP potentiates glutamate-induced $[Ca^{2+}]_i$ changes these results suggest that elevation of $[Ca^{2+}]_i$ may not be sufficient for ROS production, and are consistent with the hypothesis that Ca^{2+} uptake by mitochondria may be the critical event that ultimately results in oxidative stress.

We also monitored the effects of glutamate receptor activation on mitochondrial membrane potential using rhodamine 123 (R123). Addition of FCCP (5μM) rapidly and completely disrupts the characteristic punctate staining normally seen with R123 using confocal microscopy. The addition of glutamate (100μM) also altered R123 staining, although to a lesser degree than FCCP. This effect was mimicked by NMDA (500μM) but not kainate (100μM). The effect of glutamate was also dependent on Ca^{2+} entry, but was not reduced by Na^+ removal.

These studies suggest that neurotoxic stimulation of NMDA receptors results in an alteration of mitochondrial function which may contribute to cell death.

628.13

GLUTAMATERGIC RECEPTOR ACTIVATION AND EXCITATORY AMINO ACID RELEASE IN METABOLIC INHIBITION. G.S. Neal, W.J. Nicklas* and G. D. Zeevalk. Dept. of Neurology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

It has been suggested that ischemia-induced CNS damage is mediated by prolonged activation of glutamatergic receptors (excitotoxicity) which may result in whole or in part from increased extracellular glutamate ($_{ec}$GLU). In these studies transverse hippocampal slices from male rats were used to investigate the effects of chemically-induced hypoglycemia (IOA-iodoacetate) or hypoxia (KCN) on temporal GLU release and associated neuronal damage mediated by glutamatergic receptor (GluR) activation. Treatment with either drug for 30 min increased extracellular aspartate (ASP) and GLU and produced lesions in the CA1, CA3, CA4 and dentate gyrus (DG) regions which were attenuated by NMDA and non-NMDA antagonists. A more severe lesion was observed in slices treated for 30 min with both IOA and KCN; this was completely attenuated by combining non-NMDA and NMDA antagonists. To investigate the temporal release of GLU and activation of GluRs, slices were treated with KCN for 2.5 to 30 min. Within 2.5 min discrete CA1 neuronal swelling was seen which coincided with decreases in tissue ATP levels. These CA1 lesions were not accompanied by increases in extracellular GLU or ASP but were completely prevented by NMDA antagonists. Within 10 min there were increases in $_{ec}$GLU which coincided with extensive lesions in the CA1, CA4 and DG. Within 20 min, all regions of the hippocampus appeared severely damaged and $_{ec}$GLU was twice that of control slices. This data supports our previous studies which suggested that excitotoxicity associated with mild metabolic stress was caused by changes in the physiological state of the NMDA receptor following compromise of energy stores rather than by increased extracellular GLU or ASP. More prolonged or more severe metabolic inhibition precipitates involvement of nonNMDA receptors perhaps mediatedby increased $_{ec}$GLU.

628.14

OPEN-CHANNEL BLOCKADE OF NMDA-ACTIVATED WHOLE-CELL CURRENTS BY 9-AMINOACRIDINES. M.E. Nelson[1], R.F. Bulleit[1]*, and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD, USA 21201; [2]Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ, Brazil 21944.

In previous studies using the single-channel patch-clamp technique we have demonstrated that the bis-9-aminoacridines are potent open-channel blockers of NMDA-activated currents in cultured rat hippocampal neurons (Nelson and Albuquerque, *Mol. Pharmacol., in press*). Based on the acridine-induced reduction in single-channel open times at -80 mV, the forward blocking rate constants were found to be $1.1x10^8$ $M^{-1}s^{-1}$ and $1.4x10^8$ $M^{-1}s^{-1}$ for 1,2-propane-bis-9,9′-aminoacridine (1,2-PAA) and 1,4-butane-9,9′-aminoacridine (1,4-BAA), respectively, compared to $3.5x10^7$ $M^{-1}s^{-1}$ for 1,2,3,4-tetrahydro-9-aminoacridine (THA). However, the affinities of the bis-acridines could not be easily quantitated from the single channel data because the blocked state produced by these compounds was long in duration (in the range of 200 ms) and similar to normal channel closed times. To circumvent this problem, experiments using whole-cell patch-clamp were performed with a motorized, multi-barrel, fast perfusion system to study the unbinding kinetics of these compounds. In nominally Mg^{2+}-free solutions, the recovery from blockade by THA indicated that the dissociation was from a single blocked state ($\tau_{recover}$ = 48.7±0.9 ms, n=3), but the single-channel experiments clearly showed that a much briefer THA-blocked state (duration < 200 μs) exists that would not be detected by this means of drug application in the whole-cell experiments. The recoveries from blockade by the bis-acridines, on the other hand, were slower and were normally best described by a single-exponential function having time constants ($\tau_{recover}$) of 217.6±14.7 ms (n=5), and 167.1±8.9 ms (n=11) for 1,2-PAA and 1,4-BAA, respectively. The single-channel studies mentioned above also revealed that the interaction of the 9-aminoacridines with the channel of the receptor were at a site that is different from the Mg^{2+} binding site of the channel and suggested that the channel could be simultaneously occupied by Mg^{2+} and the 9-aminoacridines. This simultaneous occupation likely modifies the affinity of the receptor for the 9-aminoacridines. Indeed, preliminary results in the presence of 100 μM Mg^{2+} showed that the rates of association and dissociation of 1,2-PAA were increased. Support: USPHS Grant ES 05730.

628.15

NEUROPROTECTIVE EFFECTS OF SIGMA LIGANDS: INDEPENDENCE FROM PCP-RECEPTOR RELATED NMDA RECEPTOR ANTAGONISM. J.B. Long*, D.L. Yourick, M.A. DeCoster, and M.L. Koenig. Dept. of Med. Neurosci., Div. of Neuropsych., Walter Reed Army Inst. of Res., Wash., D.C. 20307.

Several σ ligands have been shown to protect against neuronal ischemic injury in vivo. We have used primary cultures of rat spinal cord neurons to evaluate the protective effects of a variety of selective σ ligands in comparison with PCP-related noncompetitive NMDA receptor antagonists during either hypoxia or brief exposure to excitotoxic (100 μM) concentrations of NMDA or kainic acid (KA). In addition, the effects of these compounds on NMDA-induced changes in intraneuronal calcium concentration ($[Ca^{2+}]_i$) and phosphatidylinositol (PI) metabolism were compared. After 8-10 days in culture, neurons were subjected to hypoxia or brief excitatory amino acid exposure in Locke's solution from which $MgCl_2$ and glucose were omitted. Cell damage was quantitatively assessed on the following day using a tetrazolium salt colorimetric assay. NMDA-induced changes in $[Ca^{2+}]_i$ were measured in single identified neurons (5-20/150 μm^2 field) preloaded with the Ca^{2+}-sensitive dye indo-1 using an ACAS 570C laser cytometer. The PCP receptor ligands MK-801, dextrorphan, and ketamine, and a variety of σ ligands, including DTG, (+)-pentazocine, and caramiphen, caused significant, dose-dependent protection against hypoxia and NMDA- but not KA-induced cell injury. In contrast to the PCP receptor ligands, at neuroprotective concentrations the σ ligands failed to antagonize the sustained elevations in $[Ca^{2+}]_i$ elicited by NMDA and failed to consistently alter NMDA-stimulated PI hydrolysis (200-300% above control). These results indicate that, through mechanisms distinguishable from PCP-receptor related antagonism of the NMDA receptor, σ ligands provide an effective means of preventing excitotoxic neuronal injury.

628.16

INTERACTION OF (±)ß-METHYL-α,ß-DIAMINOPROPIONIC ACID (BMAA) WITH THE STRYCHNINE-INSENSITIVE GLYCINE SITE ON THE NMDA RECEPTOR. I. OMELCHENKO AND C. N. ALLEN*. Cntr. Res. Occup. Environ. Toxicol., Ore. Hlth. Sci. Univ., Portland, OR. 97201.

BMAA is a neurotoxic monocarboxylic amino acid which in the presence of bicarbonate is an agonist at non-NMDA-type receptors. However, BMAA has also been reported have actions mediated by NMDA-type receptors. We used whole cell voltage clamp techniques on cultured hippocampal neurons to determine whether of BMAA was an agonist at the strychinine-insensitive glycine receptor. Neither L-BMAA or (±)BMAA in the presence of extracellular calcium produced membrane currents. (±)BMAA (EC_{50} = 300 μM) but not L-BMAA coapplied with NMDA produced a reversible potentiation of the NMDA (50 μM) activated currents. Currents activated by a combination of NMDA and (±)BMAA showed desensitized in a majority of neurons. (±)BMAA increased the amplitude of NMDA-activated currents but did not change their voltage dependence. The potentiation of NMDA currents by (±)BMAA and glycine were antagonized by 7-chlorokynurenic acid. Concentration jump experiments were performed to determine the kinetics of the unbinding of the BMAA. The time course of BMAA unbinding was 350±60 ms while that of glycine was 940±100 ms suggesting that the BMAA binds with a lower affinity than glycine to the strychnine-insensitive glycine site. These data suggest that (+)BMAA is capable of binding to the strychnine insensitive glycine binding site and acting as a coagonist with NMDA to activate the NMDA receptor. This work was supported by NS 19611.

628.17

ARACHIDONIC ACID METABOLITES INDUCE HSP70 IN CINGULATE CORTEX IN MK801 TOXICITY. J. Chen, J. Q. Lan, F. R. Sharp, S. H. Graham* Neurology, VAMC and University of California, San Francisco.

NMDA antagonists such as MK801 and phencyclidine injure a discrete populations of neurons in the cingulate and retrosplenial cortex. These agents produce cytoplasmic vacuoles and induce production of the stress protein HSP70. Cyclopentane arachidonic acid (AA) metabolites such as PgA2 and hepoxilin A2 have been proposed to be intracellular messengers that induce HSP70 expression in tumor cells. Accordingly, we hypothesized that arachidonic acid metabolites may signal HSP70 expression due to MK801 toxicity. MK801 1mg/kg was injected i.p. in awake rats. HSP70 mRNA transcription was studied using in situ hybridization with 35-S-oligonucleotides at 8 hrs. HSP70 protein expression was studied by immunocytochemistry at 24hrs after MK801 injection. The cyclooxygenase (COX) inhibitor indomethicin, the mixed COX/lipoxygenase inhibitor BW755C and the phospholipase inhibitor mepacrine all inhibited both HSP70 mRNA and protein expression in a dose dependent manner at 5, 10, 20, 30 and 50mg/kg i.p given 15 min prior to MK801 injection. Furthermore, the mRNA encoding the mitogen inducible cyclooxygenase (COXII) was induced in cingulate and retrosplenial cortex in regions where HSP70 was also expressed 4 hrs after MK801 injection. These data support a role for AA metabolites in the expression of HSP70 in MK801 toxicity. Further experiments will be required to determine if AA metabolism inhibitors block MK801-induced neuronal injury or if their effects are limited to HSP70 induction.

628.18

NEURON NECROTIZING PROPERTIES OF PHENCYCLIDINE TD Corso*, DF Wozniak, MA Sesma and JW Olney. Washington Univ., St. Louis MO, 63110.

Antagonists of NMDA glutamate receptors, including phencyclidine (PCP) and MK-801, acutely injure neurons and induce expression of 72 Kd heat shock protein (HSP) in the posterior cingulate/retrosplenial (PC/RS) cortices when administered sc or ip to adult rats. Sharp et al. reported that a high dose of PCP (50 mg/kg ip) causes a robust HSP response not only in PC/RS neurons but in many additional neurons in several neocortical and limbic brain regions. They proposed that HSP serves a protective function and that the robustness with which an injured neuron expresses HSP may be a measure of its ability to survive the injury. Thus, the widespread injury induced by high-dose PCP may be entirely reversible. In contrast, it is known that a high dose of MK-801 (5 mg/kg ip) kills PC/RS neurons. Silver stains have been used to detect neuronal injury induced by PCP or MK-801, but it is not known what silver staining of neurons signifies regarding cell injury vs cell death. To address these questions we treated adult female rats with a single high dose of PCP (50 mg/kg ip) and examined the brains at various posttreatment intervals (4 hrs to 4 days) using H&E paraffin, de Olmos silver or HSP immunocyto-chemical methods. We found that high dose PCP induced HSP in PC/RS neurons in layers II to V and that it killed many neurons distributed in these same layers. Occasionally, but not consistently, it induced HSP and killed neurons in other neocortical and limbic brain regions. Our findings help to validate the de Olmos silver stain for studying PCP-induced neuronal necrosis in that in each brain only those neuronal populations showing definite signs of necrosis (intense eosinophilia) in H&E sections were impregnated by the de Olmos stain. Moreover, the de Olmos stain may be a selective marker of cell death in that PC/RS neurons did not become argyrophilic until approximately 36 hrs after PCP treatment, but PCP is well known to physically injure neurons at a much earlier interval (4-12 hrs). Our findings do not confirm the view that HSP expression by PCP-injured neurons signifies recoverability. Supported by AA 07466, AG 05681, DA 05072 and RSA MH 38894 (JWO).

628.19

RATS BECOME HYPOSENSITIVE TO MK-801 NEUROTOXICITY DURING PREGNANCY. NB Farber* and JW Olney Washington Univ., St. Louis MO, 63110.

Antagonists of NMDA glutamate receptors, including phencyclidine (PCP) and MK-801, cause vacuolar injury (low dose) or kill (high dose) neurons in the posterior cingulate/retrosplenial (PC/RS) cortices when administered sc or ip to adult rats. Non-pregnant female rats are substantially more sensitive than male rats to this neurotoxic action. We now report that female rats become hyposensitive to NMDA antagonist neurotoxicity (NAN) during pregnancy. A high dose of MK-801 (5 mg/kg sc) in E17-E19 pregnant dams (n = 4) induced a vacuole reaction affecting 88.75 ± 25.8 (SEM) neurons per section compared to 212.2 ± 18.9 (SEM) neurons per section for control non-pregnant females (n =6). A similar phenomenon was witnessed for PCP (30 mg/kg sc), which caused 125 vacuolated neurons per section in a E18 pregnant dam compared to 268.5 ± 32.5 (SEM) neurons per section in control non-pregnant female rats (n = 2). This hyposensitivity extended into the early postpartum period in that a low dose of MK-801 (0.2 mg/kg sc) induced a vacuole reaction in 17.3 ± 15.8 (SEM) neurons per section in rats (n=3) within the first 2 days postpartum.compared to 119.25 ± 23.8 (SEM) for non-pregnant non-postpartum female controls (n = 12) or 90 ± 36 (SEM) neurons per section in 7-10 day postpartum females (n=4). Our findings suggest that during pregnancy, and in the immediate postpartum period, sensitivity to NAN is suppressed by some factor, presumably hormonal, that is peculiar to the peripartum state. Estrogen and progesterone and/or their metabolites are likely candidates in that: 1) Concentrations of these hormones rise during pregnancy and fall shortly thereafter. 2) These hormones or their metabolites interact with CNS $GABA_A$ and sigma receptors both of which are critically involved in the mediation of NMDA antagonist neurotoxicity. Supported by AG05681, DA05072, MH14677, RSA MH 38894 (JWO) and a NARSAD Award (JWO).

628.20

TRACING THE CIRCUITRY THAT MEDIATES NMDA ANTAGONIST NEUROTOXICITY MT Price*, NB Farber, J Labruyere, J. Foster, JW Olney Washington Univ., St. Louis MO 63110.

Antagonists of NMDA glutamate receptors, including phencyclidine (PCP) and MK-801, acutely injure (low dose) or kill (high dose) pyramidal neurons in the posterior cingulate/retrosplenial (PC/RS) cortices when administered systemically to adult rats. Our prior findings suggest that a polysynaptic circuit and at least 4 transmitter receptors (NMDA, muscarinic, GABAergic, sigma) mediate this reaction. However, it is not known where in the brain the various neural components of this circuit are located. To address this question, we administered MK-801 to adult female rats in a dose (0.5 mg/kg sc) that reliably induces a full cerebrocortical neurotoxic reaction and injected various agents into the PC/RS cortex or other brain regions in an attempt to interupt the circuit and block the PC/RS reaction. We found that the neurotoxic reaction could be prevented ipsilaterally by unilateral injection of muscimol (GABA agonist), scopolamine (muscarinic antagonist) or rimcazole (sigma antagonist) into the PC/RS cortex, or by injecting muscimol into the diagonal band (DB) region of the basal forebrain, or into the anterodorsal/anteroventral (AD/AV) nucleus of the thalalmus. Our interpretation is as follows: systemic administration of MK-801 blocks NMDA receptors that tonically drive GABA inhibitory neurons in at least three separate brain regions (PC/RS, DB, AD/AV). This results in a complex disinhibition syndrome in which: 1) DB neurons release excessive ACh at muscarinic receptors on PC/RS neurons. 2) AD/AV neurons release excessive glutamate at non-NMDA receptors on PC/RS neurons. 3) local PC/RS interneurons release an endogenous agent that modulates a sigma site in PC/RS cortex. We postulate that the proximal mechanism causing PC/RS neuronal injury is simultaneous excess release of 3 endogenous transmitters/modulators from separate pathways that convergently innervate these neurons. Supported by DA05072, AG05681, MH14677, RSA MH38894 (JWO) and a grant from NARSAD (JWO).

EXCITATORY AMINO ACIDS: EXCITOTOXICITY AND NON-NMDA RECEPTORS

629.1

KAINATE FAILS TO EVOKE MITOCHONDRIAL OXYGEN RADICAL GENERATION IN A SUBPOPULATION OF CORTICAL NEURONS SELECTIVELY VULNERABLE TO AMPA/KAINATE TOXICITY L.M.T. Canzoniero*, S.L. Sensi, L.L.Dugan, D.M.Turetsky and D.W.Choi, Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

We previously described a subpopulation of cortical neurons highly vulnerable to death induced by relatively brief AMPA or kainate exposure, identifiable by staining for kainate-stimulated cobalt uptake ("cobalt-positive cells"; Turetsky et al , Soc.Neurosci. Abstr. 18.81, 1992), a marker for cells expressing AMPA or kainate receptors linked to Ca^{2+} permeable channels. Elsewhere this meeting, we (Dugan et al.) report that toxic exposure to NMDA (but not kainate) elicits mitochondrial free radical production. We hypothesized that cobalt-positive cells might respond to kainate with mitochondrial oxygen radical production analogous to that produced by NMDA on most cortical neurons.

Cobalt-positive neurons were functionally identified by increased $[Ca^{2+}]_i$ (fura-2 videomicroscopy) in response to kainate in Na^+-free medium. Mitochondrial production of oxygen radicals was detected with dihydrorhodamine 123 (see Dugan et al.). Exposure to kainate (100 μM) for 30 min produced a marked increase in $[Ca^{2+}]i$ in cobalt-positive cells, followed by death of >50% of the cells. The increase in $[Ca^{2+}]i$ was comparable to that reached following neurotoxic exposure to NMDA (100μM for 5 min) in the general neuronal population. However the same kainate exposure failed to stimulate mitochondrial oxygen radical production in cobalt-positive cells (n=19). Failure to detect a rhodamine signal was not due to loss of the mitochondrial membrane potential as tetramethylrhodamine showed no loss of this potential in cobalt-positive cells. Present data suggest that the route of Ca^{2+} entry may be an important determinant of consequent mitochondrial oxygen radical production. Supported by NIH grants NS 30337 (DWC) and AG00599-01A1 (LLD).

629.2

IMAGING OF MITOCHONDRIAL OXYGEN RADICAL PRODUCTION IN CORTICAL NEURONS EXPOSED TO NMDA L.L.Dugan*, S.L. Sensi, L.M.T. Canzoniero, M.P. Goldberg, S.D. Handran, S.M. Rothman and D.W. Choi, Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Confocal microscopy was used to image oxygen radical generation by mitochondria in cortical neurons exposed to NMDA. The mitochondrion-specific, oxidation-sensitive dye, dihydrorhodamine 123 (DHR; 5μM) was added to murine cortical cell cultures for 30 min. A time- and dose-dependent increase in neuronal mitochondrial fluorescence was observed in response to toxic concentrations (50-300 μM) of NMDA. This signal, starting 3-5 min after onset of NMDA exposure, reflects oxygen radical-mediated oxidation of non-fluorescent DHR to its fluorescent derivative, rhodamine 123.

The signal was specific to NMDA in that 100 μM kainate (plus 10 μM MK-801), 50 mM potassium (plus 10 μM MK-801), 3 μM ionomycin, or 300 μM *t*-ACPD all failed to stimulate mitochondrial radical formation. Removal of calcium (plus 300 μM EGTA) abolished the NMDA-induced mitochondrial response. Inhibitors of mitochondrial electron transport, 10 μM rotenone or 1 μg/ml antimycin A, abolished the NMDA-stimulated mitochondrial production of radicals. An uncoupler of electron transport, FCCP (1-3 μM), mimicked the NMDA response, even in the presence of 10 μM MK-801 and 10 μM NBQX. We speculate that mitochondrial electron transport may be an important source of reactive oxygen species during excitotoxic activation of NMDA receptors (see also I. Reynolds et al., elsewhere this meeting), and that this oxygen radical generation may be due to an uncoupling of mitochondrial electron transport. Supported by NIH NS 30337 (DWC), AG0059901A1 (LLD), NS01543 (MPG).

629.3

HEK 293 CELLS EXPRESSING GLUR4 ARE VULNERABLE TO AMPA TOXICITY. D.M. Turetsky*, L.M.T. Canzoniero, S.L. Sensi, C.A. Csernansky and D.W. Choi Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Transfection of glutamate receptor subunits has been demonstrated to induce vulnerability to excitotoxicity in fibroblasts (Rogers and Heinemann, Soc. Neurosci. Abstr. 16: 544, 1990; Anegawa et al., Soc. Neurosci. Abstr. 19:1348; Bergold et al., PNAS 90:6165, 1993). We have developed a human embryonic kidney 293 cell line that is stably transfected with the AMPA receptor GLUR4, and have begun to characterize its responses to receptor stimulation. Immunocytochemistry using a polyclonal antibody to GluR4 revealed moderate levels of receptor expression in 80 - 90% of cells. Fura-2 microfluorimetry demonstrated that either AMPA (100μM) or kainate (500μM) could increase $[Ca^{2+}]_i$ approximately 2 fold over basal in this transfected line. Addition of 100 μM cyclothiazide, which blocks AMPA receptor desensitization, to the exposure medium increased the AMPA-induced elevation in $[Ca^{2+}]_i$ to about 10-fold; further increases could be achieved by raising the extracellular Ca^{2+} concentration from 1.8 mM to 10 mM.

Continuous exposure to 500μM AMPA, 100μM cyclothiazide, and 10 mM Ca^{2+} resulted in the death of 50-60% of transfected cells by 24 hrs, and about 90% of cells by 48 hrs. Death was dependent on AMPA concentration (30-1000μM), and could be blocked by 50 μM NBQX. Death could also be induced by 24 hr exposure to 500 μM kainate, but not by 48 hr exposure to 500 μM NMDA.. Supported by NIH grant NS 30337 (DWC).

629.4

SMI-32 ANTIBODY LABELS SUBSETS OF CORTICAL AND SPINAL CORD NEURONS WITH UNUSUAL, Ca^{2+}-DEPENDENT VULNERABILITY TO KAINATE TOXICITY. S.J. Burke, S.G. Carriedo, R. Lamberta, H. Yin and J.H. Weiss*. Depts. of Neurology and Psychobiology, U.C. Irvine, Irvine CA 92717

SMI-32 is a monoclonal antibody (Sternberger-Meyer Immunocytochemicals) that recognizes non-phosphorylated neurofilament epitopes, and has been reported to label subsets of CNS neurons, including populations of pyramidal neurons that degenerate in Alzheimer's disease (J Comp Neurol 301:44). We found SMI-32 immunostaining to label small subsets (2-4%) of neurons in dissociated cultures of murine cortex and spinal cord. Cortical SMI-32 (+) neurons were larger than average, frequently mulitpolar and sometimes of pyramidal morphology. Double staining in cortex revealed the majority (75-90%) of SMI-32 (+) neurons to be immunoreactive for GABA. In spinal cord, SMI-32 labeled some small neurons, as well as a set of darkly staining neurons with features characteristic of motor neurons (large soma, extensive dendritic tree and frequently a prominent axon).

In both cortex and spinal cord, most SMI-32 (+) neurons appeared to be selectively damaged by a brief kainate exposure (100 μM, 10-40 min); removal of Ca^{2+} during the exposure significantly enhanced survival. Also, a large majority (> 80%) of both cortical and spinal cord SMI-32 (+) neurons were subject to kainate-activated cobalt uptake, a histochemical procedure that marks cells with Ca^{2+} permeable AMPA/kainate channels. While further studies are necessary to identify the full range of neurons identified by SMI-32 staining in intact brain and in culture, these results suggest that unusual vulnerability to Ca^{2+}-dependent, AMPA/kainate receptor-mediated injury may be a feature of SMI-32(+) neurons in diverse CNS regions. If, as seems likely, certain cortical pyramidal neurons and spinal motor neurons are among those stained, these results could bear upon factors contributing to preferential degeneration of those neuronal populations in diseases like Alzheimer's disease and ALS. Supported by a grant from the Alzheimer's assoc.

629.5

AMPA-INDUCED DELAYED NEURONAL INJURY IN THE HIPPOCAMPAL SLICE, WITH PROTECTION WITH POST-TREATMENT DNQX. K.L. Panizzon* and R.A. Wallis, Dept. of Neurology UCLA, Los Angeles, CA 90024 and Sepulveda VAMC, Sepulveda, CA 91343.

Prevention of delayed neuronal injury is a prime objective in the acute treatment of stroke and head trauma. To investigate potential non-NMDA excitotoxic mechanisms of delayed neuronal injury, we examined the effect of sub-lethal AMPA exposure upon CA1 neurons in hippocampal slices. In these studies, paired rat hippocampal slices taken from the same dissection were placed in recording chambers perfused with ACSF containing 2.4 mM Ca^{2+}, 1.3 mM Mg^{2+} and 4 mM glucose. One slice of each pair was exposed to 25 μM AMPA until disappearance of the orthodromic CA1 population spike (PS). In three paired trials, the AMPA exposure averaged 3.7 ± 0.9 mins. in duration. AMPA treated slices regained an average of 98% ± 2 of their original orthodromic PS within a mean of 30 ± 0 min. After initial recovery, AMPA-treated slices maintained generally stable recordings for several hours and then abruptly collapsed. Control slices maintained PS significantly longer. A 3mV CA1 PS amplitude was used as the criterion for slice viability. AMPA treated slices maintained a PS of greater than 3 mV for an average of 6.3 ± 1.4 hrs while paired control slices maintained an PS greater than 3 mV for 16.5 ± 1.1 hrs. Post-AMPA treatment of 100 μM DNQX extended the duration of CA1 PS recordings from 7.7 ± 0.4 hrs to 18.6 ± 1.4 hrs. These findings suggest that activation of non-NMDA receptors can induce delayed neuronal dysfunction, and that protection from this injury can be afforded by DNQX treatment given after that receptor activation.

Supported by the VA Research Service and the American Epilepsy Society.

629.6

DOMOIC ACID ACTION ON HIPPOCAMPAL NEURONS OF THE NEONATAL RAT. D. Xi, T.B. Taylor, L. Matsuda* and J.S. Ramsdell, Marine Biotoxins Program, U.S. National Marine Fisheries Service and Marine Biomedical and Environmental Sciences, Medical University of South Carolina, Charleston, SC 29412.

Domoic acid (DOM) is a rigid analog of glutamate demonstrated to be an agonist on non-NMDA receptors. We previously determined that DOM given i.p. to mice induces the c-fos immediate response gene and causes degeneration in hippocampal CA1 and CA2 pyramidal cells. We have now investigated the acute effects of DOM on neonates, using rats aged postnatal (P) day 1, 5, & 10. DOM-induced behavioral changes include immobility and ataxia, repetitive scratching and finally generalized clonic or tonic/clonic seizures. P1-P5 rats were more sensitive than P10 (EC_{50}=0.3 vs 0.7 mg/kg). Scratching occured within 20 min and seizures within 30 min at 0.2 mg/kg, twice as long at 0.1 mg/kg and did not occur at 0.02 mg/kg. We next localized the *in vivo* action of DOM to hippocampal pyramidal cells of CA2 and CA3 using c-Fos immunohistochemistry. Primary hippocampal cultures were then used to evaluate the ionic effects of DOM. 30 μM DOM elevated $[Ca^{2+}]_i$ and caused membrane depolarization measured using FURA-2 and bisoxinol. The NMDA antagonist AP-5 (100 μM) totally prevented DOM elevated $[Ca^{2+}]_i$ and depolarization. Pretreatment of P5 rats with 1 mg/kg AP-5 also prevented the induction of c-Fos by DOM, but failed to prevent DOM-induced symptoms. These results indicate that neonatal rats are highly sensitive to the toxic effects of DOM. The ionic and neuroexcitatory actions of DOM on pyramidal cells appear to require involvement of NMDA receptors, yet the behavioral changes appear to occur independently of the NMDA receptor subtype.

629.7

NON-NMDA RECEPTOR ANTAGONIST ACTIVITY AGAINST EXCITOTOXIC INJURY IN IMMATURE RAT BRAIN. W.H. Trescher* and M.V. Johnston. Kennedy Krieger Institute, Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Antagonists of non-N-methyl-D-aspartate (NMDA) receptors have been shown to be protective against cerebral hypoxic-ischemic injury in the adult brain. Therefore, we investigated the non-NMDA receptor antagonist GYKI 52466, activity in two models of injury in the immature rat brain. In a model of cerebral hypoxic-ischemic injury, postnatal day (PND) 7 rats underwent a unilateral carotid ligation followed by 2 hours of hypoxia. In a model of selective excitatory animo acid (EAA)-induced injury, PND 7 rats received an intrahippocampal injections of specific EAA anologues. Damage was assessed one week after the injury. Intraperitoneal GYKI 52466 provided only minimal protection against injury in the model of cerebral hypoxic-ischemic injury in the immature rat brain. Against excitotoxic injury, GYKI 52466 failed to protect against AMPA- or kainate-induced injury to the hippocampus in the neonatal rat brain. In preliminary experiments, however, GYKI 52466 provided partial protection against NMDA-induced hippocampal injury. The data suggest that GYKI 52466 neuroprotection may be partially through an NMDA dependent mechanism.

629.8

MELATONIN PROTECTS PRIMARY CULTURES OF CEREBELLAR GRANULE NEURONS FROM KAINATE BUT NOT FROM NMDA EXCITOTOXICITY. M. Gusella,[1] M. Lipartiti,[1] D. Milani,[1] P. Giusti,*[1] and H. Manev.[2] Dept. Pharmacology, Univ. Padova, Italy[1]; ASRI, Medical College of Pennsylvania, Pittsburgh, PA 15212 [2]

We and others have shown that in primary neuronal cultures, neuroprotective agents which do not block glutamate receptors protect from excitotoxcity by selectively affecting mechanisms associated with either N-methyl-D-aspartate (NMDA) receptors or non-NMDA receptors (*Brain Res* 1993, 624: 331). Thus, non-NMDA, but not the NMDA, excitotoxicity can be attenuated with an analog of vitamin E, suggesting that free radical formation plays a role in the latter type of toxicity (*Brain Res* 1994, 639: 102). Recently, it was proposed that melatonin might be a potent endogenous hydroxyl radical scavenger (*Endocrine J* 1993, 1: 57). In addition, melatonin has been shown to protect neurons from photochemically induced oxidative stress (Manev et al., this meeting). In this study, we used 7-9-day-old primary cultures of rat cerebellar granule neurons. Cultures were exposed to kainate (30 min), glutamate (15 min), or NMDA (without magnesium; 60 min) and then returned to the culture-conditioned medium. Viability was measured with the MTT technique (*Neuropharmacology* 1990, 29: 1103) 16-18 hrs later. Co-treatment with melatonin (500 μM) protected neurons completely from the toxicity of kainate (up to 1 mM), and shifted the LD50 for glutamate from 55 ± 2.6 to 97 ± 3.6 μM. It was ineffective in protecting from NMDA toxicity. When melatonin was added to cultures only before or after kainate treatment, there was no resultant protection from kainate toxicity. The neuroprotective effect of melatonin does not appear to be related to the direct action of melatonin on non-NMDA glutamate receptors. That is, the kainate-stimulated increase in free cytosolic calcium (measured at the single-cell level with a digital imaging fluorescent microscopy with fura-2) was not affected by melatonin; the binding of 3H-glutamate to rat cerebellar membranes was likewise not affected. Further studies are needed to evaluate the pharmacological relevance of the neuroprotective action of melatonin.

629.9

L-α-AMINOADIPIC ACID-INDUCED ASTROGLIAL LESIONS ENHANCE GLUTAMATE EXCITOTOXICITY IN STRIATUM. M. Garcia-Osuna, C. Davolio & J.T. Greenamyre*. Dept of Neurology, University of Rochester, Rochester, NY, 14642

Excitotoxicity has been implicated in the pathogenesis of Huntington's disease (HD). There is reason to believe that normal astroglial function may be essential for resistance against glutamate (GLU) toxicity, but the role of astroglia in excitotoxicity *in vivo* has not been studied. The selective glial toxin, L-α-amino adipic acid (L-AAA), was used to ablate striatal astrocytes and the effect of this treatment on GLU excitotoxicity was examined. Rats received stereotaxic injections of buffer or L-AAA into striatum on day 1, and injections of GLU or buffer into the same site on day 2. On day 5, animals were killed and the brains were processed for histology. Rats that received buffer/glutamate injections had a lesion volume of 1.65 ± 0.40 mm^3, and those injected with L-AAA/buffer had a lesion volume of 1.24 ± 0.08 mm^3. In contrast, rats that received L-AAA/GLU had a lesion volume of 5.50 ± 0.47 mm^3, which was significantly larger than the other treatments alone ($p < 0.0001$) or summed ($p < 0.002$). GLU toxicity was mediated by both NMDA and non-NMDA receptors: MK-801 reduced lesion volume by 43%, NBQX (a non-NMDA antagonist) by 71%, and MK-801 + NBQX by 87%. NBQX and NBQX + MK-801 provided significantly greater protection than MK-801 alone ($p < 0.05$), suggesting that most of the toxicity is mediated by non-NMDA receptors. The results suggest that astroglial cells protect against glutamate toxicity, which is particularly intriguing in light of reports of glial cell loss in HD. *(Supported by an unrestricted grant-in-aid from Fisons Pharmaceuticals).*

629.10

SYSTEMIC ADMINISTRATION OF KAINIC ACID SELECTIVELY DECREASES GLUCOCORTICOID RECEPTOR mRNA EXPRESSION IN THE DENTATE GYRUS OF THE RAT HIPPOCAMPUS. D. O'Donnell*[1], S. Kar[1], J.R. Seckl[2], and M.J. Meaney[1]. [1]Develop. Neuroendo. Lab., Douglas Hosp. Res. Ctr.; McGill University, Montreal, Canada. [2]Dept. Medicine, Western General Hosp., University of Edinburgh, Edinburgh, Scotland.

Kainic acid is a glutamate analogue which possesses potent excitotoxic properties. Systemic administration of kainic acid causes severe pyramidal neuron loss in the CA1 and CA3 cell fields, but with no apparent detrimental effects to the granule cells of the dentate gyrus. Glucocorticoids have been shown to exacerbate neuronal cell loss in excitatory amino acid mediated insults. The present study examined the effects kainic acid treatment on glucocorticoid receptor (GR) mRNA expression. Male Sprague Dawley rats were intraperitoneally administered kainic acid (12 mg/kg body weight) or vehicle (0.9% sodium chloride) and sacrificed 12 hours following the injection. Brains were snap-frozen and processed for *in situ* hybridization using a ^{35}S-labeled GR cRNA probe. Saline treated controls exhibited the classical hippocampal distribution pattern of GR mRNA expression. In contrast, animals injected with kainic acid showed a 30-35% decrease in GR mRNA levels in the dentate gyrus with no significant changes observed in either CA1 or CA3 regions. This selective decline in GR expression in the granule cells of the dentate gyrus may account for their sparing following exposure to an excitotoxin.

629.11

DOWN-REGULATION OF A CALCIUM-PERMEABLE NON-NMDA RECEPTOR BY KAINATE. S.Allcorn and P.Mobbs*. Dept. of Physiology, University College London, Gower St., London, WC1E 6BT, England.

Kainate, a non-NMDA receptor agonist, acts as a potent neurotoxin in the adult retina. Consistent with this, acute exposure (12 hours) of primary cultures of chick embryonic day 8 retinal cells to 100μM kainate caused 59.6±0.3% of the cells to die when applied at 6 days *in vitro* (DIV). In contrast the number of cells in cultures grown in the presence of 10, 100 and 500μM kainate from 1 DIV did not differ significantly from those grown in the absence of kainate. This lack of effect of kainate on cell survival does not appear to result from receptor desensitization since whole-cell patch clamp recording showed that cells grown in the presence of kainate for 6 days continue to exhibit a current which is suppressed by the non-NMDA receptor antagonist CNQX. We have utilised the cobalt staining technique of Pruss et al. (1991, Neuron 7, 509) to determine the number of cells that express the calcium permeable form of the AMPA/kainate receptor in retinal cultures. We found that the number of cells expressing this receptor in control cultures rose from 5% at 2 DIV to a maximum of 50% at 5 DIV. However, in cultures grown in the presence of 10-500μM kainate the number of cells stained by cobalt was reduced by as much as 90% at 5 DIV. The effects of kainate on the expression of the receptor could be prevented by the addition of 20μM CNQX, but not by 20μM AP5 or 20μM diltiazem and nifedipine. When kainate was removed from the culture medium at 4 DIV the number of cells expressing the receptor at 7 DIV was restored to normal. A down-regulation of the Ca^{2+}-permeable non-NMDA receptor will decrease Ca^{2+} influx into the cells and this may account for their survival when grown in the presence of excitotoxic concentrations of kainate.

Supported by the Wellcome Trust and the M.R.C

629.12

NON-NMDA RECEPTOR ANTAGONISTS PROTECT AGAINST NEUROTOXIC SPINAL CORD INJURY IN THE RAT S. Liu, G.L. Ruenes, H.A. Dancausse*, R.P. Yezierski. Dept. of Neurological Surgery and The Miami Project, University of Miami, Miami, FL 33136.

The effects of the non-NMDA receptor antagonists NBQX and MCPG on quisqualate (QUIS), AMPA or ACPD induced spinal cord injury were evaluated. Twenty-eight male rats were divided into seven groups: 1) QUIS; 2) QUIS+MK-801; 3) QUIS+NBQX; 4) AMPA; 5) AMPA+NBQX; 6) ACPD; and 7) ACPD+MCPG. Drugs were intraspinally injected between spinal segments T13-L5. Animals were perfused transcardially with 10% formalin 1-10 days after injection. Spinal segments with injection sites were blocked and cut (75μm) on a freezing microtome. Cross-sections were stained with cresyl violet and evaluated with light microscopy. The rostrocaudal extent of cell loss in the gray matter and the area of gray matter damage at the epicenter of injection sites were evaluated.

The longitudinal extent of cell loss in AMPA+NBQX or ACPD+MCPG injected animals was significantly less than animals injected with AMPA or ACPD alone ($P<0.05$). The area of gray matter damage in AMPA+NBQX animals (1,922.38 ± 782.45μm^2) was significantly less than AMPA animals (11,058.13 ± 1,445.45μm^2, $P<0.001$). The difference in gray matter damage between ACPD animals (15,474.17 ± 1,742.01μm^2) and ACPD+MCPG animals (1,963.17 ± 1,176.35μm^2) was also significant ($P<0.001$). Although there was no significant difference between QUIS and QUIS+NBQX animals, NBQX showed some blockage of QUIS effects. MK-801 produced no protective effects on non-NMDA induced spinal cord damage. The results suggest that non-NMDA (including metabotropic) receptors may have an important role in mediating the excitotoxic effects of excitatory amino acids in spinal cord injury. This work was supported by NS28059 and by The Miami Project.

629.13

INTRINSIC OPTICAL SIGNALLING IN THE HIPPOCAMPAL SLICE EVOKED BY THE EXCITOTOXIN DOMOIC ACID. Trevor M. Polischuk* & R. David Andrew. Dept. of Anatomy & Cell Biology, Queen's Univ., Kingston, Ontario, K7L 3N6.

As cells swell, light transmittance increases across brain slices (Lipton, *J. Physiol. 231*, 365; Andrew and MacVicar, *Neuroscience* in press). Also, as shown by Adams and Andrew (this meeting), increases in light transmittance induced by glutamate agonists can be imaged in real time in the entire hippocampal slice. Here we studied the effects of the potent neurotoxin and glutamate analogue, domoic acid (DOM), in the rat hippocampal slice. We attempted to characterize 1) which hippocampal regions were affected, 2) which sub-class of receptors were involved, and 3) if cell swelling played a role in the response. A 1 min exposure of 10 μM DOM (22°C) elevated light transmittance by up to 58% in the dendritic regions of CA1 and of upper dentate gyrus. The response slowly reversed during a 30 min wash (n = 10). No significant changes were observed in the CA3 region nor in the lower blade of the dentate gyrus. The response to DOM was reversibly blocked by the non-NMDA receptor antagonists kynurenate (1mM; n = 5), CNQX (50 μM; n = 5) or DNQX (50 μM; n = 7) but not by the NMDA receptor antagonist AP-5 (50 μM; n = 5) nor the kainate receptor antagonist GAMS (100 μM, n = 5). Tetrodotoxin (1 μM) blocked action potentials and the evoked CA1 population spike but light transmittance increases were maintained and therefore were not associated with neuronal discharge. Extracellular tissue resistance measured across CA1 stratum radiatum increased rapidly in response to DOM and slowly fell over 30 min, which paralleled the light transmittance response. We conclude that brief DOM exposure elicits a prolonged, post-synaptic swelling in the CA1 dendritic region primarily mediated by AMPA receptors. This imaging technique allows a real-time view of events preceding neuronal death and so may prove useful in assessing potentially therapeutic glutamate antagonists that combat excitotoxicity.

Supported by the Canadian MRC

629.14

IN VITRO AND IN VIVO EFFECTS OF β-OXALYLAMINO-L-ALANINE (BOAA) ON NADH-DEHYDROGENASE ACTIVITY IN MOUSE BRAIN. M.J. Sabri*, D. N. Roy, B. Lystrup, C. N. Allen, and P. S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland OR 97201.

Experimental primate studies have shown that chronic ingestion of the excitatory and neurotoxic amino acid BOAA, disrupts motor neuron function in a manner similar to that seen in the human neurodegenerative disease , lathyrism. BOAA is known to exert its neurotoxic effects at μM concentrations by over-stimulation of non-NMDA receptors, especially the AMPA receptor. A recent study (Brain Res., 621:215, 1993) has reported that a very low (0.1 pM) concentration of BOAA selectively inhibits the activity of the mitochondrial enzyme NADH-dehydrogenase (NADH-DH; Complex I), while 1 pM BOAA promotes LDH release into the incubation media indicating neuronal cell death. This study investigates the effect of pure BOAA on mouse brain NADH-DH both *in vitro* and *in vivo*. For *in vitro* studies, transverse brain slices (500 μm) from adult male CD-1 mice (25-34 g) were incubated in Krebs Ringer (3.0 ml/slice) bubbled with 95% O_2 + 5% CO_2 with and without BOAA for 1h at 37^0 C. The specific activity of NADH-DH in control slices after 1h incubation was 12.1 ± 0.85 *n* moles NADH oxidized/min/mg protein (n=15). Treatment of slices with BOAA showed a concentration-dependent inhibition of NADH-DH, but only at μM concentrations (16, 24, 33, and 37% inhibition at 10, 100, 500 and 1000 μM BOAA, respectively). Release of LDH into the incubation media was observed only in slices treated with 500 μM and 1000 μM BOAA. Young (8-day old) and adult (4-month) mice treated ip with 500 and 700 mg/kg BOAA, respectively, showed behavioral signs of BOAA toxicity. Young mice had severe convulsive seizures while adult animals exhibited drowsiness and lethargic movement. The NADH-DH activity in brain homogenates and mitochondria from BOAA-treated animals did not differ with that in age-matched animals treated ip with an equivalent volume of saline. These findings are in contrast to the reported suggestion that BOAA is a highly potent inhibitor of mitochondrial energy metabolism. Supported by NIH grant NS 19611 and MRF of Oregon.

629.15

DELAYED ANTAGONISM OF AMPA/KAINATE RECEPTORS REDUCES LONG-TERM FUNCTIONAL DEFICITS RESULTING FROM SPINAL CORD TRAUMA. Jean R. Wrathall* and Yang Dong Teng. Neurobiology Division, Dept. of Cell Biology, Georgetown Univ., Washington, DC 20007.

Ionotropic glutamate receptors appear to play a significant role in secondary injury processes after spinal cord trauma. We previously reported that NBQX, a potent and highly selective antagonist of the AMPA/kainate subtypes of glutamate receptors, focally administered at 15 min after a standardized traumatic spinal cord injury (SCI), results in a dose-dependent increase in the speed of recovery and reduction in long-term hindlimb deficits. There was a correlated, dose-related, sparing of gray and white matter at the injury site, and increased serotonergic innervation below the level of the injury. We have now examined the effects of delaying administration of NBQX. A weight-drop device was used to produce a standardized incomplete thoracic SCI in rats, and NBQX (15 nmoles), or vehicle alone, administered focally at the injury site at 4 hrs post-injury (p.i.). Behavioral tests of hindlimb functional deficits were performed at 1 day and weekly thereafter for 8 weeks. The group treated with NBQX demonstrated a reduction in long-term functional deficits that was significant from 4 through 8 weeks. There was improved performance in tests of a number of hindlimb reflexes, and, most importantly, in use of the hindlimbs in locomotion. However, administration of NBQX at 4 hrs p.i. did not increase the speed of recovery, as had been observed when the same dose was given at 15 min p.i.. The results indicate that this antagonist of AMPA/kainate receptors may be effective in a clinically-relevant time frame. They confirm the importance of long-term analyses of functional deficits in evaluating the potential of drugs that may mitigate the effects of SCI. In addition, they suggest that NBQX can act differently, perhaps due to alterations in the presence or sensitivity of cellular targets, when administered at different times after SCI. [Supported by NIH-NS28130 and PVA-SCRF # 1232].

630.1

CYCLOTHIAZIDE POTENTIATES INTRACELLULAR CALCIUM INCREASE IN RESPONSE TO AMPA IN CULTURED RAT HIPPOCAMPAL NEURONS. M. Okada, K. Ohno, M. Shimizu-Sasamata* and T. Yamaguchi. Neuroscience/Gastrointestinal Research Lab., Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

AMPA receptors are characterized by a fast desensitization which is considered to be a possible mechanism for the termination of excitatory synaptic transmission. The present experiments are aimed to evaluate the ability of cyclothiazide(CYT), a drug which reduces desensitization of AMPA responses, to alter the intraneuronal Ca^{2+} concentration ([Ca]i) after exposure to AMPA.

Experiments were performed in primary rat hippocampal neurons grown for 10-16 days in cultures. Changes in [Ca]i were measured by microfluorometrical monitoring of the fluorescence intensities from individual neurons loaded with fura-2. In most neurons, application of AMPA induced a very small increase of [Ca]i. CYT markedly potentiated the responses to 30μM AMPA in a dose-dependent manner (EC_{50}=3.2μM). In the presence of 10μM CYT the EC_{50} for AMPA was 12.5μM. The AMPA-CYT-induced [Ca]i increase was inhibited by competitive AMPA receptor antagonists, NBQX and YM90K(6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione monohydrochloride), and by a non-competitive antagonist, GYKI52466. Under the conditon of 10μM CYT and 20μM AMPA, IC_{50} values for NBQX, YM90K and GYKI52466 were 0.19, 0.30 and 9.9μM, respectively. From these results, it is demonstrated that CYT potentiates the action of AMPA in terms of intraneuronal calcium mobilization as well.

630.2

INTERACTIONS OF GYKI 52466 AND CYCLOTHIAZIDE: PATCH CLAMP STUDIES. G. Rammes[1], W. Müller[1], D. Swandulla[1], C.G. Parsons[2], and G.L. Collingridge*[3] Dept. Molecular Pharmacol., Univ. Erlangen, Germany[1], Dept. Pharmacol., Merz + Co., Frankfurt, Germany[2], Dept. Pharmacol., Univ. of Birmingham, England[3].

The effects of GYKI 52466 and cyclothiazide on isolated AMPA receptor-mediated EPSCs and AMPA-induced inward currents were investigated with whole cell patch clamp recordings from the CA_1 region of hippocampal slices and cultured superior colliculus neurones respectively. Cyclothiazide concentration-dependently slowed the decay of AMPA-EPSCs (10μM, 157%; 30μM, 198%; 100μM, 291%; 330μM, 306% of control τ=23ms) with an EC_{50} of 31μM and produced a variable increase in the amplitude of AMPA-EPSCs (up to 133% of control). GYKI 52466 (10-30μM) concentration-dependently reduced the amplitude of AMPA-EPSCs without affecting response kinetics, both in the absence and the presence of cyclothiazide (330μM). Control inward currents to AMPA 100μM rose rapidly (τ=4.8ms) to a peak of 973pA and then desensitized (τ=21ms) to a plateau of 187pA. Cyclothiazide concentration-dependently potentiated the plateau component to a greater degree than the peak component (10μM, peak 195%, plateau 698%; 30μM, peak 223%, plateau 926%; 100μM, peak 252%, plateau 1114% of control). This effect was accompanied by a reduction in the rate of desensitization (10μM, τ=593ms) with little effect on the rate of onset (τ=7ms) or offset (τ=162ms cf. 167ms control) of AMPA responses. GYKI 52466 antagonized both peak and plateau components of control AMPA response (IC_{50}s Peak=12.9μM, plateau=10.9μM) whilst having little effect on response kinetics. Cyclothiazide 10μM shifted the GYKI 52466 concentration-response curve to the right in a manner suggestive of a common site of action for the two substances (GYKI 52466 Plateau IC_{50}=57.9μM). However, in the presence of cyclothiazide 10μM, GYKI 52466 was unable to reintroduce desensitization and concentration-dependently slowed the onset kinetics and enhanced the offset kinetics of AMPA responses (GYKI 52466 30μM, τ onset 19ms, τ desensitization 966ms, τ offset 83ms). In conclusion, cyclothiazide may be an agonist and GYKI 52466 an antagonist at a common regulatory site on AMPA receptors but the two compounds mediate very divergent effects on receptor activation kinetics. Furthermore, these data provide supportive evidence that the offset kinetics of AMPA receptor-mediated EPSCs are partially governed by desensitization.

630.3

CONCONAVALIN A RECRUITS CEREBELLAR NEURONS RESPONDING TO GLUTAMATE, OVERCOMES THE QUISQUALATE ANTAGONISM OF THE KAINATE RECEPTOR AND PRODUCES A GLIA-LIKE EFFECT, by P. McCaslin* and A. R. Aroor, Ochsner Medical Foundation, Division of Research, 1516 Jefferson Highway, New Orleans, LA 70121

Glutamate is the predominant excitatory neurotransmitter in the CNS, and when directly applied on neural tissue, it excites most central neurons. The effects of Conconavalin A (Con A) on the pharmacology of glutamate were examined in cultures of cerebellar granule cells using digital and ratio Ca^{2+} imaging. In Mg^{2+} buffer, glutamate, quisqualate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) activate the same neuronal subpopulation (10-15% of the neurons), whereas kainate activates all neurons and NMDA activates none. Glutamate, AMPA and quisqualate reverse the kainate-induced elevations of $[Ca^{2+}]_i$ in cells unresponsive to glutamate (85-90%). The treatment of cells with Con A (1) reverses the quisqualate inhibition of the kainate response, (2) recruits the percentage of neurons responding to glutamate, AMPA and quisqualate without effecting kainate and decreasing NMDA responses, (3) and results in the pharmacologic expression of a specific population of receptors. Finally, Con A-like effects are seen in co-cultures of neurons and glia. Contrary the assumption that glutamate is universally excitatory to neurons, these results indicate that the neuronal response to glutamate is not apparent in most neurons in cerebellar neuronal cultures and that glia, like Con A, can change the glutamate pharmacology.

630.4

STABLE EXPRESSION OF GLUR6 AND GLUR2(Q) IN BHK CELLS. C. K. Tygesen, J. S. Rasmussen, O. Thastrup, M. Jørgensen and P. H. Andersen*. Departments of Molecular Pharmacology, Molecular Biology II and BioImage Satellite, Bioscience, Novo Nordisk, DK-2880 Bagsvaerd, Denmark.

Rat GluR6 was cloned by PCR using rat hippocampal derived 1.strand cDNA as template and sequence specific oligonucleotides as primers. Rat GluR2flip(Q) was constructed from previously cloned rat GluR2flip(R) using PCR. Each cDNA was transfected into BHK cells under control of a constitutively active metallothionine promoter. Following addition of selection agent, subclones were isolated showing binding of either ^{3}H-kainate (GluR6) or ^{3}H-AMPA (GluR2). Western blots using antibodies specific to either GluR6 or GluR2 revealed bands of approximately 100 kd each. The pharmacological profiles of the receptors were studied by binding of either ^{3}H-kainate (GluR6) or ^{3}H-AMPA (GluR2). For GluR6 the rang order of potency was: domoate>kainate»quisqualate>L-glutamate» AMPA and for GluR2(Q): quisqualate> AMPA»L-glutamate»kainate. Functional properties of the receptors were studied by Ca^{2+}-imaging using Fura-2. With both GluR6 and GluR2(Q), application of kainate resulted in a marked increase in intracellular Ca^{2+}.

630.5

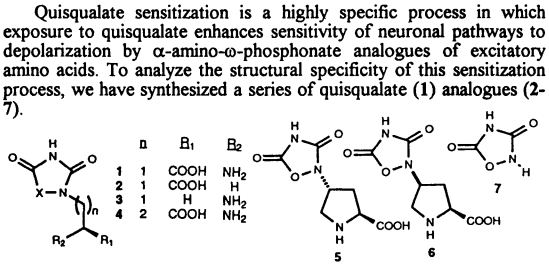
UNIQUE PROPERTIES OF AMPA RECEPTOR AGONISTS ACTING AT GLUR1-FLOP RECEPTORS EXPRESSED IN *XENOPUS* OOCYES. P. Wahl, U. Madsen, Banke T., Krogsgaard-Larsen P., and A. Schousboe. PharmaBiotec Res. Center, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark.

A series of AMPA derivatives were evaluated for activity at homo-oligomeric GluR1-flop receptors expressed in *Xenopus* oocytes, using two electrode voltage clamp. (RS)-2-Amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA) EC_{50}, 2.4 μM) a homologue of AMPA with a carboxylate group as the terminal acidic functionality was five times more potent than (RS)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate (AMPA) (EC_{50}, 12 μM) and 20 times more potent than kainate (KA) (EC_{50}, 46 μM). (RS)-2-Amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl)propionic acid (Tri-F-AMPA) in which an electrophilic trifluoromethyl group has been substituted for the methyl group on the isoxazole ring in the AMPA structure was three times more potent than AMPA, whereas (RS)-3-hydroxy-4,5,6,7-tetrahydro-isoxazolo 5,4-*c* pyridine-5-carboxylic acid (5-HPCA), a homologue of AMPA with highly restricted structural mobility was 10 times less potent than AMPA. Responses evoked by near saturating concentrations of the agonists varied in amplitude by greater than 7-fold. The sequence of efficacy was ACPA = KA > Tri-F-AMPA > AMPA > 5-HPCA. Moreover, when a saturating concentration of Tri-F-AMPA and KA was co-applied the response was significantly larger than if the agonists were applied seperately. The potency of the antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quino-xaline (NBQX) (estimated K_B, ~ 0.2 μM) at blocking currents mediated by GluR1-flop receptors was similar for all the agonists tested in this study. These results indicate that simple changes in the molecular structure of AMPA are associated with marked effects on potency and efficacy. In particular, it is suggested that the acidity of the terminal group plays a major role in determining the degree of receptor activation in the steady-state state.

630.6

UTILIZATION OF QUISQUALIC ACID ANALOGUES TO DEFINE THE SPECIFICITY OF THE QUIS SENSITIZATION SITE IN RAT HIPPOCAMPUS. M.K. Schulte, R.J. Roon*, S. Venkatraman, R.L. Johnson and J.F. Koerner. Depts. of Biochemistry and Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455-0347.

Quisqualate sensitization is a highly specific process in which exposure to quisqualate enhances sensitivity of neuronal pathways to depolarization by α-amino-ω-phosphonate analogues of excitatory amino acids. To analyze the structural specificity of this sensitization process, we have synthesized a series of quisqualate (**1**) analogues (**2-7**).



Compounds **2**, **3**, **6**, and **7** produced no sensitization at the highest levels tested whereas compounds **4** and **5** were 1/10 and 1/1000, respectively, as active as **1** in sensitization of hippocampal CA1 neurons to depolarization by L-AP6. Since cellular uptake has been implicated in QUIS-sensitization, we compared the uptake of **1** and **4** into hippocampal slices and found that the rate of uptake of **4** is ~50% of that of **1**. (Supported by NIH NS 17944).

630.7

2,3-BUTANEDIONE MONOXIME SUPPRESSES KAINATE-INDUCED CURRENTS OF MURINE VENTROMEDIAL HYPOTHALAMIC NEURONES. J.H.Ye* and J.J. McArdle. Dept. Pharmacology/Toxicology and Anesthesiology, New Jersey Medical School (UMDNJ), Newark, NJ 07103-2714.

The effects of the "chemical phosphatase" 2,3-butanedione monoxime (BDM) on kainate-induced current were studied in hypothalamic neurones acutely dissociated from young mice. Drug containing solutions were rapidly applied to activate current in neurons subjected to the nystatin perforated patch or conventional whole cell recording techniques. When applied simultaneously with kainate, BDM produced a rapid, reversible and dose-dependent (IC_{50} 25 mM) suppression of agonist-induced current. This acute blocking effect of BDM was neither voltage- nor use-dependent. Including 500 μM ATP-gamma-S in the recording pipette did not prevent the acute effect of BDM. Furthermore, H-7 (200 μM), a non specific protein kinase inhibitor, did not prevent the rapid recovery of the kainate response following washout of BDM. These findings suggest that the acute blocking action of BDM on the kainate response of hypothalamic neurone does not require a "chemical phosphatase" action. The most likely mechanism of action is a direct block of channels from the outside surface of the neurolemma. However, preincubating neurons with 30 mM BDM reversibly reduced the current recorded with the perforated patch technique when neurons were subsequently exposed to kainate alone. This persistent effect of BDM was not seen for neurons dialyzed with a solution containing ATP-gamma-S during conventional whole cell recording. Furthermore, exposure to H-7 prevented recovery of the kainate response suppressed by preincubation in BDM. These findings suggest that BDM causes sustained suppression of the kainate response of hypothalamic neurons via a "chemical phosphatase" action. Support from the NIH (NS31040) and the NIAAA (AA08025) made this work possible.

630.8

PARADOXICAL EFFECT OF A NON-NMDA ANTAGONIST ON THE KAINATE-INDUCED ELEVATION OF CYCLIC GMP (cGMP). S. Oh* and P. McCaslin[1], Dept. Pharmacol. & Toxicol., University of Miss. Med. Ctr, Jackson, MS 39216, and [1]Alton Ochsner Medical Foundation, Div. Res., 1615 Jefferson Hwy., New Orleans, LA 70121

It is well known that N-methyl-D-aspartate (NMDA) and kainate (KA) result in the elevation of cGMP. These studies were designed to examine the long-term effects of NMDA and KA on cGMP elevation in primary cultures of cerebellar granule cells, and also to determine if this elevation has functional significance. Both NMDA and KA result in a concentration-dependent elevation of cGMP with maximal elevations of cGMP occurring at 200 μM NMDA and 50 μM kainate after a 3 min exposure. The cGMP concentration-response to kainate or NMDA is markedly blunted and shifted to the left if cells are incubated for an hr when compared to the above 3 min exposure. As expected, an NMDA antagonist blocked the elevation of cyclic GMP and the release of glutamate induced by NMDA with both short-term and long-term exposures. However, a non-NMDA antagonist produced paradoxical results by augmenting the kainate-induced elevation of cGMP. While 6,7-dinitroquinoxaline-2,3-dione blocked the kainate-induced elevations of cGMP with short-term incubations, it augmented the response with long-term incubations; moreover, in both cases it blocked the kainate-induced release of glutamate. These results suggest a more complex agonist-antagonist receptor interaction with the kainate receptor than is seen with the NMDA receptor.

630.9

KAINATE-INDUCED Ca^{2+} SIGNALING IN ASTROCYTES REQUIRES Na^+ AND Ca^{2+} FLUX VIA THE Na^+/Ca^{2+} EXCHANGER. W. T. Kim, M. G. Rioult, and A. H. Cornell-Bell*. Dept. of Cell Biology, Yale Univ. Sch. of Med., New Haven, CT 06510.

In primary cultures of rat hippocampal astrocytes, the binding of ionotropic glutamate receptors with kainate (KA, 100 μM) induces a rise and sustained elevation of $[Ca^{2+}]_{in}$ as well as regenerative intercellular Ca^{2+} waves. Using time-lapse confocal scanning laser microscopy and Fluo-3 we found that the removal of Na^+ or Ca^{2+} from the bath abolished these $[Ca^{2+}]_{in}$ changes; therefore, external Na^+ and Ca^{2+} are both necessary for the KA-induced response (N = 7 experiments). The involvement of Na^+ and Ca^{2+} in the ionotropic response is not via influx through voltage-dependent channels. Blocking Na^+ channels with TTX (10 μM) did not affect the incidence, latency, or spatio-temporal characteristics of KA-induced $[Ca^{2+}]_{in}$ changes (N = 3 experiments). Similarly, blocking L-type Ca^{2+} channels with Nifedipine (10 μM) and Nimodipine (1 μM) did not affect the $[Ca^{2+}]_{in}$ elevation or waves (N = 4 experiments). However, inhibiting the Na^+/Ca^{2+} exchanger with Benzamil (100 μM) completely abolished the $[Ca^{2+}]_{in}$ elevation and intercellular Ca^{2+} waves (N = 7 experiments). The inhibition of Ca^{2+} signaling by Benzamil appears to result from its effects on the Na^+/Ca^{2+} exchanger in particular rather than to its non-specific effects on Na^+ and Ca^{2+} channels and the Na^+/H^+ antiporter. The more selective Na^+/H^+ antiporter inhibitor 5-(N,N-dimethyl)-amiloride (100 μM) did not affect KA-induced $[Ca^{2+}]_{in}$ changes (N = 3 experiments).

630.10

Excitatory amino acid responses in acutely isolated cerebellar Purkinje neurons

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We have examined the effects of agonists and antagonists of excitatory amino acid receptors in acutely isolated cerebellar Purkinje cells from 6-9 day old rats. Neurons were whole cell voltage clamped at -70mV with a KCl based pipette solution and compounds applied by bath perfusion. Kainate (KA,10-300μM) AMPA (10-50μM), domoate (10-100μM) and L-glutamate (10-100μM) induced inward currents in a concentration-dependent manner. Applications of NMDA (100μM in Mg free/10μM glycine solutions) failed to evoke inward currents at either -70 or -40mV (n=4). Kainate (100mM)-induced currents were antagonized by NBQX (IC_{50} value approx. 0.2μM) and and 2,3-benzodiazepines, GYKI 52466 and 53655 with IC_{50} values of 5.1$\pm$1.1μM (n=3) and 1.5$\pm$0.2μM (n=3) respectively. Peak KA (100μM)-induced currents were potentiated in the presence of cyclothiazide (30μM) by 43$\pm$11% (n=5) and AMPA (50μM)-induced currents by 450$\pm$40% (n=4). Concanavilin A (10μM) had no significant effect on either KA- or AMPA-induced currents. Cobalt uptake into isolated neurons was stimulated by KA (100μM). KA-evoked cobalt uptake was prevented by NBQX (10μM) and 2,3-benzodiazepines suggesting the presence of divalent permeable AMPA/KA receptors.

630.11

MK-801-INDUCED HYPERMETABOLISM IN THE POSTERIOR CINGULATE CORTEX IS ATTENUATED BY AN AMPA RECEPTOR ANTAGONIST. T.R. Patel & J. McCulloch*, Wellcome Surgical Institute, University of Glasgow, G61 1QH, U.K.

MK-801 induces marked increases in glucose utilisation in the posterior cingulate cortex. We have examined if pretreatment with the AMPA receptor antagonist NBQX alters the pattern of glucose use seen with the administration of MK-801. Local cerebral glucose use was measured in male SD rats using ^{14}C-2-deoxyglucose autoradiography. NBQX (7mg/kg i.v.) was administered 2 min prior to the administration of MK-801 (0.2 mg/kg i.v.). The effects of NBQX and MK-801 alone (with vehicle administration at appropriate times) was also examined.

At the dose used, NBQX (7mg/kg) had no significant effect on glucose use in any area examined. MK-801 (0.2mg/kg) increased glucose use in some components of the limbic system. In the posterior cingulate gyrus, MK-801 caused a marked increase in local cerebral glucose use from 67 ± 2 with vehicle to 101 ± 9 µmoles/100g/min after MK-801. Pretreatment with NBQX attenuated the MK-801 induced increase in glucose use in the posterior cingulate cortex (69 ± 3 µmoles/100g/min after NBQX + MK-801). Pre-treatment with NBQX did not accentuate the MK-801-induced decreases in glucose use in the neocortex.

The attenuation of the MK-801 hypermetabolism in the posterior cingulate cortex indicates that there may be some interaction *in vivo* between NBQX and MK-801. The combination of these drugs may be useful in counteracting the potential adverse effects of the non-competitive NMDA receptor antagonists.

630.12

AN AUTORADIOGRAPHIC STUDY OF THE LOW-AFFINITY [^{3}H]KAINATE BINDING SITE IN RAT BRAIN

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[^{3}H]Kainate binding to rat cortical membranes can be separated into high- and low-affinity binding sites with Kd-values of 2 and 24 nM, respectively. The low-affinity binding site can be studied separately in the presence of Ca^{2+}. Saturation of [^{3}H]kainate binding to rat brain sections was examined in 10 regions in the absence or presence of 30 mM $CaCl_2$. In the absence of $CaCl_2$ both high and low-affinity [^{3}H]kainate binding sites could be detected in all the investigated regions, whereas only binding to a single receptor population (Kd = 40-90 nM) could be detected in the presence of 30 mM $CaCl_2$. Comparison of the regional distribution of the low-affinity [^{3}H]kainate binding site and the [^{3}H]AMPA binding site did not indicate that calcium insensitive [^{3}H]kainate binding represents binding to the AMPA receptor.

The non-NMDA antagonist 5-nitro-6,7,8,9-tetrahydrobenzo[*g*]indole-2,3-dione-3-oxime (NS-102), which selectively blocks the low-affinity [^{3}H]kainate binding site in rat brain and which has high affinity for the recombinant GluR-6 receptor, has been used to examine regional differences in [^{3}H]kainate binding. In the absence of $CaCl_2$ where both high and low-affinity binding sites were labelled, NS-102 only partially inhibited the binding and showed regional differences in IC_{50} values (hippocampus, CA3: 75 μM -cerebellum: 2 μM) indicating a relatively higher proportion of low-affinity binding sites in cerebellum compared to the rest of the brain. In the presence of $CaCl_2$, where only the low-affinity [^{3}H]kainate binding site is labelled, NS-102 showed an incresed affinity in all regions. However, regional differences in the IC_{50}-values of NS-102 for the low-affinity [^{3}H]kainate binding site were obtained (hippocampus, CA3: 12 μM – cerebellum: 0.7 μM), indicating that the low-affinity [^{3}H]kainate binding site may not represent a homogenous receptor population.

630.13

STRUCTURAL ANALOGS OF THE NON-NMDA RECEPTOR ANTAGONIST GYKI 52466 DEPRESS K^+ CURRENTS IN HIPPOCAMPAL NEURONS. W. Li, D. L. Czilli and L. K. Simmons.* CNS Research, Lilly Research Laboratories, Indianapolis, IN 46285.

The 2,3-benzodiazepine GYKI compounds have been characterized as potent, selective antagonists of neuronal responses mediated by non-NMDA receptors. In this study we used whole-cell voltage-clamp protocols to investigate the K^+ channel actions of structural analogs of the parent compound GYKI 52466 in embryonic rat hippocampal neurons.

Three of the four compounds evaluated blocked voltage-gated K^+ currents with a rank order potency and stereoselectivity comparable with their reported antagonist activities at non-NMDA receptors. In contrast, the most potent K^+ channel blocker of the compounds tested was GYKI 53391, a 4',3-di-N-acetyl derivative of GYKI 52466 known to be inactive at non-NMDA receptors. Kynurenate, CNQX and Ro-15-1788 failed to elicit similar responses, therefore the GYKI analogs do not appear to be modulating K^+ channel function indirectly via interactions with ionotropic EAA or benzodiazepine receptors. The results described here suggest that the K^+ channel and non-NMDA receptor actions of the 2,3-benzodiazepine GYKI compounds can be isolated so that further evaluation of structural analogs within this series may lead to the identification of more potent K^+ channel blockers.

630.14

DIFFERENTIAL RESPONSES TO PHOSPHONATE ANALOGS OF GLUTAMATE DISTINGUISH TWO SUBCLASSES OF L-AP4-SENSITIVE SITES. J.F. Koerner*, R.D. Randall and R.J. Roon. Dept. of Biochemistry, Univ. of Minnesota, Minneapolis, MN 55455.

In previous studies of hippocampal neural pathways, it has been shown that L-2-amino-4-phosphonobutanoic acid (L-AP4) is a potent inhibitor of excitatory glutamatergic projection pathways of the lateral perforant pathway (LPP) of the rat, the mossy fiber-CA3 pathway (MF) of the guinea pig, and a partial inhibitor (10-40%) of the medial perforant pathway (MPP) of the rat hippocampus. It is also a potent inhibitor of the Schaffer collateral-CA1 pyramidal pathway after (but not before) sensitization by exposure to quisqualic acid (QUIS). It was also demonstrated that the QUIS-sensitized site is pharmacologically distinct from the L-AP4 receptor of the rat LPP and the guinea pig MF. Specifically, the QUIS-sensitized site also shows high sensitivity to L-AP6 and *E*-cyclopentyl-AP4. We now wish to report that the partial sensitivity of the rat MPP also shows the pharmacological profile of the QUIS-sensitized site, not of the classical L-AP4 receptors which have low sensitivity for L-AP6 and *E*-cyclopentyl-AP4. Exposure of a slice to QUIS sensitizes the MPP for 100% inhibition by L-AP4, L-AP6, and *E*-cyclopentyl-AP4 with IC_{50} values indistinguishable from the partial inhibition observed with these compounds before QUIS. The high sensitivity of the rat LPP and guinea pig MF to L-AP4 previously made it impossible to demonstrate QUIS-sensitization of these pathways. However, using L-AP6 and *E*-cyclopentyl-AP4, QUIS-sensitization can be readily demonstrated. (Supported by NIH NS 17944).

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL CHARACTERIZATION II

631.1

BOMBESIN EXISTS IN BOTH A LEU AND PHE FORM: IMPLICATIONS FOR THE CLASSIFICATION OF THE BOMBESIN-LIKE PEPTIDES. E.R. Spindel*, J. Taylor, B.J. Barry and S.R. Nagalla. Div. Neuroscience, Oregon Rgl. Primate Research Ctr., Beaverton OR 97006 and Biomeasure Inc., Milford MA 01757.

The bombesin-like peptides are a diverse family of peptides initially characterized in frog skin and later found to be widely distributed in mammals. The bombesin-like peptides have been classically divided into the bombesin/GRP subfamily, the ranatensin/NMB subfamily and the phyllolitorin subfamily. Distinguishing the bombesin subfamily from the ranatensin subfamily is the presence of a Leu or Phe as the penultimate C-terminal amino acid. We now show that this separation is incorrect as bombesin occurs in both a Phe and Leu form. Multiple cDNAs encoding bombesin from brain of *Bombina orientalis, Xenopus laevis* and phyllolitorin from *Phyllomedusa Sauvagei* were characterized. In all three species, two forms of peptide were encoded, one containing Leu and one containing Phe - and the Phe form strongly predominated in brain. cDNAs were highly homologous differing by only a few nucleotides. In *Xenopus* and *Bombina*, Phe and Leu bombesin appear to arise from separate genes. In *Phyllomedusa*, Phe and Leu phyllolitorin appear to arise by RNA editing (*Mol. Endocrinol.*, In Press). Phylogenetic analysis showed that the GRP and bombesin prohormones strongly diverge; that the NMB and ranatensin prohormones strongly diverge; but that there is strong homology between the bombesin, ranatensin and phyllolitorin prohormones. This suggests that the bombesin-like peptides should be reclassified into the GRP subfamily, the NMB subfamily and the bombesin subfamily. Consistent with this, Nagalla et al., have found 3 classes of receptors in frogs that correspond to these 3 peptide subfamilies (Abstract, Soc. Neurosci, 1994). These findings predict that mammals also have a (as yet uncharacterized) member of the bombesin subfamily and that it will exist in both a Phe and Leu form.

631.2

CONVERSION OF MET-ENKEPHALIN-ARG-PHE TO MET-ENKEPHALIN IN HUMAN SERUM. M.K. Leung*, K. Oscar, D. Dieudonne and N. Dowell. Neuroscience Research Institute and Department of Chemistry, SUNY/Old Westbury, Old Westbury, NY 11568-0210.

Both Met-enkephalin (YGGFM) and the opioid heptapeptide, Met-enkephalin-Arg-Phe (YGGFMRF), are reported to be capable of activating the same population of immune cells in the human circulatory system. Earlier study also showed I^{125}-YGGFMRF is converted by human serum to a product with the same HPLC retention time (Rt) as I^{125}-YGGFM. The aim of this study was to verify the conversion of YGGFMRF to YGGFM in human serum. Time-course study with pooled human serum (Pel-Freez, WI) showed YGGFMRF (50 uM) was processed rapidly to three major products with HPLC Rt's corresponding to that of GGFM, YGGFM and GGFMRF. The material with the Rt of YGGFM rose to its highest level in 1 min and remained at over 70% of this level after 5 min. The identity of the peptide material in this peak was verified with three procedures. 1) It was reactive against YGGFM antibody in RIA. 2) When derivatized with PITC the peptide derivative migrated with the same Rt as the derivatized YGGFM standard. 3) Amino acid analysis of the peptide with PTC technique showed the presence of Y, G, F and M in the ratio of 1:2:1:1 ås with YGGFM. Serum protease inhibitors studies showed the conversion of YGGFMRF to YGGFM is mediated by ACE and GGFMRF and GGFM are products of the action of aminopeptidase on YGGFMRF and YGGFM. Unlike YGGFMRF, the opioid octapeptide, Met-enkephalin-Arg-Gly-Leu, is not converted to YGGFM by human serum. Our results showed YGGFMRF is converted to YGGFM in human serum and it may be serving as a part of a mechanism for sustaining YGGFM concentration in the circulatory fluid.

Supported by NIDA DA-09010, NIMH-NIDA-COR NM-17138, and NIH GM-08180.

631.3

COMPARATIVE POTENCIES OF MID-PORTION CALCITONIN GENE-RELATED PEPTIDE ANALOGUES IN PROTOTYPICAL $CGRP_1$ AND $CGRP_2$ IN VITRO BIOASSAYS. D. Lavallée[2], Y. Dumont[1], D. Jacques[1], S. St-Pierre[2], A. Fournier[2]* and R. Quirion[1] (1) Douglas Hosp. Res. Ctr., Dept. Psychiatry, McGill Univ., Verdun, P.Q., Canada, H4H 1R3. (2) INRS-Santé, Pointe-Claire, P.Q., Canada, H9R 1G6.

Alanine substituted analogues and fragments of $hCGRP\alpha$, $hCGRP_{8-37}$ (a potent antagonist) and its linear-analogue $[Cys(ACM)^{2,7}]hCGRP\alpha$ (a $CGRP_2$ preferential agonist) were synthesized in order to investigate the respective role of amino acid residues between positions 14 to 23 for their abilities to induce an inotropic effect in the guinea pig atria and to inhibit contraction of the electrically-stimulated rat vas deferens, two prototypical $CGRP_1$ and $CGRP_2$ receptor bioassays, respectively (Dennis et al., J. Pharmacol. Expt. Ther. 254: 123-128, 1990). Results demonstrated that Leu^{16} and Arg^{18} are critical for the activation of CGRP receptors in both tissues; $[Ala^{16}]hCGRP\alpha$ being inactive. Interestingly, Ala substitutions of Gly^{20} and Gly^{21} residues generated analogues having similar or greater potencies than $hCGRP\alpha$ itself in the $CGRP_2$ assay, while being less potent than the native peptide in the $CGRP_1$ bioassay. $[Ala^{17}]hCGRP\alpha$ was found to be 2.5 fold more potent than $hCGRP\alpha$ in the $CGRP_2$ preparation while being somewhat less potent (63%) than the endogenous peptide in the guinea pig atria. The two analogues $[Cys(ACM)^{2,7},Ala^{17}]hCGRP\alpha$ and $[Cys(ACM)^{2,7},Ala^{21}]hCGRP\alpha$ were slightly more potent than the unmodified analogue on $CGRP_2$ receptors while being inactive on the $CGRP_1$ receptors. Additionally, Ala substitutions of the peptide antagonist $hCGRP_{8-37}$ demonstrated that $[Ala^{17}]hCGRP_{8-37}$ and $[Ala^{20}]hCGRP_{8-37}$ had slightly higher potencies than $hCGRP_{8-37}$ in blocking $hCGRP\alpha$ in the guinea pig atria while being ineffective in the rat vas deferens. Taken together, these results demonstrate the importance of the amino acid residues in positions 16, 17, 18 and 20 for adequate CGRP recognition and activation, and should be helpful toward the development of full selective analogues.

631.4

PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) IS UP-REGULATED IN THE RAT DORSAL ROOT GANGLIA AFTER PERIPHERAL NERVE INJURIES Y-Z. Zhang*, J. Hannibal, Q. Zhao, N. Danielsen, K. Moller, H. Mulder, E. Ekblad, J. Fahrenkrug and F. Sundler, Depts. Anesth., and Hand Surg., Malmö Gen. Hosp., Univ. Lund, S-21401 Malmö, Dept. Med. Cell Res., Univ. Lund, S-22362 Lund, Sweden and the Dept. Clin. Biochem., Bispebjerg Hosp., D-12345 Copenhagen, Denmark.

The effects of sciatic nerve injuries on the levels of PACAP and its mRNA in the dorsal root ganglia (DRG) and the spinal cord were investigated in rats. The sciatic nerve on the left side was either transected or crushed in anesthetized rats. The sciatic nerve on the right side was intact. The animals were killed 14 days postoperatively. The spinal cord L4-S1 and the DRG related to the sciatic nerve were dissected and processed for immunocyto-chemistry, immunochemistry, in situ hybridization and northern blot. Immunostaining of PACAP was seen in a population of mainly small nerve cells in the DRG of the control side, confirming our previous observations in normal DRG. PACAP was more intense in the DRG on the injured sides where PACAP was also seen in many large cells. In the dorsal horn of the spinal cord, transection reduced PACAP intensity whereas crush injury revealed no overt difference as compared to the control. The results from northern blot and in situ hybridization supported the immunocyto-chemical observations. The PACAP-mRNA in the DRG increased significantly after nerve crush or transection injury.

631.5

NEUROPEPTIDE-LIKE IMMUNOREACTIVITIES IN BOVINE BRAIN CLATHRIN COATED VESICLES. W.I. Silva*, L. Martinez, and N. Rosario. Department of Pharmacology, Universidad Central del Caribe, School of Medicine, Call Box 60-327, Bayamón, Puerto Rico 00621-6032.

Clathrin coated vesicles (CCV) participate in the proteolytic maturation of neuropeptides (NP) in the trans Golgi network (TGN), and potentially in the internalization of neuropeptides and their receptors. In this study CCV from bovine brain gray matter were purified after chromatography over Sephacryl S-1000 columns. Fractions (15-25ml) were collected and analyzed for their adaptor protein profile via SDS-PAGE, for their carboxypeptidase H (CPH) activity via radioenzymatic assays, and for their NP (NPY_{1-36}, CCK-8 and SS-14) content by means of radioimmunoassays. The results reveal the expression of CPH and neuropeptide-like immunoreactivities (NP-Li: NPY-Li, CCK-Li and SS-Li) associated with clathrin coated vesicles. The NP-Li and CPH were mainly associated with the ascending portion of the CCV peak suggesting that these molecules pertain to the expectedly larger diameter CCV subpopulation associated with the TGN, recycling and/or endocytic CCV. Analysis of the adaptor protein profile of the material eluting from the Sephacryl S-1000 column demonstrates a wide distribution of the Golgi-associated AP-1 (HA-I) molecules (AP47 and AP19) in relation to the CCV peak, spanning both the ascending and descending portions of the CCV peak. This adaptor protein pattern is consistent with the inherent size microheterogeneity of TGN CCV, large diameter CCV of the regulated secretory pathway and smaller diameter CCV of the lysosomal pathway. Analysis of NP and NP processing enzymes in brain CCV will yield valuable information about the factors and conditions that influence the post-translational maturation and activation of NP. (This work was supported by NIH grants NS27259 and RR03035 awarded to WIS).

631.6

AN OXYTOCIN (OXT) ANTISENSE OLIGONUCLEOTIDE (AS) INFUSED INTO THE SUPRAOPTIC NUCLEUS (SON) RAPIDLY ALTERS THE EXCITABILITY OF OXT NEURONS IN LACTATING RATS. **I. Neumann and Q.J.Pittman*. Neuroscience, Med. Physiol., Univ. of Calgary, Calgary, T2N 4N1, Canada.**

We, recently, described a rapid effect (within 4 hours) on suckling after infusion of OXT AS into the SON. In this study we asked, if this was due to reduced excitablity of OXT neurons. The femoral vein and artery and an abdominal mammary gland of urethane anesthetized, lactating rats were cannulated. Phosphorothioated OXT AS (2.5μg/0.5μl, n=8), mixed bases (MB, n=8) or saline (SAL, n=6) were infused into both SON. A stimulation electrode was placed into the neurohypophysial stalk. Injection of synthetic OXT increased mammary pressure in an identical fashion in all groups. Electrical stimulation of the stalk (50Hz, 4sec) increased mammary pressure due to stimulated OXT secretion from the neurohypophysis, but the threshold was higher in OXT AS treated rats (34.0±3.78 V versus 14.1±2.57 V [MB] and 10.0±2.23 V [SAL]; $p<0.001$) within 1.5 - 2 hours after treatment. Extracellular recordings from OXT neurons (identified by antidromic invasion from stalk, response to CCK-8 and non-response to nitroprusside) revealed threshold for antidromic stimulation higher in OXT AS rats (29.1±2.56 V) than in MB (18.5±3.08 V) or SAL rats (14.8±1.79 V, $p<0.001$). Furthermore, in OXT AS treated rats, the antidromic threshold was higher in OXT cells than in cells identified as vasopressinergic neurons (29.1±2.56 vs. 19.1±2.28 V).

These results demonstrate a rapid, local and selective effect of OXT AS on the excitability of OXT neurons.

Supported by HFSPO, MRC and AHFMR.

631.7

SUBCORTICAL LESIONS REDUCE CORTICAL GALANIN. S.M. Gabriel, V. Haroutunian, P.J. Knott.* Departments of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 and Bronx VA Hospital, Bronx, NY 10468.

GAL is associated with multiple long tract neurons, such as the cholinergic neurons of the nucleus basalis of Meynert [NBM], the noradrenergic neurons of the locus coeruleus and the serotonergic neurons of the dorsal raphe nucleus [DRN]. Lesions of each of these systems in the rat produce significant alterations in GAL concentrations in the frontal cerebral cortex. Following NBM lesions, decreases in cortical GAL depend on the magnitude of cholinergic depletion and lesion survival time. Lesions of the anterior noradrenergic bundle result in immediate reductions in cortical GAL concentrations which are comparable to the considerable depletion in norepinephrine. In contrast, biphasic effects of DRN lesions occur with increased GAL and serotonin 1 hour after DRN lesion and decreased GAL and serotonin 7 days after injury. Six weeks after a DRN lesion, GAL concentrations recover in the cortex despite, continued depletion of serotonin. These studies suggest a complex pattern for GAL regulation within the cerebral cortex, in which a large portion of the peptide is derived from multiple classical neurotransmitter efferents.

631.8

DISTRIBUTION OF DEAMIDASE ACTIVITY IN BOVINE BRAIN H. L. Jackman*, C. H. Anderson, and E. G. Erdös. Lab. of Peptide Research, Depts. of Pharmacol., Anesthesiol., and Anat. and Cell Biol., Univ. of IL Coll. of Med., Chicago IL 60612.

Deamidase is a lysosomal serine carboxypeptidase which has deamidase and esterase activities as well. It is identical with the lysosomal protective protein and probably with cathepsin A. The lack of this protein causes genetically determined galactosialidosis with involvement of the CNS. The enzyme inactivates peptides or modulates their actions (e.g., bradykinin, endothelin, and enkephalin hexapeptides, Jackman et al., J. Biol. Chem. 265:11265, '90; 267:2872, '92). We measured the specific activity of deamidase, with Dansyl-Phe-Leu-Arg as substrate, to study its distribution in the bovine brain. Areas that had the highest specific activity (nmol/h/mg) are the choroid plexus (288), the pineal body (155), and the pituitary (131). Areas with the lowest activity are the corpus callosum (65), the fornix (64), and the caudate (50). The activity in other regions of the brain (hippocampus, occipital cortex, frontal cortex, thalamus, hypothalamus, putamen, and amygdala) is between the high and low values given above. As deamidase cleaves a number of precursors and active peptides, and the lack of it causes a degenerative brain disease, the high activity found in some areas suggests a functional importance for this enzyme. (Supported by NIH grants HL36473, HL36081).

631.9

EXPRESSION OF ANTISENSE PC1 OR PC2 IN STABLY TRANSFECTED RIN 5F CELLS SIGNIFICANTLY REDUCES CELLULAR CONTENT AND SECRETION OF CCK 8. J. Y. Yoon* and M. C. Beinfeld. Dept. Pharm. Phys. Sci. St. Louis U. Sch. Med., St. Louis, Mo. 63104

The subtilisin-like enzymes PC1 and PC2 have been implicated in the processing of a number of pro neuropeptides. Northern analysis of total RNA from several cell lines, including RIN 5F, which express CCK mRNA and process pro CCK to CCK 8 indicates that both PC1 and PC2 transcripts are present. In order to evaluate whether PC1 or PC2 is involved in pro CCK processing, stable cell lines derived from Rin 5F cells were created which express either anti PC1 or anti PC2 mRNA. Cellular extracts and media were assayed for CCK 8 by radioimmunoassay. Individual clones that express anti PC1 or anti PC2 were identified that displayed a significant reduction of CCK 8 cell content and secretion. These results suggest an important role of both PC1 and PC2 in the processing of pro CCK to CCK 8. This work supported by NS 18667 and NS 31602.

631.10

ISOLATION OF A CANDIDATE cDNA CLONE FOR RAT BRAIN CCK 8 GENERATING ENZYME (CGE) BY EXPRESSION CLONING. M.C. BEINFELD*, L.R. ALLARD, AND T.B. USDIN. Dept. Pharm. Phys. Sci., St. Louis University Medical Center, St. Louis MO, 63104 and Laboratory of Cell Biology, NIMH, Bethesda, MD.

An enzyme capable of generating CCK 8 from CCK 33 was isolated and partially purified from rat brain synaptosomes in 1992 (Viereck and Beinfeld, J. Biol. Chem. 267:19475-19481). Using the hybrid vaccinia T7 infection/transfection system and a highly sensitive assay system using batchwise ion exchange and CCK RIA, we identified a single cDNA clone from a rat cerebral cortex library which when expressed in CV1 cells displayed enzymatic activity which was identical to the isolated enzyme including pH optimum, inhibitor sensitivity, and product formation. The clone is not present in the Gene Bank. Northern Analysis of the distribution of its mRNA in a variety of endocrine tumor cells which express CCK mRNA and which process it to CCK 8 is consistent with a role in the processing of pro CCK. Additional clones were isolated which differ in inhibitor specificity or product formation. Supported by NIH grants NS 18667 and NS 31602 (to M.C.B.).

631.11

FACTORS PREDICTING AMINO ACID INCORPORATION IN THERMAL PROTEINS. LeDuc, R.L.*, D.C. Smith and S.W. Fox. Dept. of Psychology, Southern Illinois University, Carbondale IL 62901 and University of South Alabama.

Studies of the minimum excitable biological membrane depend on an understanding of the self-organizing abilities of amino acids (AA). Thermal proteins (TP) are the leading model of self-organization of amino acids. Here multiple linear regression is used to determine correlation's between individual AA and their concentration in TP polymers.

The multiple regression analysis used a previously published series of 11 TP reactions using 11 AA each, from which crude hydrolysis data were available. Data consisted of paired values of the mole percent of each of 11 AA in the reaction mixture and the resulting polymer. A stepwise multiple linear regression was performed on the reaction concentration of all AA in the mixture to predict the concentration for each AA in the crude product. Additionally, 11 multiple linear regression analyses were performed to predict the concentration of each AA using the concentrations of all the other AA in the crude TP. In each of these cases the AA in question was excluded from the regression analysis. All equations were cross-validated. Eight equations predicting AA concentration in the polymer from the reaction conditions cross-validated. In each case the reaction concentration of the given AA was the best predictor of the concentration of the AA in the polymer. Only three equations predicting AA concentration in the TP polymer from all other AA in the polymer cross-validated. Aspartic acid (Asp) and glutamic acid (Glu) covaried (Multiple R=.975) and isoleucine was predicted from valine (Multiple R=.988). Thus the best predictors of TP polymer composition are the properties of the specific AA and not the presence or absence of other AA (except for Asp and Glu). Supported by NSF-IBN-9223377 to D.C. Smith and S.W. Fox.

SEROTONIN RECEPTORS: PHYSIOLOGY

632.1

5-HT_{1D} RECEPTORS REGULATE SOMATODENDRITIC RELEASE OF 5-HT: NEUROCHEMICAL STUDIES IN THE RAT RAPHE NUCLEI. G. Piñeyro*, C. de Montigny and P. Blier. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada, H3A 1A1.

Classically, 5-HT_{1A} receptors which modulate the firing activity of 5-HT neurons have also been suggested to control the somatodendritic release of 5-HT. We have recently shown that the somatodendritic 5-HT release is regulated independently of firing frequency via 5-HT_{1D} receptors (*Neurosci. Abst. 93.4, 1993*). The present study further assessed the regulation of somatodendritic release of 5-HT. In a first series of experiments, voltammetry studies using nafion-coated carbon fiber electrodes showed that the non-selective 5-HT_1 agonist TFMPP (0.5 mg/kg, i.v.) gradually reduced extracellular 5-hydroxyindoles in the dorsal raphe over a two-hour period. This effect was prevented by mianserin (2 mg/kg, i.v.), which blocks rat 5-HT_{1D} but not 5-HT_{1B} receptors, but not by the selective 5-HT_{1A} antagonist (+)WAY 100135 which effectively blocked the reducing effect of 8-OH-DPAT (30 µg/kg, i.v.) on the voltammetric signal. The receptor subtype(s) mediating inhibitory control of somatodendritic release of 5-HT was then assessed with electrically evoked release of [^{3}H]5-HT from slices of the mesencephalon containing the dorsal and median raphe nuclei. The evoked release of [^{3}H]5-HT was Ca^{+2} dependent. Neither TFMPP (1 nM - 1 mM) nor the selective 5-HT_{1B} agonist CP 93,129 (1 nM - 10 µM) affected the evoked release of [^{3}H]5-HT. However, the non-specific 5-HT_1 agonist 5-CT and the 5-HT_{1D} agonist sumatriptan, dose-dependently reduced electrically the evoked release of [^{3}H]5-HT, attaining maximal inhibitions of 80% and 100% respectively, at 1 µM. Mianserin (1 µM) blocked the effects of both agonists. The present results suggest that 5-HT_{1D} receptors locally control extracellular 5-HT concentration in the raphe nuclei and may thus consequently play an important role in controlling 5-HT release in widespread projection areas.

632.2

MODULATORY EFFECT OF 5-HYDROXYTRYPTAMINE (5-HT) ON CRAYFISH NEUROSECRETORY CELLS. F. Sáenz, U. García and H. Aréchiga*. Dept. Fisiología, Biofísica y Neurociencias, CINVESTAV, IPN México and *División de Estudios de Postgrado e Investigación, Facultad de Medicina, UNAM, México

5-Hydroxytryptamine (5-HT) has been shown to influence the release of neurohormones from the crustacean eyestalk (see Fingerman and Nagabushanam, Comp. Biochem. Physio. 71C:195,1992). Its possible mechanism of action on neurosecretory cells, is largely unexplored. In the present work, 5-HT was applied onto neurosecretory cells in the X-organ of the crayfish eyestalk. The effects were tested in the same population of neurons, both in situ, in isolated eyestalks and in cultured cells, taken from the same population. Two effects were found: a) a long-lasting (up to 15 min) dose-dependet (within a range of 1-100 µM) depolarizing response, which could result in the firing of action potentials in previously silent neurons, or in the enhancement of ongoing activity. There was a strong desensitization. This response was blocked by methysergide. b) a long-lasting hyperpolarization, with similar temporal and pharmacological properties to the depolarizing response. Under voltage-clamp, these effects are associated to an outward current with a reversal potential around -90 mV, thus suggesting a potassium channel as the target of 5-HT effects. No differences were found in the responses of X organ cells to 5-HT when tested in situ as compared with isolated neurons in culture.

This work was supported by CONACyT grant number 0804-N9110.

632.3

THE 5-HYDROXYTRYPTAMINE (5-HT)$_4$ MEDIATED DECREASE IN THE SLOW AFTERHYPERPOLARIZATION IS MODIFIED BY CORTICOSTERONE. S. Birnstiel* and S.G. Beck. Dept. of Pharmacology, Loyola Univ. Med. Ctr., Maywood, IL 60153.

In response to stressful stimuli, corticosterone (CT) production is increased by the hypothalamic-pituitary-adrenal (HPA) axis. One site of feedback regulation is the hippocampus, which contains high densities of both mineralocorticoid (MR) and glucocorticoid (GR) receptors. Basal plasma CT levels occupy the MR; high concentrations of CT activate the MR and the GR, both of which are implicated in the feedback control of the HPA. Activation of 5-HT_4 receptors decreases the slow afterhyperpolarization (sAHP) elicited by a train of action potentials or a calcium spike. To investigate if CT modifies the 5-HT_4 receptor mediated decrease of the sAHP, intracellular recordings of the sAHP following a calcium spike were obtained from the CA1 pyramidal cell layer of transversal hippocampal slices. Male Sprague-Dawley rats were adrenalectomized (ADX) 14 days prior to recording. One ADX group was chronically treated with a CT pellet (12.5 mg) to mimic basal plasma CT levels. Another ADX group was implanted with 300 mg CT to induce chronic stress. Data for concentration-response curves were generated by addition of 5-HT in half log unit decrements (0.1-100 µM). Preliminary results suggest that the EC_{50} of the 5-HT induced decrease in sAHP amplitude is higher in stressed animals than in ADX animals. When 1 nM CT was added to the superfusion medium of slices from ADX animals, the decrease in AHP amplitude was significantly more pronounced, indicating a short term modulatory role of the MR. The modulatory effects of CT on the 5-HT mediated response may underlie feedback control of the HPA and may influence pyramidal cell excitability. Supported by NS28512 and MH00880.

632.4

RECEPTOR RESERVE FOR 5-HT_{1A} RECEPTORS IN RAT BRAIN AS ASSESSED BY NEUROENDOCRINE RESPONSES TO 8-OH-DPAT STIMULATION. W. Pinto*, T. Cabrera, Q. Li, L. Van de Kar and G. Battaglia Neuroscience Graduate Program and Dept. of Pharmacology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.

The present study investigates receptor reserve in female rats with respect to 5-HT_{1A} mediated neuroendocrine responses. While previous studies have reported a large reserve for pre- and postsynaptic 5-HT_{1A} receptors in male rats, 5-HT_{1A} receptor reserve in female rats has yet to be determined. The stimulation of postsynaptic, hypothalamic 5-HT_{1A} receptors with the full agonist 8-OH-DPAT, elicits an increase in several plasma hormones including ACTH and corticosterone. Therefore, neuroendocrine responses to 5-HT_{1A} stimulation can be used as a functional end-point to assess receptor reserve. Female, adult rats (350-400 gms) were administered a single s.c. injection of vehicle (1:1 v/v EtOH/H_2O) or 1 mg/kg EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline), an ireversible receptor inactivator. 24 hours post-treatment, rats were challenged with either vehicle (0.9% saline) or increasing doses (0.05, 0.2 and 0.5 mg/kg s.c.) of 8-OH-DPAT and sacrificed 30 mins later. There was a significant ($p < 0.001$) reduction (53%) in the Bmax of ^{3}H-8-OH-DPAT labeled hypothalamic 5-HT_{1A} receptors in EEDQ-treated rats, with no alteration in K_D. In non-EEDQ treated rats, 8-OH-DPAT elicited a significant ($p < 0.001$) dose-dependent elevation of plasma ACTH (8.4 fold at highest dose) and corticosterone (3.7 fold at highest dose). Following receptor inactivation by EEDQ, there was no significant difference in the maximal ACTH (868.3 ± 148 vs 1025 ± 188 pg/ml) or corticosterone (43.4 ± 6.3 vs 50.5 ± 8.8 µg/dl) elevation elicited by 8-OH-DPAT. These data provide evidence for a large receptor reserve (at least 53%) for hypothalamic 5-HT_{1A} receptors mediating the elevation of plasma ACTH and corticosterone levels in female rats.

(Supported by DA07741 and Loyola University Potts Foundation)

632.5

MODULATION OF RAT NEOCORTICAL HIGH-VOLTAGE SPINDLE ACTIVITY BY SEROTONIN RECEPTOR SUBTYPE SPECIFIC DRUGS. P. Jäkälä, J. Sirviö, J. Kaukua, E. Koivisto, M Björklund, L. Yavich, P. Riekkinen Sr. and P. Riekkinen Jr. *. Dept. of Neurology, Univ. of Kuopio, P.O.B. 1627, SF-70211 Kuopio, Finland. Fax: 358-71-162048.

The present experiments investigated the role of serotonergic (5-hydroxytryptamine, 5-HT) receptors in the modulation of rat neocortical high-voltage spindle activity. We administered different 5-HT_1/5-HT_2 receptor subtype specific drugs singly or in combination either systemically or via cannulae that were implanted unilaterally to the ventromedial thalamus, and measured neocortical high-voltage spindle activity of adult rats by IBM software. A mixed 5-HT_1/5-HT_2 receptor antagonist, methiothepine (i.p.), significantly increased neocortical high-voltage spindle activity, whereas another mixed 5-HT_1/5-HT_2 receptor antagonist, methysergide (i.p.), had no effect. A 5-HT_{2A}/5-HT_{2C} receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (s.c.), significantly decreased neocortical high-voltage spindle activity, and systemic methiothepine and specific 5-HT_{2A}/5-HT_{2C} receptor antagonists ketanserin (s.c.) and ritanserin (s.c.) blocked this effect. Furthermore, intrathalamic infusions of DOI dose-dependently decreased neocortical high-voltage spindle activity and systemic ketanserin completely blocked this effect. The results suggest that 1) the serotonergic system may via 5-HT_{2A}/5-HT_{2C} receptors modulate rat thalamocortical arousal as assessed by neocortical high-voltage spindle activity, 2) 5-HT_{2A}/5-HT_{2C} receptor agonists may decrease neocortical high-voltage spindle activity, and 5-HT_{2A}/5-HT_{2C} receptor antagonists may block this effect, 3) the effects of 5-HT_{2A}/5-HT_{2C} receptor subtype specific drugs on rat neocortical high-voltage spindle activity may be at least partly mediated at the thalamic level, suggesting that activation of thalamic 5-HT_{2A}/5-HT_{2C} receptors may increase the efficacy of transfer of information through the thalamus to the cortex.

632.6

EFFECTS OF 5-HT ON NMDA AND NON-NMDA RECEPTOR-MEDIATED RESPONSES IN TRIGEMINAL MOTONEURONS. **P.R. Trueblood*, S.H. Chandler, M.S. Levine, Department of Physiological Science and Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.**

Previously, we demonstrated that 5-HT potentiated both the NMDA and non-NMDA mediated EPSP evoked by Mesencephalic V stimulation in trigeminal motoneurons using an in-vitro preparation of the brainstem from guinea pigs. To further examine the mechanism by which 5-HT facilitates excitatory amino acid responses in trigeminal motoneurons, we tested the effects of .5-20 μM bath application of 5-HT to NMDA (200mM) and AMPA (5 mM) iontophoretic responses. In current clamp mode, 5-HT significantly potentiated both the NMDA and AMPA response in a dose-dependent manner. Selective agonists and antagonists were used to show that this potentiation resulted from activation of 5-HT_{1C} receptors. Furthermore, using voltage clamp technique, 5-HT enhanced both the NMDA and AMPA current, suggesting that 5-HT has a direct modulatory role to these receptors, in addition to a potentiating effect to the intrinsic properties of trigeminal motoneurons. Therefore, 5-HT may function to facilitate the afferent input to trigeminal motoneurons by potentiation of both NMDA and non-NMDA receptor-mediated synaptic processes. Supported by NIH grants DEO6193 and DEO7212.

632.7

TOLERANCE AND CROSS-TOLERANCE OF HALLUCINOGENS: ELECTROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES. R.Y. Wang*, E. Edwards and J.Y. Zhang. Dept. Psychiatry (R.Y.W. and J.Y.Z.), SUNY at Stony Brook, Stony Brook, NY 11794-8790 and Dept. Pharmacol. (E.E.), Univ. Maryland at Baltimore, Baltimore, MD 21201-1180.

We have previously shown that local bilateral injection of hallucinogens d-LSD and (-)-DOB, but not non-hallucinogenic congeners lisuride and BL 3912A, into the medial prefrontal cortex (mPFC) elicited the head twitching response (HTR). This HTR was prevented by the selective 5-HT_{2A} receptor antagonists. It was proposed that 5-HT_{2A} receptors in the mPFC could play an important role in mediating the action of hallucinogens. Iontophoresis of low currents of (-)-DOB, and to a lesser extent d-LSD, markedly potentiated glutamate-induced activation of the firing of mPFC cells. The aim of the present study was to decide whether pretreatment with mianserin or subacute treatment of d-LSD, which is known to down-regulate the 5-HT_{2A} receptors in the frontal cortex, would induce the development desensitization of mPFC cells to d-LSD and (-)-DOB. Groups of rats were treated with d-LSD (.2 mg/kg/day, i.p. for 5 days; experiments began 24 hr after the last injection), mianserin (10 mg/kg, i.p. 48 - 72 hr prior to experiment), and saline (1 µl/kg, i.p. either for 1 or 5 days), respectively. Compared to cells in the saline group, mPFC cells in rats pretreated with either d-LSD or mianserin became subsensitive to both d-LSD and (-)-DOB. Furthermore, in these drug pretreated rats, both d-LSD and (-)-DOB became less effective in stimulating the phosphoinositide hydrolysis in the mPFC. In conclusion, our results suggest that hallucinogen-induced tolerance and cross-tolerance may be accounted for, at least partly, by the functional desensitization of mPFC 5-HT_{2A} receptors at the cellular level. (Supported by USPHS grants MH-41440 and DA-07193).

632.8

A POSSIBLE ROLE OF 5-HT2 RECEPTORS FOR SYNAPSE FORMATION. N. Okado*, Y. Niitsu, and S. Hamada Lab. of Neurobiol., Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba, Ibaraki 305, Japan.

Serotonin appears to enhance formation and maintenance of synapses in developing as well as adult brain and spinal cord (J. Neurobiol. Vol. 24; Neurosci. Res. Vol. 19). The present study was undertaken to clarify the specific receptor subtype involved in synapse formation. One of serotonin antagonists specific to each serotonin receptor subtype was daily injected in eggs between embryonic day (E)11 and E17. Embryos were deeply anesthetized and perfused on E18. The density of axosomatic synapses on motoneurons in the lateral motor column of lumbar spinal cord was calculated from electron micrographs. Following injections of 5 mg (N=4) and 50 mg (N=4)/kg weight of ketanserin the synaptic density decreased by 15 % ($P<0.03$) and 40 % ($P<0.0002$) compared to that of control animals (N=4), respectively. Although further studies are necessary to conclude 5-HT2 receptors are involved in facilitation of formation and maintenance of synapses, the result of present study may explain synaptic loss and reduction of 5-HT2 receptors in the aged and Alzheimer brain.

632.9

FUNCTIONAL EVIDENCE FOR THE DIFFERENTIAL RESPONSIVENESS OF PRE- AND POSTSYNAPTIC 5-HT_{1A} RECEPTORS IN THE RAT BRAIN. P. Blier*, B. Seletti, C. Bouchard, F. Artigas and C. de Montigny. Neurobiological Psychiatry Unit, McGill Univ., Montreal, Canada H3A 1A1.

Several groups have documented the capacity of (±)pindolol to antagonize the hypothermic effect of 5-HT_{1A} agonists, including that of 8-OH-DPAT, whereas the increase in prolactin produced by the latter 5-HT_{1A} agonist is not blocked by similar doses of (±)pindolol (*Eur. J. Pharmacol.* 146: 253, 1988). In a first series of experiments, the effectiveness of pindolol to block presynaptic 5-HT_{1A} autoreceptors was examined by assessing the firing activity of dorsal raphe 5-HT neurons in rats treated for 2 days with the 5-HT reuptake blocker paroxetine (10 mg/kg/day, s.c.) alone or together with (±)pindolol (10 mg/kg/day, s.c.), both drugs being delivered by an osmotic minipump. The firing rate of 5-HT neurons under chloral hydrate anesthesia was decreased by about 75% in both groups. However, the firing rate of 5-HT neurons was within the control range in rats treated with paroxetine and 15 mg/kg/day of (-)pindolol (which is the enantiomer with 5-HT_{1A} affinity) for 2 days. In keeping with the latter observations, the suppressant effect of the 5-HT autoreceptor agonist LSD on the firing of 5-HT neurons was markedly attenuated. In a second series, the prolactin increase produced by 8-OH-DPAT (100 µg/kg, s.c.) was also prevented by (-)pindolol (15 mg/kg, i.p.) given 30 min prior to 8-OH-DPAT. The inhibitory effect of iontophoretic applications of 5-HT and 8-OH-DPAT on the firing rate of hippocampus CA_3 pyramidal neurons was however unaltered in rats treated with (-)pindolol (15 mg/kg/day X 2 days). These data indicate that a high dose of (-)pindolol can block the presynaptic 5-HT_{1A} receptor modulating 5-HT neuronal firing activity and the postsynaptic 5-HT_{1A} receptor controlling prolactin release, but not the postsynaptic 5-HT_{1A} receptor coupled to K+ channels in the hippocampus.

632.10

REGULATION OF BASAL FOREBRAIN MONOAMINE SYNTHESIS BY 5-HT RECEPTORS IN THE RAT SUBSTANTIA NIGRA PARS RETICULATA (SNr) AND VENTRAL TEGMENTAL AREA (VTA). S.Ahlenius[1]*, V.Hillegaart[1] and O.Magnusson[2]. Departments of Behavioral Pharmacology[1] and Neuropharmacology[2], Astra Arcus AB, S-151 85 Södertälje, Sweden.

The 5-HT_{1A} receptor agonist 8-OH-DPAT (0-5 µg), or 5-HT (0-40 µg), was infused into the SNr or into the VTA in awake, gently hand-restrained male Wistar rats, at a rate of 0.33 µL min^{-1} to a final volume of 0.5 µL. Upon completion of the infusion, the animals received an injection of the cerebral decarboxylase inhibitor NSD-1015 (100 mg kg^{-1} IP) and were decapitated for brain dissections 30 min later (medial prefrontal cortex, d-l neostriatum, nucleus accumbens, olfactory tubercle and amygdala). The *in vivo* rate of forebrain 5-HT and DA synthesis was estimated by measuring the accumulation of 5-HTP and DOPA (n=3-5). Stimulation of 5-HT_{1A} receptors by 8-OH-DPAT in either SNr or the VTA produced a dose-dependent, and statistically significant, decrease in forebrain 5-HT synthesis, whereas DA synthesis was only decreased in the prefrontal cortex and amygdala and only after a VTA infusion. In sharp contrast, a 5-HT infusion into the SNr or the VTA did not affect forebrain 5-HT synthesis. However, there was a marked increase in the DA synthesis of the neostriatum after SNr application, and to a lesser extent also after VTA infusion. It is suggested that 5-HT_{1B} receptors on midbrain DA somata and/or dendrites exert an inhibitory control of firing and release in dopaminergic neurons. The effects of 8-OH-DPAT demonstrate an important role of 5-HT_{1A} receptors in the SNr and VTA for the regulation of forebrain 5-HT synthesis. 5-HT_{1A} receptors in the VTA were also found to selectively affect DA synthesis in meso-cortical dopaminergic projections.

632.11

EFFECTS OF BUSPIRONE ON 5-HT TURNOVER IN DIABETIC AND NONDIABETIC RATS L.L. Bellush* and A.M. Wright . Dept. of Psychology, Ohio University, Athens, OH 45701.

Diabetic (D) rats have reduced 5-HT turnover throughout the brain. They also show attenuated hypothermia to the 5-HT1A agonist 8-OHDPAT, as well as enhanced response to stress and greater anxiety, both of which could reflect altered 5-HT functioning. However, anxiolytic effects of the 5-HT1A partial agonist buspirone (BUSP) did not differ in D and nondiabetic (N) rats, and anxiolysis was greater after chronic BUSP. Here we measured 5-HT turnover in brainstem, midbrain, hippocampus and hypothalamus after single BUSP injections or after two weeks of daily treatment. BUSP had no effect on 5-HT turnover 20 min after acute treatment BUSP (0.1 mg/kg) in either D or N. BUSP did not affect light/dark emergence either, although D were more anxious than N., as well as having lower 5-HT turnover. BUSP (0.1, 0.6 and 1.2 mg/kg) reduced 5-HT turnover in all 4 regions of N rats 30 min after drug treatment. Here, the lower doses tended to reduce anxiety, while the high dose was anxiolytic. Assessment of 5-HT turnover after chronic BUSP 0.1 and 0.6 mg/kg in D and N is now being completed.

632.12

SEROTONERGIC FIBERS ARE NOT DEVELOPED IN THE TRANSPLANTED HIPPOCAMPAL TISSUE OF THE S-100β RETARDED MUTANT MOUSE. S.Ueda*, P.M. Whitaker-Azmitia, E.C. Azmitia and M. Kawata. Dep. of Anatomy, Kyoto Pref. Univ. of Med.,Kyoto 602, Japan, Dep of Psychiatry, SUNY at Stony Brook, NY11794, and Dep. of Biology, NYU, NY10003, USA.

Our previous study (Ueda et al. 1994 Brain Res. 633,275-283) has demonstrated that the hippocampus of the homozygote of polydactyly mutant mouse (Polydactyly Nagoya, Pdn/Pdn) was markedly reduced in both S-100β positive astrocytes and serotonergic fibers as compared to the heterozygote (Pdn/+) and wild type (+/+). Since the Pdn/Pdn mice die within 2 days after birth, it is imposible to examine postnatal changes in development. To demonstrate the developmental change of Pdn/Pdn hippocampal tissue, hippocampal pieces of neonatal Pdn/Pdn and +/+ mice were transplanted into the right and left sides hippocampus of the same adult ICR mice, respectively, and immunocytochemistry was performed. Two weeks after transplantation, +/+ hippocampal tissue contained a large number of GFAP and S-100β positive astrocytes and a small number of serotonergic fibers. Pdn/Pdn hippocampal tissue contained numerous GFAP positive astrocytes, while S-100β positive astrocytes and serotonergic fibers were not observed. Two months after transplantation, GFAP and S-100β were expressed in the Pdn/Pdn hippocampal tissue similar to the +/+ tissue. Serotonergic fibers were distributed in the +/+ tissue, whereas few serotonergic fibers were observed in the Pdn/Pdn transplant tissue. On the contrary no difference was observed in the tyrosine hydroxylase positive fibers between Pdn/Pdn and +/+ graft.

632.13

5-HT_{1A} ANTAGONISTS AND TERMINAL 5-HT RELEASE. EFFECTS OF THE COMBINED TREATMENT WITH 5-HT UPTAKE INHIBITORS. F. Artigas*, L. Romero, P. Celada and N. Bel. Dept. of Neurochemistry, C.S.I.C. Barcelona, Spain

Antidepressant drugs of different types increase extracellular 5-HT in the raphe nuclei of the midbrain. As a result, somatodendritic autoreceptors are activated and terminal release decreases. Thus, the local infusion of 50 μM citalopram (CIT, a 5-HT uptake inhibitor) into the dorsal raphe nucleus (DRN) reduced *in vivo* release of 5-HT in striatum of the same animals by -47 %. Pretreatment with (-) pindolol (15 mg/kg i.p.) or (-) tertatolol (10 mg/kg i.p.) totally abolished intra-DRN effects of CIT, indicating that these reductions were mediated by 5-HT_{1A} autoreceptors. Lower doses of (-) pindolol (5 and 10 mg/kg, i.p.) induced a partial antagonism. In contrast, (+) WAY 100135 at a dose (10 mg/kg, i.p.) that antagonized the effects of 8-OH-DPAT (0.1 mg/kg, s.c.) on striatal release, was unable to prevent the CIT-induced reduction. These results indicate that the effects of 5-HT on somatodendritic 5-HT_{1A} autoreceptors are antagonized by (-) pindolol and (-) tertatolol, but not by (+) WAY 100135. The previous (1-3 days before) intra-DRN injection of pertussis toxin also prevented the effects of CIT on striatal 5-HT release. This further supports the role of 5-HT_{1A} autoreceptors in the attenuation of terminal 5-HT release elicited by 5-HT uptake inhibitors. In agreement, the combined treatment with (-) pindolol and 5-HT uptake inhibitors (paroxetine, citalopram, fluvoxamine, fluoxetine) increased terminal 5-HT release to a greater extent than the 5-HT uptake inhibitors alone (e.g., (-) pindolol + 1 mg/kg CIT elevated striatal 5-HT release by +600 % whereas the increase produced by 1 mg/kg CIT was +150 %). Thus, blockade of somatodendritic 5-HT_{1A} receptors may be a new way to potentiate the effects of low doses of 5-HT uptake inhibitors, in agreement with recent clinical data (Artigas et al., *Arch. Gen. Psychiatry* 51:248-251, 1994)

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632.14

5-HT_4 RECEPTOR STIMULATION FACILITATES THE IN VIVO RELEASE OF ACETYLCHOLINE IN RAT FRONTAL CORTEX. H. Ladinsky*, S. Arnaboldi[1], G. Russi[1], G.B. Schiavi and S. Consolo[1]. Department of Biochemistry and Molecular Pharmacology, Boehringer Ingelheim Italia, Milan 20139 and [1]Laboratory of Neuropharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Milan 20157 Italy.

The effect of the serotonergic 5-HT_4 receptor agonists BIMU 1 and BIMU 8 on the in vivo release of ACh in brain hemispheric regions of freely moving rats was investigated using the microdialysis technique. Both agonists, applied intracerebroventricularly, facilitated ACh release selectively in the frontal cortex at doses of 30-100 nmol. The agonists were ineffective in striatum, despite the high density of 5-HT_4 receptors in this region. BIMU 1, 60 nmol i.c.v., produced a significant decrease in ACh release in the dorsal hippocampus. The facilitatory effect of BIMU 1 (40 nmol, i.c.v.) in frontal cortex was prevented by the highly selective and potent 5-HT_4 receptor antagonists GR 125487 (10 and 20 nmol, i.c.v.) and GR 113808 (15 nmol, i.c.v.), which by themselves did not alter basal ACh release. The results provide evidence that serotonin facilitates ACh release in frontal cortex through stimulation of 5-HT_4 receptors that are not tonically activated. Assuming that ACh plays a role in cognitive processes, this facilitation supports the theory that 5-HT_4 sites are important in the control of learning and memory. 5-HT_4 receptor activation might thus offer a novel means of boosting central cholinergic function to overcome the cholinergic deficit in memory disorders. Financed in part by CNR, Rome, Convenzione Psicofarmacologia (S.C.).

632.15

ESTRADIOL ENHANCES THE BRADYCARDIA ELICITED BY THE CENTRAL ADMINISTRATION OF THE 5-HT_{1A} AGONIST 8-OH-DPAT. R.H. Alper* and T.M. Schmitz. Department of Pharmacology, The University of Kansas Medical Center, Kansas City, KS 66160-7417.

Activation of central 5-HT_{1A} receptors by the selective agonist 8-OH-DPAT decreases blood pressure (BP) and heart rate (HR). This study was to determine if the steroid hormone estradiol (E_2) modulates the cardiovascular responses elicited by 8-OH-DPAT when administered into the lateral cerebral ventricle (ICV). Experiments were performed in conscious, freely-moving rats implanted chronically with a guide cannula for ICV drug infusions and an arterial catheter to monitor BP and HR. 8-OH-DPAT (100 nmoles, ICV) decreased BP similarly in male and ovariectomized female (OVX) rats. There was a tendency for a greater bradycardia in the OVX rats, but this did not attain statistical significance. The experiment was repeated to include OVX rats implanted subcutaneously with time-release pellets containing E_2 (0.1 mg/21 day pellet). The BP response to 8-OH-DPAT was the same in all 3 groups: male, OVX and OVX + E_2. By contrast, 8-OH-DPAT produced a greater bradycardia in the OVX + E_2 group when compared to either male or OVX rats; OVX again tended to be greater than male. In the final experiment, graded doses of 8-OH-DPAT (3 - 100 nmoles, ICV) were administered to OVX and OVX + E_2 rats. The hypotensive effect produced by 8-OH-DPAT was not altered by hormone treatment, but the bradycardia was greater across doses in the E_2-treated rats. In summary, the cardiovascular responses elicited by 8-OH-DPAT were not significantly different between male and OVX rats. However, E_2 enhanced the bradycardia, but not the hypotension, elicited by 8-OH-DPAT, suggesting that estrogen modulates some, but not all, functional responses produced by central 5-HT_{1A} receptor activation.

[Supported by NIH Grant NS 29765]

633.1

CHARACTERIZATION OF [³H]8-OH-DPAT BINDING TO HUMAN CEREBRAL CORTEX. E.K. Nénonéné* and T.A. Reader. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Faculté de médecine, Université de Montréal (Québec) CANADA H3C 3J7.

A series of saturation and competition experiments were conducted with the selective serotonin (5-HT) agonist [³H]8-OH-DPAT to investigate the properties of the 5-HT1A receptors from the temporal region of human cerebral cortex. The binding parameters of the saturation curves were best fitted to a two-site model, thus giving two dissociation constants, *i.e.* : KH and KL of 1.22 nM ± 0.30 and of 12.63 ± 1.54 for the high- and the low-affinity binding sites, respectively. Using a 1 nM concentration of the radioligand, the pharmacological profile of the 5-HT1A receptors from human cerebral cortex was studied by competitions with 5-HT and the specific agonists 8-OH-DPAT and buspirone, as well as with the antagonists pindolol, NAN-190, and WAY 100148. In all cases, both the agonists and the antagonists competed with [³H]8-OH-DPAT binding in a biphasic manner, revealing high- and a low-affinity sites. The competition curves with these drugs gave two inhibition constants (KI) of nanomolar and micromolar values, respectively. Furthermore, kinetic experiments carried out at 1 or 10 nM concentrations of [³H]8-OH-DPAT showed that the equilibrium was reached after 30-45 min incubation at 25°C. Even though the association curves were best fitted to a one-site model, two dissociation rate constants could be documented. It can be concluded from these observations that, as was the case for the rat cortex (Nénonéné et al. 1994; *J. Neurochem.* **62**, N° 5), human cerebral cortex also contains two different and heterogenous populations of 5HT1A receptors. *[Supported by the Réseau de Recherche en Santé Mentale FRSQ / Université de Montréal].*

633.2

A NEW 5-HT$_{1A}$ RECEPTOR ANTAGONIST: MPPI. M.-P. Kung*, Z.-P. Zhuang, D. Frederick, M. Mu and H. F. Kung. Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Previously, a new radioiodinated agonist, [I-125]8-OH-PIPAT, for 5-HT$_{1A}$ receptors, was reported (Kung et al., Neuroscience Abs., 1993). In developing antagonists for 5-HT$_{1A}$ receptors, we have recently prepared several iodinated derivatives of the phenylpiperazine (Zhuang et al., J. Med Chem., in press). Among them, MPPI (4-(2'-methoxy-phenyl)-1-[2'-(n-2"-pyridiny)-p-iodobenzamido-]ethyl-piperazine) displayed high binding affinity for 5-HT$_{1A}$ receptors (Ki=1.0 nM, rat hippocampal homogenates). Low to moderate binding affinity of MPPI to D2, α1, α2, ß and 5-HT$_2$ was observed with Ki values of 19, 35, 181, 740 and 270 nM, respectively. Saturation binding studies in rat hippocampal homogenates revealed that [I-125]MPPI bound to a single population of affinity sites with a K_d of 0.18 nM (no $MgCl_2$) and a B_{max} of 320 fmol/mg protein. In parallel studies, the density of sites labeled with [I-125]MPPI was 30-40% higher than that with [I-125]8-OH-PIPAT. Guanylyl nucleotides did not affect the specific binding of [I-125]MPPI, but significantly inhibited the specific binding of the radioiodinated agonist, [I-125]8-OH-PIPAT. In addition, Mg^{+2} ion showed an inhibitory effect on MPPI binding, in contrast to the stimulatory effect observed for the agonist ligand, 8-OH-PIPAT. Both agonists and antagonists inhibited [I-125]MPPI binding with K_i values consistent with pharmacological profile of binding to 5-HT$_{1A}$ receptors. Autoradiographic studies with [I-125]MPPI in rat brain sections further illustrated the anatomical distribution of 5-HT$_{1A}$ sites. In summary, [I-125]MPPI displayed antagonist-like binding properties with high affinity and selectivity for 5-HT$_{1A}$ receptors. In combination with the potential radioiodinated agonist, [I-125]8-OH-PIPAT, they may provide powerful tools for studies of the pharmacology of the 5-HT$_{1A}$ receptor system. Acknowledgment: support from PHS (NS-24538 and MH-48125).

633.3

CHARACTERIZATION OF AGONIST AND ANTAGONIST RADIOLIGANDS AT 5-HT$_{1A}$ RECEPTORS. K. D. Burris*, H. A. Miller, J. G. Hensler, M.-P. Kung, H. F. Kung and P. B. Molinoff. Depts. of Pharmacology and Radiology, Univ. of Pennsylvania, Phila., PA 19104.

CHO cells expressing transfected 5-HT$_{1A}$ receptors (CHO-5HT$_{1A}$ cells) were used to characterize iodinated radioligands. An iodinated derivative of 8-OH-DPAT, 8-hydroxy-2-[N-propyl-N-(3'-iodo-2'-propenyl)amino]tetralin (8-OH-PIPAT), inhibited forskolin-stimulated cAMP accumulation in CHO-5HT$_{1A}$ cells. Furthermore, [^{125}I]R(+)-8-OH-PIPAT bound with high affinity (K_d = 0.1 nM) to 5-HT$_{1A}$ receptors on membranes prepared from CHO-5HT$_{1A}$ cells. An iodinated derivative of the putative 5-HT$_{1A}$ receptor antagonist, WAY100635, was examined for its ability to block 5-HT$_{1A}$ receptor activation. 4-(2'-Methoxy-)phenyl-1-[2'-(n-2"-pyridinyl)-p-iodobenzamido-]ethyl-piperazine (p-MPPI) blocked the decrease in forskolin-stimulated cAMP accumulation produced by 8-OH-DPAT in CHO-5HT$_{1A}$ cells. [^{125}I]p-MPPI bound with high affinity (K_d = 0.4 nM) to 5-HT$_{1A}$ receptors. [^{125}I]R(+)-8-OH-PIPAT labeled approximately 70% as many receptors as were labeled with [^{125}I]p-MPPI. Specific binding of [^{125}I]R(+)-8-OH-PIPAT was inhibited by Gpp[NH]p, whereas the binding of [^{125}I]p-MPPI was not affected by the addition of Gpp[NH]p. The agonists 8-OH-DPAT and 5-HT bound with approximately 40-fold greater affinity to receptors labeled with [^{125}I]R(+)-8-OH-PIPAT than to receptors labeled with [^{125}I]p-MPPI (in the presence of Gpp[NH]p). In contrast, spiperone, an antagonist at 5-HT$_{1A}$ receptors, bound with nearly 10-fold lower affinity to receptors labeled with [^{125}I]R(+)-8-OH-PIPAT than to receptors labeled with [^{125}I]p-MPPI. [^{125}I]R(+)-8-OH-PIPAT and [^{125}I]p-MPPI should prove useful in further studies of the properties and regulation of 5-HT$_{1A}$ receptors. (Supported by USPHS grants NS18591, NS09245, NS24538, MH48125)

633.4

EVALUATION OF 4-(2'-methoxy-)phenyl-1-[2'-n-2"-pyridinyl) -p-iodobenzamido-]ethyl-piperazine (p-MPPI) FOR ANTAGONIST ACTIVITY AT 5-HT$_{1A}$ RECEPTORS. A.R. Allen*, A. Singh, H.F. Kung, M. Kung and I. Lucki. Departments of Psychiatry, Pharmacology and Radiology, University of Pennsylvania, Philadelphia, PA 19104.

The lack of selective 5-HT$_{1A}$ receptor antagonists has hampered study of the functional role of these receptors. A candidate for such an antagonist, p-MPPI, is a structural analog of WAY-100,135 that binds with high affinity to 5-HT$_{1A}$ receptors (Kd=0.3 nM). p-MPPI was tested for its ability to antagonize the effects of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on two responses mediated by postsynaptic 5-HT$_{1A}$ receptors, the 5-HT syndrome and hypothermia. Male Sprague-Dawley rats were pretreated with p-MPPI (3-30 mg/kg, s.c.) 15 min prior to 8-OH-DPAT injection (1 or 2 mg/kg in the hypothermia and 5-HT syndrome studies, respectively). p-MPPI dose-dependently prevented the full 5-HT syndrome (ie, the production of four out of the six component behaviors) induced by 8-OH-DPAT. However, p-MPPI did not prevent two of these behaviors, flat body posture or hindlimb abduction. p-MPPI did not produce any of the behavioral symptoms when given alone. In rats pretreated with reserpine (1 mg/kg, 18 hours), p-MPPI (30 mg/kg) also blocked the 5-HT syndrome without producing any symptoms when given alone. p-MPPI did not produce changes in body temperature when given alone, but may only partially antagonize the hypothermic effect produced by 8-OH-DPAT. These results demonstrate that p-MPPI has antagonist properties and is devoid of any intrinsic efficacy at 5-HT$_{1A}$ postsynaptic receptors. Studies are currently underway to examine whether p-MPPI is an effective antagonist at presynaptic 5-HT$_{1A}$ receptors by examining the effects of p-MPPI, given alone and in combination with 8-OH-DPAT, on extracellular levels of 5-HT using *in vivo* microdialysis. This research was supported by USPHS grants MH 14654, MH 36262 and MH 48125.

633.5

THE 5-HT$_{1A}$ ANTAGONIST 4-(2'-METHOXY-)PHENYL-1-[2'-(N-2"-PYRIDINYL-)p-IODOBENZAMIDO-]ETHYL-PIPERAZINE (p-MPPI) COMPETITIVELY ANTAGONIZES 5-HT$_{1A}$ RECEPTOR ACTIVATION IN VIVO. R.J. Thielen* & A. Frazer. Dept. of Pharmacol., UTHSCSA and Vet. Affairs Med. Ctr., San Antonio, TX 78284.

Until recently, selective 5-HT$_{1A}$ receptor antagonists were unavailable, in that the available antagonists either lacked selectivity for the 5-HT$_{1A}$ receptor or were shown to have partial agonist activity. We report that p-MPPI (Zhuang et al., J. Med. Chem., in press), competitively antagonizes 5-HT$_{1A}$ receptor activation in the rat, as measured by hypothermia and forepaw treading. Further, on these parameters, p-MPPI exhibits no partial agonist activity. p-MPPI (1-10 mg/kg, ip) dose-dependently antagonized the hypothermia induced by a fixed dose of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (0.5 mg/kg, sc), with an IC_{50} of 5.7 mg/kg. Further, the inhibitory effect caused by a fixed dose of p-MPPI (6 mg/kg) was competitively antagonized by higher doses of 8-OH-DPAT. p-MPPI (10 mg/kg, ip) also attenuated the hypothermia induced by gepirone (10 mg/kg, sc). Forepaw treading caused by 8-OH-DPAT (2 mg/kg, sc) in reserpine-pretreated rats (1 mg/kg, sc, 18-24 hours prior to the experiment) was dose-dependently antagonized by p-MPPI (2.5-10 mg/kg, ip), with an IC_{50} of 3.3 mg/kg. p-MPPI also antagonized forepaw treading induced by 5-methoxy-N,N,-dimethyltryptamine (5-MeODMT) (5 mg/kg, sc) in reserpine-pretreated rats, and this antagonism was competitively overcome by higher doses of 5-MeODMT. These results suggest that p-MPPI is a competitive antagonist at 5-HT$_{1A}$ receptors mediating these responses and does not have intrinsic activity at these receptors; however, these data do not rule out the possibility that p-MPPI may show intrinsic activity in other tests of 5-HT$_{1A}$ receptor activation. (Supported by Research Funds from the VA and USPHS Grant MH48125).

633.6

LY206130, A CYCLOHEXYL ANALOG OF PINDOLOL, AN ANTAGONIST OF 5-HT$_{1A}$ RECEPTOR, D. T. Wong*, D. N. Mayle, N. W. Delapp, D. O. Calligaro, D. W. Robertson, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46286;

The beta-adrenergic blocking drug, pindolol, is also an antagonist to 5-HT$_{1A}$ receptor. Replacement of the N-iso-propyl in pindolol by a N-cyclohexyl as in LY206130, increases the affinity for 5-HT$_{1A}$ receptor 5 times and decreases affinity for beta-adrenergic receptor 6 times as indicated by the concentrations to inhibit 50% binding of ^{3}H-8-OHDPAT (IC_{50} of 17.4 and 3.4 nM) and ^{3}H-dihydroalprenolol (IC_{50} of 3.8 and 23 nM), respectively. LY206130 has near micromolar affinities for other receptors. At 1-1000 nM, LY206130 antagonized the agonist, 8-OHDPAT (1 μM), induced inhibition of the forskolin stimulated adenylate cyclase of rat hippocampus, while up to 10 μM LY206130 alone had no effect. 8-OHDPAT at 1 mg/kg s.c. significantly lowered 5-hydroxyindoleacetic acid (5-HIAA) levels in hypothalamus, hippocampus and cerebral cortex. LY206130 at 1 and 3 mg/kg s.c. antagonized the decrease of 5-HIAA in hypothalamus and cortex and at 1 mg/kg s.c. attenuated the hyperphagia induced by 8-OHDPAT. Thus, LY206130 has a pharmacological profile of an antagonist of the postsynaptic 5-HT$_{1A}$ receptor in projected areas and the 5-HT$_{1A}$ autoreceptors in cell bodies of 5-HT neurons.

633.7

THE 5-HT_2 ANTAGONISTS, KETANSERIN AND RITANSERIN, DISCRIMINATE BETWEEN RECOMBINANT HUMAN 5-$HT_{1D\alpha}$ AND 5-$HT_{1D\beta}$ RECEPTOR SUBTYPES: IDENTIFICATION OF "SELECTIVE" 5-$HT_{1D\alpha}$ ANTAGONISTS. J.M. Zgombick*, L.E. Schechter, S. Kucharewicz, R.L. Weinshank, P.R. Hartig and T.A. Branchek. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

Molecular cloning has demonstrated that the 5-HT_{1D} subfamily is comprised of two closely related subtypes termed 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. These receptors display a high transmembrane homology (~75%) which contribute to their similar pharmacological profiles. The classical 5-HT_2 antagonists, ketanserin and ritanserin, displayed moderate affinity (K_i=50-75 nM) and marked selectivity (25-70-fold) for the cloned human 5-$HT_{1D\alpha}$ subtype relative to the 5-$HT_{1D\beta}$ receptor. In contrast, the nonselective 5-$HT_{1/2}$ antagonist, methiothepin, exhibited similar affinities (K_i=10-20 nM) for both 5-HT_{1D} subtypes. These three compounds were evaluated for their ability to antagonize sumatriptan-induced inhibition of forskolin-stimulated cAMP accumulation in stable cell lines expressing the 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ gene. All three compounds behaved as competitive antagonists devoid of intrinsic activity in the functional assays. The apparent K_b values measured in the functional assays closely matched their K_i values obtained in binding assays. The development of selective 5-$HT_{1D\alpha}$ antagonists, devoid of crossreactivities at other biogenic amine subtypes (i.e. 5-HT_2, α_1-adrenergic), will allow for evaluation of 5-$HT_{1D\alpha}$ receptor function in intact animals and isolated tissue preparations.

633.8

KINETIC CHARACTERIZATION OF THE RESPONSES TO SUMATRIPTAN AND SEROTONIN IN CEREBRAL BLOOD VESSELS. G.P. Thalody, F.D. Yocca and H.L. Wiener*. CNS Drug Discovery, Bristol-Myers Squibb Co., Wallingford, CT 06492.

Although traditional pharmacological evaluations in isolated blood vessels have applied steady-state methods to study the responses to selective agents, and have provided a wealth of information, they fail to consider the time-dependent aspects of response generation. It is for this reason that kinetic models supersede studies performed under steady-state conditions, for they extend our knowledge of the manner by which the steady-state is achieved and allow for a quantitative analysis of the time-dependent changes which should assist in elucidating the biochemical basis of the observed physiological response (Wiener *et al.*, EJP 220: 131, 1992). The bovine middle cerebral artery was chosen as a model system to study the responses to serotonin (5-hydroxytryptamine; 5-HT) and sumatriptan, a clinically efficacious antimigraine agent, by steady-state and kinetic models. While both 5-HT and sumatriptan evoked concentration-dependent and saturable contractile responses, sumatriptan was a weak partial agonist whose intrinsic activity was approximately 20% that of 5-HT. The kinetic model described by Clancy *et al.* (JPET 242: 108, 1987) was, therefore, chosen to characterize the kinetics of competitive drug action between 5-HT and sumatriptan, and to estimate the association and dissociation rate constants for both agents. The association rate constants for 5-HT and sumatriptan were 0.55±0.08 and 2.9±1.1 $(\mu M\ min)^{-1}$, the dissociation rate constants were 0.19±0.01 and 0.0064±0.0010 min^{-1}, and the pK_a and pK_b values were 6.42±0.04 and 8.50±0.11, respectively. To the best of our knowledge, this is the first report of the association and dissociation kinetics for sumatriptan in isolated cerebral blood vessels.

633.9

[^{3}H]BMS-181101: A NOVEL HIGH AFFINITY 5-HT_{1D} AGONIST RADIOLIGAND. H.P. Nowak*, D.D. Dischino, L.G. Iben, F.D. Yocca, and C.D. Mahle. CNS Drug Discovery and Central Chemistry, Bristol-Myers Squibb Co., Wallingford, CT 06492.

Binding sites displaying high (5-HT_{1D}) and low (5-HT_{1E}) affinity for 5-carboxamidotryptamine have been described using [^{3}H]5-HT and selectively masking 5-HT_{1A} and 5-HT_{1C} receptors. Utilizing [^{3}H]5-CT (0.5 nM) as the radioligand eliminated binding to the 5-HT_{1E} sites. However, potent 5-HT_{1D} agonist compounds such as sumatriptan and BMS-181101 (a novel antidepressant candidate) continued to produce a regionally distinct biphasic displacement (K_H=20 nM, 2 nM and K_L=1 µM, 10 µM, sumatriptan and BMS-181101, respectively), suggesting a heterogeneous population of 5-HT_{1D}-like sites radiolabeled by [^{3}H]5-CT in certain mammalian brain regions. In bovine substantia nigra, both sumatriptan and BMS-181101 produced monophasic displacement of [^{3}H]5-CT binding. Therefore, this tissue was utilized initially to characterize the binding of [^{3}H]BMS-181101 to 5-HT_{1D} binding sites. In bovine substantia nigra membrane homogenates, [^{3}H]BMS-181101 binding was rapid, reversible and saturable, displaying high affinity (K_d=1 nM) and low non-specific binding. Pharmacologically, affinity values revealed the following rank order of potency: 5-CT≥5-HT>LSD=DHE> sumatriptan>methiothepin≥GTI>8-OH-DPAT. CP-93,129, mesulergine, buspirone and spiperone all displayed negligible affinity. Gpp(NH)p induced a concentration-dependent decrease in high affinity [^{3}H]BMS-181101 binding, suggesting an agonist interaction with the binding site. Given these properties, we suggest that [^{3}H]BMS-181101 will be a useful pharmacological tool in the characterization and regional localization of pre and post-synaptic 5-HT_{1D} receptors. (Supported in part by NIH Grant RR01237, National Center for Research Resources).

633.10

THE ATYPICAL NEUROLEPTIC, SERTINDOLE, IS A 5-HT_{2C} RECEPTOR ANTAGONIST. E. Meier and K. Frederiksen*. Pharmacological Research, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark.

An important new advance in the pharmacotherapy of schizophrenia has been the introduction of drugs which have antipsychotic activity without extrapyramidal side effects (e.g., dystonia, parkinsonism, and akathisia) usually seen with classical neuroleptic drugs. The exact molecular mechanism of action of atypical neuroleptics is still unknown, however, binding studies with a new atypical neuroleptic, sertindole (Skarsfeldt & Perregaard, Eur. J. Pharmacol. **182**, 1990, 613), have indicated that 5-HT_2 and σ_1 receptors may be involved (Sanchez et al., Drug Devel. Res. **22**, 1991, 239).

In the present study the effect of sertindole on the 5-HT_{2C} receptor has been characterized in binding and functional studies utilizing a NIH-3T3 cell line transfected with cloned rat 5-HT_{2C} receptors. Both sertindole (K_i= 0.51 nM) and clozapine (K_i =5.1 nM) display high affinity in receptor binding assays utilizing homogenates of 5-HT_{2C} transfected NIH-3T3 cells while the classical neuroleptic, haloperidol (K_i = 1500 nM), displays very low affinity for this 5-HT receptor subtype. The same rank order of potencies of sertindole, clozapine and haloperidol is obtained utilizing living 5-HT_{2C} cells although the binding affinities is about 100 fold lower. In functional studies of 5-HT mediated phosphoinositol turnover in 5-HT_{2C} transfected NIH-3T3 cells, sertindole (IC_{50} = 140 nM) and clozapine (IC_{50} = 720 nM) act as antagonists while haloperidol (IC_{50} > 100,000 nM) has no effect in this functional assay.

These data indicate that functional inhibition of 5-HT_{2C} receptors may be involved in the antipsychotic effect of atypical neuroleptics.

633.11

GR127935: *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF A PUTATIVE 5-HT_{1D} ANTAGONIST. F.D. Tingley,*A.W. Schmidt, H.R. Howard, and D.W. Schulz. Central Research Division, Pfizer Inc., Groton, CT. 06340.

Functional studies of 5-HT_{1D} receptors have been hampered by the limited receptor selectivity of available antagonists (e.g. methiothepin). Recently it was reported that the biphenyl carboxamide GR127935 has high affinity for the cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, displays >100-fold selectivity vs other receptor subtypes, and behaves as an antagonist based on its reversal of contralateral rotation in guinea pigs caused by intranigral administration of a 5-HT_1 agonist (Br. J. Pharmacol. 110:9P,1993). We have further characterized the functional activity of GR127935 at 5-HT_{1D} receptors in guinea pig brain by 1) measuring its effects on forskolin-stimulated adenylate cyclase activity in substantia nigra and 2) examining changes in 5-HT turnover in guinea pig hypothalamus following peripheral administration.

Adenylate cyclase studies revealed that GR127935 is a full agonist at 5-HT_{1A} receptors and a partial agonist at 5-HT_{1D} receptors (50-60% efficacy , relative to 5-HT). EC_{50} values (>1 µM at 5-HT_{1A} receptors, 2 nM at 5-HT_{1D} receptors) are consistent with the reported receptor selectivity profile of GR127935.

In vivo, s.c. treatment with GR127935 reversed the decrease in 5-HT turnover (5-HIAA:5-HT ratio) caused by the 5-HT_{1D} agonist CP-135,807 (see Schmidt et al., Soc. Neurosci. Abstr., 1994). When administered alone, GR127935 dose-dependently increased 5-HT turnover in guinea pig brain, suggesting an antagonism of endogenous 5-HT acting at inhibitory 5-HT_{1D} terminal autoreceptors.

633.12

CP-135,807: A SELECTIVE 5-HT_{1D} AGONIST WITH CENTRAL ACTIVITY. A.W. Schmidt*, F.D. Tingley, H. Rollema, J.E. Macor, and D.W. Schulz. Central Research Division, Pfizer Inc., Groton, CT. 06340-1596.

The use of sumatriptan to study central 5-HT_{1D} receptors is limited by its poor brain penetration. Here we describe the functional properties of CP-135,807 (3-[pyrrolidin-2R-ylmethyl]indole), a novel 5-HT_{1D} agonist with improved potency and CNS penetration.

CP-135,807 (3-[pyrrolidin-2R-ylmethyl]indole) binds with high affinity (K_i = 1.3 nM) to brain 5-HT_{1D} receptors (Koe et al., Soc. Neurosci. Abstr., 1994). Like sumatriptan, CP-135,807 behaves as an agonist by inhibiting adenylate cyclase activity at both 5-HT_{1D} (guinea pig substantia nigra, EC_{50}=1.0 nM) and 5-HT_{1A} receptors (guinea pig hippocampus, EC_{50}=41 nM). Sumatriptan has a similar receptor selectivity profile, but is much less potent (5-HT_{1D} EC_{50}=97 nM).

Using *ex vivo* binding, it was determined that CP-135,807 readily crosses the blood-brain barrier, while sumatriptan was undetectable in brain after a subcutaneous (s.c.) dose of 5.6 mg/kg. As expected of a compound that activates 5-HT_{1D} terminal autoreceptors, s.c. CP-135,807 dose-dependently inhibited 5-HT turnover in guinea pig brain, decreasing 5-HIAA:5-HT ratios by up to 50%. Consistent with this mechanism of action, *in vivo* studies demonstrated that perfusions with 0.2-10 µM CP-135,807 through a microdialysis probe in the hypothalamus caused a dose-dependent decrease in extracellular 5-HT. The centrally-mediated effect of CP-135,807 on 5-HT turnover is consistent with its ability to cause hypothermia in guinea pigs (Seymour et al., Soc. Neurosci. Abstr., 1994).

633.13

CP-135,807, A NOVEL 5-HT1D AGONIST INDUCES HYPOTHERMIA IN THE GUINEA PIG. P.A. Seymour*, D.W. Schulz, F.D. Tingley and J.E. Macor. Departments of Neuroscience and Medicinal Chemistry, Central Research Division, Pfizer Inc, Groton, CT 06340.

Central serotonergic systems have long been implicated in thermoregulation. The 3-(pyrrolidin-2R-ylmethyl)indole, CP-135,807, has recently been characterized as a novel 5-HT1D agonist *in vitro* with greater than 30-fold functional selectivity vs. 5-HT1A receptors as measured by inhibition of adenylate cyclase activity (Schmidt *et al.*, Soc. Neurosci. Abstracts, 1994). To investigate the functional correlates of 5-HT1D receptor activation *in vivo*, the effect of CP-135,807 on body temperature was evaluated in guinea pigs, a species known to have central 5-HT1D receptors. Rectal temperatures were measured prior to and 30, 60, 120 and 240 min after either s.c. or p.o. administration. It was found that CP-135,807 profoundly and dose-dependently lowers body temperature after either route of administration, with greater potency obtained after the s.c. route. This effect appears to be centrally mediated since sumatriptan and CP-123,803, an analog with a similar binding profile that does not readily enter the CNS, failed to produce this response. In addition, the putative 5-HT1D antagonist, GR-127935, antagonizes this effect when given 60 min prior to agonist administration. These data suggest that 5-HT1D receptors play a thermoregulatory role in guinea pig, and that this response may be useful in the characterization of drugs that act at 5-HT1D receptors.

633.14

NOVEL TRYPTAMINE DERIVATIVES WITH A 5-(3-NITRO-2-PYRIDYLAMINO) MOIETY BIND PREFERENTIALLY TO 5-HT1D RECEPTORS. B.K. Koe,* L.A. Lebel, C.B. Fox, and J.E. Macor. Central Research Division, Pfizer Inc, Groton, CT 06340

The recent introduction of serotonergic drugs (5-HT reuptake inhibitors as antidepressants; 5-HT1A agonists as anxiolytics and antidepressants; a 5-HT1D agonist as antimigraine agent) as well as the chemical description of new and distinct 5-HT receptors have spurred interest in finding selective agonists for these receptors. Our approach is based on modifying the serotonin molecule by introducing novel substituents at the indole C5 and C3 positions. Examples from our laboratories include a selective 5-HT1B agonist, CP-93,129 (Macor et al., 1990), and a potent ligand at 5-HT1A, 5-HT1B, 5-HT1D receptors and the DA transporter, CP-110,330 (Koe et al., 1992; Nowakowski et al., 1993). We now report that the 5-(3-nitro-2-pyridylamino) moiety (R) confers 5-HT1D binding selectivity to tryptamine derivatives (IC50 nM):

Compound	C5 Moiety	C3 Moiety	5HT1D	5HT1A
Serotonin	HO	CH2CH2NH2	3.0	5.2
Sumatriptan	MeNHSO2CH2	CH2CH2NH2	61	640
CP-113,113	R	CH2CH2NMe2	10	73
CP-135,807	R	N-Me-2-pyrrolidinylmethyl(r)	3.1	33
CP-123,803	R	2-pyrrolidinyl-methyl(r)	6.2	120
CP-124,439	R	N-Me-3-pyrrolidinyl	15	320

SEROTONIN RECEPTORS: BEHAVIOR

634.1

WAY-100635, A POTENT AND SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST, INCREASES SEROTONERGIC NEURONAL ACTIVITY AND BLOCKS THE ACTION OF 8-OH DPAT IN BEHAVING CATS. C. A. Fornal *, C. W. Metzler, R. A. Gallegos, S. C. Veasey, A. C. McCreary and B. L. Jacobs. Prog. Neurosci., Princeton Univ., Princeton, NJ 08544.

We have recently demonstrated that pharmacological blockade of somatodendritic 5-HT_{1A} autoreceptors increases the basal firing rate of central serotonergic neurons in awake cats. These results were obtained using spiperone, a non-selective 5-HT_{1A} autoreceptor antagonist. The present study examined the effects of WAY-100635, a selective 5-HT_{1A} receptor antagonist (Fletcher et al., Br. J. Pharmacol. 112: 91P, 1994), on spontaneous serotonergic neuronal activity and 8-OH DPAT-induced neuronal suppression. Serotonergic neurons in the dorsal raphe nucleus (DRN) were recorded and identified using methods previously described (Fornal et al., Am. J. Physiol. 259: R963-R972, 1990). Systemic administration of WAY-100635 (25-500 µg/kg, i.v.) significantly increased the basal firing rate of serotonergic DRN neurons, while producing no appreciable effects on behavior or polygraphic parameters. The stimulatory action of WAY-100635, like that of spiperone, was apparent during wakefulness (when neurons display a relatively high level of activity) but not during sleep (when neurons display little or no spontaneous activity). Pretreatment with WAY-100635 at doses as low as 100 µg/kg i.v. completely blocked the inhibitory action of 8-OH DPAT, indicating that WAY-100635 has a strong antagonist action at 5-HT_{1A} autoreceptors. These results confirm the general findings obtained with spiperone, and further support the hypothesis that 5-HT_{1A} autoreceptor-mediated feedback inhibition operates under physiological conditions when cells are firing at a relatively high rate. Supported by grants from the AFOSR (90-0294) and the NIMH (MH 23433).

634.2

EFFECTS OF 5-HT_{1A} AUTORECEPTOR BLOCKADE WITH WAY-100635 ON SENSORY-EVOKED ACTIVITY OF SEROTONERGIC NEURONS IN BEHAVING CATS. R. A. Gallegos*, C. A. Fornal, C. W. Metzler, J. C. Horvitz, and B. L. Jacobs. Program in Neurosci., Princeton University, Princeton, NJ 08544.

Somatodendritic 5-HT_{1A} autoreceptors are thought to be part of a local negative feedback mechanism and may modulate the responsiveness of central serotonergic neurons to sensory stimulation. The present study examined the effects of autoreceptor blockade with systemic WAY-100635 (Fletcher et al., Br. J. Pharmacol., 112: 91P, 1994) on sensory-evoked responses of serotonergic dorsal raphe neurons. Single-unit responses to repeated presentation of a simple auditory stimulus (click: 100 dB, 1 msec; 100 trials) and to complex arousing stimuli (e.g. door opening) were obtained both before (baseline) and after administration of WAY 100635 (100 µg/kg, i.v.). This dose has been shown to block 5-HT_{1A} autoreceptors in cats (see Fornal et al., preceding abstract). Unit activity was stored on a computer and used to construct peri-stimulus time histograms (PSTH). Of the 15 cells studied, 11 (73%) showed excitation and 4 (27%) showed inhibition in response to phasic auditory stimulation. Onset of excitatory and inhibitory responses occurred approximately 40-60 msec and 20-40 msec, respectively, after the stimulus and lasted for about 100 msec. WAY-100635 increased basal serotonergic unit activity and attenuated excitatory responses (8 of 11 cells), but had no appreciable effect on inhibitory responses. In contrast, WAY-100635 potentiated the increase in unit activity produced by arousing stimuli. These results provide preliminary evidence that 5-HT_{1A} autoreceptors exert an inhibitory influence on neuronal responses to arousing stimuli. Supported by grants from the AFOSR (90-0294) and the NIMH (MH 23433).

634.3

After acute and repeated treatment with the 5-HT_{1A} receptor antagonists (-)UH-301 and WAY100135, tolerance develops to a 5-HT_{1A} receptor-mediated functional response. L. Rényi* and P. Jimenez. CNS Preclinical R& D, Department of Behavioural Pharmacology, Astra Arcus AB, S-151 85 Södertälje, Sweden.

After acute treatment with the 5-HT_{1A} agonist 8-OH-DPAT tolerance develops to several 5-HT_{1A} receptor-mediated responses. These include the inhibition of the cage-leaving response (ICLR) induced 24 h later by a subsequent dose of this agonist (Ross et al. Naunyn-Schmiedeberg´s Arch Pharmacol 1992;346:138). A similar result was obtained following acute treatment with the 5-HT_{1A} antagonists (-)UH-301 and WAY100135 (3.0 mg/kg s.c.). These compounds (1.0 mg/kg s.c.), when administered 30 min before 8-OH-DPAT (0.1 mg/kg s.c.), blocked the 8-OH-DPAT-induced ICLR, but tolerance was evident when the rats were challenged 24 h later with the same dose of 8-OH-DPAT. The development of tolerance was blocked by the non-competitive NMDA receptor antagonist, dizocilpine. Furthermore, the rats did not leave their cages after 10.0 mg/kg of (-)UH-301. This response could not be blocked by any receptor antagonist investigated in the study. However, (-)UH-301 induced tolerance to itself already after the second administration when given every third day, and after the third administration when given once a week. Simultaneously, wet-dog shakes induced by the 5-HT_2 receptor agonist DOI (0.3 mg/kg s.c.) were strongly potentiated.

Further studies are in progress to establish whether the effects of (-)UH-301 are pre- or postsynaptically mediated.

634.4

S 15535, A NOVEL AND SELECTIVE ANTAGONIST AT POSTSYNAPTIC 5-HT_{1A} RECEPTORS AND AGONIST AT 5-HT_{1A} AUTORECEPTORS: ACTIONS IN MODELS OF ANTIDEPRESSIVE, ANXIOLYTIC AND PROMNESIC ACTIVITY. M.J. Millan, M. Brocco, R. Schreiber, J.-M. Rivet, S. Hjorth, P. Lacroix, R. Samanin, R. Jaffard, S. File, M. Spedding and J.-L. Peglion*, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, France.

S 15535 is a novel, potent antagonist at postsynaptic 5-HT_{1A} receptors which retains agonist actions at 5-HT_{1A} autoreceptors. Here, we evaluate its potential therapeutic utility. Doses are in mg/kg. All animals were male. In the learned helplessness paradigm in rats, S 15535 (0.63 - 40.0, p.o.) abolished escape failures from the first day of treatment whereas, in the forced-swimming test, S 15535 was inactive. In the pigeon conflict test, S 15535 (0.04 - 0.63, i.m.) selectively augmented punished responses, an action prevented by the 5-HT_{1A} antagonist, (-)-alprenolol (10.0, i.m.). Similarly, S 15535 (0.3 - 3.0, s.c.) increased punished responding in a Geller-Seifter test in rats. Further, S 15535 (0.63 - 10.0, s.c. and 2.5 - 40.0, p.o.) reduced aggression in isolated mice and, though it did not show anxiolytic activity in the elevated plus-maze, WAY 100,135 and buspirone were also inactive under these conditions. Consistent with potential antidepressant and anxiolytic activity, S 15535 (0.16 - 10.0, s.c.) reduced release of 5-HT in dialysates of hippocampus and blocked the firing of raphe nuclei in rats. Microinjection of S 15535 (1 µg) into the hippocampus of rats attenuated the inhibitory influence of hippocampal 8-OH-DPAT (5 µg) upon working memory in a water maze. Further, S 15535 (0.16 and 0.63, s.c.) significantly improved the working memory of mice in a radial maze. Up to 40.0, s.c. and p.o., S 15535 did not elicit a serotonin syndrome or ataxia, did not modify spontaneous or amphetamine-induced locomotion and did not alter the release of dopamine in dialysates of the accumbens and striatum. In conclusion, S 15535 possesses a distinctive profile of antidepressant, anxiolytic and promnesic properties in rodents.

634.5

EFFECTS OF PSYCHOSOCIAL STRESS ON $5HT_{1A}$ RECEPTORS IN THE BRAIN. G. Flügge* and E. Fuchs. German Primate Center, 37077 Göttingen.

Normal neurotransmission in the brain requires normal numbers of receptors. Perturbing situations such as sustained psychosocial stress (PSS) may disturb the functional balance of the transmitter systems. In the present study, we investigated the effects of PSS on $5HT_{1A}$ receptors in the brains of tree shrews (*Tupaia belangeri*). Using quantitative in vitro autoradiography with ^{3}H-8-hydroxy-2(di-n-propylamino)tetralin (^{3}H-DPAT), high numbers of $5HT_{1A}$ receptors were detected in the dorsal raphe nucleus, the dorsal tegmental nucleus, in the hippocampal CA1 region, the basal nucleus of the amygdala, and the claustrum. In the frontal cortex, the moderate number of ^{3}H-DPAT binding sites can differ by up to 30% between the left and right hemisphere. In subordinate male tree shrews, whose physiology is characterized by a constantly elevated activity of the HPA-axis, the hippocampal ^{3}H-DPAT binding sites are diminished in number after 21 days of social conflict. In other brain areas, the number of binding sites increases with the duration of PSS. After 30 days of perpetual social confrontation, subordination is accompagnied by an elevation of low affinity ^{3}H-DPAT binding sites in the dorsolateral frontal cortex, in the dorsal raphe, and the dorsal tegmental nucleus. These data indicate that prolonged PSS induces an up-regulation of $5HT_{1A}$ recognition sites in the raphe nuclei and some raphe projection fields.

634.6

EFFECTS OF 5-HT AND 8-OH-DPAT ON SPONTANEOUS OPEN-FIELD MOTOR ACTIVITY AFTER LOCAL APPLICATION INTO THE SUBSTANTIA NIGRA PARS RETICULATA (SNr) AND VENTRAL TEGMENTAL AREA (VTA) IN THE RAT. V. Hillegaart* and S. Ahlenius. Department of Behavioral Pharmacology, Astra Arcus AB, S-151 85 Södertälje, Sweden.

The $5\text{-}HT_{1A}$ receptor agonist 8-OH-DPAT (0-5 μg), or 5-HT (0-40 μg), was infused into the SNr or into the VTA in awake, gently hand-restrained male Wistar rats, at a rate of 0.33 μL min^{-1} to a final volume of 0.5 μL. Ten minutes upon completion of the injection, the animals were transferred to the open-field arena (0.5 m^2) for 30 min photocell monitoring of patters of movement [locomotor activity (LMA), rearing (R), forward locomotion (FL) and peripheral activity (PA)]. Repeated measurements were made on four groups (n=13-15) given the different doses of 8-OH-DPAT or 5-HT in the SNr or VTA, respectively, in a change-over design. 5-HT, locally applied into the SNr produced a decreased LMA, FL and R with a shift to the periphery of the arena. A more stereotyped pattern of activity was obtained after the VTA infusion of 5-HT, primarily characterized by an increased LMA and loss of habituation. The local application of 8-OH-DPAT into the SNr produced the characteristic pattern of spontaneous motor activity seen after its systemic administration *i.e.* a decrease in LMA and R, and an increased proportion of FL and PA. A similar, but less conspicuous, pattern of effects was obtained after 8-OH-DPAT infusion into the VTA.

In conclusion, serotonin receptors in the SNr and VTA are important targets for effects on spontaneous motor activity by serotonergic drugs, and 8-OH-DPAT in particular, after their systemic administration in the rat.

634.7

5-HT1A RECEPTOR ANTISENSE OLIGONUCLEOTIDE, MDMA AND 8-OH-DPAT TREATMENT EFFECTS ON COCAINE INHIBITION OF DORSAL RAPHE 5-HT CELLS. R.T. Windh*, R. De La Garza II, E.J.Mah, M.L. Thomas and K.A. Cunningham. Dept. Pharmacol., Univ. Texas Med. Branch, Galveston, TX 77555.

We have reported that cocaine inhibits dorsal raphe nucleus (DR) serotonin (5-HT) neuronal activity through 5-HT1A and D1 receptor mechanisms. In order to further investigate 5-HT1A involvement in cocaine-induced inhibition of DR 5-HT activity, the sensitivity of DR 5-HT cells to cocaine was determined after chronic 8-OH-DPAT (DPAT, 1 mg/kg, sc, qd, 7 days), 5-HT1A antisense nucleotide (ASN, 5 ug, icv, bid, 7 days), or (+)MDMA (10 mg/kg, sc, bid, 4 days) treatment. In addition, the presence and severity of flat body posture (FBP), forepaw treading (FPT), forward locomotor activity (FLA) and lower lip retraction (LLR) in response to DPAT (0.5 mg/kg, sc) were used as an independent assay of 5-HT1A function. Drug naive, male Sprague-Dawley rats (200-300 g) were scored for DPAT behavior, treated with one of the 3 chronic regimens, and then re-scored for DPAT behaviors or used for single barrel extracellular recording. Chronic DPAT treatment (n=6) nearly eliminated FPT and reduced FBP and FLA, whereas 5-HT1A ASN treatment (n=4) prevented FBP and FLA and reduced FPT and LLR. MDMA treatment had no effect on any DPAT-induced behaviors (n=5). The ED_{50} for cocaine-induced inhibition of DR 5-HT cell firing was 0.6 mg/kg in drug-naive, chloral hydrate-anesthetized rats (n=6), as determined by intravenous cumulative dose-response analysis. MDMA (n=3) and 5-HT1A ASN (n=3), but not DPAT (n=9), treatment significantly reduced the potency of cocaine to inhibit DR 5-HT cells compared to untreated controls (ED_{50}s =1.3, 1.2 and 0.8 mg/kg, respectively). Thus chronic ASN and DPAT treatments differentially alter DPAT-induced behaviors and cocaine inhibition of DR 5-HT firing, suggesting that 5-HT1A-induced FBP and FLA may be more closely associated with cocaine mechanisms of DR 5-HT cell inhibition and less easily desensitized than others. Additionally, the integrity of DR 5-HT forebrain projections may be important in mediating cocaine inhibition of these cells. These results are currently being replicated and extended. Supported by DA 06511.

634.8

5-HT$_{1A}$ RECEPTOR MODULATION OF THE HYPERACTIVITY INDUCED BY ACUTE COCAINE ADMINISTRATION. *R. De La Garza II* and K. A. Cunningham.* University of Texas Medical Branch, Department of Pharmacology and Toxicology, Galveston, Texas 77555-1031.

Important interactions between 5-HT and DA systems occur in brain regions known to be critically involved in drug abuse. Moreover, specific manipulations of $5\text{-}HT_{1A}$ receptors may modulate the output of cocaine-induced behaviors (i.e., pretreatment with the partial $5\text{-}HT_{1A}$ agonist gepirone enhanced the locomotor activation induced by cocaine). To more definitively clarify the potential role of $5\text{-}HT_{1A}$ receptors in this behavior we sought to study the full dose-response relationship for cocaine in the presence of multiple doses of the selective and full agonist 8-OH-DPAT (DPAT). To this end, experimentally naive rats (n=32) were pretreated with DPAT (0, 0.1 or 0.2 mg/kg) 15 min prior to an injection of cocaine (0, 10 or 20 mg/kg). Similar levels of horizontal activity were observed in animals pretreated with DPAT (0.1 or 0.2 mg/kg) or saline, although vertical activity was reduced by DPAT. Pretreatment with DPAT (0.1 mg/kg) had no effect on cocaine-induced horizontal activity but significantly decreased rearing elicited by cocaine (10 and 20 mg/kg). At a higher dose (0.2 mg/kg), DPAT significantly increased horizontal, and decreased vertical, activity induced by cocaine (10 and 20 mg/kg). Thus, a similarity in the ability of the full (DPAT) and partial (gepirone) $5\text{-}HT_{1A}$ agonists to enhance cocaine-induced locomotor activity is observed. If 5-HT inhibits DA function, it is possible that stimulation of somato-dendritic $5\text{-}HT_{1A}$ autoreceptors by DPAT or gepirone and a subsequent reduction in terminal 5-HT release might diminish this inhibitory 5-HT role, thereby enhancing the DA-mediated behavioral effects of cocaine. ***Supported by DA 06511 & DA 05708***

634.9

Hallucinogenic drug interactions with recombinant cells expressing $5HT_{2A}$ or $5HT_{2C}$ receptors: evidence further implicating the $5HT_{2A}$ receptor as the brain site-of-action. C. Casey*, K. Herrick-Davis, K.J. Miller[@], B.J. Hoffman[@], R.A. Glennon[#], and M. Teitler Dept. of Pharmacology and Toxicology (C.C., K.H.D., M.T), Albany Medical College, Albany, N.Y. 12208;[@]Lab. Cell Biol., NIH, Bethesda, MD 20892; [#]Dept. Med. Chem., Virginial Commonwealth University, Richmond, VA 23298

Evidence from radioligand binding, animal drug-discrimination, and human trials have been presented as implicating the $5HT_{2A}$ receptor as the "LSD receptor" in brain; i.e. that LSD and related hallucinogenic phenylisopropylamines stimulate this receptor in initiating the cascade of events producing the hallucinogenic experience (1). Recently evidence has been presented indicating that lisuride, a purported non-hallucinogenic congener of LSD, stimulates $5HT_{2A}$ receptors and not $5HT_{2C}$ receptors (2). Thus it has been proposed that the $5HT_{2C}$ receptor is more likely to be the "LSD receptor". In order to further investigate the interaction of lisuride with $5HT_{2A}$ and $5HT_{2C}$ receptors we tested the ability of lisuride and LSD to stimulate PI metabolism in recombinant cells stably expressing $5HT_{2A}$ or $5HT_{2C}$ receptors. We have found lisuride potently stimulates IP_3 production in cells expressing $5HT_{2C}$ receptors, as well as stimulating IP_3 production in cells expressing $5HT_{2A}$ receptors. These data indicate that lisuride is a non-specific $5HT_2$ receptor agonist and is consistent with reports that lisuride does have hallucinogenic activity. Data concerning the effects of other hallucinogens in stimulating PI metabolism in these recombinant cells will be presented.

1. Glennon et al, Life Sci., 35, 2505-2511, 1984
2. Burris et al, J.P.E.T., 258, 891-896, 1991

634.10

USE OF SEVERAL $5\text{-}HT_{2A/C}$ ANTAGONISTS TO DIFFERENTIALLY SEPARATE THE DOI-INDUCED $5\text{-}HT_{2A}$- AND $5\text{-}HT_{2C}$ RECEPTOR FUNCTION IN MICE. N.A. Darmani* and C.F. Gerdes. Dept. of Pharmacol., Kirksville Col. of Osteo. Med., Kirksville, MO 63501.

The serotonergic $5\text{-}HT_{2A}$ receptor subtypes consist of $5\text{-}HT_{2A}$ (classical $5\text{-}HT_2$), $5\text{-}HT_{2B}$ ($5\text{-}HT_{2F}$) and $5\text{-}HT_{2C}$ ($5\text{-}HT_{1C}$) sites. The $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors have similar structures and use inositol phosphate hydrolysis as their signal transduction mechanism. Relative to the classical $5\text{-}HT_{2A/C}$ antagonists, the new $5\text{-}HT_{2A}$ antagonists (SR-46349B and AMI-193) are reported to bind more selectively to $5\text{-}HT_{2A}$ versus $5\text{-}HT_{2C}$ as well as other monoamine receptors. The purpose of this investigation was to compare the effects of the new antagonists with the classical $5\text{-}HT_{2A/C}$ antagonists (e.g. ketanserin, ritanserin and spiperone) on the DOI-induced behaviors such as the $5\text{-}HT_{2A}$-mediated head-twitch response (HTR) and the $5\text{-}HT_{2C}$-mediated ear-scratch response (ESR) in mice. Different groups of mice were treated with either vehicle (n=12) or the cited antagonists (0.001 - 0.5 mg/kg, n=5-8, i.p.) 30 min prior to DOI (1.0 mg/kg, i.p.) administration. The DOI-induced HTR and ESR frequencies were counted for 20 min following its injection. The antagonists reduced the HTR frequency with similar ID_{50} values (mg/kg) in following order: SR-46349B (0.12); ketanserin (0.14); spiperone (0.15); ritanserin (0.17) and AMI-193 (0.27). They were more potent (2-12 times) in reducing the ESR frequency relative to the HTR score with the following respective ID_{50} values: 0.021; 0.012; 0.037; 0.035 and 0.115. Thus, the potency order of the cited antagonists to differentiate between the DOI-induced HTR and ESR is ketanserin > SR-46349 > ritanserin > spiperone > AMI-193. None of the cited antagonists appear to be selective for the $5\text{-}HT_{2A}$- versus $5\text{-}HT_{2C}$-mediated behaviors.

634.11

CLOZAPINE AND THE PUTATIVE, ATYPICAL ANTIPSYCHOTICS, RISPERIDONE, SERTINDOLE AND MDL 100,907, BLOCK DOI-INDUCED DISCRIMINATIVE STIMULUS EFFECTS AND HEAD-TWITCHES IN MALE RATS. M. Brocco, R. Schreiber, V. Audinot* and M.J Millan, Institut de Recherches Servier, 125, Chemin de Ronde, 78920 Croissy, France.

The present study evaluated the contribution of serotonin (5-HT)$_{2A}$ receptors to the *in vivo* actions of clozapine and several, putative atypical antipsychotics. In a drug discrimination paradigm employing animals trained to discriminate the 5-HT$_{2A/2C}$ agonist, DOI (0.63 mg/kg. i.p.), from saline, the discriminative stimulus (DS) effects of DOI were dose-dependently and completely blocked by the 5-HT$_{2A/2C}$ receptor antagonists, ritanserin, ICI 169,369 and mianserin (ID$_{50}$s: 0.32, 0.26 and 0.15 mg/kg, s.c., respectively), the preferential 5-HT$_{2A}$ antagonist, ketanserin (0.06) and the novel, highly selective 5-HT$_{2A}$ receptor antagonist, MDL 100,907 (0.001). Like MDL 100,907, both clozapine and two putative, atypical antipsychotics possessing marked affinity at 5-HT$_{2A}$ receptors, risperidone and sertindole, blocked the DS effects of DOI (0.05, 0.03 and 0.32, respectively). All compounds abolished the head-twitches (HTWs) evoked by DOI (2.5 mg/kg, s.c.): ID$_{50}$s: 0.28, 12.1, 0.02, 0.06, 0.005, 0.04, 0.025 and 1.0, respectively. The dopamine D$_2$ antagonist, haloperidol, failed to block the DS effects of DOI (dose-range tested: 0.01 - 0.16), but abolished DOI-induced HTWs (ID$_{50}$: 0.07). In conclusion, these data demonstrate the role of 5-HT$_{2A}$ receptors in mediating both the DS effects and the HTWs elicited by DOI, though the latter response is also modulated by D$_2$ receptors indicating the superior selectivity of the drug discrimination paradigm. The marked activity of clozapine, risperidone, sertindole and MDL 100,907 in these models supports the concept that 5-HT$_{2A}$ receptor antagonism contributes to the *in vivo* actions of atypical antipsychotics.

634.12

ANTISENSE KNOCKOUT OF THE 5-HT$_{2A}$ RECEPTOR *IN VIVO*: ANXIOLYTIC EFFECTS AND INCREASED 5-HT$_{2C}$-MEDIATED RESPONSES. D. BENJAMIN*, M. TOTH, K.R. GOLDSTEIN, E.I. SAIFF, AND L.A. POHORECKY. Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855, Cornell University Medical College, New York, NY 10021, Ramapo College of New Jersey, Mahwah, NJ 07430.

Antisense (A) phosphorothioate oligonucleotides complimentary to 5-HT$_{2A}$ receptor mRNA were administered in media to cultured C6 glioma cells and ICV (intracerebroventricular) to rats. In C6 cells, significant decreases in 5-HT$_{2A}$ mRNA and spiperone-sensitive [^{125}I]-LSD binding were evident after 3d treatment with A but not control (C) sequences (20-200 nM). In male Long-Evans rats, treatment with A (50 ug ICV twice daily x 4d; tests were 24 h after final dose), but not C, resulted in a significant *decrease* in 5-HT$_{2A}$ receptor mediated headshakes induced by the 5-HT$_{2A/C}$ agonist DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride); conversely, a significant *increase* in DOI-induced, 5-HT$_{2C}$-mediated, skin crawls was observed. A significant anxiolytic-like effect was observed in A but not in C-treated rats tested in the elevated plus-maze. Autoradiographic and saturation analysis of 5-HT$_{2A}$ and 5-HT$_{2C}$ binding from the rats in the behavioral studies indicated significant, 50-72% *decreases* in 5-HT$_{2A}$ binding, and *no change* in 5-HT$_{2C}$ binding. These results demonstrate that 5-HT$_{2A}$ receptor expression and function can be selectively attenuated using A sequences, and suggest that the 5-HT$_{2A}$ receptor inhibits agonist-induced 5-HT$_{2C}$ receptor-mediated responses. (Supported by the Smithers Foundation and the Charles and Johanna Busch Foundation).

634.13

TREATMENT WITH 5-HT$_{2A/2C}$ RECEPTOR AGONIST DISRUPTS INITIAL DEVELOPMENT OF SPATIAL NAVIGATION STRATEGIES IN RATS. M. Riekkinen*, J. Sirviö and P. Riekkinen Jr. Dept. of Neurology, Univ. of Kuopio, P.O.B. 1627, FIN-70211, Kuopio, Finland. Fax: 358-71-162048.

The present study investigates the effects of 5-HT$_{2A/2C}$ receptor stimulation on spatial and cue navigation, and visual discrimination. Daily s.c. pre-training treatment with 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) at 3 mg/kg, but not at 1 mg/kg, impaired initial development of water maze (WM) spatial navigation strategies (hidden platform), but had no effect on the development of non-spatial navigation strategies (visible platform). Pre-training injections of ketanserin (0.1, 0.4 and 2 mg/kg, i.p.), a 5-HT$_{2A/2C}$ receptor antagonist, had no effect on WM performance, but blocked the impairing effect of DOI 3 mg/kg on WM spatial navigation. Spatial reference and working memory were not disrupted by pre-training DOI (1 and 3 mg/kg) treatment if the rats had learned the reference memory rule before drug treatment. 5-HT$_{2A/2C}$ receptor stimulation did not impair consolidation as post-training DOI at 1 and 3 mg/kg had no effect on the development of spatial navigation strategies. Visual discrimination learning was not impaired by pre-training DOI 1 and 3 mg/kg. The present results suggest that 5-HT$_{2A/2C}$ receptor stimulation impairs initial development of spatial navigation strategies in rats.

634.14

Acute and Chronic Effects of M-Chlorophenylpiperazine on Food Intake and Brain 5-HT$_2$ and 5-HT$_{1b}$ Binding Sites, M.A. Blackshear.* Department of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561.

The acute and chronic effects of m-chlorophenylpiperazine (m-CPP) on serotonin (5-HT) induced decreased food intake on brain 5-HT$_2$ and 5-HT$_{1b}$ binding sites were investigated. Male Sprague-Dawley rats received a single dose of 10 mg/kg of m-CPP or 5 mg/kg twice daily for seven days. Given acutely, 10 mg/kg of m-CPP produced a marked decrease in food consumption at 1-hour and at 4-hours. Maximal inhibition occurred at 1-hour with a significant reduction in eating behavior still present at 4-hours. Acute m-CPP administration had no effect on the affinity (kD) or maximal number of binding sites (Bmax) of 5-HT$_{1b}$ or 5-HT$_2$ binding sites in the striatum and frontal cortex, respectively. In contrast to the acute effects on eating behavior, chronic injections of m-CPP failed to modify food intake when compared to saline controls. Unlike acute m-CPP, repeated m-CPP injections caused a marked decrease in [^{3}H]-ketanserin binding to 5-HT$_2$ sites in the frontal cortex. A small, but significant increase in the kD was observed also, However, chronic m-CPP treatment had no effect on the binding of [^{3}H]5-HT to 5-HT$_{1b}$ sites in the striatum. These findings confirm previous reports that adaptive mechanisms in 5-HT receptor systems following repeated agonist treatment may be different for 5-HT receptors and 5-HT binding sites. Further, considering that m-CPP has been widely used as a probe in various psychiatric disorders to evaluate central 5-HT function, this study provides additional information on the chronic effects of m-CPP on brain 5-HT function.
(Supported by NIH-RCMI Grant #5G12 RR03033)

SEROTONIN RECEPTORS: LOCALIZATION

635.1

IN VIVO STUDIES OF 5-HT2 RECEPTORS IN MONKEYS USING F-18-ALTANSERIN. C.A. Mathis*, Y. Choi, N.R. Simpson, M.A. Mintun. PET Facility, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

Altanserin is a closely related structural analogue of ketanserin and has a high in vitro affinity and selectivity for 5-HT2 receptors. We examined the in vivo properties of this radioligand in monkeys as a candidate for imaging brain 5-HT2 receptors using positron emission tomography (PET). Three rhesus monkeys received 8-10 mCi of high specific activity (1100-2500 Ci/mmol) F-18-altanserin. In addition, one monkey received a second 10 mCi injection of low specific activity (13 Ci/mmol) F-18-altanserin. Twenty scans of increasing duration were acquired over a 120 min time period. Arterial blood samples were obtained and the plasma radioactivity was corrected for the presence of radiolabeled metabolites. In two monkeys, ketanserin and altanserin (1 mg/kg) were injected 2 h after the injection of F-18-altanserin to displace specifically bound radioactivity.

At times greater than about 30 min, regionally selective retention of radioactivity was observed in cortical areas while cerebellum, caudate, and thalamus had low radioactivity levels. The ratio of frontal cortex-to-cerebellum was 2.5±0.3 in the high specific activity studies and decreased to 1.5 at 2 hours after the tracer injection in the low specific activity study. A two compartment fit to the dynamic PET data yielded kinetic rate constants K1, k2, and distribution volume (DV). Estimates of DV, which is proportional to the Bmax/Kd, were 3.4±1.2 for frontal cortex, 2.1±0.8 for caudate, and 1.7±0.5 for cerebellum. The DV's varied regionally in a manner consistent with the known distribution of 5-HT2 receptors. Both ketanserin and altanserin displaced specifically bound radioactivity in cortical areas with displacement half-times of 23 and 37 min, respectively. Unmetabolized altanserin comprised 58±1% of the total plasma activity at 2 h post injection. These results indicate that F-18-altanserin is a useful agent to image and quantitate 5-HT2 receptors in vivo using PET.

635.2

LOCALIZATION OF 5-HT$_{2A}$ RECEPTOR mRNA IN NEURONS OF THE HUMAN BRAINSTEM. *M.C. Austin*, J.A. Weikel and J.J. Mann.* Labs of Neuropharmacology, Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213.

A previous receptor autoradiography study, using [^{3}H] LSD as a ligand, reported the anatomical localization of serotonin receptors in the human brainstem (Palacios et al. 1983). This study found that the highest levels of binding occurred predominantly in the raphe nuclei, thus revealing the localization of serotonin receptors of the 5-HT$_1$-like subtype. Given the recent cloning of the human 5-HT$_{2A}$ receptor (Chen et al. 1992), the present study was designed to examine the distribution of neurons expressing 5-HT$_{2A}$ receptor mRNA in the human brainstem using *in situ* hybridization histochemistry (ISHH). Postmortem human brainstem tissue was obtained at autopsy, dissected and frozen. All cases (N=4) had negative toxicological results and no known neurologic or psychiatric disorders. Coronal tissue sections (20 µm) of the brainstems were cut, thaw-mounted on gelatin-coated slides and processed for ISHH. At the level of the medulla, neurons in the hypoglossal nucleus, the dorsal motor nucleus of vagus and the gigantocellular reticular nucleus all contained abundant levels of 5-HT$_{2A}$ receptor mRNA. In the pons, dense silver grains corresponding to 5-HT$_{2A}$ receptor mRNA were localized in neurons in the motor trigeminal nucleus and in pigmented and small, non-pigmented locus coeruleus neurons. A small, discrete population of neurons in the caudal dorsal raphe, bordering the fourth ventricle, expressed hybridization signal. Scattered mRNA-positive cells were also found in the oral pontine nucleus. A large population of neurons expressing 5-HT$_{2A}$ receptor mRNA was found in the diffuse sector of the pedunculopontine tegmental nucleus, and several mRNA-positive neurons were located in the caudal portion of the substantia nigra. These findings provide important information regarding the postsynaptic localization of 5-HT$_{2A}$ receptors and the relevant nuclei in the human brainstem that receive a putative serotonergic innervation. (Supported by MH30915, MH46745)

635.3

5-HT1A RECEPTOR LOCALIZATION IN THE RAT, CAT AND PRIMATE SPINAL CORD. [1]N.M. Kheck,* [2]P.J. Gannon, [3]B.L. Jacobs and [1]E.C. Azmitia. [1]Dept Biology, New York Univ, NY, NY10003; [2]Dept Otolaryngology, Mt Sinai Sch Med., NY, NY10029; [3]Neuroscience, Princeton Univ, Princeton, NJ 08544

The anatomic distribution of 5-HT1A receptors in rat brain and spinal cord has been defined by radioligand binding studies and quantitative autoradiography using the specific, high affinity radioligand, ^{3}H-8-OH-DPAT. We are currently using a synthetic antipeptide antibody to characterize the localization of the 5-HT1A receptor protein in the mammalian spinal cord. This polyclonal antibody was raised in rabbit against amino acid sequence 170-186, a transmembrane region of the 5-HT1A receptor that is structurally linked to the ligand binding site, and has no sequence homology with other known proteins.

In the spinal cords of rats, cats and macaques we observed the highest density of 5-HT1A-IR in the superficial layers of the dorsal horn on primary afferent sensory fibers, and in nerve fibers surrounding the central canal. This pattern of 5-HT1A receptor-IR corresponds precisely to the autoradiographic localization of ^{3}H-8-OH-DPAT described in the rat spinal cord. The most notable pattern of 5-HT1A receptor -IR that we observed was on the axon hillocks of motoneurons throughout the ventral horn. This novel site of 5-HT1A receptor localization is not detected with ^{3}H-8-OH-DPAT, suggesting possible heterogeneity of 5-HT1A receptor signal transduction mechanisms. A dense population of 5-HT1A receptors on the motoneuron axon hillock indicates an important role in the regulation of motor output. Species differences in 5-HT1A receptor-IR were noted: for example, variation in density of fiber labeling in the dorsal horn, the pattern of 5-HT1A-IR around the central canal, and the intense somatodendritic labeling of spinal serotonergic (raphe) cells in the primate cervical spinal cord. (Research supported by NIA # P01 AG10208)

635.4

DISTRIBUTION OF 5-HT1A RECEPTORS IN THE PRIMATE BRAINSTEM [1]P.J. Gannon*, [2]N.M. Kheck, and [2]E.C. Azmitia. [1]Dept Otolaryngology, Mt Sinai Sch Med, NY NY10029; [2]Dept. Biology, New York Univ. NY NY10003.

We used an 5-HT1A receptor antipeptide (aa170-186) antibody to investigate the distribution of this serotonin receptor subtype in the brainstem of the Old World monkey *Macaca fascicularis.*

Three adult monkeys were fixed by transcardiac perfusion, then 40μm brainstem sections incubated in primary antibody at 1:2000 for 72 hours at 5°C followed by visualization using an ABC-peroxidase kit (Vector).

Three main types of cell labelling patterns were apparent throughout the brainstem: 1) on neurons of both the serotonergic raphe nuclei and the noradrenergic locus ceruleus, dense labelling was distributed diffusely throughout the soma and dendritic tree. Similarly labelled large neurons were scattered throughout the reticular formation; 2) Most neurons in the brainstem motor nuclei showed a dense label localized specifically to the axon hillock. This axon hillock pattern was also seen throughout the brainstem on some neurons of both sensory and reticular groups. Sub-populations of vestibular nuclei neurons were also labelled; 3) A diffuse general label was present on astrocytes throughout the brainstem.

The somatodendritic label we observed may represent receptor populations acting as autoreceptors. The dense axon hillock label may represent populations of 5-HT1A receptors involved in temporally dynamic regulation of motor output at this critical cellular location. During primate evolution, basal mammalian motor systems evolved and changed to subserve the more sophisticated communicative repertoire present in primates. These evolutionary advances in brain function involved incorporation of the 5-HT1A receptor as a key player.

(Work supported in part by NIA Grant# PO1 AG10208)

635.5

DISTRIBUTION OF 5-HT1A RECEPTOR mRNA IN THE HUMAN HIPPOCAMPUS J.F. López*, D.T. Chalmers, A.A.F. Sima, and S.J. Watson University of Michigan-MHRI, , Ann Arbor, MI 48109-0720

The present study examined the comparative distribution of 5-HT1a receptor mRNA and 5-HT1a binding in human hippocampus using in situ hybridization with a specific human cRNA probe and receptor autoradiography with the selective ligand [3H]8-OH-DPAT. In agreement with previous binding studies, the highest levels of 5-HT1a binding in the human hippocampus were observed in the CA1 subfield and in the subiculum. High levels of binding were also observed in the granular and molecular layers of the dentate gyrus. Intermediate levels were present in the pyramidal and molecular layers of CA2 and CA3 subfields. In contrast to 5-HT1a binding, the highest concentration of 5-HT1a mRNA expression was observed in the granular layer of the dentate gyrus. A positive signal was also detected in the pyramidal cell layer of CA1, CA2 and CA3 subfields, although the mRNA levels were lower than in the dentate gyrus. No 5HT1a mRNA was detected in the stratum oriens, radiatum or moleculare. The subiculum and the external layers of the parahippocampal gyrus also expressed 5HT1a mRNA at concentrations similar to the CA subfields. In conclusion, there is a concordance between the areas expressing 5-HT1a mRNA with the areas showing specific 5-HT1a binding in human hippocampus. However, there are differences in distribution and concentration between mRNA and receptor sites within specific anatomical regions. These differences are probaly due to the cytoarchitectural distibution of the neuronal cell bodies, where the 5-HT1a mRNA is transcribed, versus the apical and basal dendrites, where the 5HT1a receptors are localized. Supported in part by a grant from the American Suicide Foundation.

635.6

DISTRIBUTION OF THE SEROTONIN-1A RECEPTOR, SEROTONIN UPTAKE SITE AND TRYPTOPHAN HYDROXYLASE-LIKE IMMUNOREACTIVITY (TpH-LI) IN THE HUMAN DORSAL RAPHE. L.A. Shapiro, C.A. Stockmeier*, M.T. Lowy and J.F. Singer, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

The anatomical distribution of serotonin-1A (5HT-1A) receptor binding and 5HT uptake sites, in relation to TpH-LI, was measured in serial sections in subnuclei of the human dorsal raphe nucleus. Midbrains from 7 male subjects dying of natural causes were collected at autopsy, frozen, and 10 μm thick sections were cut. The ranges in ages and postmortem intervals were 46-67 years and 11-24 hr., respectively. Quantitative receptor autoradiography was used to measure the binding of [^{3}H]8-hydroxy-DPAT to 5HT-1A receptors and [^{3}H]paroxetine to the 5HT uptake site in serial sections. Immunohistochemical localization of TpH-LI was also done in post-fixed sections to localize 5HT-synthesizing neurons in relation to radioligand binding. Subnuclei within the DR were identified according to Baker et al. (1990, 1991). 5HT-1A receptor binding was significantly greater in the ventrolateral (VL) subnucleus than the ventral (V), interfascicular (IF) or dorsal (D) subnuclei. 5HT-1A receptor binding closely paralleled cell bodies stained for TpH-LI. In contrast, [^{3}H]paroxetine binding was homogeneously distributed among the subnuclei. Age was negatively correlated with 5HT-1A receptor binding in only the VL, and postmortem interval was negatively correlated with 5HT-1A receptor binding in only the V subnucleus. Immunohistochemical localization of TpH-LI is an important tool for identifying 5HT-1A receptor binding and 5HT uptake sites within subnuclei of the human dorsal raphe nucleus. MH45488 and MH41684.

635.7

EXPRESSION OF 5-HT$_{1a}$,5-HT$_{2a}$ and 5-HT$_{2c}$ RECEPTORS ON ASTROCYTES. W.D. Hirst, M.A.N. Rattray, G.W. Price and G.P. Wilkin, (SPON: Brain Research Association),Department of Biochemistry, Imperial College of Science, Technology and Medicine. London SW7 2AZ, England.

The evidence to date for the presence of serotonin receptors on astrocytes *in vitro* has mainly been dependent on ligand binding and second messenger assays. We have demonstrated the expression of serotonin receptor subtype mRNAs using *in situ* hybridisation. cDNA clones of 5-HT$_{1a}$, 5-HT$_{2a}$ and 5-HT$_{2c}$ subcloned into suitable vectors were used to generate specific ^{35}S-labelled riboprobes. These were hybridised to neonatal rat cortical astrocyte cultures. Results show that the astrocytes *in vitro* express mRNAs for the above three receptor subtypes. Data from binding experiments with receptor specific ligands and second messenger assays complement the *in situ* hybridisation results,showing that the astrocytes express the receptor protein and that it is functional.

We have also addressed the question of serotonin receptor subtype expression on astrocytes in adult rat brain sections using a combination of *in situ* hybridisation of receptor subtype specific cRNAs and immunohistochemical localisation of the astrocyte specific marker GFAP. We found little evidence of co-localisation of receptor message with GFAP positive astrocytes, suggesting that few astrocytes express these serotonin receptors in the adult brain. We are currently investigating the possibility of *in vivo* receptor expression during development or following brain damage to interpret our *in vitro* findings.

635.8

TWO POPULATIONS OF [^{3}H]8-OH-DPAT BINDING SITES IN BRAIN: REGIONAL DIFFERENCES IN BINDING PARAMETERS AND RESPONSE TO GTP. A.Y. Korneyev*, O.B. Belonogoff and S. B. Seredenin, Institute of Pharmacology, 8 Baltiyskaya Str. Moscow, Russia.

The heterogeneity of [^{3}H] 8-OH-DPAT binding is attributed to an independent population of binding sites, 5-HT transporter, or to the multiple affinity states of a 5-HT$_{1A}$ receptors. The situation is complicated by the high tendency of 8-OH-DPAT and 5-HT to artefactual, nonreceptor binding, when incubation medium is not supplemented with ascorbic acid.

In the present study the profiles of [^{3}H] 8-OH-DPAT binding to 5-HT$_{1A}$ receptors in rat brain cortex and brain stem were compared in the presence of ascorbic acid. In both brain regions [^{3}H] 8-OH-DPAT specific binding was stimulated by a number of divalent cations (most potent Mn^{2+}) and inhibited by Cu^{2+} and GTP. The high affinity binding component (K_d = 0.2 - 0.3 nM) represents 62 ± 14% of the binding capacity in the brain cortex membranes, whereas the low affinity component (K_d = 8 -12 nM) predominates in brain stem (79 ± 13% of binding capacity). In the presence of GTP the fraction of the high affinity component decreases to 24 ± 6% in brain cortex membranes and to less than 6% in brain stem membranes. GTP-insensitive [^{3}H] 8-OH-DPAT binding may occur as a result of oxidation of -SH groups on the 5-HT$_{1A}$ receptor/G protein complex or other posttranslational modifications. Since the membranes from the two tissues were isolated and incubated in identical conditions and in the presence of ascorbic acid, the detected regional differences have apparently preexisted in the rat brain. These data indicate that the fraction of high affinity [^{3}H] 8-OH-DPAT binding sites, not sensitive to GTP negative modulation, is about 4 times higher in brain cortex, than in brain stem.

635.9

LOCALIZATION OF 5-HT6 RECEPTOR mRNA IN THE RAT BRAIN BY IN SITU HYBRIDIZATION HISTOCHEMISTRY. R. P. Ward[1], M. W. Hamblin[2], B.J. Hoffman[3], J. E. Lachowicz[4], D. R. Sibley[4*], and D. M. Dorsa[1]. [1]Dept. of Pharmacology, Dept of Psychiatry and Behavioral Sciences, Univ. of Washington, Seattle, WA 98195; [2]GRECC, Seattle VAMC, Seattle, WA 98108; [4]Molecular Neuropharmacology Section, Experimental Therapeutics Branch, NINDS, NIH Bethesda, MD 20892; [3]Laboratory of Cell Biology, NIMH , NIH Bethesda, MD 20892.

The Serotonin receptor subtype 5-HT6, which raises intracellular cAMP via stimulatory G-proteins, has been recently cloned and characterized. To determine the CNS distribution of 5-HT6 mRNA, *in situ* hybridization was performed in coronal sections of rat brain. An ^{35}S labeled riboprobe, complimentary to the 5 prime noncoding region of the 5-HT_6 RNA, and a ^{33}P labeled riboprobe complimentary to its 3 prime noncoding region were used for hybridization.

5HT-6 receptor message was found in serotonin projection fields, rather than regions of serotonin containing cell bodies, suggesting that the receptor is mainly postsynaptic. Hybridization signal was highest in olfactory tubercle, as well as prominent in the striatum, nucleus accumbens, dentate gyrus, and CA1, 2, and 3 of the hippocampus. Less intense hybridization was observed in cerebellum, some diencephalic nuclei, the amygdala, and layers two, three, four, and six of the cortex, including prefrontal cortex. This pattern of hybridization was observed with both probes, but not when sense transcripts were used.

Given the 5-HT_6 receptor has a high affinity for the atypical antipsychotic clozapine, and the importance of striatum and nucleus accumbens as proposed sites of antipsychotic drug effects, the data suggests that this receptor may play an important role in mediating the effects of the atypical antipsychotic agents.

635.10

SEXUAL DIFFERENCES IN mRNA EXPRESSION AND BINDING DISTRIBUTION OF 5-HT_{1A} AND 5-HT_2 RECEPTORS IN RAT HIPPOCAMPUS. L.Zhang, W.Ma, J.L.Barker and D.R.Rubinow. BPB, NIMH, Lab.of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

Sexual differences in the expression of mRNA and concentration of 5-HT_{1A} and 5-HT_2 receptors were studied by *in situ hybridization* and autoradiography ([^{3}H]DPAT and [^{3}H]ketanserin binding)in rat hippocampus. 5-HT_{1A} receptor mRNA showed distinct expression patterns in female and male rats. In males little 5-HT_{1A} receptor mRNA was detected in CA3 and CA4, while in females the receptor expression was relatively uniform throughout the hippocampal formation. However, there were no significant sexual differences in the distribution of 5-HT_{1A} receptor binding sites. Unlike 5-HT_{1A}, 5-HT_2 receptor mRNA was expressed in CA3 and CA4 with little expression detected in CA1 and CA2. However, the concentration of the 5-HT_2 receptor measured with [^{3}H]ketanserin was significantly higher in females than in males. To our knowledge, this is the first report of the sexual differences in the transcript and receptor levels of hippocampal 5-HT receptors. The data complement a growing literature demonstrating functional and anatomical difference in the 5-HT system in males and females.

NEUROTRANSMITTERS AND OTHER AMINES/PURINES

636.1

PHARMACOLOGICAL CHARACTERIZATION OF THE EFFECT OF EXTRACELLULAR ADENOSINE 5'-TRIPHOSPHATE ON THE CONCENTRATION OF FREE CYTOPLASMIC CALCIUM IN RAT PHEOCHROMOCYTOMA PC12 CELLS. E. Adamec* and G. Koski. Dept. of Anesthesia, Massachusetts General Hospital, Boston, MA 02114.

In PC12 cells, ATP causes substantial elevation of $[Ca^{++}]_i$ ($[Ca^{++}]_i$max =1400 nM, EC_{50}=30 μM). Administration of ATP in Ca^{++}-free buffer caused a $[Ca^{++}]_i$ rise of approximately 10% of the total response indicating a small contribution of Ca^{++} release from internal stores to the total $[Ca^{++}]_i$ rise. The majority of ATP-induced $[Ca^{++}]_i$ rise was therefore caused by Ca^{++} influx. Increasing $[Ca^{++}]_e$ increased peak $[Ca^{++}]_i$ response to ATP with half-saturation at 3.5 mM reflecting high permeability of ATP-activated ion channels to Ca^{++}. ATP-induced $[Ca^{++}]_i$ increase positively correlated with the concentration of free, uncomplexed, ATP (ATP^{4-}) suggesting that ATP^{4-} is the agonist at the ATP receptor in this cell type. Administration of UTP also increased $[Ca^{++}]_i$ ($[Ca^{++}]_i$max=322 nM, EC_{50}=36 μM). This elevation again consisted of a combination of Ca^{++} influx and Ca^{++} release from internal stores. The $[Ca^{++}]_i$ response to ATP and UTP administered together was not additive and pre-exposure to one nucleotide decreased the response to the other indicating that, in PC12 cells, ATP and UTP share a common receptor (P_{2N}). Since the EC_{50} values for the ATP- and UTP-induced $[Ca^{++}]_i$ rise were not significantly different, but ATP caused much larger maximal $[Ca^{++}]_i$ rise, it appears that ATP acts as a full agonist and UTP as a partial agonist on the P_{2N} receptor in PC12 cells.

636.2

Distinct cellular responses are evoked by ATP and UTP in PC12 cells.

L. de Souza[1], H. Moore[2], J. K. Reed[1,2] and J. Fullard*[3]. Depts. of Biochemistry[1], Chemistry[2] and Zoology[3], University of Toronto, Erindale College, Mississauga, ONT. L5L 1C6, Canada.

Extracellular ATP is a modulator in many cell types including those of neuronal origin. The intracellular responses of ATP are mediated by purinergic P_2 receptors, of which several sub-types have been identified based on the ATP analogue efficacy profiles. We and others have identified a P_{2N} sub-type in PC12 cells that recognizes both ATP and the pyrimidine, UTP and mediates the increase in $[Ca^{2+}]i$ and IP_3 (Raha et al., J. Cell. Physiol., 154: 623-630, 1993).

We have now identified additional signalling pathways in PC12 cells that are mediated by ATP but not by UTP. Specifically, ATP but not UTP depolarizes PC12 cells (measured with bisoxonol and [^{3}H]TPP$^+$). The ATP-mediated depolarization is not desensitized by preincubation with UTP.

The effect of extracellular nucleotides on pH_i in PC12 cells was measured using BCECF. 100 μM ATP induces a rapid initial acidification from an basal pH_i of 7.0, followed by a gradual amiloride-sensitive alkalinization; 100 μM UTP evokes only a delayed alkalinization. The degree of ATP-mediated acidification is concentration dependent.

In addition, ATP but not UTP (up to 400 μM) induces secretion of endogenous dopamine. Taken together, these observations support the view that purine and pyrimidine nucleotide triphosphates activate distinct signalling pathways in PC12 cells.

This research was supported by NSERC.

636.3

Facilitation by zink of ATP-activated currents and dopamine release in PC12 cells. K. Inoue*, K. Nakazawa, K.Koizumi, M.Ikeda and K.Inoue, Div. Pharmacology, NIHS, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

We reported that ATP stimulates P2 purinoceptor, activates Ca-permeable cation channels, stimulates Ca influx and dopamine (DA) release in PC12 cells (a revew in NIPS, April, 1992). We have also reported that endogeneous transmitters, dopamine (Eur.J. Pharmacol., 215, 321-324, 1992 ; Pflügers Arch., 422, 458-464, 1993), 5-HT (Pflügers Arch., in press, 1994) , and adenosine (Br.J.Pharmacol., in press, 1994; Eur.J.Pharmacol., in press, 1994) regulate the ATP-evoked responses. We report here that zink also regulates ATP-activated currents and DA release from PC12 cells.

Zink (3 to 300 μM) potentiated ATP(30μM)-evoked inward current in a concentration dependent manner without change the maximal response. The mechanism of the facilitation by zink was different from that by dopamine and high dose of adenosine. Zink also potentiated ATP(30μM)-evoked dopamine release in the same manner as that of the currents. Zink (10μM) shifted the concentration-response curve of ATP-evoked release to the left without affecting the maximal response. It is suggested that the facilitation is dependent on the calcium influx through ATP-activated cation channels since it was dependent on external calcium, was blocked by an ATP-receptor blocker suramin, and was parallel to the increase of intracellular free calcium concentration.

636.4

UPREGULATION OF A_1 ADENOSINE RECEPTORS IN CEREBELLAR GRANULE CELLS IN RESPONSE TO CHRONIC CAFFEINE EXPOSURE. **B.D.Hettinger-Smith, M.Leid and T.F.Murray*. College of Pharmacy, Oregon State University, Corvallis, OR 97331.**

Adenosine is a ubiquitous neuromodulator acting in the central nervous system at A_1, A_2 and A_3 adenosine receptors. Caffeine and related methylxanthines act as nonspecific antagonists at A_1 and A_2 adenosine receptors. Chronic antagonism of these G-protein coupled receptors results in tolerance to the stimulatory effects of caffeine which may, in part, be due to A_1 adenosine receptor upregulation. The effect of chronic caffeine exposure in cerebellar granule cell cultures was examined.

Cerebella were obtained from eight day old Sprague Dawley rat pups and primary cultures of the granule cell neurons were established. Binding in membranes derived from granule cell neurons was examined using the A_1 adenosine receptor antagonist [^{3}H] 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Nonspecific binding was determined in the presence of 100 μM N^6-cyclopentyladenosine (CPA). Receptor expression was analyzed at day 5,7,9,11 and 13 post culture. Maximum [^{3}H]DPCPX binding was present at day 13. In order to determine the caffeine concentration needed to obtain maximal upregulation, cells were incubated with final concentrations of 10^{-4} to 10^{-9} caffeine and [^{3}H]DPCPX binding was analyzed. Maximal upregulation of [^{3}H]DPCPX binding was obtained with 100 μM caffeine. Time course studies revealed that incubation with 100 μM caffeine for 7 days produced the greatest upregulation of [^{3}H]DPCPX binding. Cerebellar granule cells in primary culture appear to afford a useful model system for the study of A_1 adenosine receptor regulation.

636.5

CHARACTERIZATION AND EXPRESSION OF THE HUMAN A2a ADENOSINE RECEPTOR GENE. RA Peterfreund*, MM MacCollin, J Gusella, JS Fink, Depts. Anesthesia and Neurology, Massachusetts General Hospital, Boston, MA 02114.

Adenosine A2a receptors (A2aRs) mediate vasodepressor actions of adenosine in the CNS and periphery and behavioral actions of adenosine in the basal ganglia. To begin to define mechanisms controlling the expression of A2aRs, we isolated and characterized genomic clones from a human chromosome 22-only cosmid library which contain the A2aR sequence. Southern blot and PCR analysis confirm the location of this gene on chromosome 22. A single intron (~6 kb) interrupts the coding sequence between transmembrane domains III and IV of the cDNA. Northern blot analysis reveals that the gene encodes a principle ~2.6 kb transcript as well as a secondary ~1.4-1.8 kb transcript in some tissues. Abundant expression is observed in caudate but little or no expression is detected by Northern blot in other brain regions sampled. Heart, lung and hematopoietic tissues express significant amounts of A2aR mRNA. We conclude that the human A2aR gene is a member of the intron-containing class of G protein-linked receptor genes with regionalized expresssion in discrete brain and peripheral tissues. The findings support roles for A2aRs in basal ganglia, cardiopulmonary and immune function.

636.6

INTERACTIONS BETWEEN ETHANOL AND ADENOSINE IN HIPPOCAMPAL BRAIN SLICES. L. Diao* and T.V. Dunwiddie . Dept. Pharmacology, UCHSC & VA Med. Res. Service, Denver, CO80262.

A number of studies have strongly suggested that adenosine may be an important mediator of the effects of ethanol(EtOH) both in isolated systems as well as intact animals. These adenosine-mediated effects of EtOH might result from increases in extracellular adenosine which occur as a result of the inhibition by EtOH of adenosine uptake via the nucleoside transporter. Although this effect has been demonstrated biochemically, there have been few functional studies of EtOH-adenosine interactions. In the present study, we tested whether EtOH inhibits the uptake of adenosine in vitro by measuring the extracellularly recorded fEPSP response in the rat hippocampal slice preparation. When tested alone, EtOH(20mM and 100mM) produced variable effects on the fEPSP response. In about one third of slices, EtOH had a biphasic effect with an initial excitation followed by inhibition. In the remainder of the slices, EtOH had no effect or produced a slow decline the response, but the overall effect was not statistically significant. However, on the one hand, when slices were pretreated with competitive adenosine receptor antagonists such as theophylline(250μM) and 8-cyclopentyltheophylline(1μM), both types of responses to EtOH were blocked, and there was a 2 to 6-fold reduction in the standard deviation of responses to EtOH. On the other hand, when slices were pretreated with the adenosine uptake inhibitor dipyridamole(5μM), exogenous adenosine(10μM and 30μM), and the selective A1 adenosine receptor agonist N^6-cyclohexyladenosine(1μM), ethanol caused significant decreases in fEPSP amplitudes that were reversible upon washout or application of theophylline(250μM-1mM). However. when slices were pretreated with EtOH, this had no effect upon subsequent responses to either dipyridamole(5μM) or adenosine(10μM and 30μM). These results suggest that the ability of EtOH to modulate synaptic transmission at these synapses is dependent on the prior activation of adenosine receptors, but this does not appear to involve an inhibition of adenosine uptake by EtOH.

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636.7

ACETATE DOES NOT INDUCE ADENOSINE-MEDIATED INHIBITION IN THE DENTATE GYRUS OR HIPPOCAMPAL CA1 REGION. J.M. Brundege and T.V. Dunwiddie*. Dept. of Pharmacology, University of Colorado Health Sciences Center, and Veterans Admin. Medical Res. Service, Denver, CO.

Acetate is the primary breakdown product of ethanol metabolism and can accumulate in the brain following ethanol consumption. It has been suggested that acetate can be metabolized to adenosine within cells in the CNS and release of this adenosine may cause neuronal inhibition. In order to determine the effects of extracellular acetate on neurons, we applied exogenous sodium acetate (0.5 to 2 mM) to hippocampal slices from adult male rats. The acetate caused no significant effect on the resting membrane potential, input resistance, or EPSP amplitude in CA1 pyramidial neurons and had no effect on extracellular field EPSPs in either the CA1 region or the dentate gyrus. To determine the effects of intracellular acetate, we compared the effects of the adenosine receptor antagonist theophylline on cells impaled with intracellular microelectrodes containing 2.5M K^+-acetate, 1M KCl, or 2M K^+-methylsulfate. With all three intracellular filling solutions, theophylline produced small but statistically significant increases in input resistance, consistent with the presence of low concentrations of extracellular adenosine. However, the response to theophylline with acetate-filled electrodes was not different from the other filling solutions. **These results suggest that the presence of acetate, either in the extracellular solution or within the intracellular electrode, does not induce a significant adenosine response in the hippocampus.** Supported by NS 29173 and the Veterans Administration Medical Research Service.

636.8

ADENOSINE REDUCES ACCOMMODATION AND A TRANSIENT OUTWARD CURRENT IN RAT SPINAL DORSAL HORN NEURONS IN CULTURE. Y.S Pak* and M.W Salter, Div. Neurosci., Hosp. for Sick Children, Dept. Physiol., Univ. Toronto, Toronto, Ont. Canada M5G 1X8.

Studies *in vivo* have indicated that adenosine may be a chemical mediator in the spinal dorsal horn (Salter *et al.* Prog. Neurobiol. 41;125-156, 1993). To investigate the effects of adenosine further we made recordings from dorsal horn neurons *in vitro* using perforated patch and whole-cell techniques. Recording electrodes contained the following (in mM): for perforated patch - K_2SO_4 90, KCl 35, EGTA 11, Ca^{2+} 1, HEPES 10; for whole-cell - KCl 140, HEPES 10, EGTA 11, $MgCl_2$ 2, ±ATP 4, ±GTP 0.5. Adenosine (100-500 μM; 30-120 s) had no effect on the resting membrane potential or resting conductance during current clamp recordings (n=9 cells). However, adenosine caused a 50-100% increase in the rate of action potential discharge evoked by depolarizing intracellular current pulses. This effect was reversible and reproducible. To investigate the underlying mechanism we examined the effect of adenosine on voltage-activated K currents. Tetrodotoxin (1 μM) was included in the extracellular medium for these experiments. Currents were evoked by voltage steps from -110 to +30 mV; a hyperpolarizing pulse (-120 mV; 100 ms) was given prior to each step. Typically, a rapidly-activating transient outward current was elicited beginning near -60 mV and a sustained outward current was recruited at -20 mV. With 34 of 38 cells adenosine reduced the amplitude of the transient outward current but had no effect on the sustained current; neither current was affected in the remaining cells. The transient outward current appeared to be an A-type current because it was blocked by 4-AP (6 mM) but was insensitive to TEA (10 mM). Our results suggest a novel effect of adenosine to reduce accommodation by depressing an A current in dorsal horn neurons. (Supported by Canadian MRC).

636.9

ADENOSINE RECEPTOR-MEDIATED MODULATION OF CHOLINERGIC NEURONES OF THE LATERODORSAL TEGMENTAL NUCLEUS OF THE RAT, *IN VITRO*. D.G. Rainnie*, H.C.R. Grunze, R.W. McCarley, and R.W. Greene, Dept of Psychiatry, Harvard Medical School and VAMC, Brockton, MA 02401.

Increased discharge activity of mesopontine cholinergic neurones plays a key role in the production of electroencephalographic (EEG) arousal. We recently reported that mesopontine cholinergic neurones are under the tonic inhibitory control of endogenous adenosine (AD; Rainnie et. al., Science 1994). Here we extend this study to examine factors regulating the muliphasic response of laterodorsal tegmental nucleus (LDT) neurones to exogenous adenosine application.

Whole cell patch-clamp recordings were made from LDT neurones using a standard *in vitro* brainstem slice preparation. Application of AD (5-100 μM) resulted in a dose-dependent hyperpolarization-depolarization-hyperpolarization, in 76% of all neurones tested (n=67). The hyperpolarization was associated with a decrease in membrane input resistance that was mediated by activation of an inwardly rectifying potassium conductance. The hyperpolarization was mimicked by the A_1 agonist N^6-cyclohexyladenosine (CHA, 1 μM) and blocked by the A_1 antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPX; 500nM) suggesting that A_1 purinergic receptors mediate this response. In the presence of 500 nM CPX, no response was observed following application of the specific A_2 receptor agonist CGS-21860, suggesting that activation of A_{2a} receptors does not mediate the intervening depolarization. Pressure ejection of AD (1 mM, 300 ms) during the depolarization evoked an equivalent hyperpolarization to that obtained before AD application, suggesting that the depolarization does not result from a desensitization and subsequent resensitization of A_1 receptors. The depolarization persists in the presence of specific AD uptake inhibitors, NBTI (10 μM) and dipyridamole (1 μM). This data also suggests that activation and subsequent saturation of the AD uptake pump cannot account for the membrane depolarization. Additional experiments are being conducted to determine the mechanism underlying the depolarizing response.

636.10

PROTOCOL FOR IN VIVO MICRODIALYSIS OF ADENOSINE IN THE BRAINSTEM AND BASAL FOREBRAIN OF FREELY MOVING CATS. C.M.Portas*, M. Thakkar, D. Rainnie, R. Greene and R.W. McCarley Lab. Neurosci., Dept. Psychiatry, Harvard Med. School/ VAMC, Brockton, MA 02401.

Considerable evidence implicates mesopontine and basal forebrain cholinergic neurons in control of EEG and behavioral arousal. Our recent in vitro work indicates these neurons are under tonic inhibitory control of adenosine (Rainnie et al., Science, 1993). This raises the question of whether increased brain metabolism during prolonged wakefulness might increase extracellular adenosine concentration, thereby promoting sleep. This abstract describes the methodology for in vivo microdialysis detection of adenosine levels, and preliminary data on effects of adenosine perfusion. Following a two week postoperative recovery period, a 2 mm tip probe (CMA 10) was inserted into the target area 20-24 hours before starting perfusion with artificial CSF at a flow rate of 1μL/min (CMA 100 microdialysis pump). Perfusate was collected via a dual channel microdialysis swivel (Insteach Laboratories PA) incorporated in a 24 channel electrical swivel (Airflyte Electronics Company N.J.) used for the polygraphic recordings. Analysis of 20 uL samples was via a HPLC system (Waters Pump 501), coupled to a UV detector (Waters 486). The column used was a Waters ODS bonded phase and the mobile phase consisted of Na phosphate buffer 8mM, methanol 9%, pH ~5. Chromatographic data were recorded and concentrations determined by a Shimadzu (CR3A, Japan) integrator using an external standard calibration curve. RESULTS: The detection limit for adenosine was 100 f-moles/sample. The baseline waking level in the mesopontine LDT was about 15 nM (0.3 pmoles/sample), while in basal forebrain it was about 50nM (1pmole/sample). Since in vitro tests have shown probe recovery to be about 8%, this implies extracellular concentration levels of about 120nM in the LDT and 400nM in basal forebrain. Measurements of state-related adenosine alterations are incomplete. However, preliminary data show dramatic changes from baseline state values during 2 h of adenosine (1mM) perfusion via the microdialysis probe into the basal forebrain: there were marked increases in SWS (140%) and REM sleep (290%) and a 70% decrease in waking.

636.11

TRH ANALOGUES AND ADENOSINE AGONISTS ELICIT VOMITING IN THE CAT. James B. Lucot*. Dept. Pharmacol., Wright State Univ., Dayton, OH 45435

During the testing of novel receptor actions which theoretically could decrease motion sickness, some receptor selective drugs unexpectedly elicit vomiting. Describing novel emetics has value in (1) offering new tools for research into emetic mechanisms, (2) having potential for new insights into the emetic pathways and (3) identifying possible clinical side effects missed by studies with anesthetized subjects or in species which do not vomit. All drugs were administered SC in a volume of 0.1 ml/kg. Tests lasted 30 min or 15 min after the last vomit, whichever occurred later. The disubstituted stable TRH analogue MK 771 elicited vomiting with an ED50 of 3.1 μg/kg and the monosubstituted analogue CG 3703 did so with an ED50 of 1.9 μg/kg. The adenosine A_1 agonist CHA elicited vomiting with a steep dose-response curve. Vomiting elicited by 10 μg/kg was blocked by 300 μg/kg of the A_1 antagonist CPT, reduced by 100 μg/kg of the nonselective antagonist PD 115199 and not affected by up to 300 μg/kg of the A_{2a} antagonist CGS 15943. The adenosine A_{2a} agonist CV 1808 elicited vomiting with a shallow dose-response curve. The vomiting elicited by 300 μg/kg was not altered by the above doses of CPT, CGS 15943 or up to 100 μg/kg of the A_2 antagonist DPMX, leaving questionable the emetic site of action.

636.12

THE HISTAMINE ACTION ON ACUTELY DISSOCIATED NEURONS OF THE RAT NEOSTRIATUM. M. Munakata* and N. Akaike. Dept. of Bio-Plasticity, Kyushu Univ. Fac. of Med., Fukuoka 812, Japan

The neostriatum is innervated by histamine fibers. Histamine receptors have been detected in this region by the radioligand binding study and also involved in pathological conditions such as acathisia and catalepsy. However, the functional role of histamine receptors in the neostriatum remains unknown. We investigated, therefore, the effects of histamine in neurons acutely dissociated from the neostriatum of 2-week-old Wistar rats, using *perforated patch clamp technique*. In current-clamp mode 10^{-5}M histamine slowly depolarized the membrane with increased firing activities, and in voltage-clamp mode it evoked a net inward current accompanied by decreased conductance at a V_H of -44 mV. This response may be restricted in interneurons. We clarified pharmacologically that this histamine-induced response was mediated by both the H_1 and H_2 receptors. H_1-mediated response arised with a threshold concentration of 10^{-8} M and reached the maximal response at 10^{-6} M, whereas H_2-mediated response was in the range between 10^{-6} and 10^{-5} M. Both H_1 and H_2 receptor-mediated currents resulted from a decreased K^+ conductance, which may increase neuronal excitability. The co-existence of H_1 and H_2 receptors with a wide concentration range may result in extensive modulation of the functional activities in the neostriatum.

636.13

GENOMIC CLONING AND CHARACTERISATION OF A RECOMBINANT HUMAN HISTAMINE H1-RECEPTOR. W.H.M.L. Luyten*, M.D. De Backer, P. Van Gompel, K. De Loore, W. Gommeren and J.E. Leysen. Department of Biochemical Pharmacology, Janssen Research Foundation, Beerse, B2340 Belgium.

A human histamine H1-receptor (H1-R) gene lacking introns was isolated by screening a human genomic library with a bovine H1-R probe (BBRC **197**: 1601-1608 (1993)). The coding region of the human H1-R was sequenced and subcloned into the pSVL expression vector (Pharmacia) yielding pSVL/H1-R. Membranes from COS-cells transiently transfected with pSVL/H1-R showed saturable specific binding of [^{3}H]pyrilamine with a K_D of 1.2 nM and a B_{max} of 3400 fmol/mg protein (compared to a K_D of 0.30 nM and B_{max} of 480 fmol/mg protein for guinea-pig cerebellum membranes). Radioligand binding was inhibited by histamine and by several H1-R antagonists at comparable concentrations for membranes of pSVL/H1-R-transfected COS-cells and of guinea-pig cerebellum. Untransfected COS-cells or COS-cells transfected with pSVL showed very little specific [^{3}H]pyrilamine binding (< 50 fmol/mg protein). However, histamine (10^{-6}M-10^{-4}M) concentration-dependently induced inositol phosphate (IP) formation to a similar extent in transfected and untransfected COS-cells, and this effect could be progressively inhibited by increasing pyrilamine concentrations. This suggests that an endogenous H1-R in COS-cells couples much more efficiently to the IP signal transduction cascade than the transfected H1-R.

CHO-K1 cells were permanently transfected with a H1-R construct in pRc/CMV (Invitrogen). Membranes prepared from transfectants showed specific [^{3}H]pyrilamine binding ranging from 100 to over 2000 fmol/mg protein. Histamine concentration-dependently induced IP formation more than ten-fold over background in transfected but not in untransfected CHO-cells, and this effect could be progressively inhibited by increasing pyrilamine concentrations.

Sequencing of the genomic human H1-R clone upstream of the start-codon revealed several sequence motifs with known regulatory function. Their functional relevance is being assessed by cloning 5'-flanking regions of various size into pGL2 luciferase reporter vectors (Promega) and measuring luciferase activity after transfection.

636.14

MOLECULAR MODELLING OF THE H2 HISTAMINE RECEPTOR

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A 3-D model of the canine H2 receptor (1) was constructed and analysed. This model was defined using primary sequence comparisons and three-dimensional homology building, using bacteriorhodopsin as a template. Experimental data from a variety of sources were used to localise the ligand binding site and to identify residues likely to be responsible for receptor affinity, selectivity and efficacy.

Molecular mechanics of the receptor model with tautomeric forms and folded and extended conformations of histamine were studied.

The potential energies of this model are consistent with the binding data obtained from mutagenesis studies (2) On the basis of our results we have postulated that Glu-270 in transmembrane VII would be an important residue to target in future mutagenesis studies.

Finally, we have studied a potent H2 antagonist receptor, famotidine. The complex energy for this antagonist was 50 Kcal/mol more stable than for the histamine complex. The principal interactions were the cation guanidine chain, that corresponds to the protonated amine in histamine, with Asp-98, the amino group of the sulphamoyl amidine chain with Asp-186 and a weak interaction between the oxygen sulphonamide and Thr 190.

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2.-I. Gantz, J del Valle, L. Wang, T. Tashiro, G. Munzert, Y. Guo, Y Konda and T. Yamada, J. Biol. Chem., **267**, 20840, (1992)

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636.15

HISTAMINE H_3 RECEPTOR SUBTYPE CHARACTERIZATION IN ISOLATED GUINEA PIG TISSUES. C.A. Rizzo, S. Tozzi, M.E. Monahan, D. Krobatsch and J.A. Hey.* Schering-Plough Research Institute, Kenilworth, NJ 07033.

The histamine H_3 receptor subtypes involved in the H_3 modulation of electrical field stimulated (EFS) neurogenic responses in pulmonary artery sympathetic and ileum parasympathetic preparations were characterized to determine whether H_{3A} or H_{3B} receptors are present in these tissues. Simultaneous measures of EFS-evoked [^{3}H] overflow and tension in [^{3}H]-norepinephrine-loaded pulmonary artery were sensitive to tetrodotoxin (300 nM) and insensitive to hexamethonium (100 μM), with only evoked tension significantly antagonized by prazosin (100 nM). R-α-methylhistamine's inhibition of evoked tension and [^{3}H] overflow were both dose-dependently antagonized by thioperamide (30 - 100 nM). In the pulmonary artery the rank order potency of the H_3 antagonists thioperamide (pA_2 = 8.1 ± 0.1), burimamide (pA_2 = 7.1 ± 0.2), and impromidine (pA_2 = 6.7 ± 0.1) vs R-α-methylhistamine (pD_2 = 7.1) suggests a predominant H_{3A}-like receptor population on postganglionic sympathetic neurons. In the ileum H_3 receptor assay, the rank order potency of the H_3 antagonists thioperamide, burimamide, and impromidine, with pA_2 estimates of 8.7 ± 0.1, 7.3 ± 0.1 and 7.1 ± 0.1, respectively, vs R-α-methylhistamine (pD_2 = 8.2), also suggests a predominantly H_{3A}-like receptor population.

636.16

CLOZAPINE-SENSITIVE BINDING TO HISTAMINE H_3 RECEPTOR IN RAT FRONTAL CORTEX. A Rodrigues, J Goldfarb and GD Prell* Dept Pharmacology, Mount Sinai Medical Center, New York, NY 10029

There is evidence suggesting that histaminergic activity is elevated in brains of schizophrenic patients (Soc. Neurosci. Abstr. 18: 446; Lancet 335: 1351; Biol Psychiat. 30: 349). For example, in patients refractory to conventional neuroleptic drugs, CSF levels of histamine's primary metabolite were higher than in controls and correlated with severity of symptoms. In cortex of patients with chronic schizophrenia, H_1 receptors were downregulated, consistent with overstimulation by histamine. H_3 autoreceptors regulate synthesis and release of histamine; H_3 heteroreceptors influence release of dopamine and 5-HT. Neuroleptics bind to H_1 and H_2 receptors. We examined their interactions with H_3 receptors. The latter, in rat cortical membranes prepared in Na/K-phosphate buffer (50 mM, pH 7.4), were labeled with ^{3}H-Nα-methylhistamine. Saturation binding (non-specific binding defined with either 1 μM thioperamide or R(-)α-methylhistamine) yielded a K_D of approx 0.5 nM and a B_{MAX} of 150-170 fmol mg^{-1} protein. In competition studies using 0.8 nM ^{3}H-NαMeHA (spec. binding ≈90% of total) typical neuroleptics of many chemical classes (e.g. haloperidol, chlorpromazine, thiothixene, pimozide, spiperone) showed low affinity binding (IC_{50}s 3-50 μM). The atypical neuroleptic clozapine displayed much higher affinity, producing shallow competition curves consistent with more than one binding site. Preliminary data suggest a high affinity component (approx 30% of total specific binding) with a K_I (assuming competitive inhibition) in the 3-10 nM range, consonant with the range of plasma levels seen clinically. Clozapine's interactions with H_3 receptors may have therapeutic relevance. (NS-28012)

636.17

HISTAMINERGIC NEURONS MEDIATE RESTRAINT STRESS-INDUCED CHANGES IN ACTIVITY OF SELECTED CENTRAL CATECHOLAMINERGIC AND 5-HYDROXY-TRYPTAMINERGIC NEURONS IN THE RAT A.E. Fleckenstein*, K.J. Lookingland and K.E. Moore, Dept. Pharm./Tox., Michigan State Univ., E. Lansing, MI 48824

The role of histaminergic (HA) neurons in mediating stress-induced changes in activity of selected catecholaminergic and 5-hydroxytryptaminergic (5HT) neuronal systems was examined in male rats. Dopaminergic (DA), noradrenergic (NE) and 5HT neuronal activity was estimated by measuring concentrations of neurotransmitters and metabolites in brain regions containing terminals of these neurons. Placement of rats within restraining tubes increased dopamine metabolism in the nucleus accumbens, decreased dopamine metabolism in the intermediate lobe of the pituitary, and was without effect in the striatum and median eminence. These data indicate that restraint stress increases mesolimbic, decreases periventricular-hypophysial, and is without effect on nigrostriatal and tuberoinfundibular DA neuronal activity. Neither depletion of neuronal histamine by α-fluoromethylhistidine (αFMH), blockade of H_1 receptors by mepyramine, nor blockade of H_2 receptors by zolantidine prevented stress-induced increases in dopamine metabolism in the nucleus accumbens suggesting that HA neurons are not major contributors to stress-increased mesolimbic DA neuronal activity. In contrast, treatment with both αFMH and mepyramine, but not zolantidine, prevented stress-induced decreases in dopamine metabolism in the intermediate lobe indicating that HA neurons mediate stress-induced decreases in periventricular-hypophysial DA neuronal activity through an action at H_1 receptors. Stress increased norepinephrine and 5-hydroxytryptamine metabolism in the hypothalamus, and 5-hydroxytryptamine metabolism in the nucleus accumbens. Both αFMH and mepyramine antagonized, whereas zolantidine did not prevent these increases suggesting that HA neurons contribute to stress-induced increases in 5HT and NE neuronal activity through an action at H_1 receptors. (supported by NIH grant NS15911 and a Pharmaceutical Manufacturers Association Foundation fellowship)

636.18

SIGMA RECEPTOR LIGANDS REGULATE MELATONIN SYNTHESIS IN RAT PINEAL GLAND. L. Steardo[1]*, P. Monteleone[2] M.R. Carratù[1], A.Tortorella[2], M. Persichella[1], M.Maj[2] and V. Cuomo[1]. Dept. Pharmacology Medical School Univ. of Bari[1], Dept of Psychiatry Medical School Univ. of Naples[2].(Italy)

Into rat pineal gland blood-derived tryptophan is sequentially converted to serotonin (5HT), N-acetyl-serotonin and finally to melatonin. N-acetyltransferase (NAT), the enzyme catalyzing the acetylation of 5HT, is believed to be the rate limiting factor in this biosynthetic pathway. Recently sigma receptors have been reported to be highly concentrated in rat pineal gland, but at moment, their physiological role remains to be understood. Therefore the effects of sigma receptor ligands on NAT activity and melatonin content in rat pineal gland were investigated. In a first set of experiments , +N-allylnormetazocine (+SKF 10047), the prototype agonist of sigma binding sites, at doses of 0.5; 2.5 and 5.0 mg\kg i.p. was able to induce clear-cut increases in both pineal NAT activity ($F_{3,16} = 3.864$ $p< 0.03$ two way ANOVA) and melatonin content ($F_{3,16} = 9,229$ $p<0.001$ two way ANOVA) at nigth, but not during the day. In a second set of experiments 1,3 - di (2tolyl) guanidine (DTG), a potent and selective sigma agonist, at doses of 5; 10 and 20 mg\kg i.p., markedly enhanced NAT activity and melatonin synthesis stimulated by beta 1 adrenoreceptor activation induced by 0.3 and 1 mg\kg of i.p. isoproterenol ($F_{3,48} = 2.478$ $p< 0.03$ two way ANOVA) ($F_{3.48} = 3.551$ $p< 0.007$ two way ANOVA), respectively. The present results suggest that sigma receptor stimulation is involved in the regulation of melatonin production in rat pineal gland.

636.19

MELATONIN MODULATES CHOLINERGIC SYNAPTIC TRANSMISSION IN ENTERIC NEURONS. B. Prieto-Gómez*[1], R. Espinosa-Luna[2] and C. Barajas-López[3]. [1]Department of Physiology, Faculty of Medicine, UNAM, México [2,3]Department of Biomedical Sciences, McMaster University, Ontario, Canada.

Melatonin, a hormone produced and released by the pineal gland, is also synthesized by cells of the gastrointestinal wall, where it might be a local regulator of gut functions. In this study, we investigate the actions of melatonin as a modulator of synaptic transmission in the submucosal plexus from the guinea-pig ileum. Intracellular recordings were made in submucosal neurons to measure the amplitude of nicotinic excitatory postsynaptic potentials (EPSPs). Melatonin (0.03-3 mM) reversibly decreased the amplitude of nicotinic EPSPs in a concentration dependent manner (EC_{50}=332 μM). Maximal effects were observed within 4 min after the arrival of the melatonin-containing solution and they persisted for as long as melatonin was present (up to 15 min). Melatonin actions were not modified by the presence of idazoxan and atropine (1μM) indicating that they are not mediated by endogenous release of ACh or noradrenaline or by direct activation of α_2-adrenergic or muscarinic receptors by melatonin. The superfusion of melatonin (1μM) also blocked the nicotinic depolarizations induced by locally applied ACh. These observations indicate that melatonin might be a local modulator of synaptic transmission in the enteric nervous system and that at least part of its effects are postsynaptic. This work was supported by the MRC, OMH and CONACYT.

636.20

ADENOSINE INCREASES THE SIGNAL TO NOISE RATIO IN FASCIA DENTATA BY A PRE-SYNAPTIC MECHANISM. T.H. Swanson*. Departments of Neurology and Neurosciences, Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

Adenosine analogs increase or decrease perforant path stimulation evoked dentate granule cell action potential firing depending on concentration. We have previoulsy reported that 1.0 nanomolar of the stable A1 adenosine analog cyclopentyladenosine (CPA) increases granule cell action potential firing for a given population PSP amplitude. To explore the mechanism of this novel finding, we performed whole-cell current clamp recordings of rat dentate granule cells were performed in 400 μM thick hippocampal slices. Resting membrane potential (-65±4 mV), input resistance (136 ±32 MΩ), and action potential threshold (-33 ±7 mV) during perforant path stimulation were compared in the presence and absence of 1.0 nanomolar CPA in seven experiments. No significant change in any of the measured variables could be detected during CPA application. In other experiments, we measured glutamate release from perforant path stimulated granule cells using a sensitive HPLC assay with PITC derivitization (resolution limit 10 picomoles). Average basal glutamate release was 170 ±65 pM / ml, rising to 450 ± 50 pM / ml during one Hz perforant path stimulation. No change in glutamate release was detected during CPA application. We conclude that low concentrations of adenosine analogs increase perforant path / granule cell synaptic efficiency without affecting intrinsic post-synaptic membrane properties or altering glutamate release. These data support a pre-synaptic mechanism for this novel action in accord with the documented ability of adenosine to inhibit polysynaptic but not monosynaptic IPSP's in similar preparations.

NEUROTRANSMITTER INTERACTIONS: SEROTONIN

637.1

EVIDENCE FOR INVOLVEMENT OF MEDIAN, BUT NOT DORSAL, RAPHE NEURONAL 5-HT_{1A} AUTORECEPTORS IN THE MEDIATION OF THE ANTICATALEPTIC EFFECTS OF 8-OH-DPAT IN THE RAT. M-L Wadenberg* and V. Hillegaart. Dept of Behavioral Pharmacology, Astra Arcus AB, S-15185 Södertälje, Sweden.

The systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT has been shown to counteract the catalepsy induced by DA D_2/D_3 receptor blocking agents like raclopride or haloperidol. 8-OH-DPAT also produces this effect after icv, but not intrathecal, administration, suggesting a supraspinal mediation of the effect. The dorsal and median raphe nuclei have both been implicated as the target for effects by 8-OH-DPAT, but there are divergent opinions on the relative importance of these nuclei in this regard.

The present study was designed to clarify the functional role of median versus dorsal raphe 5-HT nuclei in the mediation of the anticataleptic effect of 8-OH-DPAT. Animals were observed for catalepsy on an inclined (60°) grid at different time intervals. Degree of catalepsy was scored according to a square root transformation of raw data. It was found that 8-OH-DPAT (0.5 or 2.5 μg, -10 min) locally injected into the median raphe nuclei produced a dose-dependent and statistically significant antagonism of raclopride-(16 mg kg^{-1} sc, -1h) induced catalepsy. 8-OH-DPAT (0.5 or 2.5 μg) locally injected into the dorsal raphe nuclei, however, had no effect on raclopride-(16 mg kg^{-1} sc) induced catalepsy.

In conclusion: The results provide evidence for a specific involvement of 5-HT_{1A} autoreceptors of the median raphe in the mediation of the antagonism by 8-OH-DPAT of catalepsy induced by dopamine receptor blocking drugs.

637.2

STRIATAL C-FOS INDUCTION BY CONCOMITANT STIMULATION OF 5-HT1A-5-HT2, D1-5-HT1A AND D1-5-HT2 RECEPTORS. J. Gervais[1], S. Bovetto[3], D. Richard[3] and C. Rouillard*[1,2]. [1]Lab. of Neurobiology, [2]Dept. of Pharmacology and [3]Physiology, Laval University, Québec, Canada G1J 1Z4.

We have previously demonstrated that the release of 5-HT by fenfluramine (FEN) induces Fos-like immunoreactivity (Fos-LI) in the dorso-medial part of the striatum (STR) as well as in other brain structures receiving a dense 5-HT innervation. The immediate-early gene response is under the control of both DA and 5-HT. However, we were unable to reproduce the effects of FEN in the STR with the administration of selective 5-HT receptor subtypes agonists. This markedly contrated with c-fos expression in cortex where 8-OH-DPAT (5-HT1A) and DOI (5-HT2) reproduced the effects of FEN. Therefore, the main objective of the present study was to investigate the possibility of interaction between different 5-HT receptor subtypes or between 5-HT and DA receptor subtypes. To investigate this possibility, seven groups of animals, each consisting of at least 3 rats were used in this study. Agonists were administrated i.p. and animals were sacrified 120 min later. Brains were removed, sliced and processed for Fos-LI. Treatments were a) Saline+Saline, b) Saline+8-OH-DPAT (2.5 mg/kg), c) Saline+DOI (2.5 mg/kg), d) Saline+SKF38393 (2.0 mg/kg), e) SKF38393+DOI, f) SKF38393+8-OH-DPAT and g) 8-OH-DPAT+DOI. Our results show that all three agonist combinations induce Fos-LI expression in the medial part of the STR, the nucleus accumbens and the cortex. The 8-OH-DPAT+DOI combination was the most effective, followed by SKF 38393+DOI and SKF38393+8-OH-DPAT combinations. No Fos-LI was found in the lateral part of STR. This study strongly suggest the possibility of interaction and synergism between 5-HT receptor subtypes and between DA and 5-HT receptors in the STR. The immediate-early gene expression is restricted to a striatal territory related to associative functions while the striatal territory related to motor functions did not show any Fos-LI. (Supported by NSERC and FRSQ)

637.3

THE EFFECT OF SEROTONIN ON TYROSINE HYDROXYLASE ACTIVITY: AN *IN VIVO* MICRODIALYSIS STUDY. Richard S.J. Nassar* and Matthew P. Galloway, Cellular & Clinical Neurobiology Program, Dept Psychiatry & Behavioral Neurosciences, Wayne State Univ Sch Med, Detroit, MI 48202.

We have shown previously that striatal perfusion of 5-HT agonists increases extracellular levels of DA. As an extension of these studies, the potential role of serotonin (5-HT) in modulating tyrosine hydroxylase (TH) activity in striatal DA nerve terminals was monitored by *in vivo* microdialysis. To ascertain TH activity extracellular DOPA levels were measured during the continual perfusion of the decarboxylase inhibitor, difluoromethyldopa (100 μM, DFMD, MDL 71,801), in artificial CSF (2μL/min). Once a baseline of DOPA was established, pharmacological manipulation of TH activity was evaluated. To test the responsivity of TH under these experimental conditions either the D_2 antagonist haloperidol or the D_3 preferring agonist 7-OHDPAT was administered systemically. 7-OHDPAT (0.5 mg/kg, i.v.) decreased DOPA levels by 40% and haloperidol (1 mg/kg, i.v.) increased DOPA levels 82% over baseline levels. Further, haloperidol reversed the decrease in DOPA levels brought on by 7-OHDPAT. Co-perfusion of DFMD and 5-HT (100 μM, 10 min) induced a 79% increase in DOPA levels with a return to baseline levels after removal of 5-HT. Moreover, when the 5-HT agonist m-chlorophenylpiperzine (mCPP, 100 μM, 10 min) was co-perfused with DFMD, DOPA levels increased 80% ± 4% (n=6) with a return to baseline after mCPP was removed. In summary, the local application of 5-HT or a 5-HT agonist caused a reversible increase in DOPA levels indicating that 5-HT may modulate TH activity. Supported by NIDA-04120 and the Joe Young Sr. research Fund.

637.4

ENDOGENOUS SEROTONIN MODULATES HISTAMINE RELEASE IN THE RAT HYPOTHALAMUS MEASURED BY IN VIVO MICRODIALYSIS. K.S.M. Laitinen*, J.T. Laitinen and L. Tuomisto. Departments of Pharmacology & Toxicology and Physiology, University of Kuopio, POB 1627, FIN-70211 Kuopio, Finland.

Histamine (HA) acts as a neurotransmitter/modulator in the mammalian brain. HA is thought to modulate circadian functions, including food intake, arousal, body temperature and hormone secretion. In the anterior hypothalamus (AHy), HA content is high in the suprachiasmatic nuclei (SCN). A major non-photic input to the SCN is the serotonergic projection from the midbrain raphe nuclei. Hypothalamic serotonin (5-HT) is also implicated in the regulation of circadian rhythms, including feeding behavior. We used in vivo microdialysis to study possible interactions of HA and 5-HT in the AHy. Male Wistar rats were anesthetized and a microdialysis probe was implanted into the AHy (aimed at the SCN). The probe was perfused with aCSF (2 μl/min) and samples were collected every 30 min. The dialysate concentration of HA was analyzed by HPLC with fluorescence detection and that of 5-HT by HPLC-EC. Local perfusion with 5-HT (10 or 100 μM) increased HA release significantly and dose-dependently. Methysergide (10 mg/kg ip.), a $5\text{-HT}_{1c/2}$ blocker, suppressed basal HA release by 30 %. Dexfenfluramine, a 5-HT releaser and reuptake inhibitor, via the probe (10 μM), increased HA release up to 160 %. With the same dose of dexfenfluramine, 5-HT release increased 10-fold in the same brain area. These data suggest that endogenous serotonin modulates histamine release in the AHy and histamine may participate in the anorexic effect of dexfenfluramine.

637.5

5-HT_2 RECEPTORS ARE NOT INVOLVED IN THE 5-HT INDUCED INCREASE OF EXTRACELLULAR DOPAMINE IN THE PREFRONTAL CORTEX OF AWAKE RATS. R.N. Iyer* and C.W. Bradberry, Department of Psychiatry, Yale Univ. Sch. of Med. and Veterans Administration Medical Center, Box 116/A2, 950 Campbell Avenue, West Haven, CT 06516.

Interactions between central DA and 5-HT pathways have been proposed to play a role in either the pathophysiology of schizophrenia or its amelioration by antipsychotics and there is increasing interest in the prefrontal cortex (PFC) as a site of dysfunction in schizophrenia. We have previously shown that the local application of 1-10 μM 5-HT through a microdialysis probe facilitates DA release in a dose-dependent manner in the PFC. Because previous work suggested that a 5-HT_2 receptor sub-type partially mediated a 5-HT induced DA release in the nucleus accumbens, we determined if there is 5-HT_2 mediation of DA release in the PFC. Infusion of the selective $5\text{-HT}_{2/1c}$ agonist DOI through a microdialysis probe at a concentration of 500 μM did not cause any increase in extracellular DA and co-perfusion of the selective 5-HT_2 antagonist MDL-907 (100 nM) with 3 μM 5-HT failed to significantly attenuate the 5-HT induced enhancement of extracellular DA. These results demonstrate the absence of 5-HT_2 receptor involvement in 5-HT induced DA release in the prefrontal cortex of the rat. Supported by DA 08073, MH 44866, DA 08227, the West Haven Veterans Administration Center Grant for the study of PTSD, and a NARSAD Young Investigator Award to CWB.

637.6

PET AND *IN VIVO* MICRODIALYSIS STUDIES OF SR 46349B, A NEW AND SELECTIVE 5-HT_2 ANTAGONIST. S.L. Dewey*, P. Tan, G.S. Smith, P. King, N. Pappas, R. MacGregor, Y.-S. Ding, C. Shea, D. Alexoff, T. Martin, S. J. Gatley, J.S. Fowler, and A.P. Wolf. Chem. and Med. Dep't, BNL, Upton, NY 11973.

Our previous PET studies with labeled raclopride have demonstrated 5-HT's inhibition of striatal DA, using altanserin (5-HT2 antagonist) and citalopram (5-HT2 reuptake inhibitor), and we have replicated these findings with *in vivo* microdialysis studies. To measure the ability of DA to modulate 5-HT in cortical, striatal and limbic areas, we sought to develop a selective radiotracer for the 5-HT2 receptor. The utility of the new and highly selective antagonist, SR 46349B was investigated as a potential PET radiotracer and microdialysis studies were performed to measure the effects of SR 46349B on extracellular dopamine. Serial PET studies using 11C-SR 46349B (11C-SR) were carried out in baboons for 90 minutes with a two hour time interval between studies. Test/retest variability was excellent (<10%). Radioactivity peaked at 20 mins in all cortical areas. Cerebellum, thalamus, and striatum peaked at 10 mins. At 60 mins the frontal cortex to cerebellum ratio was 1.5. Pretreatment with altanserin reduced this ratio to 1.0. Citalopram administration reduced 11C-SR binding both cortically and subcortically suggesting its sensitivity to changes in endogenous 5-HT. HPLC of mouse brain homogenate after 11C-SR showed >94% of the C-11 was parent compound. Microdialysis in freely moving rats after injection of SR 46349B showed an average peak increase in striatal DA of 375% which is higher that the 150% effect noted with altanserin. Supported by NARSAD award to SLD and GSS, USDOE/OHER, NIH Grants MH-49165, NS-15638, NS-15380.

637.7

5-HT_4 RECEPTORS ARE INVOLVED IN THE ENHANCEMENT OF STRIATAL DOPAMINE RELEASE INDUCED BY SEROTONIN IN VIVO. U. Spampinato, N. Bonhomme, M. Le Moal and P. De Deurwaerdère. Université de Bordeaux II, INSERM U. 259, Domaine de Carreire, 33077 Bordeaux Cedex, France. (SPON: European Brain and Behaviour Society).

The role of different serotonin receptor subtypes (5-HT_1,5-HT_2, 5-HT_3, 5-HT_4) in the control of striatal dopamine (DA) release exerted by serotonin (5-HT) was studied by using *in vivo* intracerebral microdialysis. A microdialysis probe (CMA 11/3 mm) was implanted in the striatum of rats anesthetized under a mixture of halothane-NO_2/O_2 and perfused with an artificial CSF (NaCl 145, KCl 2.7, $CaCl_2$ 1.2, $MgCl_2$ 1 mM, pH = 7.4) at a constant flow rate of 2 μl/min. Two hours after the onset of perfusion, 15 min fractions were collected over 150 min period of time and analysed by H.P.L.C. coupled with electrochemical detection. In control animals the resting DA release was stable during the experience.

Drugs used were locally applied by means of the microdialysis probe. One, 2.5 and 5μM 5-HT significantly enhanced DA release in a dose-dependent manner up to 157, 253 and 446% of basal value respectively. The effect induced by 1μM 5-HT was not blocked in the presence of 10μM (-)pindolol, a 5-HT_1 receptor antagonist; 1μM ketanserin or 10μM cinanserin, both 5-HT_2 antagonists, as well as 1 or 10μM ondansetron (GR 38032F), a selective 5-HT_3 antagonist, were also ineffective. In contrast, 10 or 100μM DAU 6285, a $5\text{-HT}_{3/4}$ antagonist, significantly reduced the effect of 5-HT on DA release (-20% and -60% respectively). Moreover, 100μM BIMU8, a selective 5-HT_4 agonist, enhanced DA release (+ 85%) and this effect was reduced by 100μM DAU6285 (-40%).

These results demontrate that *in vivo* 5-HT exerts a facilitatory influence on striatal DA release and that 5-HT_4, but not 5-HT_1, 5-HT_2 or 5-HT_3, receptor subtypes are implicated in this effect.

637.8

SEROTONIN RECEPTOR STIMULATION MODULATES BASAL GANGLIA NEUROPEPTIDE mRNAS FOLLOWING 6-HYDROXYDOPAMINE LESIONS. I.G. Capodilupo*, W.A. Wolf & P.D. Walker, Departments of Anatomy & Cell Biology and Psychiatry, Wayne State University School of Medicine, Detroit, MI 48201 and VA Medical Center, Allen Park, MI 48101.

Inhibition of dopamine (DA) neurotransmission lowers preprotachykinin (PPT) and raises preproenkephalin (PPE) mRNAs within the rodent striatum. Since serotonin (5-HT) is also a potential transsynaptic regulator of striatal neuropeptide gene expression, we sought to determine the effects of 5-HT receptor stimulation on PPT and PPE mRNA levels following lesions of the nigrostriatal pathway. Adult male Sprague-Dawley rats (175-200g) received unilateral injections of vehicle or 6-hydroxydopamine (6HD; 10μg) into the left ventral midbrain. After 3 weeks, each vehicle or 6HD-lesioned animal received daily intraperitoneal injections of saline, DOI (5-HT2 agonist; 1 mg/kg), or 8-OH-DPAT (5-HT1 agonist; 1 mg/kg) for 7 days followed by sacrifice on the day of the last injection. Within the saline or DPAT-treated 6HD groups, nigral DA levels ipsilateral to the DA lesion were only depleted by approximately 50% compared to contralateral controls. Not surprisingly, these two 6HD groups containing mild lesions exhibited no change in striatal PPT or PPE mRNAs. However, 6HD-lesioned rats treated with DOI had significantly lower nigral DA levels (below 10% of controls) and expressed the "classic" striatal neuropeptide pattern (decreased PPT, increased PPE) signifying complete DA depletion. These results suggest that 5-HT2 receptor stimulation may contribute to the development of neuropeptide transcriptional abnormalities within the striatum following removal of dopamine. Supported by the Michigan Parkinson's Foundation and a 1993 WSU Research Grant.

637.9

SEROTONIN ENHANCES DOPAMINE-INDUCED INHIBITION OF VENTAL TEGMENTAL AREA (VTA) NEURONS RECORDED *IN VITRO*, M.S. Brodie and E.B. Bunney*, Dept. Physiology and Biophysics and Program in Emergency Medicine, University of Illinois at Chicago, Chicago, IL 60612.

Dopaminergic neurons of the ventral tegmental area (VTA) have been implicated in a number of pathological processes, including drug abuse and schizophrenia. The VTA receives serotonergic innervation from the median and dorsal raphe nuclei, and recent studies have demonstrated that serotonin has both excitatory and inhibitory effects on VTA neurons. We have recently reported that serotonin potentiates ethanol-induced excitation of VTA neurons; our objective in the present study was to determine whether serotonin alters dopamine-induced inhibition of VTA neurons.

Coronal brain slices containing the VTA were prepared from young adult F344 rats. Dopamine (1 μM - 5 μM) inhibited all neurons tested, and was administered in the absence and presence of serotonin (5 - 25 μM). Serotonin alone produced small increases or decreases in the baseline firing rate. The mean percent inhibition of firing produced by 2 μM dopamine was increased from 38.8% to 48.3% by 5 μM serotonin, and was increased to 53.4% in 10 μM serotonin. Similar effects were seen on the inhibitory potency of 1 and 5 μM dopamine; 5-HT enhancement was statistically significant as determined by ANOVA ($P<0.05$). This increase in potency was not explained by an additive effect of serotonin and dopamine, since 1) potentiation of dopamine effects was seen regardless of whether serotonin alone increased or decreased the firing rate, and 2) when serotonin did cause a slight decrease in firing rate, the effect of dopamine was superadditive with that of serotonin. These data suggest that serotonergic neurotransmission in the VTA may serve to enhance the inhibitory action of dopamine in the VTA. Grant Support: PHS AA-09125; UIF Helen M. Thomsen Research Fund.

637.10

THE EFFECT OF SEROTONERGIC AGENTS ON THE EXPRESSION OF D_1 AGONIST MEDIATED REPETITIVE JAW MOVEMENTS (RJM). Helen Rosengarten*and Arnold J. Friedhoff. Department of Psychiatry, Millhauser Labs. NYU School of Medicine, New York, N.Y. 10016 USA.

Selective D_1 receptor agonists are capable of inducing repetitive jaw movements (RJM) in rats. SKF 38393 appears to be selective for the D_1 receptor, however, at higher concentrations it is capable of interacting with $5HT_{1C}$ and $5HT_2$ receptors. To explore a possible interaction between serotonergic and dopaminergic systems in the mediation of RJM, we studied the effect of a series of 5HT agonists and antagonists on SKF 38393 inducible behavior. It was demonstrated that the mixed $5HT_{1C}$ and $5HT_2$ receptor agonist, mCPP, and $5HT_2$ antagonists, ritanserin and cyproheptadine, are capable of enhancing SKF 38393-induced RJM by 180%, 240% and 217%, respectively, while the $5HT_{1A}$ agonist, 8OH-DPAT, the $5HT_{1B}$ agonist, CGS 120066, and the $5HT_2$ agonist, DOI, had no effect. The results of this study demonstrate that there is an interaction between DA and 5HT systems in the expression of RJM. This study was supported by NIMH Grants 06818 and 35976.

637.11

SEROTONIN INHIBITS $GABA_A$ RECEPTOR-MEDIATED CURRENT VIA ACTIVATION OF $5\text{-}HT_7$ RECEPTOR IN CULTURED SUPRACHIASMATIC NEURONS. H. Katsuki*, F. Kawahara and H. Saito. Dept. Chemical Pharmacol. Fac. Pharmaceutical Sci., The Univ. of Tokyo, Tokyo 113, Japan.

The suprachiasmatic nucleus (SCN) neurons receive extrinsic and intrinsic neural inputs, the majority of which utilizes GABA as a neurotransmitter. They also receive serotonergic input from midbrain raphe nuclei, which is known to influence SCN neuronal activity through as yet unidentified mechanisms. In the present study, whole-cell voltage-clamp recordings were made from postnatal rat SCN neurons in dissociated cell culture, in order to test possible modulation by 5-HT of GABA-activated current (I_{GABA}). With $[Cl^-]_o$ and $[Cl^-]_i$ of 149 and 133 mM respectively, and at a holding potential of -60 mV, GABA activated bicuculline-sensitive inward current in the SCN neurons, with the EC_{50} value of 6.6 µM. 5-HT reversibly inhibited I_{GABA} in a concentration-dependent manner (100 nM to 1 µM), without changing the reversal potential of I_{GABA}, which was almost equal to the Cl^- equilibrium potential. 8-OH-DPAT, a $5\text{-}HT_{1A,7}$ agonist, mimicked the effect of 5-HT at concentrations of 100 nM to 10 µM. 5-CT ($5\text{-}HT_{1,7}$ agonist, 1 µM) also inhibited I_{GABA}, whereas a $5\text{-}HT_2$ agonist DOI (1 µM) had no significant effect. The effect of 100 nM 8-OH-DPAT on I_{GABA} was virtually abolished by co-application of ritanserin ($5\text{-}HT_{2,7}$ antagonist, 100 nM) but not by pindolol ($5HT_{1A}$ antagonist, 100 nM). The effect of 100 nM 5-HT was also suppressed by co-application of ritanserin, but not by pindolol or ketanserin ($5\text{-}HT_2$ antagonist, 100 nM). External application of 8-bromo-cAMP (1 mM) or forskolin (50 µM) suppressed I_{GABA}, the magnitude of which was similar to that of 5-HT. Furthermore, the effect of 100 nM 5-HT on I_{GABA} was significantly suppressed by a protein kinase A inhibitor H-8 (1 µM). These results suggest that 5-HT inhibits I_{GABA} in the SCN neurons, which involves the activation of $5\text{-}HT_7$ receptor and its coupled cAMP-dependent system. This inhibition of $GABA_A$ receptor function may be involved in the regulation by 5-HT of SCN neuronal activity.

637.12

REGULATION OF SEROTONIN RELEASE IN THE INTERMEDIATE AREA OF THE RAT THORACIC SPINAL CORD BY COEXISTING NEUROCHEMICALS: SUBSTANCE P AND THYROTROPIN-RELEASING HORMONE. L.Yang* and C.J.Helke. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Serotonin (5-HT), substance P (SP) and thyrotropin-releasing hormone (TRH) coexist in ventral medullary neurons which project to the intermediolateral cell column (IML) of the thoracic spinal cord (Sasek *et al.*, Neuroscience, 35:105, 1990) and are involved in the spinal regulation of sympathetic nervous system. [^{3}H]5-HT is released from the intermediate area (which includes the IML) and the release of [^{3}H]5-HT is regulated by presynaptic inhibitory $5\text{-}HT_{1B}$ autoreceptors (Yang *et al.*, Synapse, in press, 1994). We used in vitro superfusion of the microdissected intermediate area of the rat thoracic spinal cord to study the effects of SP and TRH on the release of [^{3}H]5-HT. A neurokinin 1 (NK_1, the preferred receptor for SP) selective agonist, GR 73632 (1 µM), significantly increased the basal release of [^{3}H]5-HT. The increased release of [^{3}H]5-HT induced by GR 73632 was blocked by a selective NK_1 antagonist, GR 82334 (1 µM). GR 82334 (1 µM) did not change the basal release of [^{3}H]5-HT itself. SP (1 µM) also increased the basal release of [^{3}H]5-HT. A TRH agonist, MK-771 (1 µM), had no effect on the basal or K^+-stimulated release of [^{3}H]5-HT. The present study suggests that the release of [^{3}H]5-HT at the intermediate area could be regulated by SP, but not by TRH. The excitatory effect of SP on the release of 5-HT appears to be mediated by NK_1 receptor. These findings will be helpful in understanding the complex roles of coexisting neurochemicals in the spinal regulation of the sympathetic nervous system. (Supported by NIH grant R01 NS24876).

637.13

SEROTONIN INTERACTIONS WITH SUBSTANCE P AND ENKEPHALIN IN THE HYPOGLOSSAL NUCLEUS AND THE SPINAL CORD VENTRAL HORN OF THE RAT. D.A. Jackson*, K.M. Imel and S.R. White. Dept. of Pharmacol., Univ. of Washington, Seattle, WA 98195 and Dept. of Vet. & Comp. Anat., Pharm. & Physiol., Washington State Univ., Pullman, WA 99164.

Many serotonin-containing fibers that terminate on or near motoneurons in the ventral horn of the spinal cord and in the hypoglossal nucleus contain colocalized substance P. A smaller population of fibers in the same regions appear to contain colocalized serotonin and enkephalin. Rapidly firing serotonin cells appear to corelease both serotonin and neuropeptides but little is known about how these substances interact to affect postsynaptic cells. The present study used electrophysiological techniques to examine how serotonin may interact with these neuropeptides to alter motoneuron excitability in the lumbar spinal cord and in the hypoglossal nucleus. Microiontophoretic application of serotonin or substance P enhanced glutamate-evoked firing of all motoneurons that were tested in the spinal cord and the hypoglossal motor nucleus. Application of serotonin and substance P together had additive effects on motoneuron excitability until a maximum firing rate was reached. In contrast, microiontophoretic application of enkephalin inhibited glutamate-evoked firing of the same cells that were facilitated by serotonin. Concurrent application of enkephalin and serotonin diminished the facilitatory effects of serotonin alone. Therefore, it appears that cells which contain colocalized serotonin and enkephalin may either enhance or inhibit motoneuronal excitability depending upon the firing rate of the cell.

637.14

EFFECT OF THE α_2-ADRENOCEPTOR ANTAGONIST REMERON ON RAT 5-HT NEUROTRANSMISSION. N. Haddjeri*, P. Blier and C. de Montigny. Neurobiological Psychiatry Unit, McGill Univ., Montréal, Canada H3A 1A1.

Remeron (ORG 3770) is a mianserin derivative having antidepressant activity in humans. Using an *in vivo* electrophysiological paradigm in male Sprague-Dawley rats, the aim of the present study was to assess the effect of racemic remeron [(±)RM] and of its (-)isomer on pre- and postsynaptic α_2-adrenoceptors and to characterize their putative modulation of 5-HT neurotransmission in the hippocampus. Previous studies in our laboratory have shown that the activation of α_2-autoreceptors on norepinephrine (NE) terminals by a low dose of clonidine (10 µg/kg, i.v.) enhances the effectiveness of the electrical stimulation (200 pulses: 0.5 ms, 300 µA, 1 Hz) of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons, whereas a high dose (100 or 400 µg/kg, i.v.) activates the α_2-heteroreceptors on 5-HT terminals thereby reducing the effectiveness of the stimulation. The injection of (±)RM (25 µg/kg, i.v.) significantly enhanced the effectiveness of the stimulation and prevented the effects of subsequent i.v. administration of both low and high doses of clonidine. (-)RM (25 µg/kg, i.v.) did not prevent the enhancing effect of the stimulation induced by the low dose of clonidine, but antagonized the reducing effect of the high dose of clonidine. Furthermore, (±) and (-)RM (500 µg/kg, i.v.) blocked the suppressant effect of microiontophoretically-applied NE on the firing activity of CA_3 hippocampus pyramidal neurons. In the dorsal raphe, only (±)RM dose-dependently (10 to 250 µg/kg, i.v.) enhanced the firing activity of the 5-HT neurons, and only (±)RM (100 µg/kg, i.v.) antagonized the suppressant effect of clonidine (10 µg/kg, i.v.) on 5-HT neuron firing activity. In conclusion, these results suggest that (±)RM is an antagonist of both α_2-adrenergic auto- and heteroreceptors, whereas (-)RM is selective for the α_2-heteroreceptors.

637.15

IS TRYPTAMINE SYNTHETIZED BY SEROTONERGIC NEURONS?

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Tryptamine (T) is present in the Central Nervous System (CNS) in very low concentration. T is synthetized from Tryptophane (Trp) by the aminoacid aromatic decarboxilase (AADC), enzime that is present in all monoaminergic neurons and also in glial cells. Localization of T in serotonergic neurons is not yet clear.

An immunocytochemical technique (ICt) was applied to CNS of adult rat, using specific anti-T or anti-serotonin as primary antibody. To increase the concentration of both indoleamines in the CNS, pretratment of rats with Trp and pargiline was performed 2 hr prior to fixation. T immunoreactivity was restricted to soma and processes of neuronal cells of raphe nucleus, substantia nigra (pars reticulata), and pons giganto cellularis nuclei. We discarded, therefore, that T be synthetized by glial and other cells in the CNS.

Distribution of serotonergic cells does not lay on top of tryptaminergic cells in the CNS, detected by ICt, so not all serotonergic neurons are able to synthetize T.

Immunoreactivity for both indoleamines was strongly reduced by inhibition of serotonin synthesis with para-chlorophenilalanine pre-treatment. We postulate that synthesis of T is not an alternative route for Trp utilization when Trp-hydroxylase (limitant enzime of serotonin synthesis) is inhibited.

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637.16

ANTIPSYCHOTIC-INDUCED CHANGES IN MONOAMINE SYNTHESIS AND TURNOVER: DIFFERENCES BETWEEN CLOZAPINE AND HALOPERIDOL.

R.E. Arthur, Jr.* and W.A. Wolf. V.A. Medical Center, Allen Park, MI 48101 and Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State University, Detroit, MI 48201.

Haloperidol and clozapine differ in their clinical profile and propensity for inducing extrapyramidal side effects. One reason for this may be their effects on the activity of serotonin (5-HT) and dopamine (DA) neurons subserving behavior and motor control. To assess 5-HT and DA synthesis and turnover in vivo, animals were administered haloperidol (0.5 mg/kg, i.p.) or clozapine (5-20 mg/kg, i.p.) followed by the decarboxylase inhibitor, m-hydroxybenzylhydrazine (100 mg/kg, i.p.). Animals were killed and brain regions were dissected and processed for HPLC analysis. Clozapine-treated animals had higher levels of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the midbrain raphe than vehicle-treated animals (6.28 ± 0.41 and 4.06 ± 0.11 pmoles/mg wet wt, clozapine and vehicle, respectively). This effect was evident at the lowest dose of clozapine (5 mg/kg). Raphe 5-HIAA levels in haloperidol-treated animals were similar to control. 5-HIAA in substantia nigra was also higher with clozapine, but not haloperidol, treatment (3.96 ± 0.19 and 2.53 ± 0.13 pmoles/mg wet wt, clozapine and vehicle, respectively). 3,4-dihydroxyphenylalanine (DOPA) accumulation was elevated in substantia nigra only in haloperidol-treated animals (4.76 ± 0.31, 2.79 ± 0.19 and 3.06 ± 0.10 pmoles/mg wet wt, haloperidol, clozapine and vehicle, respectively). In caudate and nucleus accumbens, low dose clozapine (5 mg/kg) increased DA turnover 2-4 fold, but not DOPA accumulation. High dose clozapine (20 mg/kg), like haloperidol, increased both DA synthesis and turnover. Additional data will be presented. The present results suggest that clozapine affects 5-HT and DA synthesis and turnover in a manner different from haloperidol.

NEUROTRANSMITTER INTERACTIONS: CHOLINERGIC/GABA

638.1

DIHYDREXIDINE RELEASES ACETYLCHOLINE AND IMPROVES COGNITIVE PERFORMANCE IN RATS.

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Neurochemical and behavioral studies have elucidated extensive interactions between dopaminergic and cholinergic systems in brain areas associated with movement and cognition. The initial goal of these studies was to evaluate the effect of the anti-Parkinson drug dihydrexidine (DHX), a full D_1 agonist, on brain acetylcholine (ACh) release using *in vivo* microdialysis techniques. Moderate doses (3 & 10 mg/kg) of DHX produced an approximate 50% increase in striatal ACh release that was blocked by the D_1 antagonist SCH23390 (0.3 mg/kg). A higher dose (17.5 mg/kg) was less effective in raising striatal ACh, possibly due to D_2 receptor activation. In frontal cortex, DHX (10 mg/kg) evoked a more robust increase (200%) in ACh release that was blocked by SCH23390 (0.3 mg/kg). Since elevations in brain ACh are associated with cognitive improvement, the effectiveness of DHX in a passive avoidance model of learning and memory was evaluated. These studies revealed a significant improvement in performance by 0.3 mg/kg DHX in scopolamine-amnestic rats. These results provide support for the hypothesis that DHX improves cognitive performance as a consequence of ACh release in relevant brain regions. Further, D_1 agonists may have novel therapeutic potential in the treatment of dementia.

638.2

RELEASE OF [^{3}H]ACETYLCHOLINE FROM SLICES OF RAT STRIATUM AND CEREBRAL CORTEX BY 5-HT_3 RECEPTOR ANTAGONISTS.

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Controversial results have been reported on the ability of 5-HT_3 receptor ligands to modulate acetylcholine (ACh) release from rat entorhinal cortex and also from other brain regions. We have studied the effect of 5-HT_3 receptor stimulation and blockade on basal and K^+-evoked [^{3}H]ACh release from superfused slices of rat striatum and cerebral cortex. K^+-evoked release consisted of two stimulations (S_1 and S_2, 30 min apart) with KCl (20 mM, 6 min) with drugs added 15-20 min before S_2 and calculation of changes in S_2/S_1. The 5-HT_3 antagonist ondansetron (0.01-10 μM) increased in a concentration-dependent manner both basal and K^+-evoked [^{3}H]ACh release from striatal slices, the latter effect being more marked. A lower though significant [^{3}H]ACh release by ondansetron was also found in superfused cortical slices. However, basal rather than K^+-evoked [^{3}H]ACh release was influenced by this 5-HT_3 antagonist in the cerebral cortex. Similar effects were observed with higher concentrations of granisetron in both brain regions. The 5-HT_3 agonist 2-Me-5-HT (1 μM) did not modify by itself [^{3}H]ACh release and a moderate decrease was only observed in the presence of the 5-HT_2 antagonist ritanserin. Interestingly, 2-Me-5-HT blocked the increase in both basal and K^+-evoked [^{3}H]ACh release induced by ondansetron. The results suggest that 5-HT_3 receptor antagonists may influence acetylcholine release from specific brain regions.

638.3

PRETREATMENT WITH THE NORADRENERGIC NEUROTOXIN DSP-4 *IN VIVO* REDUCES ACETYLCHOLINE (ACh) RELEASE IN THE RAT PREFRONTAL CORTEX *IN VITRO*.

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A deficiency in the locus coeruleus-noradrenergic system (LC-NA) has been proposed to be an important contributory factor in the pathogenesis and progression of central neurodegenerative disorders including Alzheimer's disease (Colpaert 1994, in *Noradrenergic Mechanisms in Parkinson's Disease*, CRC Press, Boca Raton, pp. 225-254). To examine the influence of the LC-NA on cortical ACh release, male SD rats were injected with DSP-4 (40 mg/kg i.p.) preceded 30 min by an injection of citalopram (10 mg/kg). Three days later, slices of the prefrontal cortex (PFC) were prepared, incubated with [^{3}H]choline, superfused and stimulated by exposure to increasing concentrations of K^+. The DSP-4 treatment reduced endogenous noradrenaline levels in cortical tissue by 71-94%. In slices of PFC from DSP-4 treated rats, the [^{3}H] overflows which were stimulated by 20, 35 and 45 mM K^+ were reduced by 18%, 21% and 42%, respectively, compared to slices prepared from vehicle treated animals. These results indicate that disruption of the LC-NA reduces a facilitative input to the cortical cholinergic system, and suggests that injury to or loss of LC-NA neurons might contribute to the cortical cholinergic dysfunction in Alzheimer's disease.

638.4

CHRONIC EFFECTS OF CAFFEINE, NICOTINE AND ETHANOL ON CENTRAL ADENOSINE AND CHOLINERGIC RECEPTORS AND CALCIUM CHANNELS: BEHAVIORAL CORRELATES IN MICE.

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Chronic caffeine increases density of central A_1-adenosine (cortex, striatum), nicotinic, muscarinic, serotonin and $GABA_A$ergic receptors. Central A_{2a}-Adenosine, α-adrenergic, dopaminergic, and NMDA-glutaminergic receptors are unchanged and the densities of β-adrenergic receptors are decreased. Theophylline and theobromine also increase density of A_1-receptors. The changes in A_1-, β_1- and muscarinic receptors occur after 3-4 days of chronic caffeine and are reversed after 4-7 days of withdrawal. Chronic nicotine increases density of nicotine receptors, but has no effect on adenosine receptors or muscarinic receptors. Chronic ethanol increases density of A_1-receptors. Both caffeine and ethanol, but not nicotine, increase density of L-type calcium channels. Behavioral sensitivity (open field locomotor) to adenosine agonists increases after caffeine. Behavioral sensitivity to nicotine decreases after either chronic nicotine and caffeine, in spite of upregulation of receptors. Behavioral sensitivity to the muscarinic antagonist scopolamine is enhanced after chronic caffeine. Adenosine and cholinergic systems appear interrelated behaviorally as are L-type calcium channels.

638.5

INFLUENCE OF VENTRAL PALLIDAL DOPAMINE ON FOREBRAIN AND MIDBRAIN CHOLINERGIC SYSTEMS. T.C. Napier*, F. Rehman and L.K. Gorman. Dept. Pharmacol., Loyola Univ. Chicago, Sch. Med., Maywood, Il 60153; and Dept. Anesth., The Johns Hopkins Univ., Baltimore, MD 21218.

Ventral pallidal regions of the basal forebrain (VP) contain cholinergic neurons that project to the prefrontal cortex (pfC), frontal cortex (fC) and amygdala (AMG) and are involved in cognition. Cholinergic neurons of the midbrain pedunculopontine nucleus (PPN) are directly innervated by non-cholinergic neurons of the VP that mediate motoric function. Our previous work demonstrated that intra-VP injections of dopamine (DA) and its receptor-subtype selective agonists alter motor function, but do not effect working memory (i.e., choice accuracy on the radial arm maze). It is not known whether cholinergic neurons within the VP, or cholinergic neurons impinged upon by VP efferents, are affected by these treatments. In the present study, changes in hemicholinium (HC-3) binding, a marker for high affinity choline uptake, were measured in pfC, fC, AMG and PPN tissue homogenates as an indicator of cholinergic neuronal activity. Intra-VP microinjection of DAergic agonists was performed in rats previously implanted with guide cannulae, and the cholinergic regions were dissected 30 min post-treatment. DA, SKF82958 (D1 agonist), and quinpirole (D2/D3 agonist) injected in doses that change motor behavior (i.e., 0.05μmole /0.5μl per VP) did not alter HC-3 binding. Larger doses of DA (0.5μmole/0.5μl per VP) increased binding by 230% in the PPN. In contrast, 6OHDA-induced lesions of the ascending DA projection increased HC-3 binding in the pfC and AMG 300% and 240%, respectively. These data indicate that the various cholinergic systems influenced by the VP are differentially sensitive to 1) DA receptor stimulation within the VP, and 2) the compensatory mechanisms evoked upon removal of endogenous DA. Work supported by MH45180 to TCN.

638.6

SYNAPTIC INTERACTIONS INVOLVING ACETYLCHOLINE, GLUTAMATE AND GABA IN RAT NEOCORTEX. Raju Metherate* and John H. Ashe Depts. of Neuroscience and Psychology, Univ. of California, Riverside, CA 92521.

Prior studies of cholinergic modulation of cortical activity have generally used exogenously applied cholinergic agonists. We have extended these studies utilizing extracellular and whole-cell intracellular recordings *in vitro* to examine how endogenous ACh modifies cortical synaptic activity mediated by glutamate and GABA. Recordings were from layer III of rat auditory cortex brain slices maintained *in vitro*. Single stimulus pulses to deep layer VI elicited a highly consistent sequence of four field potentials (A, B, C, D) that resembled four intracellular potentials, an early-EPSP (nonNMDA), early-IPSP (GABA-A), late-EPSP (NMDA), and late-IPSP (GABA-B), respectively, in terms of latency, time course, recruitment with stimulus intensity, and sensitivity to receptor antagonists. Bath application of the anticholinesterase eserine (1-50 μM) reduced the amplitude of A and B by about 10-30%; this effect was mimicked by the cholinergic agonist carbachol (25 μM) and antagonized by the muscarinic antagonist atropine (0.3-1.0 μM). The degree of component A reduction was greater at low stimulus intensities (that elicited A alone) than at higher intensities (that elicited B as well). This differential suppression was not seen in the presence of the GABA-A antagonist picrotoxin (1 μM), suggesting that normally muscarinic suppression of B disinhibits A and limits net suppression of A. To examine cholinergic actions on responses mediated by nonNMDA vs NMDA glutamate receptors, eserine was applied in the presence of APV (50 μM) or CNQX (10-20 μM), respectively. Eserine-induced suppression of responses occurred in the presence of APV, but not in the presence of CNQX.

Cholinergic suppression of excitatory synaptic transmission that varies inversely with the strength of afferent drive and the degree of NMDA receptor activity may be an important facet of cortical information-processing. Supported by NSF (IBN 9008818, IBN 9118872).

638.7

ADRENOCEPTOR ANTAGONIST TREATMENT ALTERS MUSCARINIC ACETYLCHOLINE RECEPTOR (mAChR) AFFINITY AND DENSITY FOLLOWING CHOLINERGIC DENERVATION OF THE RAT HIPPOCAMPUS. L.E.Harrell*, D.S.Parsons, K.Kolasa. Alzheimer's Disease Center, Dept.Neurology, V.A. & Univ.Alabama Med. Ctr., Birmingham, Al, 35294

After medial septum lesions (MSL), peripheral sympathetic adrenergic fibers, originating from superior cervical ganglia, appear in the hippocampus (hippocampal sympathetic ingrowth;HSI). We have previously reported that HSI is detrimental to learning in rats and that treatment with phentolamine (PHENT), α-adrenergic antagonist, ameliorated this deficit. However, biochemical studies of HSI have revealed changes in the hippocampal cholinergic system. To determine whether PHENT treatment might mediate its effects through muscarinic cholinergic (mAChR) receptors, we assessed the effect of PHENT treatment on hippocampal mAChR. Animals underwent 1 of 4 surgeries: control [sham MSL + sham ganglionectomy (Gx)]; HSI(+) [MSL + sham Gx]; HSI(-) [MSL + Gx]; GX [sham MSL + Gx]. Half of the animals in each group were treated with either phentolamine (20 mg/kg; IP) or vehicle daily for 5 weeks. After completion of treatment, the hippocampi were dissected into the dorsal and ventral regions and membrane binding of [3H]-QNB, a non-selective mAChR antagonist, was assessed. PHENT treatment was found to 1) decrease the binding affinity of mAChR in HSI(+) animals in both hippocampal regions (Kd: 1.06 + 0.52; 1.08+0.31nM) in comparison to controls (Kd: 0.48+0.06; 0.54+0.05) and 2) increase the number of mAChR in dorsal hippocampus of HSI(+) animals (Bmax: 1111+416 fmol/mgP) in comparison to control (Bmax: 409+50.7).These results sugest that PHENT treatment may mediate its effect through the mAChR perhaps by altering the release of acetylcholine from intrinsic cholinergic neurons and indicate that balance between noradrenergic and cholinergic system may be important for mediating the behavioral effects of HSI.

638.8

CALRETININ IMMUNOREACTIVITY IN THE ADULT RAT SEPTO-HABENULAR PATHWAY. J.A. Wilson and M.D. Kawaja* Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The medial habenula (MHb) consists of a densely packed population of neurons, the majority of which stain immunohistochemically for choline acetyltransferase (ChAT). The cholinergic MHb is innervated by axons that originate from posterior septal neurons; these axons course caudally via the stria medularis (*sm*). To date, one of the few neurochemicals to be identified within the *sm* is calretinin, a calcium- binding protein. The purpose of this investigation is to determine the normal patterns of connectivity of calretinin-positive fibers in the MHb, as a prelude to our examination of *sm* innervation of fetal habenular grafts placed within the adult habenula. A dense plexus of calretinin-immunoreactive axons was evident throughout the rostro-caudal extent of the normal MHb. Following a unilateral lesion of the *sm*, calretinin immunostaining of fibers was completely abolished in the ipsilateral MHb; a small population of calretinin-positive neurons situated between the medial and lateral habenulae was revealed. At the electron microscope level, calretinin immunoreactivity was associated with myelinated and unmyelinated axons, as well as with numerous terminals containing spherical vesicles. These immunoreactive terminals formed asymmetric synaptic contacts with dendritic processes of MHb neurons. Double immunohistochemical staining was used to simultaneously demonstrate calretinin-positive fibers among ChAT-positive neurons within the ventral two-thirds of the MHb. Data will be presented concerning the ultrastructural organization of ChAT and calretinin immunoreactivities within the normal adult rat MHb and within implants of fetal rat habenula grafted into the mature rat brain. (Supported by funds from the Faculty of Medicine, Queen's University)

638.9

INTERACTION OF SOMATOSTATIN AND CARBACHOL ON HIPPOCAMPAL M-CURRENTS. P. Schweitzer*, S. Madamba and G.R. Siggins. Department of Neuropharmacology, The Scripps Research Institute, LA JOLLA CA 92037.

The M-current (I_M) is a non-inactivating potassium current that persists at slightly depolarized potentials. In CA1 hippocampal pyramidal neurons, somatostatin (SS) increases I_M whereas carbachol (CCh) decreases it. To further study the ionic mechanisms of I_M, we investigated the interaction of these two substances on I_M using intracellular voltage-clamp recordings in the rat hippocampal slice preparation. We recorded from 26 neurons with a mean resting membrane potential of -68 ±1 mV. Superfusion of CCh 1 μM decreased I_M to 25-45% of control, an effect greater than previously reported. We attempted to reverse the CCh inhibition of I_M by adding SS 1 μM together with CCh: SS had no effect and I_M remained inhibited. Reversing the order of agonist application gave different results. When SS 1 μM was superfused first, I_M was increased by 55-75%, as previously reported. Then adding CCh 1 μM to the SS-augmented I_M returned its amplitude back to 85-95% of control. As CCh did not decrease I_M below control in the presence of SS as compared to CCh alone, SS thus provides a protective effect. However, when we applied a high concentration (30 μM) of CCh in the presence of SS, the I_M amplitude greatly decreased (15-25% of control), an effect identical to the effect of CCh 30 μM alone. Thus, in rat hippocampus CCh prevails over SS for the control of I_M. However, SS can protect some of the M-channel pool against low (1 μM) but not high (30 μM) concentrations of CCh. Since CCh concentration and sequence of superfusion determines the end-effect, the interaction between these two agonists could take place at the second messenger level.
Supported by NIMH (MH 44346) and NIAAA (AA 07456).

638.10

IMIDAZENIL AND ABECARNIL, TWO ANXIOSELECTIVE $GABA_A$ RECEPTOR MODULATORS REDUCE STRESS-INDUCED RELEASE OF ACETYLCHOLINE AND DOPAMINE IN THE RAT BRAIN. L. Dazzi, C. Motzo, A. Imperato, M. Serra and G. Biggio*. Departments of Experimental Biology and Neuroscience, University of Cagliari, 09123 Cagliari, Italy

The effect of the partial agonist imidazenil (IM) and the selective agonist abecarnil (AB), was studied on the basal and stress-stimulated release of acetylcholine and dopamine in the rat hippocampus and cerebral cortex using the microdialysis technique on freely mooving rats. The effect of these drugs was compared with that of diazepam (DZ) and alprazolam (AZ), two benzodiazepines full agonists. AB (0.05 - 1 mg/kg i.p.), IM (0.05 - 1 mg/kg i.p.), DZ (2.5 - 10 mg/kg i.p.) and AZ (1 - 10 mg/kg i.p.) produced a dose-dependent decrease of basal acetylcholine release in the hippocampus. On the other hand only DZ and AZ significantly decreased dopamine release in the c. cortex. These effects were antagonized by flumazenil. Foot-shock (0.2 mA/500 ms per s.) produced a sudden and marked (+ 75%) increase of hippocampal acetylcholine output. This effect lasted for 30 min and returned to basal values in about 60 min. Foot shock enhanced also dopamine release in the c. cortex. The maximal effect (90%) was obtained in 20 min and in 30-40 min dopamine release returned to control values. The previous administration of AB or IM in a dose unable to modify the basal release of acetylcholine and dopamine prevented the effect of stress. An effect mimicked by much higher doses of DZ and AZ. The differential efficacy of IM and AB on acetylcholine and dopamine release is consistent with the existence of multiple $GABA_A$ receptor subtypes with different sensitivity to these drugs.
Supported by CNR subproject SP4 stress CNR 93.00592.PF41.

638.11

ADENOSINE A_{2A} RECEPTOR STIMULATION BLOCKS DOPAMINE D_2 RECEPTOR-MEDIATED INHIBITION OF GABA RELEASE IN SLICES OF RAT GLOBUS PALLIDUS. R.D. Mayfield*, R.A. Orona, and N.R. Zahniser. Dept. Pharmacol., Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Behavioral and biochemical studies suggest that a negative interaction exists between adenosine (ADO) A_2 and dopamine (DA) D_2 receptors in the brain and that this interaction underlies the opposing effects of ADO and DA receptor agonists *in vivo*. ADO A_{2a} receptors are localized to GABAergic striato-pallidal neurons, and we have shown that activation of these receptors increases electrically-evoked endogenous GABA release in micropunches of rat globus pallidus (GP). We examined the functional significance of A_{2a} and D_2 receptor subtypes in modulating electrically-evoked GABA release and whether agonist stimulation of these receptors produces opposing actions on pallidal GABA release. Consistent with previous findings, GABA release was increased 35-40% by the selective A_{2a} receptor agonist CGS 21680 (10 nM). The selective D_2 receptor agonist N-0437 (1-100 nM) resulted in a concentration-dependent decrease in evoked GABA release in micropunches of GP as well as in striatal slices (containing GP). Maximum inhibition of release was approximately 35% in both tissue preparations. However, in the presence of 10 nM CGS 21680, N-0437 (1-100 nM) had no effect on evoked GABA release. These results demonstrate that agonist stimulation of ADO and DA receptor subtypes has opposing actions on pallidal GABA release and that the stimulation of A_{2a} receptors abolishes the effects of D_2 receptor stimulation. It is suggested that this functional interaction between A_{2a} and D_2 receptor subtypes represents an important mechanism by which GABAergic striato-pallidal output is differentially modulated by ADO and DA. (Supported by NS 26851)

638.12

DIFFERENTIAL MODULATION OF STRIATAL GABA EFFLUX IN VIVO BY DOPAMINE AND GLUTAMATE SELECTIVE AGONISTS. E.M. Byrnes* and J.P. Bruno. Dept. of Psychology and Neuroscience Program, Ohio State Univ., Columbus, OH 43210

There are two known sources of neuronal GABA within the striatum, interneurons and recurrent collaterals of medium spiny projections. Electrophysiological data suggest that corticostriatal glutamatergic neurons converge on these neurons, increasing firing rates. The effects of nigrostriatal DA afferents on these GABAergic neurons are more complex, interacting with the DA receptor subtype involved. Activation of D1 receptors hyperpolarizese these neurons. The effects of glu and DA ligands on GABA release have been largely confined to in vitro slices. Increases in extracellular K^+ enhance striatal GABA release and this effect is potentiated by activation of D1 receptors. We have recently begun to characterize the in vivo modulation of striatal GABA efflux by glu- and DA-containing afferents. Striatal GABA efflux was measured using in vivo microdialysis in awake rats. All drugs were perfused into the striatum via the dialysis probe. Under these conditions, basal GABA efflux was decreased by 52% following TTX (10 uM) and increased by 1000% following the GABA-uptake blocker nipecotic acid (0.5 uM). The glu agonist AMPA (100 uM) produced a significant (100%), transient increase in GABA efflux and a long-lasting behavioral activation. The full D1 agonist SKF 81297 (10 uM) decreased basal GABA and only modestly reduced the stimulating effects of AMPA.

638.13

Zn++ MODULATES GABAERGIC TRANSMISSION IN ORGANOTYPIC SLICE CULTURES OF RAT HIPPOCAMPUS. T.N. Ricciardi* and A.T. Malouf. Neurological Surgery, RI-20, University of Washington, Seattle, WA 98195.

Intracellular recordings of CA3 pyramidal cells (PCs) were used to study the actions of Zn^{++} in slice cultures of hippocampus. Zn^{++} (100-300 μM) caused spontaneous and stimulus-evoked giant depolarizing potentials (GDPs) consisting of a burst of action potentials riding on top of a prolonged depolarization (see Xie and Smart (1993) J.Physiol. 460:503). Paired recordings of neighboring PCs showed simultaneous GDPs, suggesting synchronous synaptic drive. Blockade of synaptic transmission using either 1 μM TTX or 300 μM cadmium prevented the appearance of GDPs. Glutamate and GABA receptor antagonists were used to examine the contribution of these neurotransmitters to GDP initiation and topography. The glutamate receptor antagonists CNQX or kynurenic acid did not abolish the bursts of action potentials but partly attenuated the late depolarizating phase of spontaneous GDPs, indicating that glutamate plays a role in GDP topography. The NMDA receptor antagonist APV had no effect on the appearance of evoked or spontaneous GDPs. Application of Zn^{++} in the presence of APV, CNQX, and the $GABA_B$ antagonist CGP-35348, resulted in the appearance of spontaneous and evoked depolarizations. The depolarizing potentials appear to be $GABA_A$ mediated, since they were reversible near resting membrane potential. Application of Zn^{++} in the presence of APV, CNQX, and the $GABA_A$ antagonist bicuculline methiodide, resulted in the appearance of large spontaneous and evoked hyperpolarizations. These large hyperpolarizations were blocked by CGP-35348, suggesting they were $GABA_B$ receptor-mediated IPSPs. These data suggest that Zn^{++} enhances $GABA_A$ and $GABA_B$ mediated neurotransmission in organotypic cultures of hippocampus. The appearance of GDPs appears to result primarily from the enhancement of $GABA_A$ transmission. (Supported by NIH-NS28650 and NIH-T32NS-07144-15.)

638.14

COLOCALIZATION OF GLUTAMATE DECARBOXYLASE (GAD), SEROTONIN (5HT) AND TYROSINE HYDROXYLASE (TH) IN RAT MEDIAL PREFRONTAL CORTEX (mPFC). J.B. Taylor* and F.M. Benes. Department of Psychiatry and Program in Neuroscience, Harvard Medical School and Lab for Structural Neuroscience, McLean Hospital, Belmont, MA 02178.

Recent evidence has suggested that GABAergic cell bodies in rat pyriform cortex receive a convergence of monoaminergic inputs (Gellman and Aghajanian, 1993). To obtain corroborative microscopic support for this idea, a fluorescent immunocytochemical technique has been developed for the colocalization of GAD, TH and 5HT in single sections of rat mPFC. The primary antisera included a polyclonal antibody raised in rabbit against GAD_{67}, a monoclonal antibody raised in mouse against TH and a polyclonal antibody raised in goat against 5HT. Secondary antibodies were raised in donkey against rabbit (FITC), mouse (TRITC), and goat (AMCA), respectively. A digital confocal microscopic system equipped with appropriate filters was used to visualize the three fluorescent emissions. For a given cell of interest, serial Z axis planes were corrected for background flare using a deconvolve subroutine followed by co-registration of the three images. While TH *and* 5HT varicosities were found in apposition with GAD immunoreactive (IR) cell bodies, ghosts of pyramidal neurons also showed a "trivergence" of TH-, 5HT- and GAD-IR varicosities/bouton. Interactions of TH-, 5HT- and GAD-IR elements with one another were also commonly observed pre-synaptic to pyramidal cell bodies. These findings are consistent with the idea that monoaminergic afferents converge on GABAergic and pyramidal cell bodies. Moreover, pre-synaptic interactions of monoaminergic varicosities with GABAergic elements may play a significant role in the inhibitory modulation of pyramidal cell output from mPFC. Supported by MH42261, MH00423, MH31154 and the Stanley Foundation.

638.15

COLOCALIZATION OF GABA AND GLYCINE IN RAT CUNEATE NUCLEUS. A. Popratiloff.*[1] J.G. Valtschanoff.[1] P.S. Bernardi.[2] R.J. Weinberg[1] and A. Rustioni.[1] [1]Dept. of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599, USA, and [2]Dept. Fisiologia Bioch., Univer. di Milano, Milano 20133, Italy.

Recent evidence suggests that GABA and glycine are released by the same local circuit neuron in the spinal cord (Todd and Sullivan, *J. Comp. Neurol.* 296:496-505, 1990). Among the relevant questions raised by this possibility is whether all GABAergic neurons may also release glycine, and what role these two amino acids may play in mechanisms of pre- and post-synaptic inhibition, respectively. We have approached these questions in the dorsal column nuclei of the rat because: i) projecting neurons, and local circuit neurons are easily distinguishable, ii) local circuit neurons in these nuclei are mainly GABAergic, iii) they contain a large number of axo-axonic synapses thought to mediate presynaptic inhibition. We employed post-embedding immunocytochemistry and found that of all neurons that stain for GABA and/or glycine in the cuneate nucleus, 29% stain for GABA, 29% stain for glycine, and 42% stain for both.

Electron microscopy showed that GABA colocalizes with glycine at both axodendritic and axoaxonic synapses, supporting a role for glycine in pre-synaptic as well as in post-synaptic inhibition. Most GABAergic terminals were also glycinergic but some terminals were clearly enriched in GABA and not glycine, and vice versa. We are now studying quantitative differences in the enrichment of GABA and glycine in various classes of terminals in the cuneate nucleus.

638.16

INCREASED INTERACTION OF DOPAMINE (DA) VARICOSITIES WITH GABA CELLS OCCURS PROGRESSIVELY IN RAT MEDIAL PREFRONTAL CORTEX (mPFC) FROM BIRTH TO ADULTHOOD. F.M. Benes*, S.L. Vincent, R. Molloy. Dept. of Psychiatry and Program in Neuroscience, Harvard Medical School and Lab for Structural Neuroscience, McLean Hospital, Belmont, MA 02178.

A recent study has demonstrated non-random interactions between DA-immunoreactive (IR) varicosities and GABA-IR cell bodies in rat mPFC (Benes et al., 1993). Because both the DA and GABA systems of cortex have been shown to undergo progressive postnatal development, the current study has explored the possibility that increases in the interaction of these neural elements might also occur postnatally. In the present study, a progressive linear increment in the percentage of GABA cell bodies having DA-IR varicosities in apposition has been observed to occur between birth and postnatal day 60 ($r = 0.75$, $N = 19$, $p < 0.0005$). The number of DA-IR varicosities in contact with each GABA cell also shows a curvilinear increase during the post-weanling period ($r = 0.81$, $N = 19$, $p < 0.0005$). When these latter two parameters are multiplied to generate an index of interaction, a 2.5 fold increase is observed in post-weanling rats when compared to similar data for the pre-weanling period. Overall, these findings are consistent with the idea that late postnatal changes in the interaction of DA and GABA cells progressively occur. Such changes could potentially play a role in the onset of a neuropsychiatric disorder, such as schizophrenia, in which there is a defect of GABA neurotransmission and a typical onset during late adolescence and early adulthood. Supported by MH00423, MH31154 and the Stanley Foundation.

638.17

COLOCALIZATION OF GABA-, GLYCINE-, AND GLUTAMATE-LIKE IMMUNOREACTIVITY IN THE INNER NUCLEAR LAYER OF THE POSTHATCH CHICKEN RETINA. H. Sun and W.J. Crossland*, Dept. Anat. & Cell Biol., Wayne State Univ. Schl. Med., Detroit MI 48201

We previously reported the localization of immunoreactivity to the amino acid transmitters GABA, glutamate (GLU), and glycine (GLY) in the chicken retina. We report here on the colocalization of these transmitters in the chicken retina inner nuclear layer.

Posthatch chicks were deeply anesthetized (halothane), decapitated, and their retinas fixed by immersion in mixed aldehydes. Small strips of central retina were embedded in epon and sectioned transversely at 1.5 µm. Adjacent serial sections were mounted on different slides to compare sections through the same cell immunoreactive to primary antibodies; (Chemicon) GLU, GABA, or GLY; visualized using an ABC kit (Vector).

Horizontal cells were GLU+. Most GLU+ cells were also GABA+. A few GABA+ cells were GLY+. Bipolar cells were virtually all GLU+ and those which were both GLU+ and GLY+ were often adjacent to horizontal cells. Amacrine cells stained heterogeneously. GLU+ cells were most numerous followed by GLY+. Fewer of the GLU+ cells were GABA+, but most GABA+ cells were also GLU+ or GLY+, suggesting that at least some of these cells may contain all 3 transmitters.

The colocalization of GABA and GLY in some horizontal cells, the colocalization of GLU in GLY+ bipolar cells, and the probable colocalization of all 3 among some amacrine cells raises the question of possible functional and morphological differences among these cells.

(Partial support for W.J.C.: Mich. Eye Bank & Transplant. Center)

NEUROTRANSMITTER INTERACTIONS

639.1

THE EFFECTS OF SUBSTANCE P (SP) AND SEROTONIN (5-HT) ON DOPAMINE (DA) RELEASE IN THE STRIATUM: MICRODIALYSIS STUDIES IN VIVO. Camille S. Suchowski* and Matthew P. Galloway, CCN Program, Dept. of Psych & Behav Neurosciences, Wayne State Univ Sch of Med, Detroit, MI 48202.

Several studies have demonstrated that 5-HT facilitates DA release in vivo, however the 5-HT receptor subtype and the synaptic organization remain to be determined. Using microdialysis, we analyzed the potential involvement of 5-HT1b receptors and the effects of the neurokinins, SP and senktide (a NK3 agonist) on extraneuronal striatal DA levels. Local perfusion with 10uM 5-HT significantly increased DA levels approximately 7-fold above baseline (655±71%, n=6). After perfusion with 100uM cyanopindolol (CYP), a 5-HT1b antagonist, baseline DA levels increased 91%. In the presence of CYP, 10uM 5-HT no longer had a significant effect on DA levels (n=5). Perfusion of 100uM SP did not significantly alter extracellular striatal DA levels (n=7) nor did local perfusion of 100 uM senktide (n=6). To determine whether SP interacted with the effect of 5-HT, tissues were perfused with 100uM SP or senktide followed by a 10uM pulse of 5-HT. SP or senktide pretreatment did not alter 5-HT's effect on extracellular DA levels (n=11). Preliminary microdialysis experiments measuring extracellular DOPA after perfusion difluoromethyldopa, a decarboxylase inhibitor, indicated that 100uM senktide increased DOPA levels by 89% (n=3). In summary, 5-HT induced DA release was blocked by CYP. Perfusion of SP and senktide did not significantly alter DA release or alter the 5-HT facilitation of DA release. Supported by NIDA 04120 and the Joe Young Sr. Research Fund.

639.2

EFFECTS OF CCK-4, CCK-8S AND PENTAGASTRIN ON DOPAMINE RELEASE IN THE STRIATUM: STUDIES WITH MICRODIALYSIS IN VIVO Mark D. Barsamian* and Matthew P. Galloway. Cellular and Clinical Neurobiology Program, Dept. of Psychiatry and Behavioral Neurosciences, Wayne State University Sch of Med, Detroit, MI 48202.

Clinical studies have demonstrated that both Cholecystokinin (CCK) fragment 30-33 unsulfated (CCK-4) and pentagastrin (PG) induce panic attacks in panic disorder patients. Since dopamine (DA) neurons are exquisitely activated by mild stress, we investigated the effects of CCK-4, CCK-8S (CCK 26-33) or PG on DA release in the striatum. Microdialysis techniques and HPLC-EC were employed to measure extracellular levels of striatal DA in male Sprague Dawley rats anesthetized with chloral hydrate. Perfusion of artificial CSF (2uL/min) with 100uM CCK-4 (1, 20-min pulse, n=4) or 100uM CCK-8S (3 successive pulses, n=4) had no significant effect on levels of extracellular striatal DA. Similarly, intravenous administration of CCK-4 (0.6 mg/kg and 2.10 mg/kg, n=2), CCK-8S (1 mg/kg,n=4), or pentagastrin (0.5 mg/kg,n=2) had no significant effect on striatal DA release. Pentagastrin could not be reliably examined via perfusion studies due to its solubility properties; further studies are in progress. Taken together, these results indicate that CCK does not have a robust influence on DA release from the striatum. However, the diffusion efficiency of peptides through the dialysis membrane may influence the bioavailability of CCK or PG. Supported by NIDA and the Joe Young Sr. Research Fund.

639.3

EXCITATORY AMINO ACID MODULATION OF DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX: A MICRODIALYSIS STUDY. H. P. Jedema and B. Moghaddam*, Dept. of Psychiatry, Yale Univ. Sch. Med. and VA Med. Ctr. 116A/2, West Haven, CT 06516.

In the present study, we have investigated the effects of various classes of excitatory amino acid agonists on the release of dopamine in the medial prefrontal cortex of conscious rats. Bilateral microdialysis probes were used to assess extracellular levels of dopamine and to infuse excitatory amino acid agonists locally. Infusion of 20 µM kainate resulted in behavioral stimulation (e.g., sniffing) and a sustained, robust, nearly 7-fold increase of in the extracellular levels of dopamine. Local application of 20 µM AMPA had a less profound effect, causing an increase of 2- to 3-fold in extracellular levels of dopamine. Higher concentrations of AMPA (100 µM) increased dopamine levels by nearly 10-fold. However, this increase was associated with convulsions in most animals tested. Local infusion of 20 µM NMDA did not affect extracellular dopamine levels. A much higher concentration of NMDA (200 µM) led to a 3- to 5-fold increase in dopamine levels. In contrast to AMPA or kainate, higher concentrations of NMDA did not cause convulsions. These results indicate that excitatory amino acid agonists facilitate the release of dopamine in the prefrontal cortex *in vivo*, and that non-NMDA receptor agonists are more potent than NMDA receptor agonists in so doing. This finding is in agreement with our previous studies showing that non-NMDA receptor antagonists can attenuate the release of dopamine in the prefrontal cortex during stress. Supported in part by MH48404 and MH44866 and the Scottish Rite Foundation.

639.4

DOPAMINE RELEASE IN ANTERIOR MEDIAL STRIATUM IS CONTROLLED BY THE PREFRONTAL CORTEX: EVIDENCE FOR TONIC AND PHASIC MODULATION. M. Karreman* and B. Moghaddam, Dept. of Psychiatry, Yale University School of Medicine and West Haven VA Med. Ctr. 116A/2, West Haven, CT 06516.

In the present study, we have investigated whether the prefrontal cortex exerts a modulatory effect on dopamine release in the anterior medial striatum of conscious rats. Two concentric microdialysis probes were used in each animal: one placed in the medial prefrontal cortex, used primarily for drug infusion, and the other placed in the contralateral anteromedial striatum, used to measure extracellular levels of dopamine. Infusion of TTX (5 µM) into the prefrontal cortex caused a decrease in extracellular dopamine levels in the anterior medial striatum, indicating that neuronal events in the prefrontal cortex exert a tonic excitatory effect on striatal dopamine release. Infusion of bicuculline (100 µM) into the prefrontal cortex resulted in an increase in the release of dopamine in the striatum, while application of amphetamine (50 µM) in the prefrontal cortex attenuated dopamine release in the striatum. These data suggest that GABAergic, as well as monoaminergic, systems in the prefrontal cortex control striatal release of dopamine. Further pharmacological characterization of tonic and phasic cortical mechanisms that modulate the release of dopamine in the striatal complex are currently underway.

Supported in part by PHS grants, MH48404 and DA08227

639.5

THE 5-HT2 ANTAGONIST AMPEROZIDE POTENTIATES AMPHETAMINE-STIMULATED DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX. E.A. Pehek* and H.Y. Meltzer. Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The pharmacological profile of the putative atypical antipsychotic drug amperozide includes 5-HT2 antagonism coupled with the ability to block dopamine (DA) uptake. One or both of these properties may be the basis for the reported ability of amperozide to attenuate amphetamine (AMPH)-stimulated DA release in the dorsal and ventral striatum. Since previous research has demonstrated significant differences between the mesostriatal and mesocortical DA pathways, it is important to compare the regulation of DA release between these systems. The present study thus examined the effects of amperozide administration on AMPH-induced DA release in the prefrontal cortex (PFC). Male rats were implanted with chronic indwelling guide cannulae above the medial PFC. 2-3 days later, microdialysis probes were lowered through these cannulae into the PFC of awake rats. Following collection of baseline samples, amperozide (1.0, 5.0 or 10.0 mg/kg i.p.) or saline were administered 30 min before d-AMPH (5.0 mg/kg i.p.). AMPH increased basal extracellular DA levels from 0.68 pg/20 ul to 4.35 pg/20 ul 30 min following injection. Amperozide administration did not attenuate this increase. Rather, injections of amperozide potentiated AMPH-stimulated DA release in a dose-dependent manner. Relative to AMPH alone, administration of 1.0, 5.0 and 10.0 mg/kg amperozide produced additional increases in DA efflux of 1.06, 2.44 and 4.05 pg/20 ul, respectively. These results may be due to actions of amperozide on the cortical 5-HT2 receptor coupled with effects of AMPH on the DA transporter. Furthermore, these data add to previous work demonstrating differences between the mesocortical and mesostriatal systems in the regulation of DA release.

639.6

BASAL GANGLIA SUBSTRATES OF MK-801-INDUCED STIMULATION OF STRIATAL DOPAMINE RELEASE. D.W. Miller* and E.D. Abercrombie. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Glutamate and dopamine (DA) interactions are important in regulating the behavioral outputs of the basal ganglia. Previously, we have reported that systemic administration of the non-competitive NMDA antagonist MK-801 significantly increases extracellular DA in striatum, measured with *in vivo* microdialysis in awake rats. MK-801 (0.5 mg/kg) increased extracellular DA from 5.1 pg/20µl sample to 7.3 pg (n=7). This increase was TTX-sensitive. These data support electrophysiological studies from other laboratories showing that systemic MK-801 increases activity of nigrostriatal DA neurons. We hypothesize that blockade of excitatory glutamatergic drive to striatum could increase DA cell firing by decreasing the activity of GABAergic projections to the SNC (direct striatonigral path) and/or via disinhibition of globus pallidus (GP) projections (indirect striatonigral path) to SNR neurons which in turn inhibit the SNC. The present experiments analyzed the effects of intrastriatal application of kainic acid (KA; 2 µg/µl) on MK-801-induced striatal DA release. Two weeks following the administration of KA, striatal DA levels were significantly lower in KA-lesioned (n=6) vs non-lesioned (n=7) animals. Although MK-801 increased extracellular DA from 1.8 pg to 2.4 pg in KA-lesioned animals, this increase was significantly less than in non-lesioned animals. To control for the likelihood that GP was damaged by an intrastriatal injection of KA as well as to differentiate the role of both the direct and indirect striatonigral paths in MK-801's stimulatory effects on striatal DA release, selective lesions of GP were carried out using ibotenic acid (IBO; 10 µg/µl, 0.1 µl/injection, 3 injections in the same dorsal/ventral plane) two weeks prior to the dialysis experiment. Preliminary data suggest that basal striatal DA levels are unaltered in IBO-lesioned animals (n=2). The role of GP in MK-801-induced striatal DA release has yet to be determined, but preliminary data also suggest that GP-lesions may decrease MK-801's stimulatory effects on striatal DA release. Thus, the efferents of striatum and GP may have differential roles in regulating both basal and MK-801-induced striatal DA release. [Supported by USPHS Grant NS 19608]

639.7

FLUOXETINE AND (+)-MDMA-INDUCED DOPAMINE RELEASE IN VIVO: THE ROLE OF ENDOGENOUS SEROTONIN

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(+)-MDMA (3,4-methylenedioxymethamphetamine, "Ecstasy"), an amphetamine related drug of abuse, is a potent releaser of serotonin (5-HT) and causes toxicity to 5-HT neurons after repeated exposure. (+)-MDMA also releases dopamine (DA), although with less potency. Since we have shown previously that the intrastriatal application of 5-HT facilitates DA release via an axo-axonic mechanism, we hypothesized that increased release of striatal 5-HT after (+)-MDMA may influence extracellular levels of DA. To elucidate the effect of (+)-MDMA on 5-HT and DA release, both in vitro (striatal slices) and in vivo (microdialysis) studies were used. In vivo, (+)-MDMA (4.7µmol/kg,i.v.) increased extracellular DA levels 401% ($p<0.01$, $n=12$). In the presence of fluoxetine (FLX, 14.4µmole/kg, s.c.), which prevents (+)-MDMA effects on 5-HT release, the (+)-MDMA-induced increase in DA was significantly less (275%, $p<0.05$, vs no FLX, $n=8$). In vitro studies with striatal slices, to test drug selectivity, showed that (+)-MDMA (0.3-3µM) increased extracellular levels of both DA and 5-HT in a dose-dependent manner. FLX (3µM) completely blocked the effects of (+)-MDMA on 5-HT release, but did not alter (+)-MDMA-induced DA release in vitro. The selective DA transport inhibitor GBR-12909 (1µM), blocked (+)-MDMA's effect on DA release. Surprisingly, the 5-HT_2 antagonist ketanserin (1µM) greatly attenuated (+)-MDMA's effect on 5-HT release in vitro. It is concluded that 5-HT release after (+)-MDMA treatment partially contributes to (+)-MDMA's effect on DA release in vivo. Further, the DA transporter appears to be essential in mediating the direct effect of (+)-MDMA on DA release. Supported by NIDA-04120 and the Joe Young Sr. Research Fund.

639.8

STRIATAL EXTRACELLULAR DOPAMINE IS REGULATED BY EFFERENT PROJECTIONS OF MEDIAL PREFRONTAL CORTEX. J.M. Finlay*, M.J. Zigmond and A.F. Sved, Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15260

Excitatory amino acid containing projections from the medial prefrontal cortex (mPFC) may regulate the activity of nigrostriatal dopamine (DA) neurons and disruption of this interaction has been proposed to play a role in schizophrenia and Tourette's syndrome. We have examined the impact of ibotenic acid lesions of mPFC on DA efflux in the striatum (STR) using *in vivo* microdialysis. Lesions of neurons intrinsic to the mPFC were produced by local application of ibotenic acid (5 µg/0.5 µl/side). Following a 2 week recovery period, microdialysis probes (2.5 mm length, 250 µm O.D.) were implanted in the STR and approximately 18 h later, basal extracellular DA levels were determined in freely moving rats. Basal DA levels (determined as pg/20 µl in 15 min samples) were not affected by ibotenic acid lesions of mPFC [control: 11.7 ± 0.8 (n=17); lesion: 11.7 ± 1.0 (n=13)]. The effect of amphetamine (1.5 mg/kg, i.p.) on extracellular DA was attenuated in lesioned rats relative to control rats (+462 ± 70% and +603 ± 82% from baseline, respectively) whereas 30 min of tail pressure elicited a greater increase in extracellular DA in lesioned than control rats (+32 ± 3% and +12 ± 3% from baseline, respectively). These data support the hypothesis that efferent projects of mPFC regulate the activity of nigrostriatal DA neurons. *[This work is supported by the Scottish Rite Schizophrenia Research Program, Tourette Syndrome Association, National Alliance for Research on Schizophrenia and Depression and USPHS grants MH45156 and MH43947.]*

639.9

EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS SHELL IS ALTERED BY 6-OHDA LESIONS OF MEDIAL PREFRONTAL CORTEX. D. King*, M.J. Zigmond, and J.M. Finlay. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

We previously reported that neither basal nor evoked extracellular dopamine (DA) in neostriatum is altered by medial prefrontal cortex (mPFC) DA depletion (King et al., Soc. Neurosci. Abst. 19:1829). In the present study, the impact of such lesions on extracellular DA in nucleus accumbens subregions, shell and core, were examined using *in vivo* microdialysis. Bilateral 6-OHDA infusions (1 µg/2 µl/side) into mPFC were performed 30 min after treatment with desipramine (25 mg/kg). Two weeks later, a microdialysis probe (150 µm O.D., 1.5-2 mm active length) was placed into shell or core. Approximately 18 hours after implantation of the dialysis probe, basal extracellular DA levels were determined at 15 min intervals (all data expressed as pg per 20 µl). In rats sustaining lesions of mPFC (60 ± 5 % DA and 24 ± 7 % NE loss), basal levels of DA in shell were increased relative to control rats [7.2 ± 0.6 (n=11) and 5.0 ± 0.7 (n=8), respectively]. In contrast, basal DA levels were not different in core of lesioned and control rats (9.0 ± 1.0 (n=5) and 8.4 ± 0.7 (n=6), respectively). Systemic d-amphetamine (1.5 mg/kg, i.p.) produced a similar increase of extracellular DA in shell of both lesioned and control rats (28.6 ± 3.1 and 29.0 ± 4.0, respectively). In contrast, mPFC lesions attenuated the effects of d-amphetamine in core relative to controls (33.0 ± 1.0 and 44.8 ± 5.8, respectively). Tail pressure (30 min) produced a similar increase of extracellular DA in core of lesioned and control rats (10.4 ± 1.4 and 9.4 ± 0.9, respectively). In contrast, 6-OHDA lesions of mPFC potentiated the tail pressure-induced increase of extracellular DA in shell compared to controls (8.5 ± 0.8 and 5.9 ± 0.8, respectively). These data suggest that the mesoaccumbens DA neurons terminating in shell but not core may be regulated by mesocortical DA neurons especially under conditions of stress. *[Supported by Tourette Syndrome Association, Scottish Rite Schizophrenia Research Program, National Alliance for Research on Schizophrenia and Depression and USPHS grants MH45156 and MH43947.]*

639.10

GLUTAMATE POTENTIATES THE STRESS-EVOKED INCREASE IN STRIATAL DOPAMINE SYNTHESIS. S.L. Castro*, A.F. Sved, and M.J. Zigmond. Departments of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

The present study examined the role of glutamate in the regulation of stress-evoked changes in tyrosine hydroxylation (TH) in neostriatum. Adult male rats were exposed to 30 min of intermittant tail shock in the presence of an aromatic amino acid decarboxylase (AAAD) inhibitor, NSD-1015 (100 µM), administered locally through a dialysis probe implanted into the striatum, and the concentration of DOPA in the dialysate was measured. Stress was applied beginning either 15 min or 75 min after the onset of NSD treatment, corresponding to the initial rate or steady-state phase of the DOPA accumulation curve, respectively. Stress significantly increased the steady-state levels of extracellular DOPA (+40%). However, no change was observed in the initial rate of DOPA accumulation unless animals also received the D_2 antagonist, eticlopride (50 nM). The impact of stress on the steady-state level of DOPA could be attenuated by the D_2 agonist quinpirole (100 µM). Similar results were obtained using APV (100 µM) or CNQX (100 µM), glutamate antagonists acting at NMDA or AMPA receptors, respectively. These results suggest that acute stress normally has little effect on TH in striatum due to the inhibitory influence of DA present in the extracellular fluid. However, when that influence is absent (e.g., during long-term inhibition of AAAD or DA receptor blockade) stress does increase TH, an effect that is mediated by glutamate. Thus, glutamate released from corticostriatal projections may serve to stimulate DA synthesis and thereby restore dopaminergic activity under conditions in which the availability of DA for release has temporarily been compromised. (Supported in part by USPHS grants MH45156, NS19608, MH43947, and MH00058.)

639.11

DOPAMINE-SOMATOSTATIN INTERACTIONS IN THE RAT STRIATUM: AN IN VIVO MICRODIALYSIS STUDY. A. Kastellakis#, J. Radke, Y. Anagnostakis, K. Thermos and C. Spyraki*. Lab of Pharmacology, Dept. of Basic Sciences, Sch. of Medicine and #Dept. of Psychology, Sch. of Social Sciences, Univ. of Crete, Crete, Greece.

The neuropeptide somatostatin is found throughout the central nervous system where it is believed to act as a neurotransmitter. Pharmacological and behavioural studies have provided evidence suggesting a role for somatostatin in dopamine mediated behaviours. To assess such an interaction between the two systems, our first attempts focused on the elucidation of the effect of dopaminergic agents on somatostatin release, using in vivo microdialysis. Apomorphine administered subcutaneously, and directly infused in the striatum, as well as the selective dopamine antagonists SCHE 23390 and sulpiride, failed to elicit somatostatin release (1). In the present work we examined the effect of different concentrations (10-4M, 10-5M, 10-6M) of somatostatin-14 and somatostatin-28 on dopamine release in the striatum.Vertical designed probes were implanted bilaterally in the striatum and used for the infusion of somatostatin, as well as the collection of the dialysate at 15 min intervals (1 ul/min). Dopamine and metabolites were measured prior to and 120 min after administration of somatostatin. A net dose-dependent increase of dopamine was observed, without significant alterations in the levels of the metabolites, HVA and DOPAC. Rats exhibited signs of stereotypy and grooming.
These results, taken together, show that although the induced alterations in dopamine transmission failed to influence somatostatin levels, somatostatin did modulate dopamine release. This suggests that a unidirectional interaction exists between somatostatin and dopamine in the striatum, at the neurochemical level.
1). J.M. Radke, C. Spyraki and K. Thermos. Effect of dopaminergic drugs on the release of somatosttin in the rat striatum. Soc. Neurosci. Abstr., 19 (1993) 1173.

639.12

BEHAVIORAL EVIDENCE FOR CHOLECYSTOKININ-D1 INTERACTIONS. J. M. VanKampen, H. Frydryszak & A. J. Stoessl*. Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada, N6A 5A5.

The neuropeptide cholecystokinin (CCK) has been shown to have modulatory effects on dopaminergic activity in several areas of the central nervous system, including the striatum. In light of this, it has been proposed that abnormalities of CCK may play a significant role in the development of dyskinesias. We examined the effects of CCK on the behavioral responses to a dopamine D1 agonist in the rat. Peripheral administration of the selective D1 agonist SKF 38393 (5 mg/kg s.c.) resulted in significant increases in vacuous chewing movements (VCMs) and stereotypic grooming, which were significantly attenuated by the peripheral administration of CCK-8S (50 μg/kg i.p.). Elucidation of the specific CCK receptor subtype involved in this effect was approached in two ways. The preferential CCK-B agonist CCK-4 (10-50 μg/kg i.p.) failed to suppress SKF-induced VCMs. Suppression of SKF-induced VCMs and stereotypic grooming by CCK-8S (50 μg/kg i.p.) was significantly attenuated by the CCK-A antagonist devazepide (0.1-0.3 mg/kg i.p.) but not by the CCK-B antagonist L-365,260 (0.1-0.3 mg/kg i.p.). CCK-8S appears to suppress some dopamine-mediated behaviors via post-synaptic mechanisms linked to the D1 receptor, via stimulation of CCK-A receptors. CCK agonists may prove useful in alleviating dyskinesias in humans.

639.13

NEUROKININ-1 TACHYKININ RECEPTOR-DOPAMINE INTERACTIONS IN THE RAT. M. Brackstone, C. J. Gibson* & A. J. Stoessl, Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada, N6A 5A5.

There is anatomical evidence for a dense substance P (SP) projection from the striatum to the substantia nigra (SN). Electrophysiological, neurochemical and behavioural studies all suggest a functionally significant interaction between tachykinins and dopamine (DA) neurons of the SN, but this structure is devoid of SP receptors. Pharmacological and molecular studies have demonstrated that SP and related compounds act through a family of tachykinin receptors, termed neurokinin (NK) 1-3. Autoradiographic studies have demonstrated the presence of NK-3 receptors on nigral DA neurons and stimulation of nigral NK-3 receptors leads to classical DA-mediated behavioural responses of locomotion and rearing. In contrast, stimulation of nigral NK-1 receptors elicits grooming in the absence of locomotion and rearing. We explored the possibility that NK-1-induced grooming might reflect *dendritic* release of DA which could then stimulate local DA D1 receptors. Nigral infusion of the selective NK-1 agonist [AcArg^6,Sar^9,Met(O$_2$)^{11}]SP$_{6-11}$ (0-1 nmol) elicited dose-dependent stereotypic grooming. This response was significantly attenuated by peripheral administration of the DA D1 antagonist SCH 23390 (100 μg/kg s.c.), but not by the DA D2 antagonist sulpiride (30 mg/kg i.p.). Grooming induced by nigral infusion of the NK-1 agonist was unaffected by either nigral or striatal SCH 23390 (0.1-5 μg), but was blocked by bilateral 6-hydroxydopamine lesions. Our findings indicate that stimulation of nigral NK-1 receptors elicit grooming which is dependent upon release of DA and stimulation of D1 receptors in an as yet unidentified site.

639.14

CHRONIC L-DOPA TREATMENT ALTERS LIGAND BINDING TO STRIATAL NMDA RECEPTORS AND DOPAMINE UPTAKE SITES IN RATS WITH NIGROSTRIATAL INJURY. S.J. O'Dell*, F.B. Weihmuller, and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Growing evidence suggests that alterations of brain dopamine (DA) activity can change corticostriatal transmission and striatal glutamate (GLU) receptors. We previously found large decreases (75-80%) in [3H]mazindol binding to DA transporters and substantial increases (22-46%) in [3H]GLU binding to NMDA receptors in postmortem striatal tissue sections from Parkinsonian patients. However, it was not certain whether these changes resulted from the disease process or long-term dopamine replacement therapy. To investigate this, rats were given unilateral 6-OHDA injections along the nigrostriatal DA projections. One month later, these lesioned rats and unlesioned control rats were given 2 daily injections of Sinemet (100 mg L-DOPA + 25 mg carbidopa/kg, ip) or vehicle for 21 days. Three days after the last injection, their brains were processed for in vitro autoradiography. The nigrostriatal 6-OHDA injection alone reduced ipsilateral striatal [3H]mazindol binding by 90% but failed to increase striatal [3H]GLU binding to NMDA receptors. In contrast, 6-OHDA-lesioned rats treated chronically with Sinemet showed a 30% increase in NMDA receptor binding ipsilaterally. Unexpectedly, chronic Sinemet also altered striatal [3H]mazindol binding in both lesioned and control animals. Thus, the receptor changes measured in postmortem Parkinsonian striatum may be strongly influenced by DOPA therapy.

639.15

STRIATAL DOPAMINE LEVELS INCREASE FOLLOWING PERIPHERAL SCOPOLAMINE INJECTIONS AND FOLLOWING APPLICATION OF SCOPOLAMINE TO THE TEGMENTAL PEDUNCULOPONTINE NUCLEUS. J.R.Blackburn1*, C.A.Chapman1, C.D.Blaha2 and J.S.Yeomans3. Depts. of Psychology, 1McMaster University, Hamilton, Ontario; 2University of British Columbia, Vancouver, B.C.; and 3University of Toronto, Toronto, Ontario, Canada.

The cholinergic cells of the tegmental pedunculopontine nucleus (PPT) monosynaptically excite dopaminergic (DA) neurons of the substantia nigra. Further, DA-dependent behaviours are enhanced following peripheral or central PPT injections of antimuscarinic drugs, consistent with a disinhibition of PPT cholinergic cells via blockade of their inhibitory autoreceptors. Rat dorsal striatal DA levels were monitored chronoamperometrically (1sec 350mV pulses) with bilateral, chronically implanted, stearate-modified graphite paste electrodes. I.p. injections of scopolamine (1,3,10mg/kg) resulted in bilateral dose-related increases in DA oxidation currents in the striatum. Unilateral microinjections of scopolamine into the PPT (10,50,100μg/.5μl) also resulted in short-latency, bilateral dose-related increases in striatal DA oxidation currents which usually peaked 50-80 min post-injection. Carbachol (4μg/.5μl) injected in the PPT 20 min prior to 100μg PPT scopolamine attenuated these increases. These results indicate that elevated striatal DA levels following the systemic scopolamine treatments may be mediated by the PPT. This study further demonstrates a powerful excitatory effect of Ch5 PPT cells on the nigrostriatal DA system.

639.16

CHRONIC BLOCKADE OF DOPAMINE RECEPTORS AFFECTS BINDING TO NMDA RECEPTORS IN RAT CORTEX AND BASAL GANGLIA. J. Ulas*, F. B. Weihmuller[1], S.J. O'Dell[1], J. F. Marshall[1] and C.W. Cotman. *IRU in Brain Aging and [1]Dept. of Psychobiology, University of California, Irvine, CA 92717, USA.*

We have previously shown that chronic haloperidol treatment increases binding to NMDA receptors in the outer layers of parietal cortex. To further elucidate this phenomenon, we investigated the effect of long-term (3 weeks) blockade of dopamine receptors (D1, D2 or both) on binding to NMDA receptors in rat cortex, hippocampus and striatum. SCH23390 (0.5 mg/kg/day, s.c.), eticlopride (0.5 mg/kg/day, i.p.), or combination of both drugs was used to block D1, D2, or both subtypes of dopamine receptors, respectively. As revealed by quantitative autoradiography, eticlopride treatment significantly increased L-[^{3}H]glutamate binding to NMDA receptors in the outer layers of frontal cortex, and the pyramidal layer of CA3 hippocampal region, while significantly attenuated binding to NMDA receptors in the lateral and ventral caudate-putamen, and nucleus accumbens. SCH23390 alone generally had no effect on NMDA receptor binding. On the other hand, simultaneous blockade of D1 and D2 receptors resulted in a slightly smaller increase (than that evoked by eticlopride alone) in binding to NMDA receptors in the frontal cortex, an increased binding in the deep layers of parietal cortex and CA3 region, and further reduced binding to NMDA receptors in the striatum. These results suggest that *i)* dopamine modulates NMDA receptor function differently in basal ganglia *vs* various cortical regions, *ii)* although SCH23390 alone does not affect binding to NMDA receptors, chronic blockade of D1 receptor may modify the effects of D2 receptor inhibition, and *iii)* the effect of haloperidol on NMDA receptors cannot be completely explained by blockade of D2 receptors.

639.17

DOPAMINE NEURONS MAKE TWO KINDS OF TERMINALS. D. Sulzer and S. Rayport*. Depts Psychiatry, Neurology, Anatomy & Cell Biology, and Ctr Neurobiology & Behavior, Columbia Univ; Dept Neuropathology, NYS Psychiatric Institute, NY 10032.

Morphological studies suggest that ventral midbrain dopamine neurons form dopaminergic as well as non-dopaminergic synaptic terminals in target areas at which glutamate may be the cotransmitter (Hattori, *Neurosci Res*, 1993); similarly, serotonergic raphe neurons appear to release glutamate (Johnson, *Neuron*, 1994). Electrophysiological studies of single identified dopamine neurons show that the cells frequently make glutamatergic autaptic EPSPs (Sulzer and Rayport, *Soc Neurosci Abstr*, 1993). We now report that individual dopamine neurons immunostained for tyrosine hydroxylase show significant variation in neurite staining. In single neuron microcultures, where all processes arise from a single dopamine neuron, some thin neurites — which are most likely axonal — have varicosities that stain darkly and others have varicosities that stain lightly or are unstained. Double staining for synaptophysin shows putative release sites that do not stain for tyrosine hydroxylase. Double immunostaining with a polyclonal antiserum directed against glutamate (the glutaraldehyde-conjugate) shows that dopamine neurons are often glutamate-positive, while GABA neurons are rarely glutamate-positive. Taken together, these observations are consistent with the possibility that dopamine neurons release glutamate as a cotransmitter and may do so at separate terminals from where they release dopamine. If so, this may account in part for glutamate dependence of dopamine synaptic plasticity and neurotoxicity.

639.18

NEUROPEPTIDE Y INCREASES CHOW INTAKE AND DOPAMINE CONCENTRATIONS IN MICRODIALYSATE FROM THE NUCLEUS ACCUMBENS OF RATS IN A FOOD CHOICE PARADIGM. R. L. Corwin* and J.N.Crawley. SBN, ETB, NIMH, NIH, Building 10, Room 4N212, Bethesda, MD 20892.

Neuropeptide Y (NPY) stimulates food intake when injected centrally (1). This effect of NPY on feeding may be direct or may be mediated by other neurotransmitters. Previous reports have demonstrated a high density of NPY neurons in the nucleus accumbens (NA) of the rat (2,3), some of which are in synaptic contact with tyrosine hydroxylase-like immunoreactive terminals (4). In the present study, rats were provided continuous access to rat chow and water, and limited access to a wet cookie mash, during *in vivo* microdialysis in the anterior NA. NPY (5 µg/5 µl) or vehicle (Ringer's, 5 µl) was administered intraventricularly. Food intake and dopamine (DA) concentration in microdialysate samples were measured every 20 min for 3 hours. DA concentration was quantitated using HPLC-EC. NPY increased chow intake and DA concentration, while having no effect on cookie mash intake in most of the rats tested. No increase in DA was seen in rats who did not consume chow but did consume cookie mash. These results suggest that NPY may play a role in food choice and that this effect may involve mesolimbic DA. 1. Clark, et al., 1984, Endocrinology 115:427. 2. De Quidt and Emson, 1986, Neuroscience 18: 545. 3. Salin, et al., 1990, Exp. Brain Res. 81:363. 4. Aoki and Pickel, 1988, Brain Research 459:205.

639.19

NEUROPEPTIDE Y RELEASE FROM PC12 CELLS IS REGULATED BY DOPAMINE X. Chen*, S.P. Han and T.C. Westfall Department of Pharmacological and Physiological Science, St. Louis University Health Science Center, St. Louis, MO 63104.

We have previously demonstrated that NPY is co-localized and co-released with dopamine from pheochromocytoma PC12 cells by potassium-induced depolarization (J. Neurochem. Vol. 61, suppl., 1993). This study was designed to further investigate the mechanisms regulating NPY release from the peripheral sympathetic nervous system using PC12 cell line as a model system. PC12 cells grown in cell culture inserts were differentiated with nerve growth factor for 5 days. NPY content in the cell and in the releasing buffer was determined by radioimmunoassay as described previously (DiMaggio, 1992). KCl (50 mM) induced a 1-2 fold increase in NPY release over basal levels. KCl-stimulated NPY release was greatly attenuated by the activation of dopamine receptors on PC12 cells with apomorphine, a non selective D_1 and D_2 dopamine receptor agonist. The inhibitory effect of apomorphine on NPY release was dose dependent and could be partially reversed by eticlopride, a D_2 dopamine receptor selective antagonist.

These results suggest that NPY release from PC12 cells is regulated by dopamine which acts as a cotransmitter of NPY in this model of a peripheral sympathetic neuron. This work is supported by HL26319; HL35202 and NS02254.

639.20

EFFECTS OF HALOPERIDOL AND CLOZAPINE ON AMPHETAMINE INDUCED DOPAMINE RELEASE MEASURED WITH *IN VIVO* MICRODIALYSIS IN FREELY MOVING RATS. M.R. Kasten, M.A. Clinton, A.M. Ulrich, D.L. Alexoff, G.S. Smith, C.R. Ashby, Jr*, and S.L. Dewey. Chem. and Med. Dep't, BNL, Psych. Dep't, NYU, Upton, NY 11973.

Using PET we have demonstrated that clozapine altered the ability of 5-HT or GABA to modulate cortical and subcortical ACh while haldol altered the ability of 5-HT and GABA to modulate striatal DA in the primate brain (Dewey, et al., 1993). More importantly, these alterations appeared dependent upon the duration of neuroleptic exposure. To investigate the mechanisms underlying this altered responsiveness, we performed studies in rats using *in vivo* microdialysis and a similar drug treatment protocol. Consistent with our PET findings, rats exposed to haldol (1.0 mg/kg) for 1 week demonstrated a potentiated responsiveness of the DA system to an amphetamine challenge when compared to saline controls. Unlike our PET findings this potentiated response was evident in rats exposed to haldol for 5 weeks. Similar findings were observed in rats exposed to clozapine. Taken with our previous PET studies, these data suggest that alterations in the ability of 5-HT to modulate DA following *acute* exposure, may result primarily from changes in the DA system while alterations observed in animals exposed *chronically* may be linked primarily to changes in 5-HT or its ability to interact with DA. These data suggest that drug induced alterations may occur with different temporal profiles in specific neurotransmitter systems resulting from either direct or trans-synaptic alterations. Supported by NARSAD award to SLD and GSS, USDOE/OHER, NIH Grants MH-49165, NS-15638, NS-15380, USDOE/SERS.

NEUROTRANSMITTERS: GLUTAMATE/NITRIC OXIDE

640.1

THE MESOPONTINE NADPH-DIAPHORASE SYSTEM IN THE JAPANESE QUAIL G.C. Panzica*, A. Garzino. Dept. Human. Anatomy & Physiology, Univ. of Torino, I-10126 Torino, Italy

The distribution of NADPH-diaphorase activity (ND), an enzyme recently identified as the nitric oxide synthase, was histochemically investigated in the Japanese quail brainstem. A large number of ND-positive neurons were observed at all levels of the brainstem. In particular, they were located within the substantia nigra (SN), the area ventralis tegmentalis (AVT), the substantia grisea centralis, the nucleus interpeduncularis (IP), the locus coeruleus (LoC) and the subcoerulean nuclei (SCon). Scattered elements were present in other regions such as the vestibular nuclei or the nucleus of the solitary tract (S). This distribution partly overlaps with catecholamine (CA) neurons evidenced both with histochemical and immunohistochemical techniques. In most mammalian species, ND-positive neurons also show immunoreactivity for cholineacetyltransferase (ChAT) and they are intermingled but distinct from the CA-synthesizing elements. In the present study, we performed a double staining for ND, and ChAT (antiserum provided by M.Epstein), or tyrosine hydroxylase (TH), or serotonin (5HT) in order to analyze the degree of coexistence of these systems in the quail brainstem. At the level of LoC and SCon, the large majority of the ND positive neurons is also expressing ChAT, but the ND and TH systems are almost totally segregated in different parts of the nuclei. On the contrary, in the more rostral areas, at the level of SN, AVT and IP, ND positive neurons are essentially distinct from the ChAT immunopositive neuron and a large majority of them is expressing TH immunoreactivity. In contrast with the findings reported in rodents, there is only a rare or no coexpression of both ND and 5HT along the entire brainstem. In conclusion these data demonstrate that, within the mesopontine ND systems, it is possible to identify neuronal populations containing different neurotransmitters (CA, acetylcholine, 5HT). Moreover, the demonstration that the TH immunopositive neurons of SN and AVT are largely positive for ND suggests that the nitric oxide could have an important role in regulating the target areas connected to these regions. *Supported by grants of CNR and MURST 60%.*

640.2

SIMULTANEOUS VOLTAMMETRIC DETECTION OF STIMULATED DOPAMINE AND NITRIC OXIDE RELEASE IN RAT BRAIN SLICES M.Iravani, J.Millar & Z.L.Kruk*, Departments of Pharmacology and Physiology (JM), Queen Mary & Westfield College, Mile End Rd, London E1 4NS UK.

We have recently described a voltammetric method which uses a carbon fibre electrode for real time detection of nitric oxide (NO; Iravani et al 1993, J.Physiol., 496: 48p), and we have used this to examine a NO like signal in rat striatum. Pressure ejection of NMDA, or electrical stimulation with trains of pulses in a 350µM rat striatum slice, gave rise to complex voltammetric signals which could be resolved into components attributable to the presence of dopamine (DA) and NO. The time courses of the DA and NO signals were different. The signal attributed to NO could be reduced by addition of the NOS inhibitor L-NMMA to the perfusion fluid. In the presence of the endogenous NO like substance, release of DA by electrical stimulation was attenuated, indicating that NO may have a role in local regulation of dopamine release.

640.3

SIMULTANEOUS MEASUREMENT OF ENDOGENOUS GLUTAMATE RELEASE AND NITRIC OXIDE PRODUCTION IN VIVO IN THE RAT HIPPOCAMPUS. K.M. Mitchell*[1], G.S. Wilson[2] and E.K. Michaelis[1]. Depts. of Pharmacology & Toxicology[1] and Chemistry[2], Univ. of Kansas, Lawrence, KS 66045.

Glutamate activation of NMDA receptor ion channels and the subsequent influx of Ca^{2+} is known to result in the activation of nitric oxide synthase (NOS) and enhancement of nitric oxide (NO) production. While glutamate and NO are known to be involved in the excitotoxic process of neuronal degeneration the exact mechanisms of this process are still unclear. The objective of this research was a first step towards the goal of further elucidation of the role of these transmitters in the excitotoxic process. This initial step entails the application of electrochemical detection methods to the measurement of evoked glutamate and NO overflow in the extracellular fluid (ECF) of the rat hippocampus. Evoked glutamate overflow was monitored using a dual enzyme biosensor developed in our laboratories. NO was measured using a nickel porphyrin based sensor developed by Malinski and colleagues (Nature 358:676-678, 1993). Glutamate and NO overflow were evoked by the focal injection of depolarizing levels of K^+ into the hippocampus. Potassium evoked glutamate release was correlated with local NO production. Local administration of nitro-L-arginine methyl ester (L-NMAE), an inhibitor of NOS, resulted in a decrease in NO production as well as a decrease in glutamate overflow. Furthermore, application of L-arginine in addition to L-NAME reversed this effect. Our results indicate that NO and glutamate in the ECF can be detected simultaneously in vivo using electrochemical methods. In addition, NO production appears to result in the enhanced overflow of glutamate in confirmation of similar findings reported previously (Buisson, et al., J. Neurochem. 61:690-696,1993).

640.4

NITRIC OXIDE INHIBITS NEUROGENIC NON-ADRENERGIC, NON-CHOLINERGIC CONTRACTIONS BUT NOT CHOLINERGIC CONTRACTIONS OF CIRCULAR MUSCLE FROM GUINEA PIG ILEUM. J.J. Galligan* and A.M. Yunker Dept. Pharmacology and Toxicology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824

Nitric oxide (NO) is released from enteric neurons resulting in relaxation of gastrointestinal (GI) smooth muscle. NO may also inhibit release of excitatory neurotransmitters such as substance P (SP) and acetylcholine (ACh) from enteric nerves. This hypothesis was tested using circular muscle-myenteric plexus preparations (CMMPPs) from guinea pig ileum. In the presence of scopolamine and guanethidine, stimulation (1-50 Hz, 0.01 TPS) of CMMPPs produced non-adrenergic, non-cholinergic (NANC) contractions. These contractions were blocked by tetrodotoxin and were attenuated in a concentration-dependent manner by CP96345-1, a neurokinin-1 receptor antagonist. The nitric oxide synthase (NOS) antagonists N_ω-nitro-L-arginine, N_ω-nitro-L-arginine methyl ester, and N^G-methyl-L-arginine potentiated the amplitude of the NANC contractions in a concentration-dependent manner. The D-stereoisomers of these NOS antagonists did not alter the NANC contractions. Oxyhemoglobin binds extracellular NO and caused a concentration-dependent increase in the amplitude of NANC contractions. Neither the NOS antagonists nor oxyhemoglobin altered the concentration-response curve for substance P methyl ester, suggesting that GI smooth muscle was not the site of action for these drugs. Finally, sodium nitroprusside attenuated NANC contractions in a concentration-dependent manner. Neither the NOS antagonists nor oxyhemoglobin affected cholinergic contractions generated in the presence of guanethidine and CP96345-1. These data suggest that NO inhibits SP but not ACh release, thus providing a second mechanism by which NO can inhibit motility of GI smooth muscle. (Supported by DK 40210 and NS 07279)

640.5

NITRIC OXIDE DEPOLARIZES PARAVENTRICULAR NUCLEUS NEURONS IN VITRO. J. S. Bains* and A. V. Ferguson. *Department of Physiology, Queen's University*, Kingston Ontario, Canada K7L 3N6.

The presence of nitric oxide synthase, the enzyme necessary for the production of nitric oxide (NO), within neurons in the paraventricular nucleus of the hypothalamus (PVN) has lead to the suggestion that NO may play a role in neurotransmission within this structure. Recent *in vivo* experiments have demonstrated that microdialysis of NO directly into PVN elicits decreases in blood pressure. This physiological change is accompanied by an increase in the release of excitatory amino acids such as glutamate within this nucleus (Horn et al., AJP, 1994). In this study, we have used whole-cell patch clamp recording techniques to investigate the potential effects of NO on PVN neurons. Using patch electrodes filled with potassium-gluconate, recordings were made from PVN neurons in coronal brain slices (400 μm thickness) of adult, male Sprague-Dawley rats. Bath application of NO ($5x\ 10^{-10}$- $5x\ 10^{-8}$ M for 1-3 min.) induced a variable (8.2 mV to 17.9 mV) membrane depolarization in 6 of the 7 cells tested (mean depolarization ± SEM: 10.6 ± 4.1 mV). This NO induced depolarization was also accompanied by a mean decrease in membrane resistance of 28 ± 17 %. These results indicate that NO, either itself, or through influencing the release of other neuronal mediators acts to excite PVN neurons.

640.6

EFFECTS OF NMDA ON NITRIC OXIDE AND NEUROTRANSMITTER RELEASE IN THE RAT STRIATUM. R. Guevara-Guzman*, P.C. Emson and K.M. Kendrick. Dept. Neurobiology, BBSRC Babraham Institute, Cambridge CB2 4AT, U.K. *Dept. Physiology, Faculty of Medicine, Mexico University, Mexico 04510, DF.

We have shown previously that nitric oxide (NO) stimulates *in vivo* release of acetylcholine (ACh), 5-HT, glutamate (GLU), taurine (TAU) and GABA in the rat striatum and that these effects are mimicked by cGMP. Dopamine (DA) release is inhibited by NO and increased by cGMP suggesting that this NO effect is mediated indirectly. The present study has investigated the effects of NMDA on NO and classical transmitter release in the presence and absence of the NO synthase inhibitor nitroarginine (NARG) using *in vivo* microdialysis.

The experiments were conducted in urethane anaesthetised rats and microdialysis probes were placed in the medial striatum. Krebs Ringer, with 2μM neostigmine, was passed through the probes at 1.5μl/min and a liquid switch was used to deliver different substances into the probe. ACh, DA, 5-HT, GLU, TAU and GABA were measured by HPLC. Nitrite (NO_2) and citrulline (CIT) were used as indicators of NO. NO_2 was measured using the Greiss reaction and a microplate reader (550nm) and CIT using HPLC. Results showed that a 15 min infusion of NMDA (150μM), GLU (1mM) or KCl (100mM) evoked simultaneous release of NO_2 and CIT. NMDA (1-100μM) evoked a dose-dependent release of CIT and all the transmitters measured, although for the transmitters a clear dose-response relationship was mainly seen with the lowest NMDA concentrations (1-10μM). With a 50μM NMDA dose, when a second application (S2) was given 3h after the first (S1) then evoked release of transmitters was reduced in S2 compared to S1, with the exception of DA which was increased in S2. If NARG (100μM) was applied for 2h before S2 by retrodialysis, or given peripherally, then the transmitter response to NMDA after S2 was signficantly increased, except in the case of DA where it was decreased.

Our results indicate that NO is released in response to doses of GLU and NMDA which are not toxic. NMDA also evokes transmitter release in the striatum, although inhibiting NO-release with NARG potentiates this release. This provides further support for a neuroprotective role for NO. The fact that effects of NMDA on DA release either in the presence or absence or NARG are opposite to those on the other transmitters measured probably reflects differential levels of inhibition of DA release produced by NMDA activation of ACh and GABA.

640.7

HEPATIC ENCEPHALOPATHY: INTERACTIONS OF NMDA RECEPTORS AND DOPAMINE RELEASE IN THE STRIATUM AND FRONTAL CORTEX. S.S. Oja*, H.D. Borkowska, J. Albrecht and P. Saransaari. Tampere Brain Res. Ctr., Univ. Tampere, Finland, and Med. Res. Ctr., Polish Acad. Sci., Warsaw, Poland.

Hepatic encephalopathy (HE) is often accompanied by motor abnormalities which are thought to reflect dysfunction of the striatal dopaminergic system. The release of dopamine (DA) from nigrostriatal neurons is presynaptically regulated by glutamate receptors. We studied now the effects of HE on the interactions of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor with DA release in the striatum and frontal cortex of male Wistar rats. The precomatose stage of HE was induced with intraperitoneal injections of hepatotoxin, thioacetamide. NMDA (0.1 mM) potentiated the release of newly-loaded [^{3}H]DA from slices from both brain regions studied, the effects being similarly abolished by the non-competetive receptor antagonist dizocilpine (MK-801). HE diminished the stimulation of NMDA by 46 % in striatal slices but had no significant effect in frontal cortical slices. In membranes prepared from the cerebral cortex of HE rats the maximal binding capacity B_{max} of dizocilpine was decreased when compared to controls. The binding constant K_D of dizocilpine to striatal membranes was not altered but B_{max} was decreased in rats with HE. B_{max} for N-[1-(2-thienyl)cyclohexyl]-piperidine (TCP) binding was not markedly altered by HE in the cerebral cortex but was significantly diminished in the striatum. The function of NMDA receptors seems thus to be attenuated in HE, which apparently diminishes DA release in the striatum. This phenomenon may be a factor causative of the motor disturbances of HE in vivo.

640.8

GLUTAMATE - SOMATOSTATIN INTERACTIONS IN THE RAT STRIATUM: AN IN VIVO MICRODIALYSIS STUDY. J.M. Radke, C. Spyraki and K. Thermos*. Lab of Pharmacology, Dept. of Basic Sciences, Sch.of Med., Univ. of Crete, Heraklion, Crete, Greece.

In the rat striatum, somatostatin is contained within a specific population of medium sized aspiny interneurons, where it is believed to act as a neurotransmitter. The corticostriatal (glutamergic) pathway may play an important role in somatostatin's function. To improve our understanding of somatostatin's role in the striatum, we investigated the interactions of this peptide with the excitatory amino acid system.

Using the technique of in vivo microdialysis we have successfully measured neuronally released somatostatin in the striatum. Administration of N-methyl-D-aspartate (NMDA) at doses of 10-4M and 10-5M significantly increased the release of somatostatin. This increase was blocked by the administration of the NMDA antagonists 2-AVP and MK801, but was unaffected by the administration of the sodium channel blocker tetrodotoxin (TTX). Administration of kainic acid also increased somatostatin release, in a dose dependent manner (10-5M to 10-7M). This increase was attenuated by the kainic acid receptor antagonist 6,7 DNQA but only partially blocked by the adminstration of TTX. Kainic acid at the dose of 10-4M resulted in a decrease in somatostatin release. This effect is believed to be due to the lesioning effects of high doses of the drug. Quisqulate (10-4M) had no effect on the release of somatostatin.

These results indicate that excitatory amino acids interacting with NMDA and kainate receptors, but not quisqulate receptors, regulate the release of somatostatin in the stritatum.

640.9

REGULATION OF THE PHOSPHORYLATION STATE OF DARPP-32 IN THE HALOTHANE-ANAESTHETIZED RAT. J. Ingum[1], I. Sefland[2] and S.I. Walaas*[2]. [1]Natl. Inst. of Forensic Toxicology, Oslo, Norway. [2]Neurochemical Laboratory, University of Oslo, Norway.

Results obtained in vitro indicate that the phosphorylation state of DARPP-32, a cytosolic protein that is highly enriched in the medium-sized spiny neurons of the striatum, is regulated by several neurotransmitters. The present study determined the phosphorylation state of DARPP-32 obtained in vivo by injection of drugs i.p. or by perfusing microdialysis probes inserted into the striatum of halothane anaesthetized rats with various neuroactive substances. Dopamine levels in the microdialysates were determined by HPLC, and the phosphorylation state of DARPP-32 was measured by Western blotting with phosphorylation state specific monoclonal antibodies.

Injection of amphetamine (1-10 mg/kg) i.p. significantly increased the state of phosphorylation of DARPP-32, as did intrastriatal perfusion with carbachol (2.5 mM). In contrast, perfusion with NMDA (0.5 mM) for 1 h dephosphorylated DARPP-32 significantly. Forskolin (0.1 mM) (an activator of adenylate cyclase) and Rp-cAMP (0.1 mM) (an inhibitor of protein kinase A) only moderately influenced the phosphorylation state of DARPP-32. Despite their differential effects on DARPP-32 phosphorylation, carbachol and forskolin both increased the extracellular levels of dopamine 8-10 fold. These results suggest that, under the experimental conditions employed, DARPP-32 phosphorylation is regulated in the intact brain by both muscarinic and NMDA receptor-regulated mechanisms, in addition to previously the described D1 receptor-cAMP-PKA signal pathway.

640.10

THE μ OPIOID AGONIST DAMGO DIFFERENTIALLY MODULATES THE NMDA COMPONENT OF EPSPs IN NUCLEUS ACCUMBENS (NAcc) NEURONS VIA PRE- AND POSTSYNAPTIC MECHANISMS G. Martin, Z. Nie and G.R. Siggins* Dept.of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

We previously showed that DAMGO decreases evoked EPSP amplitude in the NAcc slice (Yuan et al., Neurosci. Lett. 134: 223, 1992). However, the mechanism of this effect was unclear. In the present study, we investigated the action of DAMGO on pharmacologically-isolated NMDA components of evoked EPSPs. We recorded from a total of 44 NAcc cells in a slice preparation. In current-clamp, their mean resting membrane potential was -84 mV; mean input resistance was 43 MΩ and spike amplitude, 124 mV. In addition, these cells showed a strong inward rectification with a small sag in the hyperpolarized direction and a depolarizing ramp in response to positive currents. DAMGO 1 μM did not alter membrane potentials or input resistance in any cell tested (n = 34). In the presence of CNQX (10 μM) and bicuculline (15 μM), EPSPs evoked by local stimulation displayed the characteristics of an NMDA component: 1) long duration; 2) increase in amplitude with membrane depolarization; 3) total block by the NMDA receptor antagonist d-APV (60 μM; 9 of 9 cells). DAMGO 1 μM significantly decreased NMDA-EPSP amplitude by up to 50% of control (ANOVA: $p < 0.001$; 12 of 12 cells), with reversal of this effect by the opiate antagonist naloxone (1 μM; 9 of 9 cells). To assess a possible postsynaptic DAMGO action, we superfused the slices with TTX (1 μM) and evoked NMDA currents by pressure application from pipettes. Under these conditions, DAMGO 1 μM markedly enhanced (by 75%) the NMDA currents (4 of 5 cells). These results suggest that DAMGO modulation of the NMDA components of glutamatergic synaptic transmission is complex: DAMGO may decrease the NMDA-EPSP amplitude through a reduction of glutamate release, yet increase it via a direct postsynaptic action. Supported by NIH grants DA03665 and AA06420.

640.11

MUSCARINIC MODULATION OF NMDA RESPONSES IN NEOCORTEX. V. B. Aramakis*, I. H. Ashe and A. E. Bandrowski. Depts. of Neuroscience and Psychology, Univ. of California, Riverside, CA 92521.

Muscarinic actions of acetylcholine facilitate the amplitude of glutamate-induced membrane depolarizations (DPs) in rat auditory cortex (Cox et al, Synapse, 16:123, 1994). To assess the contribution of NMDA receptors to this facilitation, the actions of the cholinergic agonist, methacholine (MCh) were tested upon glutamate-induced DPs elicited in the presence of the AMPA/kainate antagonists CNQX or NBQX (20 μM). The actions of MCh upon the late-EPSP (NMDA-mediated) were also examined. Whole-cell recordings were obtained from layer III cortical neurons of the *in vitro* auditory cortex. Iontophoretic application of MCh (1M, 50-150 nA, 1-3 min) or glutamate (1M, 5-60 nA, 20 sec) was also to layer III within 50 μm of the recording electrode. During MCh, the amplitudes of glutamate DPs were consistently suppressed in either the presence or absence of CN/NBQX. On the other hand, following MCh the amplitudes of glutamate DPs were largely facilitated; about 75% in the absence of CN/NBQX, and about 300% in the presence of CN/NBQX. Durations of facilitation could last up to 35 min. MCh was also tested upon the isolated late-EPSP, i.e., elicited in the presence of CN/NBQX at 10-20 μM to block AMPA/kainate receptors, and to also minimize the contribution of the IPSPs (Metherate & Ashe, in press). Under these conditions, the isolated late-EPSP was consistently suppressed by MCh. Moreover, the isolated late-EPSP amplitude was usually enhanced above pre-MCh levels, following the initial suppression. Thus, the actions of MCh on glutamate-induced DPs and the isolated late-EPSP suggests an involvement of NMDA receptors in cholinergic modulation of glutamatergic transmission. Supported by NSF (IBN 9310582).

640.12

EFFECT OF NMDA RECEPTOR BLOCKADE ON REGIONAL DOPAMINERGIC ACTIVITY. Gina M. Fadayel, Vicki L. Taylor, Geoffrey Metcalf* and Christopher J. Schmidt, Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215

A dopaminergic bias within the glutamate/dopamine (DA) balance of the basal ganglia is believed to contribute to the symptoms of schizophrenia. The psychotomimetic MK-801, a channel blocker at the NMDA-glutamate receptor complex, is used to model this imbalance by reducing glutamate activation of NMDA receptors. Although MK-801 can produce its behavioral effects in monoamine-depleted animals, this occurs at higher doses than are necessary to elicit behavior in intact animals. MK-801 is also reported to increase the firing rate and bursting pattern of midbrain DA neurons. These observations suggest that increases in DA activity as well as decreased glutamatergic activity may contribute to the behavioral effects of MK-801. To further explore this question, the effect of MK-801 on DA release was determined in several brain regions using microdialysis in awake, freely moving rats. High doses of MK-801 (2 mg/kg, s.c.) caused a rapid increase in extracellular concentrations of DA in both the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAS). DA concentrations reached 360% of basal levels in the mPFC and increased to approximately 150% in the NAS. Transmitter levels remained elevated for 3 h. Concentrations of the DA metabolites DOPAC and HVA rose in delayed sequence. MK-801 also increased dopamine synthesis in both regions as assessed by DOPA accumulation following decarboxylase inhibition. In contrast to the results in the mPFC and NAS, striatal DA and its metabolites decreased following MK-801 administration. These observations are consistent with the hypothesis that alterations in DA activity contribute to the behavioral effects of MK-801. The results also suggest that the mesolimbic and mesostriatal DA systems differ significantly in their interaction with the NMDA-glutamate system.

640.13

MECHANISM OF THE SYNERGISTIC ENHANCEMENT OF GLUTAMATE INDUCED CALCIUM INCREASE BY NORADRENALINE IN CULTURED VISUAL CORTICAL NEURONS. B. Yang*, Y.H. Wang and M.S. Cynader. Department of Ophthalmology,University of British Columbia, 2550 Willow St., Vancouver B.C., Canada V5Z 3N9.

In our previous studies, noradrenaline was found to be able to synergistically enhance glutamate induced intracellular free calcium ($[Ca^{2+}]_i$)increases in cultured visual cortical neurons(Neurosci. Abst. 205.13,1993). To further address the mechanism underlying this interaction, we employed antagonists of a variety of receptor subtypes to investigate the cellular pathways responsible for the modifying effect. We found that the dose dependent glutamate induced calcium increase was blocked by 25 μM APV. It was interesting to find that this NMDA receptor antagonist also completely blocked the synergistic increase in $[Ca^{2+}]_i$ observed when both glutamate and noradrenaline were coapplied. This result indicates that the noradrenaline enhanced increase in calcium require at least an initial activation of NMDA receptor channels. Further possible sources of calcium mobilization include facilitated NMDA receptor channels, voltage gated calcium channels, internal calcium stores or any combination of these potential routes. We also tested three major subtypes of adrenoceptors with respective antagonists, propranolol (β), prazosin (α1) and rauwolscine (α2). Since we were dealing with a mixed culture of cortical neurons, these antagonists showed varying effectiveness in blocking the synergistic enhancement induced by noradrenaline in different individual neurons. Analyses of data obtained thus far indicate that propranolol is capable of blocking the synergism more completely on more cells than rauwolscine. Prazosin showed relatively weak effects. This implies that both cAMP and PI turnover pathways may be involved in the synergistic interaction between these transmitters. This result is being further studied by manipulating intracellular cascades that may be involved.

640.14

LOCALIZATION OF MYOMODULIN-LIKE IMMUNOREACTIVITY IN THE LEECH CNS. H. H. Keating and C. L. Sahley*. Dept. of Biological Sci., Purdue University, West Lafayette, IN 47907.

The distribution of myomodulin-like peptides in the leech CNS was determined using an antiserum raised against Aplysia myomodulin (Miller et. al., 1991). Numerous immunoreactive neurons were found in the nervous system. Also, immunoreactive varicosities were found throughout the neuropil. Two types of immunoreactive fiber staining were observed in the connectives between ganglia and in the nerve roots, 1) fibers with smooth contours and 2) fibers with periodic swellings. Double-labeling experiments are in progress to identify the labeled neurons. Preliminary results suggest that myomodulin-like peptides are present in the Anterior pagodas, AE motorneurons and the Leydig cells; components of the shortening reflex which can be modified by learning. Kuhlman et al., (1985) have previously demonstrated FMRF-amide immunoreactivity in these cell types as well. Specific staining was abolished by preabsorption of antiserum with synthetic myomodulin but not with FMRF-amide. It is likely, therefore, that these cells contain multiple neuromodulatory peptides. HPLC elution profile data are consistent with the presence of myomodulin in the leech CNS.
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641.1

HYPOTHYROIDISM IMPROVES THE STRESS RESPONSE AND MODIFIES ANXIETY BEHAVIOR IN RATS.

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Learned Helplessness (LH), an animal model that determines the response of rats to footshock stress, has been used to screen pharmacologic agents for use in depression. In addition to tricyclic antidepressants, low dose T3 administration and GABA-mimetic agents have been useful in improving performance in this paradigm. Low doses of T3 may actually cause brain hypothyroidism by decreasing pituitary secretion of thyrotropin, thereby inhibiting endogenous thyroid hormone secretion. We tested this hypothesis by studying hypothyroid rats for LH. Hypothyroid rats had a 25% lower incidence of LH and a 20% increase in LH-resistant behavior compared to euthyroid controls ($p<0.05$). There were no differences in activity level by open field testing between hypothyroid and euthyroid rats. Hypothyroid rats also displayed anxiolytic behavior relative to euthyroid rats in the elevated plus maze, a behavioral assessment of GABAergic activity, although on retesting 1 month later, hypothyroid LH rats displayed anxiogenic activity. Hypothyroid rats did not display differences in $GABA_A$ receptor sensitivity compared to euthyroid controls, as assessed by $GABA_A$ receptor-mediated chloride ion influx. Euthyroid LH-resistant rats did manifest an increase in $GABA_A$ receptor sensitivity compared to euthyroid LH controls. We conclude from these studies that hypothyroidism decreases the incidence of LH behavior, and the antidepressant activity of T3 may actually be due to its induction of secondary brain hypothyroidism. This effect may be mediated in part by a hypothyroidism-induced enhancement of GABAergic transmission by a mechanism other than $GABA_A$ receptor sensitivity.

641.2

EFFECTS OF THE CARBAMATE PESTICIDE CARBARYL ON THE REPEATED ACQUISITION OF RESPONSE SEQUENCES IN RATS. J. Cohn*[1] and R.C. MacPhail[2]. [1]Curriculum in Toxicology, UNC, Chapel Hill, NC; [2]US EPA, Research Triangle Park, NC.

Carbaryl is a commonly used carbamate pesticide that inhibits acetylcholinesterase. Cholinergic neurotransmission is known to play an important role in cognitive function. This experiment was therefore undertaken to determine the effects of acute carbaryl administration on the repeated acquisition of response sequences in rats. Repeated acquisition is a method by which learning can be continuously assessed in single subjects. Adult male Long-Evans rats, maintained at 300 g, performed under a multiple schedule of RA and performance (P) in a standard operant chamber equipped with three response levers. The RA component required rats to learn a new 4-member sequence of responses during each experimental session (Center Right Left Right, RLCL, RCRL, etc.); the correct sequence of responses for the P component remained constant across sessions. Components alternated twice during a session. After baseline training, rats were given vehicle (5% Emulfor, 5% ethanol in saline), 3, 6.5, 7.9, 10 and 17.4 mg/kg carbaryl. Injections were given i.p. (1 ml/kg) 20-min presession, and no more than twice weekly. Significant decreases in both accuracy and response rate were produced by doses of 6.5 mg/kg and greater. Generally, results indicated that carbaryl's effects on RA were non-specfic in that both the RA and P components were equally affected. Some rats, however, exhibited specific effects of carbaryl on RA. Microanalysis of response patterning indicated that errors consisted primarily of pseudorandom lever presses ("skipping" errors) rather than perseverative responses on single levers.

641.3

DOES SEASONAL VARIATION AFFECT RESULTS OF THE MIRRORED CHAMBER BEHAVIORAL ASSAY? S. J. Ciric and N. L. Katz*. Dept. Pharmaceutics and Pharmacodynamics, U. Ill. at Chicago, Chicago, IL 60612.

We fashioned several mirrored chambers described by Toubas et al. (Pharmacol. Biochem. Behav. 35: 121, 1990). The mirrored chamber test is a behavioral assay based on an approach-avoidance response when a mirror is placed into a mouse's environment. The sight of an animal reflected in the mirror is a source of anxiety presumably preventing entry into the chamber. In the present studies, male Balb/cByJ mice were used. Their ages, at the onset of the studies, were 7-8 weeks. Each mouse was allowed to explore the chamber for 5 min. The time to first entry into the mirrored chamber (T), total time spent in the chamber (TT), and number of entries (NE) were quantitated. Control studies were run during the first 2 weeks of October (1993), first 2 weeks of December, first 2 weeks of February (1994), and last week of April. Latencies to entry were 182 (N=30), 102 (N=47), 101 (N=17), and 236 sec (N=10), respectively. In the winter months, results seemed heterogenous and inconsistent, and base line (percent of animals entering the chamber) ranged from 50-75%. During this season, cholecystokinin tetrapeptide (CCK_4), in doses ranging from 3-30 μg/kg, produced a dose-dependent increase in T, and decreased both TT and NE. The CCK_B antagonist PD135158, 1 mg/kg, reversed CCK_4-induced changes in T and TT. PD135158 alone was without effect. Results obtained in April were most similar to those reported by Toubas et al. (1990). Diazepam, 1 mg/kg, decreased T, and increased TT and NE. PD135158 had no effect.

641.4

NCS-382 REVERSES HYPERTHERMIA, NOT HYPOTHERMIA, INDUCED BY GAMMA-HYDROXYBUTYRATE IN THE RAT. R. Godbout,[1]* P.-P. Rompré,[1] M. Schmitt[2] and J.-J. Bourguignon.[2] [1]Dép. psychiatrie, U. de Montréal, Montréal (Québec) H4J 1C5; [2]Labo. pharmacochim. molécul., CNRS 421, Strasbourg France

Gamma-hydroxybutyrate (GHB) is an endogenous fatty acid recognized for its sedative-hypnotic effect. Since thermoregulatory mechanisms are bound to be involved in this effect, we measured central temperature before and after administration of GHB at various doses and we tested the newly synthesized GHB receptor antagonist, NCS-382. Body temperature was monitored by mean of a rectal probe; baseline readings were first taken, then each 15 min for the 1st hour following an i.p. injection of saline or GHB, and each hour thereafter. When NCS-382 was used, it was co-administered with GHB, at the same dose. Low GHB doses (10 mg/kg) were associated with increased temperature (max = +0.67°C, 45 min post injection) whereas hypothermia was observed from 20 to 250 mg/kg (max at 250 mg/kg = -0.95°C, 30 min post injection). NCS-382 blocked the hyperthermic effect of GHB 10mg/kg but not the hypothermia induced by GHB 250mg/kg. These results suggest that the pyrogenic effect of GHB is mediated by high affinity specific GHB receptors and that GHB-induced hypothermia involves a more complex mechanism. Supported by FRSQ and CNRS

641.5

ESTABLISHMENT OF MELATONIN DISCRIMINATION IN PIGEONS. J. A. Stanley* and A. A. Ortiz. CNS Psychobiological Disorders, Bristol-Myers Squibb Company, Wallingford CT 06492.

Melatonin (5-methoxy-N-acetyltryptamine) is a pineal neurohormone that controls circadian patterns of behavior (e.g., sleeping, eating, drinking, and reproduction) in many species by modulating the "biologic clock," a cluster of cells found in the suprachiasmatic nuclei (SCN). In order to permit exploration of the functional effects of melatonin, pigeons were trained to use the interoceptive cue produced by peripheral administration of this hormone as the discriminative stimulus for differential responding using a drug discrimination procedure.

Ten minutes before daily fifteen-minute sessions, White Carneaux pigeons were given melatonin (0.3 or 0.56 mg/kg, i.m.) or vehicle (0.5% DMSO in normal saline) and placed in a darkened, two-key operant chamber. Responding on the appropriate key was reinforced (FR-30); whereas, responding on the incorrect key had no consequence other than resetting the FR value.

Pigeons learn to discriminate a systemically administered dose of melatonin from its vehicle at doses that have no effect on response rate. When doses of melatonin from 0.01 and 3.0 mg/kg, i.m., are substituted for the training dose (0.3 or 0.56 mg/kg, i.m.), the drug-key is selected demonstrating complete stimulus generalization.

In rats melatonin discrimination has been demonstrated only at doses which are sedative and more than 100 times those used in circadian rhythm studies. Our results, demonstrating the exquisite sensitivity of the pigeon to melatonin, suggest the bird may be the subject of choice in discrimination studies investigating the effects of melatonin at doses comparable to those used in circadian rhythm studies.

641.6

RESTORATIVE EFFECTS OF FIBROBLAST GROWTH FACTOR, GIVEN TEN DAYS AFTER FIMBRIA-FORNIX SEVERANCE, ON SPATIAL MEMORY IMPAIRMENT IN RATS H.Amino*, N.Nishiyama and H.Saito Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan.

Fimbria-fornix (F-F) is a major cholinergic afferent from medial septum to hippocampus, which is suggested to play a critical role in spatial learning. We have previously reported that both acidic and basic fibroblast growth factor (a, bFGF) accelerated the acquisition of position discrimination reversal learning in Y-maze test in naive rats. In the present study, we studied the effects of FGFs on spatial memory deficits induced by F-F lesion. Rats with a normal spatial learning ability had been selected, based on the results of Y-maze test training. Guide cannulas were planted into bilateral cerebroventricle, and F-F was severed with a blade at the same time. From 10 days after the operation, spatial learning performances of the rats were observed in Y-maze test, and subsequently in water maze test. FGFs (100-400 ng/rat, i.c.v.) were daily injected 30 min prior to each learning session. F-F lesion induced severe and long-lasting spatial memory deficits in Y-maze test (correct response: intact 89.6 ± 8.2 %, lesioned 37.5 ± 8.5 %) and in water maze test (escape latency: intact 9.4 ± 1.5 sec, lesioned 69.5 ± 15.1 sec). Both aFGF and bFGF significantly ameliorated the spatial memory deficits induced by F-F lesion in dose dependent manners(correct response: 400 ng of aFGF 85.4 ± 6.0 %, 400 ng of bFGF 75.0 ± 6.9 % escape latency: 400 ng of aFGF 11.0 ± 1.2 sec, 400 ng of bFGF 12.8 ± 1.2 sec). Reduction of hippocampal choline acetyltransferase activity caused by F-F lesion was not affected by the injection of aFGF and bFGF. In conclusion, we suggest that delayed application of aFGF and bFGF improve the surgically induced spatial memory disorders without affecting the hippocampal cholinergic transmission. Further investigations would differentiate the long-term (neurotrophic) and short-term (neuromodulatory) actions of FGFs on learning and memory.

641.7

EFFECTS OF CHRONIC ddC ADMINISTRATION ON LOCOMOTOR ACTIVITY IN RATS. H.D.Davis[1*], D.E.Morse[1] and N.E.Grunberg[2]. [1]FDA, Antiviral Research Laboratory, Rockville, MD 20857. [2]Uniformed Services University of the Health Sciences, Medical Psychology Department, Bethesda MD 20814.

Dideoxycytidine (ddC) is a nucleoside analogue used in the treatment of HIV infection. Unfortunately, the long-term use of ddC has been associated with an apparent peripheral neuropathy and debilitating pain in a subset of HIV patients. Recently, we reported on the value of a behavioral paradigm in rats to study the neurotoxicity of the anti-HIV nucleosides. We found that locomotor activity was altered in a U-shaped dose-effect relationship following increasing doses of ddC (IG) to rats. The present experiment examined the effects of chronic ddC administration (PO, 0.1-10.0 mg/ml in the drinking water) on the locomotor activity of female Sprague-Dawley rats. Activity was monitored for one hour each day for eight consecutive days during a period of continuous drug administration. Body weight, food and water consumption were also monitored. Exposure to ddC at 3.0-10.0 mg/ml concentrations resulted in a significant decrement in locomotor activity beginning with day 5 of exposure. While water consumption was reduced somewhat among animals in the high drug concentration group, this occurred during days 2-3 of dosing and was not correlated with the observed locomotor decrement. Body weight, food and water consumption among all other dose groups was indistinguishable from that of the control group. Chronic dosing thus revealed a pattern of behavioral toxicity that an acute paradigm could not detect. Implications of these findings for the neurotoxicity testing of drugs used in AIDS therapy will be discussed.

641.8

THE ROLE OF THE ADENOSINE SYSTEM IN MITIGATING THE LOCOMOTOR DEFICITS PRODUCED BY THE RADIOPROTECTOR WR-151327. J.B. Hogan, C.A. Castro, K.A. Benson, T.W. Shehata, J.F. Weiss, H.M. Jacocks*, and M.R. Landauer. Department of Behavioral Sciences, Armed Forces Radiobiology Research Inst., Bethesda, MD 20889.

The methylxanthine caffeine has been shown to mitigate the locomotor deficit that follows administration of aminothiol radioprotective compounds. A likely mechanism of action for this effect is caffeine's antagonism of the adenosine (A) system. This study compared the effects of nonselective adenosine antagonists (theophylline and caffeine) with those of selective A_1 (8-Cyclopentyl-1,3-dimethylxanthine, CPT) and A_2 (3,7-Dimethyl-1-propylargylxanthine, DMPX) receptor antagonists in attenuating locomotor decrements induced by WR-151327 [S-3-(3-methylamino-propyl-amino)-propyl-phosphorothioic acid]. Locomotor activity (total distance travelled) was examined in male CD2F1 mice in an automated activity monitor for 12 hr postinjection. The effects of WR-151327 alone and in combination with adenosine antagonists on locomotor activity were examined. WR-151327 (200 mg/kg, i.p.) alone significantly reduced activity from 30 min to 3 hr postinjection. The adenosine antagonists (20 mg/kg, i.p.) were each administered 30 min prior to WR-151327 (200 mg/kg, i.p.) at which time locomotor activity monitoring began. Compared with vehicle controls, theophylline alone significantly increased activity for 1 hr, and caffeine alone for 2 hr. CPT alone increased activity for 0.5 hr, and DMPX alone increased activity for 2 hr. When combined with WR-151327, theophylline reversed the WR-151327-induced motor deficit for 1 hr, and caffeine for 2 hr. DMPX reversed the deficit for 1 hr, while CPT failed to mitigate the deficit. These results provide evidence that the A2 adenosine receptor is important in mitigating WR-151327-induced locomotor activity decrements.

641.9

EVALUATION OF DOSE-RELATED EFFECTS OF D-CYCLOSERINE IN UNMEDICATED OBSESSIVE COMPULSIVE DISORDER PATIENTS. Barr LC*, Bennett A, D'Souza DC, Price LH, Krystal JH. Yale Univ Sch of Med, New Haven, CT 06519

Functional neuroimaging studies implicate orbital frontal cortex and caudate in the mediation of obsessive compulsive symptoms. As excitatory transmission within the frontal cortex and from frontal cortex to striatum is prominent, the effects of D-cycloserine, a partial agonist at the glycine site of the NMDA receptor, on obsessive compulsive symptoms was assessed. **Method:** 11 unmedicated Obsessive Compulsive Disorder (OCD) patients (mean ± SD age= 39.6 ± 11.2;7 male, 5 female) received either a placebo, a 50 mg or 500 mg oral dose of D-cycloserine on three test days each separated by 7 days. A 15 point scale was used to assess obsessions and compulsions. Possible effects of D-cycloserine on frontal and memory function were assessed using the following: Hopkins Verbal Learning Test, Nonverbal Selective Reminding Test, Rey Taylor Complex Figure Test and a test of verbal fluency. As 50 mg D-cycloserine has been shown to increase cortisol in healthy subjects, cortisol was measured at baseline and following D-cycloserine. Data were analyzed using RM-ANOVA. **Results**: No dose or dose X time effects were identified for clinician or patient-rated obsessions and compulsions and no clinically apparent changes in symptom severity were observed. Neuropsychological testing also did not identify medication effects. Data comparing the hormonal response of these patients with healthy subjects will be presented. **Conclusions**: Putative facilitation of excitatory transmission produced by D-cycloserine may not markedly effect OC symptoms or neuropsychological deficits sometimes observed in these patients.

641.10

DISULFIRAM CAUSES SUSTAINED BEHAVIORAL AND BIOCHEMICAL EFFECTS IN RATS. M.A. Rahman, N.E. Grunberg* and G.P. Mueller. Neuroscience Program, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

Disulfiram is the most commonly used pharmacologic treatment for alcoholism. Despite its established use, the research literature is sparse regarding the central neurochemical or behavioral toxicology effects of this important medication. We recently observed (Mueller, Husten, Mains, & Eipper, 1994) that disulfiram causes a marked inhibition in the biosynthesis of α-amidated peptides which persists for weeks after the cessation of treatment. More than half of the peptides used in intercellular communication are α-amidated and in nearly all cases this modification is required for receptor binding and biologic activity. The present experiment was designed to evaluate the effects of disulfiram administration on behavioral measures of nociception (hot plate and tail flick), peripheral muscular performance (grip strength), motivated performance, balance, and coordination (rotorod); body weight; and peptidylglycine α-hydroxylating monooxygenase (PHM) activity in the hypothalamus, neuro-intermediate lobe of the pituitary (NIL), adenohypophysis, and atrium; and α-MSH levels in the neurohypophysis. Adult, male, Sprague-Dawley rats were treated daily for one week with disulfiram (0, 50, or 150 mg/kg, s.c.) and were evaluated for two weeks after cessation of treatment. Disulfiram significantly increased hot plate response latencies, decreased grip strength, decreased rotorod performance, and decreased body weight in a dose-response manner. These effects persisted through the two week post-treatment period and paralleled the time course of the inhibition of PHM previously reported. In addition, disulfiram significantly altered PHM activity in tissue samples from the NIL and atrium, and significantly decreased amounts of α-MSH. These findings indicate that disulfiram may have several untoward effects that are relevant to therapeutic use and warrant further investigation.

641.11

TYROSINE REVERSES COLD-STRESS INDUCED IMPAIRMENT OF DRL PERFORMANCE IN RATS. J. R. Thomas*, D. Shurtleff, J.Schrot, and R. Moran. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Acute exposure to cold stress has been reported to impair performance on a variety of tasks, including an increase in the speed with which behavioral and neural operations occur. Exposure to a temperature of 2°C increases rate of responding on a time-based differential-reinforcement-of-low-rate (DRL) reinforcement schedule, without affecting attentional, sensory, or motor aspects. Administration of the amino acid, l-tyrosine, has been shown to improve behavioral impairments induced by exposure to cold. The present study examined the effects of tyrosine in rats performing on a DRL schedule that were exposed to a temperature of 2°C for one hour. Consistent with previous research and relative to 22°C exposure sessions, during the 2°C exposure response rates on the DRL schedule increased markedly. Administration of tyrosine (50,100,200 mg/kg i.p.) returned DRL response rates during cold to within control values. Pretreatment with valine (100 mg/kg), an amino acid that competes with the transport system for tyrosine, prevented the DRL response rate-reversing effects of tyrosine during cold exposures, suggesting a rather direct effect of tyrosine on cold-stress impairment of DRL performance.

641.12

A TEST OF THE CLASSICAL CONDITIONING MODEL OF THE PLACEBO EFFECT WITH CARISOPRODOL AS UNCONDITIONED STIMULUS M.A. Flaten, T. Simonsen, K.K. Waterloo and H. Olsen, Dept. Psychology, Univ. Tromsø, N-9037 Tromsø, Norway.

Two groups (N=10) received pairings of a distinct environment, the laboratory, and administrations of 700 mg of the muscle relaxant carisoprodol (Carisoprodol group), or pairings of the same environment with administrations of an inactive agent (Control group) for a total of three sessions, each spaced one week apart. The laboratory was hypothesized to constitute a conditioned stimulus (CS) for the drug unconditioned stimulus (US) in the Carisoprodol group. In the fourth session the Carisoprodol group received the inactive agent in the laboratory, and the Control group received carisoprodol in the laboratory. Dependent variables were blink reflexes and carisoprodol serum consentration. Results showed facilitated blink reflex amplitudes in the Carisoprodol group when an inactive agent was administrated in the laboratory, which is opposite to the effects of carisoprodol. This indicates that a conditioned drug antagonistic response was elicited by the CS in this group. Moreover, blink reflex amplitudes were inhibited in the Control group when it received carisoprodol, indicating that an antagonistic response was not elicited in this group.

SPON: European Brain and Behaviour Society

641.13

DISCRIMINATIVE-STIMULUS EFFECTS OF THE POTASSIUM CHANNEL BLOCKER 4-AMINOPYRIDINE IN RATS. R. Brandsgaard*, S. Rosenzweig-Lipson, J. Willetts and J. E. Barrett. American Cyanamid, Lederle Laboratories, CNS-Biology, Pearl River, NY 10965.

In the present study, rats (N=12) were trained to discriminate the voltage-gated potassium channel blocker 4-aminopyridine (4-AP) (1.0 mg/kg, n=9 or 1.7mg/kg, n=3, s.c., 30 minute pretreatment) from saline in a two-lever drug discrimination procedure. Rats responded differentially on the left and right levers under a 30 response fixed-ratio schedule of food presentation depending on whether 4-AP or saline was administered. 4-AP served as a discriminative-stimulus in all the rats, showing dose-dependent (0.1-1.7 mg/kg) increases in drug lever responding up to the training dose. Administration of saline resulted in <1% drug lever responding. There was no effect on response rate for the rats with a training dose of 1.0 mg/kg, and a moderate dose-related decrease in response rate for rats with a training dose of 1.7 mg/kg. Once the discrimination was established, the discriminative-stimulus effects of 4-AP were compared to several known potassium channel blockers, a calcium channel opener and an acetylcholinesterase inhibitor. The structurally related potassium channel blocker, 3,4 diaminopyridine (0.3 - 17.8 mg/kg) fully substituted at doses which moderately decreased response rate in 4 out of 4 rats trained at 1.0 mg/kg. 3,4 diaminopyridine did not substitute in 2 rats that were trained at 1.7 mg/kg. Other potassium channel blockers, including quinine (30.0 - 100.0 mg/kg) and amodiaquin (30.0 - 56.0 mg/kg), a Ca^{2+} channel opener, Bay K 8644 (0.3 - 1.0 mg/kg), and an acetylcholinesterase inhibitor, 9-amino-1,2,3,4-tetrahydroacrdine hydrochloride (THA) (1.0 - 5.6 mg/kg), generally did not substitute up to doses that decreased response rate. The present results demonstrate that 4-AP can serve as a discriminative-stimulus in rats. In view of the substitution of 3,4 diaminopyridine, but not quinine or amodiaquin, the present results suggest that the discriminative-stimulus effects of 4-AP are mediated by a subset of voltage-gated potassium channels.

641.14

HORSE SERUM BUTYRYLCHOLINESTERASE PROTECTS AGAINST MEPQ-INDUCED RESPONSE SUPPRESSION IN RATS . R.F. Genovese*, R. Larrison and B.P. Doctor, Divisions of Neuropsychiatry and Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

New therapies against organophosphorus (OP) toxicity have focussed on the administration of enzyme "scavengers", in order to prevent deleterious effects by neutralizing the OP *before* target areas are affected. We examined the ability of horse serum butyrylcholinesterase (HS-BChE) to attenuate the effects of the OP, MEPQ, in rats trained to lever press under a variable interval 56" schedule of food reinforcement. Under baseline conditions, the schedule produced constant and relatively fast rates of responding throughout the 60 min sessions. MEPQ (32μg/kg, IP) (n=5) produced complete or nearly complete response suppression in rats pretreated with vehicle. Rats treated with 7500 U HS-BChE (IP) (n=5) approximately 18 h before MEPQ injection, however, responded at nearly 70% of control rates. Blood BChE activity was elevated in rats receiving HS-BChE (34-68 U/ml) as compared to rats receiving vehicle (0.8-1.6 U/ml), but decreased when measured 1 h following MEPQ administration. These results demonstrate that administration of HS-BChE can attenuate the toxic effects of MEPQ, and merits consideration as a prophylactic therapy against OP exposure. These results also confirm and extend previous reports demonstrating that protection against OP toxicity can be conferred by a number of cholinesterases.

641.15

NEUROPEPTIDE-Y ALTERS THE ACQUISITION OF RESPONSE SEQUENCES IN RATS. J. Schrot* and J.R. Thomas. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Administration of neuropeptide-Y (NPY) has been shown to both improve and impair working memory as measured with a delayed matching-to-sample (DMTS) procedure. The present study used a repeated acquisition (RA) procedure to investigate the effect of NPY (2.0 to 34.0 ug/kg, icv) on the acquisition of three-member response sequences in rats. Each incorrect response in this procedure results in a brief timeout from reinforcement. Sessions were divided into a learning and a performance phase by imposing a five-minute delay at the mid point. A biphasic response pattern of acquisition, followed by performance, was observed during control sessions. The average number of error responses was 296 during the 1st half and 155 during the 2nd half of each session. Administration of NPY slightly improved performance at a dose of 8.6 ug/kg and markedly disrupted performance at a dose of 34.0 ug/kg. The 1st half vs 2nd half incorrect responses at the 8.6 ug/kg dose were 208 and 116, while the same measures with the 34.0 ug/kg dose were 502 and 214, respectively. A dose of 2.0 ug/kg had no effect on performance. The RA procedure contains elements of both working and reference memory. Working memory is requisite for acquiring the novel sequence of lever presses each day, while reference memory is invoked by the timeout stimulus. NPY administration altered both working and reference memory aspects of the procedure. These results are consistent with and extend the finding that NPY altered working memory in a DMTS procedure.

OSMOTIC REGULATION

642.1

PERSISTENT ELEVATION OF VASOPRESSIN MRNA AFTER SALT-LOADING. M.D. Fitzsimmons*, M.M. Roberts, A.G. Robinson Department of Endocrinology, University of Pittsburgh, PA 15261

Based on computer modeling, we have suggested that pituitary vasopressin (AVP) levels depleted after chronic salt-loading recover to age-matched control simply by translating mRNA that accumulates during the period of salt-loading (**AmJPhysiol** 262: R1121, **Endocrinology** 134:1874). The amount of mRNA needed for such recovery depends on the assumptions in the model, but the smallest reasonable estimate would require a 4-fold increase over baseline. Experimental measurements in our lab with RNase protection assay of whole hypothalamus show that salt-loading increases AVP mRNA expression by only 1.5-2.5 times, a range consistent with other recent reports. These results might be interpreted to indicate that translational regulation plays a role in the regeneration of posterior pituitary vasopressin levels. Alternatively, mRNA may remain elevated after the stimulus is removed. Evidence from Zingg et al (**JBiolChem** 261:12956) and Arnauld et al (**NeurosciLetters** 149:177) supports the latter. We tested the impact of stimulus duration on vasopressin mRNA levels after salt-loading. Both 3 days and 5 days of salt-loading result in a significant increase in mRNA versus control (1.6-fold and 2.0-fold respectively, $p<0.001$), with 5 days being significantly greater ($p<0.01$). AVP mRNA showed no detectable decrease after two days of recovery in both the 3 day and the 5 day groups; the 3 day group remains elevated even 4 days after recovery ($p<0.01$). Plasma sodium levels in the salt-loaded groups increased significantly ($p<0.001$), but were not different from baseline in the recovered groups. These results demonstrate that the length of salt-loading affects the magnitude of AVP mRNA increases and that the post-stimulus elevation of mRNA is independent of stimulus duration. Whether the persistence of AVP mRNA is due to "momentum" in the transcriptional regulation of the gene or to increased mRNA stability will be determined with intronic *in situ* hybridization. In either case, the unexpectedly prolonged mRNA elevation probably contributes to the recovery of pituitary vasopressin content to age-matched levels after salt-loading.

642.2

OXYTOCIN (OT) AND VASOPRESSIN (AVP) EXPRESSION IN THE HYPOTHALAMUS OF THE HYPOTHYROID OSMOTICALLY-STIMULATED RAT. J.A. Amico, A. Thomas, M.M. Roberts*. Dept. of Med., Univ. of Pittsburgh, Sch. of Med. and Oakland VA Med. Ctr., Pittsburgh, PA 15261.

Sustained hyperosmolality enhances the abundance of OT and AVP mRNAs in the rat hypothalamus. Gonadal steroids modify this response. Gonadectomy prevents, and gonadal steroids restore, OT and AVP expression to osmotic challenge. The OT gene is influenced by other hormones, including thyroid hormone(T4). We questioned whether T4 deficiency would also alter OT and AVP mRNA abundance in the paraventricular nucleus (PVN) of the osmotically-challenged rat. Male Sprague-Dawley rats (7 wks of age) were sham thyroidectomized (sham TX) or TX, implanted 3 wks later with empty (TX+sham) or T4-filled (TX+T4) capsules, randomized to 2% NaCl solution or tap H_2O for 5-10 days and sacrificed at the end of the NaCl challenge. Serum T4 levels were significantly lower in the TX vs. sham TX and TX+T4 groups ($P< 0.001$). All NaCl groups developed hypernatremia and depleted pituitary stores of AVP. Northern blot hybridization showed that salt loading increased PVN OT and AVP mRNAs in all groups compared to their respective tap H_2O cohorts. In contrast to the effect of gonadectomy, hypothyroidism does not alter osmotically stimulated-accumulation of OT and AVP mRNAs in the rat PVN.

642.3

BC1 RNA IN THE RAT POSTERIOR PITUITARY: AXONAL LOCALIZATION AND REGULATION UPON DEHYDRATION AND REHYDRATION. A. Trembleau* and F. E. Bloom. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Brain cytoplasmic 1 (BC1) RNA, a small non-translated RNA-polymerase III transcript, has recently been detected in the posterior pituitary (PP) using a low resolution radioactive *in situ* hybridization (ISH) approach, and it has been hypothesized that this RNA might be axonally compartmentalized (Tiedge et al., J. Neurosci., 13:4214-4219, 1993). In the present study, we aimed to determine whether BC1 was truly located within axons, rather than in the glial cells of the PP, and further to analyse the possible regulation of this RNA upon dehydration (chronic salt-loading, providing 2% of NaCl as exclusive source of water for 3, 5 and 7 days) and rehydration (return to regular water begining after day 7 of salt-loading, for 1, 2, 3, 4 and 7 days). Using a Northern blot assay, we were able to detect BC1 RNA in both the anterior and neurointermediate lobes of the pituitary. Non-radioactive ISH of BC1 RNA performed on PP at both the light and electron microscopic levels revealed that BC1 RNA was axonally compartmentalized. Using a semi-quantitative ISH approach, we have measured and compared the changes in BC1 RNA and vasopressin (AVP) mRNA during dehydration and rehydration. During dehydration, BC1 RNA was significantly increased, but it already reached a maximum at day 3 of salt-loading, while AVP mRNA was more progressively increased during the full period of salt-loading, peaking at day 7. The increase in BC1 RNA labeling (2.5-fold) was however modest compared to the increase in AVP mRNA labeling (around 11-fold). Upon the rehydration, the BC1 RNA content in the PP was reversed to control value as early as 1 day after the onset of rehydration, while AVP mRNA slowly decreased to reach the control value at day 7. In conclusion, like AVP mRNA, BC1 RNA is transported in axons of the hypothalamo-neurohypophyseal system, and its axonal transport is similarly regulated. Therefore, we propose that BC1 RNA might be involved in either the axonal targetting and/or transport of AVP or other axonal mRNAs.

642.4

DOES GLUTAMATE REGULATE VASOPRESSIN CONTENT IN HYPOTHALAMIC NEUROENDOCRINE CELLS? R.B. Meeker, R.S. Greenwood*, J. Stewart, Y-H. Shih and J.N. Hayward, Dept. Neurology, University of North Carolina, Chapel Hill, NC 27599.

Although many lines of data indicate that glutamate receptors play a prominent role in the excitatory control of vasopressin neuroendocrine cells, little is known about how these excitatory events might translate into control of vasopressin synthesis. To evaluate the effects of glutamate receptor activation on vasopressin regulation, we measured VP content and VP mRNA in cultured neuroendocrine cells stimulated in vitro with glutamate, AMPA, kainate or NMDA. VP content was measured by radioimmunoassay and mRNA levels by agarose gel electrophoresis of a 179bp or 437bp PCR product spanning Exon B and Exon C (179bp) or Exon A through Exon C (437bp) of the vasopressin gene. Glutamate stimulation resulted in a small decrease in cellular VP content and a corresponding small supression of VP mRNA levels. AMPA stimulation gave results similar to glutamate. NMDA stimulation resulted in less VP mRNA than controls or glutamate-stimulated cultures while kainate caused an almost complete loss of detectable VP mRNA. Thus, each subtype of ionotropic glutamate receptor appears to be coupled to a mechanism which down regulates VP mRNA levels and VP production. These changes may reflect a greater turnover of VP and VP mRNA in response to increased release but also indicate that excitatroy drive alone may not be sufficient to support the increased mRNA levels observed with physiological stimuli. Thus, independent, parallel mechanisms are likely to be present to insure the activation of new synthesis in response to VP secretion. Supported by NIH Grants NS30923 and NS13411.

642.5

Testosterone (T) Enhances Vasopressin Messenger Ribonucleic Acid (AVP mRNA) Abundance in the Hypothalamus of Osmotically-Stimulated Female Rats. J.A. O'Keefe*,N.B. Kim,J.A. Amico, Dept. of Medicine, University of Pittsburgh School of Medicine and VA Medical Center, Pittsburgh, PA 15261

Gonadectomy (gdx) prevents enhanced accumulation of AVP mRNA in the hypothalamic supraoptic nucleus (SON) of osmotically-challenged male and female rats and T and dihydrotestosterone (DHT) restore this response in the male rat (Soc N.S. Abs. 530.2:1280,1993). We questioned whether AVP neurons in the SON of osmotically-stimulated rats display a sexually dimorphic response to gonadal steroid replacement. We examined the effects of various gonadal steroid replacement regimens upon AVP mRNA accumulation in the SON of osmotically-stimulated female rats. Female rats were gdx or sham gdx (intact) and implanted with sham, T or estradiol(E_2) capsules and randomized one week later to 2% NaCl solution or tap H_2O x 5 days. NaCl groups lost weight, developed hypernatremia and depleted their pituitary stores of AVP compared to tap H_2O groups ($p<.05$, Fisher's PLSD). Gdx-T and gdx-E_2 treated animals had mean T and E_2 concentrations of 1.5 ng/ml and 60 pg/ml, respectively. Intact and gdx-T-replaced rats, but not gdx or gdx-E_2 rats, receiving 2% NaCl increased hypothalamic AVP mRNA accumulation compared to respective tap H_2O controls ($p<.05$, Scheffe F-test). The data suggest that similar to male rats, T, but not E_2 modulates AVP mRNA accumulation in the SON of osmotically-challenged female rats.

642.6

DISTRIBUTION OF ARGININE-VASOTOCIN (AVT) mRNA AND AVT-CONTAINING NEURONS IN THE BRAIN OF JAPANESE QUAIL. N. Aste°^*, E. Mühlbauer^, G.C. Panzica°, and R.Grossmann^. °Dept Human. Anatomy & Physiology, Univ. of Torino, I-10126 Torino, Italy and ^Inst. for Small Animal Res., 3100 Celle, Germany.

The distribution of AVT containing neurons in quail brain has been studied in detail by immunocytochemistry (ICC). However no data are available for this species on the gene level. Up to now the chicken represents the only avian species in which chicken AVT gene expression has been investigated by specific cDNA probes. Thus we have used a gene probe directed to the 5' part of the AVT mRNA in order to detect the distribution of AVT expressing neurons in chicken and quail brain. Northen and Southern analysis demonstrated the specificity of this probe for quail AVT mRNA. 20µm thick cryostat sections from chicken and quail brain were collected on different slides to obtain adjacent slices. Consecutive series were fixed by 4% paraformaldehyde and processed for *in situ* hybridization (IH) and ICC respectively. The results indicate a common pattern in the distribution of AVT neurons containing mRNA and the peptide. In particular a wide population of perikarya was observed close to the pial surface of the preoptic region (lateral or supraoptic division), in the periventricular hypothalamic region (paraventricular nucleus) and scattered along the lateral forebrain bundle. Few neurons were observed by IH and ICC in extradiencephalic areas. Quail and chicken AVT mRNA expressing neurons showed a common pattern of distribution, confirming the suitability of the probe. Considerably more AVT expressing neurons were detected in the hypothalamus of the chicken if compared to the quail. Furthermore the hybridization signal was more intense in the brain of chicken than in quail. In conclusion our studies show that 1) the probe we used is suitable to investigate AVT gene expression in quail, 2) the localization of AVT neurons is comparable between chicken and quail, 3) the basal activity of the AVT system in chicken is higher than in quail.

Supported by grants of CNR. N.A. was granted by a ENP short term fellowship.

642.7

DIFFERENTIAL DISPLAY PCR TO IDENTIFY REGIONAL SPECIFIC GENES IN RAT HYPOTHALAMUS. X. WANG* and W. S. YOUNG, III. Lab. Cell Biology, NIMH, NIH, Bethesda, MD 20892

Hyperosmolality induced by drinking 2% saline has long been known to induce expression of many genes in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. We used a PCR technique ("differential display") to screen differentially expressed genes there. The mRNAs isolated from various brain regions from animals after drinking 2% saline or water were reverse transcribed using a TTTTTTTTTTTTGC (T12GC) primer. The PCR products amplified by pairing the T12GC primer with several 10-base primers were displayed on a 6% polyacrylamide gel. About 50 distinct bands ranging from 100-400bp were seen for each pair of primers. This technique provided a sensitive way to detect differentially expressed genes, and both up- or down-regulated genes could be compared side-by-side on the gel. Several clones that appeared to be preferentially expressed in SON/PVN were isolated. The regional specific expressions were examined by hybridization histochemistry using ^{35}S-UTP labeled riboprobes. Differential display still suffers from a lack of specificity that we are still addressing. However, one of the clones was highly expressed in the SON and PVN and was used to screen a rat hypothalamic library. A cDNA of 2.1 kb was sequenced and analyzed.

642.8

AP-1 DNA BINDING ACTIVITY INDUCED BY HYPEROSMOLALITY IN THE RAT HYPOTHALAMIC SUPRAOPTIC AND PARAVENTRICULAR NUCLEI. Z. Ying*, D. Reisman, and J. Buggy. Depts. of Physiology and Biological Science, University of South Carolina, Columbia, SC 29208

The immediate early gene (IEG) *c-fos* is induced after hyperosmotic stimulation in the paraventricular (PVN) and supraoptic (SON) hypothalamic magnocellular nuclei. AP-1 DNA binding activity was examined in these brain regions since Fos acts as a transcription factor in other systems by binding to the AP-1 site on DNA to influence gene transcription. Rats were given hypertonic saline injection (i.p.) and nuclear proteins were extracted from PVN and SON tissue punches 2 hrs later to assess AP-1 binding activity using an electrophoretic mobility-shift assay and a labeled oligonucleotide containing the AP-1 consensus sequence. Hyperosmolality induced AP-1 DNA binding in both PVN and SON; this binding was competitively displaced by excess unlabeled AP-1. The binding of proteins to the AP-1 element was shown to be specific in gel shift assays utilizing competitor DNAs such as SP-1. Thus, AP-1 binding activity in nuclear extracts from the SON and PVN was induced after hyperosmolality suggesting that dehydration-induced IEG expression results in protein products that may function in the regulation of target gene expression.

642.9

EVIDENCE FOR A FUNCTIONAL PROJECTION TO THE LATERAL HYPOTHALAMUS FROM THE MEDIAN PREOPTIC NUCLEUS AND SUBFORNICAL ORGAN IN THE RAT. A.B. Kelly* & A.G. Watts. NIBS Program and Department of Biological Sciences, USC, Los Angeles, CA 90089-2520.

We have previously reported increased levels of CRH mRNA in the lateral hypothalamic area (LHA) in response to cellular dehydration caused by increased plasma osmolality. Here, we have combined tract-tracing techniques with *in situ* hybridization in an attempt to identify those neurons responsible for this response. Following iontophoresis of fluorogold (FG) into the LHA we identified retrogradely labeled soma in a number of cell groups, including the subfornical organ (SFO), median preoptic nucleus (MePO), bed nucleus of the stria terminalis, preoptic area, and other forebrain regions. We then combined FG retrograde tracing from the LHA with c-fos *in situ* hybridization in male rats that had been given intraperitoneal injections of 1.5M saline 30 minutes before anesthesia and perfusion; these injections rapidly increase plasma osmolality, and increase c-fos mRNA accumulation in the MePO & SFO. In the MePO and SFO we found a number of FG-labeled cells which also contained c-fos mRNA, in addition to singly-labeled cells of each type.

In a second study, anesthetized male rats were given coronal unilateral knife cut lesions at the level of the suprachiasmatic nucleus, separating the LHA from more rostral inputs, including the MePO & SFO. Two weeks after surgery rats were salt-loaded for 5 days to induce cellular dehydration, then anesthetized, perfused, and processed for CRH mRNA *in situ* hybridization. Frontal deafferentation significantly attenuated the CRH mRNA response to cellular dehydration in the ipsilateral LHA.

Taken together, these studies show that cells in the MePO and SFO send direct projections to the LHA, and that a subset of these cells are osmoresponsive. This input may be implemental in the LHA response to cellular dehydration. (Supported by NS 29728).

642.10

SEPTAL VASOPRESSIN AND OSMOTIC REGULATOIN

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Septal areas have been found to play an important role in the regulation of water intake and vasopressin (AVP) neurosecretion. Several studies have suggested that the AVP innervation of the septum may play a role in this regulation. In dogs, intraventricular injections of AVP lowered the hyperosmotic thirst threshold whereas injections of AVP antagonist raised it. In rats, hyperosmotic and hypovolemic stimuli decreased AVP content in the lateral septum, and increased AVP release from this area. AVP-immunoreactive fibers in the lateral septum (LS) arise from the bed nucleus of the stria terminalis and medial amygdaloid nucleus. AVP expression in these nuclei depends on the presence of gonadal steroids. If the AVP innervation of the lateral septum is indeed involved in water intake, one would predict that this function is influenced by gonadal steroids. Here we showed that this is the case. We found that when challenged with the same amount of hypertonic saline, injected intraperitoneally, castrated male rats drank less than intact rats and castrated rats treated with testosterone did. AVP injections into the lateral septum of castrated rats partly restored the amount of water intake. AVP injections into the lateral septum of intact rats increased, and V_{1a} antagonist injections decreased, water intake in response to hypertonic saline. We also found that hypertonic saline injections increased *c-fos* and *egr-1* expression in lateral septum neurons as well as in neurosecretory AVP neurons in the paraventricular and supraoptic nucleus. This increase was more pronounced in intact than in castrated rats suggesting that the septum may also mediate steroid effects on AVP neurosecretion.

642.11

FOS AND JUN EXPRESSION IN RAT SUPRAOPTIC NUCLEUS [SON] NEURONS AFTER ACUTE *vs.* REPEATED OSMOTIC STIMULATION. *K. Wang and J.T. McCabe**. Anatomy & Cell Biology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799

Fos and Jun, protein products of the immediate early genes c-*fos* and c-*jun*, form a heterodimeric complex that affects the transcription of genes containing AP-1 and CRE DNA-binding sites. Using a double-label immunofluorescence method (CELL & TISSUE RESEARCH, 1994, **276**:1-6), we find that intraperitoneal injection of hypertonic saline results in the appearance Fos and Jun in the nuclei of SON neurons. Cell counts of immunostained sections demonstrate the co-existent appearance of Fos and Jun occurred within 30 min (20%), peaked at 90-120 min (80%) and gradually disappeared by 4 hr (13%) after injection. At 4 hr post-saline injection, 5 rats each received a *second* injection of normal or hypertonic saline. A second injection of normal saline resulted in no Fos/Jun immunostaining 90 min later, while hypertonic saline induced Fos/Jun staining in only 17% of SON neurons. Of the remaining SON cells, 23% had staining to Fos alone and 4% of the cells stained for Jun. These results indicate SON neurons, after a second hypertonic saline injection, exhibit decreased *colocalized* Fos/Jun immunostaining, dramatically decreased Jun expression, and substantial, but attenuated, immunostaining for Fos. Supported by grants NIH NS25913 and USUHS RO70AL to JTM.

642.12

ADAPTATION OF THE BRAIN TO HYPONATREMIA IN TWO DIFFERENT SPECIES: EFFECTS OF GENDER. **Z. Vexler, T. Roberts, N. Derugin, J. Kucharczyk, Al. Arieff*. Dept. of Neuroradiology and Medicine, UCSF &VAMC, San Francisco, CA 94121**

This study was to evaluate adaptation of the brain to hyponatremia as a function of gender. Brain adaptation to vasopressin (AVP)-induced hyponatremia was studied in adult male and female cats and rabbits during 3 hours or 4 days of hyponatremia, which was induced by subcutaneous AVP and 140 mM glucose/H2O. Changes in cerebral perfusion were evaluated using contrast-enhanced high speed echo-planar MRI, using bolus IV administration of a magnetic susceptibility contrast agent (DyDTPA-BMA). An Index of cerebral perfusion (CPI) was obtained as a reciprocal of the width of the contrast transit curve. Water, sodium and potassium content were assessed in brain gray matter of both cats and rabbits. Acute hyponatremia (2 - 4.5 hrs; serum sodium fell from 155±3 to 127±3 mmol//L) resulted in a significant prolongation of contrast transit time through the brains of both male and female cats: CPI decreased from 0.28±0.02 sec^{-1} to 0.14±0.02 sec^{-1} in males and 0.16±0.02 sec^{-1} in females. Adaptation was gender dependent: brain (gray matter) water content increased from 391±8 ml/100 gm dry wt to 434±5 in females and 415±3 in males ($p<0.001$ vs control), and hyponatremic females greater than males ($p<0.02$). A gender dependent decrease of brain sodium was found, from 271±13 mmol/kg dry tissue to 222±2 in males ($p<0.005$) and to 233±5 in females ($p<0.035$). In rabbits, serum sodium was decreased from 142±1 to 122±2 mmol/L over 4 days. Brain water increased from 426±13 ml/100 gm dry wt to 498±27 in females ($p<0.01$) and 432±27 in males (p=ns). Brain sodium fell from 318±6 to 294±8 mmol/kg dry wt in males ($p<0.01$) but was unaffected in females. Brain potassium did not change. Brain swelling, however, was significantly less in hyponatremic males than in females in both species. Better adaptation of brain to hyponatremia in males of 2 species, and decreased cerebral perfusion, appears to be related to interaction between AVP and sex steroid hormones.

642.13

VENTRAL FOREBRAIN RELAYS SALT INPUT FROM GUT TO VASOPRESSIN NEURONS. Y. Shan, M. King and A.J.Baertschi*. Physiology Dept., Univ. of Virginia, Charlottesville, VA 22908, and Stetson College, FLA.

Gastric hypertonic saline infusions (600 mOs/kg, 2.5ml) elicit vasopressin (AVP) release in conscious rats via noradrenergic pathways ascending from A1/A2 (Brain Res. 580:81-91,1992). Microinjections of 6-OHDA (4µg/400nl) into magnocellular hypothalamic nuclei, caudal MPA or MnPO does not affect this AVP response, but 6-OHDA lesion of the ventral vertical limb of the diagonal band of Broca (DB) reduces it by 60.9% ($p<0.05$; total n=21). The 6-OHDA lesions were then applied in 20 rats at various ventral sites from anterior 8.5 to 11 mm/ear bar, and characterized following the AVP study by immunocytochemistry for tyrosine hydroxylase (TH) and DBH. Partial or total depletion of TH/DBH from OVLT, MnPO, caudal DB, BNST or periventricular areas does not affect mean ΔAVP (over 30 min) =5.63 ± 0.40 pg/ml, whereas depletion extending to anterior 11 mm reduces mean ΔAVP to 2.02± 0.45 pg/ml ($p < 0.01$). Electrolytic lesions in ventral forebrain from anterior 9.5-11 mm (± 0.5 mm/midline) also strongly reduce the AVP response. These results suggest that salt signaling by the gut is mediated by noradrenergic projections ascending probably via MFB to ventral forebrain, and via non-catecholaminergic fibres descending to the vasopressinergic nuclei (support: NIH RO1 NS27644).

642.14

OSMOTIC ACTIVATION OF NEURONS DISSOCIATED FROM THE OVLT AND MEDIAN PREOPTIC NUCLEUS (MnPO). D.Richard* and C. W. Bourque. *Montreal General Hospital and McGill University, Montreal, P.Q. Canada.*

Neurons within the OVLT and MnPO have been implicated to play a key role in osmoregulation. To determine whether they are intrinsically sensitive to hypertonicity, patch clamp recordings were obtained from cells acutely dissociated from these regions as described by Oliet & Bourque (J. Physiol. 445, 1992). Neurons were plated on petri dishes and superfused with a HEPES-buffered medium. Recordings were obtained from phase-bright somata with a mean cross-sectional diameter ranging from 12 to 22 µm. Hyperosmotic stimuli (+10 to +30 mM Mannitol) caused a reversible and reproducible increase in the mean firing rate in 2 of 5 MnPO and 9 of 14 OVLT cells tested. These effects were associated with a membrane depolarization and a 32±7 % decrease in input resistance (n=14). Analysis of voltage-current relationships obtained before and during the osmoticaly-evoked depolarization revealed a reversal potential near - 45 mV. Furthermore, the E_{Cl} was -108 mV, suggesting the possible involvement of cationic conductance. These results demonstrate that some neurons within the OVLT and MnPO are intrinsically sensitive to hypertonicity. *Supported by the MRC and the Heart & Stroke Foundation of Canada.*

642.15

GADOLINIUM-INDUCED BLOCK OF OSMOSENSITIVITY AND MECHANOSENSITIVE CHANNELS IN RAT SUPRAOPTIC NEURONS. Stéphane H. R. Oliet* and Charles W. Bourque. *Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, P.Q. Canada H3G 1A4.*

The release of neurohypophysial hormones from supraoptic neuron terminals is regulated by plasma osmolality. Recent biophysical studies in our laboratory have suggested a role for mechanosensitive cationic channels in the sensitivity of supraoptic neurons to changes in external osmotic pressure. The involvement of such channels in osmoreception was assessed by the use of gadolinium (Gd^{3+}), a trivalent cation known to block different types of stretch-sensitive cationic channels. Whole-cell recordings obtained from isolated rat supraoptic neurons revealed a marked reduction of hyperosmotically-mediated membrane depolarization and firing rate increase in presence of Gd^{3+} (100μM; n=4). In voltage-clamp mode (n=10), bath-applied Gd^{3+} induced a reversible and dose-dependent inhibition (IC50≈20μM) of the inward current elicited at -60mV by hypertonic stimulations. This inhibition resulted from specific suppression of the cationic conductance which was previously shown to be associated with osmotic responses (E_{rev}≈-40mV). In cell-attached experiments (n=15), Gd^{3+} was also able to block mechanosensitive cationic channels when included or perfused in the recording pipette. These results confirm a role for mechanically-gated channels in the endogenous osmosensitivity of rat supraoptic neurons.

Supported by the MRC and Heart & Stroke Foundation of Canada.

642.16

FEMALE SEX HORMONES INHIBIT VOLUME REGULATION IN RAT BRAIN ASTROCYTE CULTURE. C. L. Fraser* and R. A. Swanson. University of California San Francisco, V. A. Medical Center, San Francisco, CA 94121.

To determine if sex steroids play any role in the increased morbidity associated with acute symptomatic hyponatremia in menstruant females, we studied the actions of estradiol, progesterone and testosterone on regulatory volume decrease (RVD) of brain astrocytes in culture. Using ^{3}H-3-O-D-methylglucose to determine intracellular space, cells cultured in media containing either estradiol or progesterone and those treated with ouabain were unable to volume regulate normally, whereas testosterone treated cells displayed normal RVD. After 15 seconds of hypotonic exposure, control and testosterone (100nM) treated cell volume increased by 26% and 31% respectively. Cell volume in control cells went from 1.74 ± 0.24 to 2.41 ± 0.28 ul/mg protein. At the same time, cells treated with either 10 nM estradiol or 10 nM progesterone significantly ($p<0.01$) increased their volume by 129% and 90% respectively. Both the antiestrogen agent tamoxifen, and the antiprogesterone agent RU486 blocked the effects of estradiol and progesterone. The Na-K ATPase pump, which plays an important role in cell RVD was significantly ($p<0.03$) inhibited by 32% and 21% in estradiol and progesterone treated cells, but significantly ($p<0.001$) stimulated (49%) by testosterone treatment. Taken together, these results provide a possible explanation for the increased morbidity associated with acute symptomatic hyponatremia in menstruant females.

642.17

ENHANCED VASOPRESSIN AND BEHAVIORAL RESPONSE TO METHYLPHENIDATE IN SCHIZOPHRENICS WITH WATER INTOXICATION M.B. Goldman, D.J. Luchins and G.L Robertson
Illinois State Psychiatric Institute, University of Chicago Pritzker School of Medicine, Chicago IL. 60637.

Water intoxication accounts for about 20 % of deaths in non-geriatric schizophrenics. This episodic disorder is associated with transient impairments in water excretion which often occur during exacerbations of psychosis. Many other schizophrenics remain normonatremic during such exacerbations, even if they are polydipsic. We administered the psychotimimetic, methylphenidate (0.5 mg/Kg intravenously), to matched polydipsic schizophrenics with and without a previous history of water intoxication, in order to further examine the relationship of psychosis to water imbalance.

Basal AVP was maximally suppressed in both groups, but peak post-methylphenidate levels were greater in test (time = + 15 min.: (Mn ± SD) 1.9 ± 0.7 pg/ml) than control (1.0 ± 0.7, $t = 2.21$ $df = 12$ $p < .05$) subjects, despite lower plasma osmolality in the former (T= 278 ± 5.0 mOsm/Kg , C=288 ± 5.0). AVP stimuli (e.g. nausea, abdominal discomfort, hypotension) were not present in any subjects, and parameters influencing AVP release were similar in both groups over the two hours post-methylphenidate. Desire for water was also similar and did not change. 'Positive' symptoms of psychosis increased, and negative symptoms diminished, equivalently in the two groups. Test subjects, however, were more psychotic and showed a greater increase in other psychiatric symptoms.

Methylphenidate causes greater AVP secretion and behavioral activation in schizophrenics with episodic water intoxication. These findings strengthen the link between psychotic exacerbations and vasopressin secretion, and suggest the former is not necessarily a consequence of hyponatremia, per se. Differences in limbic pathology between schizophrenics could account for the findings.

SOMATIC AND VISCERAL AFFERENTS: NOCICEPTION

643.1

NON-MYELIN-FORMING SCHWANN CELLS EXPRESS ENDOTHELIN-B RECEPTORS *IN VIVO* S.D. Rogers, J.R. Ghilardi, C.J. Allen, S.R. Vigna, J.E. Maggio, M.W. Dysken*, P.W. Mantyh. Mol. Neurobio. Lab (151) and GREEC, Mpls., MN 55417; Psychiatry Dept, Univ of Minn., Mpls., MN 55455; Dept. of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115; Dept. Cell Biology, Duke Univ. Med. Ctr., Durham, N.C. 27710

In contrast to myelin-forming Schwann cells, little is known about the factors regulating Schwann cells that do not elaborate myelin sheaths. These non-myelin-forming Schwann cells co-express several surface proteins and intermediate filaments, including glial fibrillary acidic protein (GFAP), not found in myelin-forming Schwann cells . These non-myelin-forming Schwann cells are physiologically important in that they ensheathe peptide-containing sensory C-fibers, and thus may indirectly regulate the transmission of nociceptive information and the inflammatory response in which that unmyelinated C-fibers are involved. In the present study we have used combined receptor autoradiography and immunohistochemistry to demonstrate that the same non-myelin-forming Schwann cells that are GFAP+ also express high concentrations of ^{125}I-Endothelin receptor binding sites. Pharmacological analysis suggests that these binding sites correspond to Endothelin-B receptors. These results suggest a mechanism by which a circulating factor such as endothelin may specifically, yet indirectly, affect the population of non-myelinated primary afferents that are hypothesized to be involved in transmitting chronic pain and in regulating the inflammatory response in peripheral tissues. (Supported by the NIH and VA).

643.2

TRIGEMINAL AND DORSAL ROOT GANGLION NEURONS EXPRESS α_1, α_2, β_1, β_2 ADRENERGIC RECEPTOR BINDING SITES IN THE RAT, RABBIT AND MONKEY J.R. Ghilardi, C.J. Allen, S.D. Rogers, S.R. Vigna, J.E. Maggio, L. Kruger, W.A. Raabe*, P.W. Mantyh. Mol. Neurobio. Lab (151), Mpls., MN 55417; Psychiatry and Neurology Dept, Univ of Minn., Mpls., MN 55455; Dept. of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115; Dept. Cell Biology, Duke Univ. Med. Ctr., Durham, N.C. 27710, Dept. of Anatomy and Cell Biology, UCLA, LA, CA 90024.

Noradrenaline has been shown to modulate some trigeminal and dorsal root ganglia (DRG) neurons, but the site of noradrenaline actions and the adrenergic receptor subtype(s) mediating these effects have not been explored fully. Quantitative receptor autoradiography and homogenate receptor binding was used to localize and characterize the adrenergic receptor binding sites expressed by the cells comprising the trigeminal and dorsal root ganglia in the rat, rabbit and monkey. ^{125}I-HEAT was used to label the α_1 binding sites, p-^{125}I-iodoclonidine was used to label the α_2 sites and ^{125}I-cyanopindolol was used to label the β_1 and β_2 sites. In the rat, rabbit, and monkey DRGs a high concentration of β_2 adrenergic receptor binding sites were observed, with moderate concentrations of α_2 binding sites and low but detectable concentrations of α_1 and β_1 receptor binding sites. Within the DRG, specific binding sites corresponding to all 4 subtypes of adrenergic receptors were associated with neurons and not the supporting cells, fibroblasts or endothelial cells. That all four adrenergic receptors are expressed primarily by neurons is supported by the observation that after double ligation of the sciatic nerve, build up of all four adrenergic receptors was observed proximal to the ligation. These results suggest that at least in the rabbit and monkey, the vast majority of sensory neurons, including the large mechanoreceptive cells, express α_1, α_2, β_1 and β_2 adrenergic receptors and that these adrenergic receptors are appropriately positioned to presynaptically modulate primary afferent neurons. (Supported by the NIH and VA)

643.3

Role of a Slow Ca^{2+}-dependent Afterhyperpolarization in Prostaglandin E_2-Induced Sensitization of Cultured Rat Sensory Neurons. Michael S. Gold*, Michael J. Shuster, Shahram Dastmalchi and Jon D. Levine *Depts. of Medicine and Oral Surgery, and Division of Neurosciences, UCSF, Box 0452A, San Francisco, CA 94143*

To determine if inhibition of a slow (> 1 sec) Ca^{2+}-dependent afterhyperpolarization (sAHP) contributes to prostaglandin E_2 (PGE_2)-induced sensitization of dorsal root ganglion (DRG) neurons, we have used patch-clamp electrophysiological techniques on cultured DRG neurons from the adult rat. In support of a role for the sAHP in sensitization of DRG neurons, we demonstrate that: 1) sAHP expression is restricted to a subpopulation of DRG neurons (21% of all cells studied) which also express properties associated with nociceptors, including small cell body size, a shoulder on the falling phase of the action potential (AP), and a rapid depolarization in response to capsaicin; 2) burst duration is controlled by a sAHP in these neurons; and 3) in some neurons, PGE_2 blocks the sAHP, and produces a concomitant increase in the number of APs generated in response to depolarizing current injection. However, our results also demonstrate that sAHP modulation is not sufficient to explain PGE_2-induced sensitization in the majority of DRG neurons because: 1) PGE_2 sensitized 59% of the neurons tested; 2) in many of these neurons, PGE_2 produces a decrease in AP threshold as well as an increase in the number of APs in response to current injection; and 3) the sensitizing effects of PGE_2 are dissociated from its effects on the sAHP in more than half of neurons tested. Thus, PGE_2-induced sensitization must involve the modulation of ionic currents in addition to that underlying the sAHP. *Supported by NIH grant NS21647*

643.4

CATEGORIZATION OF ACUTELY ISOLATED RAT SENSORY NEURONS BASED ON ION CHANNEL EXPRESSION AND SENSITIVITY TO CAPSAICIN. C. G. Cardenas*, L.P. Del Mar, and R.S. Scroggs. University of Tennessee, College of Medicine., Department of Anatomy and Neurobiology, Memphis, TN 38163.

Rat dorsal root ganglion cell bodies (neurons) were screened according to action potential (AP) shape, capsaicin sensitivity, and expression of several ion currents. The data for individual neurons often conformed to one of four patterns, named Type 1, 2, 3, and 4.

Type 1 neurons (N = 15) had long duration AP's (average = 12.6 ms), and were capsaicin sensitive. High-threshold Ca^{2+} current averaged 29.2% ω-conotoxin GVIA sensitive (N-type), 48% nimodipine sensitive (L-type), and 22.8% was resistant to both blockers (resistant). T-type Ca^{2+} currents averaged 260 pA . I_H current and I_{IR} current were not expressed. Soma diameter averaged 23.5 ± 3.4 SD.

Type 2 neurons (N = 7) differed from Type 1 in having shorter AP's (average = 4.8 ms), negligible T-type currents (average = 46 pA), and an I_A-like current.. Similar to Type 1, Type 2 neurons were capsaicin sensitive, lacked significant I_H and I_{IR} current, and had high threshold Ca^{2+} currents composed of 33.3% N-type, 30% L-type, and 36.7% resistant-type. Soma diameter averaged 20.1 ± 1.7 SD.

Type 3 neurons (N = 9) differed from Types 1 and 2 in having brief AP's (average = 1.8 ms), I_H current (average = 111.3 pA), I_{IR} current, and were capsaicin insensitive. Type 3 high threshold Ca^{2+} current resembled that of Types 1 and 2, ie., 22.8% N-type, 47.4% L-type, and 29.8% resistant. Similar to Type 1 neurons, T-type Ca^{2+} currents in Type 3 neurons averaged 260.7 pA. Soma diameter averaged 16.1 ± 2.1 SD.

Type 4 neurons (N = 7) differed markedly from Types 1, 2, and 3 regarding Ca^{2+} current expression. Little high threshold Ca^{2+}current was N- or L-type (11.7% and 7%, respectively), most being the resistant type (average = 81.2%). Also Type 4 cells had huge T-type Ca^{2+} currents averaging 4082 pA. Otherwise they were similar to Type 3 cells in having brief AP's (average = 1.1 ms), I_H current (average = 1035 pA), I_{IR} current, and were capsaicin insensitive. Soma diameter averaged 34.6 ± 1.6 SD.

These different phenotypes may transmit different kinds of sensory information.

643.5

MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN ISOLATED DORSAL ROOT GANGLION CELLS AFTER NERVE INJURY. M. Petersen * and R.H. LaMotte‡ Dept. of Physiology Univ. Würzburg, D-97070 Würzburg and Dept. of Anesthesiology, Yale Univ. School of Medicine‡, New Haven, CT 06510

We have studied the changes in membrane properties of freshly dissociated dorsal root ganglion (DRG) cells following a unilateral chronic constriction injury to their peripheral axons (Bennett and Xie 1988). An injury of the sciatic nerve was produced by 4 tight or 4 loose ligatures. After a survival time of 12 to 20 days, cells from L4 and L5 ganglia were recorded electrophysiologically by the whole-cell patch clamp technique.

In a subpopulation of cells, spontaneous activity could be recorded in cells of small to medium size. These cells exhibited either discharges with regular inter-spike intervals with a frequency of 1 to 3 Hz, or bursting discharge patterns. In a subpopulation of cells there was also a de novo sensitivity to norepinphrine. Most of the cells responded with action potentials and a few with a slow membrane depolarization. The threshold concentration was about 300μM. Most of the cells were small to medium in size and responded also to capsaicin (300 nM).

Phenotypically, there was a difference in the onset of outgrowth of processes from somata whose peripheral axons were injured by a tight ligation vs. from somata of uninjured cells. Seven hours after plating, 1.3% of the cells with uninjured axons developed processes vs. 19% of cells whose axons were injured 13 to 15 days earlier.

The results show the plasticity of isolated somata after nerve injury. Supported by DFG Pe 299/3-1 and by P.H.S. grants NS 14624 and PS 09871.

643.6

NOCICEPTOR EXCITATION BY PLATELETS IS NOT DUE TO 5-HT OR PROSTANOIDS RELEASED. M.Ringkamp[1], M.Schmelz[1], M.Allwang[2], A.Ogilvie[2], P.W.Reeh[1]*. [1]Dept. Physiol. & Biocyber., [2]Dept. Biochem, Univ. Erlangen-Nürnberg, D-91054 Erlangen, Germany

Accumulation of platelets is an early event in inflammation. Activated platelets release potentially algogenic chemicals, e.g. serotonin. In the rat skin-nerve preparation, ADP- activated human thrombocytes in plasma (PRP) excited 14/18 polymodal C- fiber endings (Ringkamp et al., Neurosci. Lett., in press, 1994). In a recent psychophysiological study, intradermal injection of autologous concentrated platelets in plasma induced burning pain, flare, as well as mechanical and heat hyperalgesia. In contrast, platelet poor plasma, with ADP added, was ineffective as well in rat as in human skin. To test the hypotheses that the effects of activated PRP are due to platelet derived 5-HT or prostanoids, as thromboxane A_2, in vitro experiments on identified C- fiber terminals were performed. A combination of 5-HT antagonists (methiotepin 1 μM, ketanserin 1 μM, ICS 205-930 0.1 μM) was added to ADP- activated PRP, or PRP was gained from a donor treated with 500 mg lysine-acetylsalicylate i.v. 12 hours before blood sampling. In both protocols no significant difference to previous results in percentage of polymodal C- fibers excited (9/11 and 13/17, respectively) and in magnitude of the response was found, while the onset of nociceptor excitation was clearly delayed in both conditions. Thus, nociceptor excitation by activated platelets should not be due to 5-HT or prostanoids, but seems to be accelerated by these agents. In a further study on polymodal nociceptors, it turned out that platelets suspended in a plasma free solution had lost their ability to excite nociceptors, while they were still able to aggregate (in a photometric assay). When such platelets were re-suspended in plasma, their excitatory potency was regained, which is evidence for a necessary interaction between plasma and platelets in nociceptor excitation.
Supported by the DFG, SFB 353-A3

654.7

INTRADERMAL INJECTION OF COMPOUND 48/80, SUBSTANCE P AND SEROTONIN BUT NOT HISTAMINE PRODUCES HINDLIMB SCRATCHING IN MICE. Y. Kuraishi*[1], T. Nagasawa[1], K. Hayashi[1] and M. Satoh[2]. [1]Dept. of Applied Biochem., Res. Inst. for Traditional Medicines, Toyama Med. & Pharm. Univ., Toyama 930-01, [2]Dept. of Mol. Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606-01, Japan.

As a step in investigating the peripheral mechanisms of itch sensation, we examined behavioral effects of peripheral injection of agents that are known to produce itch or pain in humans. Male ddY mice (5-6 weeks old) were given intradermal (i.d.) or subcutaneous (s.c.) injection into the rostral part of the back and scratching was observed using a video camera. An i.d. injection of compound 48/80 (10-100 μg) or substance P (100 μg), which produces itch sensation in humans and mast cell degranulation, induced scratching towards the injected site by the hindlimbs. The number of scratching was 41.8 ± 9.5 (n=8) for 10 min after 100 μg of compound 48/80. No significant behavioral effects, except transient vocalization during injection, were observed after s.c. injection of pain-producing substances such as capsaicin (30 and 100 μg) and 5 % formaldehyde. As histamine and serotonin are contained in the rodent mast cells, behavioral effects of these amines were examined. Serotonin (0.3-30 μg, i.d.) produced dose-dependent scratching, the number of which was 38.4 ± 7.6 (n=8) for initial 10 min at 30 μg, but histamine (1-100 μg, i.d.) was without such effect. Scratching induced by serotonin at doses of 141 nmol (30 μg) and 100 nmol was significantly suppressed by 15-min pretreatment with the 5-HT3 antagonist KB6933 (0.1 mg/kg) and by simultaneous injection of this antagonist (0.01 nmol), respectively. The results suggest that scratching towards the injected site by the hindlimb is due to itch rather than pain and that serotonin, but not histamine, produces scratching towards affected skin, probably at least partly through 5-HT3 receptors in the periphery.

643.8

PHOSPHOLIPASE A_2 INDUCED ELECTROPHYSIOLOGICAL CHANGES IN RABBIT LUMBAR FACET JOINT CAPSULE. AC OZAKTAY*, S KALLAKURI, S VAIDYANATHAN, OH LI, DC BLAGOEV, JM CAVANAUGH, AI KING. Bioengineering Center, Wayne State University, Detroit, MI 48202.

The aim of this study was to investigate the histological and electrophysiological effects of phospholipase A_2 (PLA_2) injected in the lumbar facet joint capsule. METHODS: 12 male rabbits were anesthetized, an L5-L6 laminectomy and ventral ramus rhizotomy were performed. The dual bipolar electrode recordings were made from split L6 dorsal roots. The L6-L7 facet joint capsule was mechanically searched for receptive fields. The units were characterized by their conduction velocities and mechanical thresholds. 750 or 1500 U of PLA_2 (*naja naja* venom, Sigma) in the buffered carrier solution (100 μl, pH 7.5) was then injected into the receptive field. The threshold and unit activity were recorded for later computer analysis. At the end of the experiment the tissue was histologically examined with H&E staining. RESULTS: *1500 U PLA_2 injections* (n=4): a) At 30 min, a significant decrease was observed in multi-unit spontaneous discharge rate (SDR) and the units were no longer mechanically responsive. b) Group II units (n=3): SDR was significantly decreased and all units disappeared before 15 min. c) A previously silent unit reached a discharge rate of 20/s and disappeared at 7 min. *750 U PLA_2 injections* (n=4): a) Multi-unit SDR was decreased at 30 min. After 30 min, the SDR rate increased in response to probing in 2 experiments. b) Group II units (n=3): SDR decreased after the injection. c) Group III units (n=8): SDR reached a higher level after the mechanical probing at 30 and 45 min. d) Silent units (n=2) were mechanically evoked after 30 min. *Control injections* (n=4) did not show any significant changes. There were also no histological changes observed in any type of injections. Supported by NIH Grant NS-28994.

643.9

DIFFERENTIAL CONTRIBUTIONS OF 5HT, BRADYKININ AND PGEs TO NOCICEPTOR SENSITIZATION. B.Y. Cooper*, Dept. of Oral Surgery, University of Florida, Gainesville, FL, 32610.

Substantial mechanical sensitization can be observed in A-delta mechanical nociceptors following injection of carrageenan (Cooper et al., 1991). This includes changes in both reactive range and mean discharge rate. It is unclear whether pro-inflammatory (PI) mediators make distinct contributions to sensitization. These studies examined the qualitative and temporal features of sensitization that follow bradykinin (BK), serotonin (5HT), and prostaglandins (PGE_1 or PGE_2). Using a force-servo stimulator, nociceptor reactivity was assessed at 5, 30, and 60 minute intervals after injection of PI agents.

Significant decreases in discharge range were observed following BK or 5HT. Changes in range were complementary, with BK producing effects within the first 5 minutes while significant effects of 5HT were delayed to the 30 and 60 minute test intervals. Neither BK or 5HT produced changes in mean discharge rate. In contrast, injections of PGEs produced delayed changes in rate of discharge (60 min) but no significant changes in reactive range. It was concluded that 5HT, BK, and PGEs made qualitatively and temporally distinct contributions to mechanical sensitization. Supported by NIH DE08701.

643.10

INTERLEUKIN-2 AND CUTANEOUS C POLYMODAL NOCICEPTORS: DOSE-RESPONSE RELATION, TACHYPHYLAXIS AND LACK OF SYNERGY WITH PROSTAGLANDIN E_2. H.A. Martin*, Division of Neurobiology, Medical School, The University of Newcastle upon Tyne, NE2 4HH, U.K.

In a previous study, we have shown that interleukin-2 (IL-2; 0.06 U/3 µl) activates broadly a third of cutaneous C polymodal nociceptors and, more rarely, other classes of nociceptors. In the present study, we have investigated whether (i) the percentage and the response characteristics (i.e., latency, intensity, duration and pattern of discharge) of activated units are dose dependent, (ii) PGE_2 induces a sensitization to IL-2.

Activity of single nociceptive fibers was recorded in ***in vivo*** saphenous nerve preparations, in nembutal anesthetized rats.

(i) Increasing doses of IL-2 (0; 4.10^{-4}; 4.10^{-3}; 4.10^{-2}; 4.10^{-1} U/µl) were injected (3 µl) in the receptive field of 28 C-polymodal nociceptors and 6 mechanonociceptors (4 C and 2 Aδ). Only 8 polymodal nociceptors were activated (28.5%). This activation appeared at 4.10^{-3} U/µl and became stronger with higher doses.

(ii) 14 C-polymodal nociceptors and 5 C-mechanonociceptors were initially treated with PGE_2 (100 ng/3 µl). Only 4 C-polymodals (28.5%) were activated by IL-2 (0.06 U/3 µl) and their response characteristics were unchanged, compared to control. Also, tachyphylaxis to subsequent injections of IL-2 (0.06 U/3 µl) was unaffected by PGE_2.

In **conclusion**, pruritus produced by high doses of IL-2 in the treatment of advanced cancer may result from the selective activation of a fraction of C-polymodal nociceptors, chemosensitive to this cytokine. The failure for PGE_2 to enhance the chemoresponsiveness to IL-2 may explain the inefficiency of steroids in the treatment of such pruritus.

643.11

AMPA AND NMDA RECEPTOR-IMMUNOREACTIVITY POST-SYNAPTIC TO PRIMARY AFFERENT TERMINALS IN THE SUPERFICIAL DORSAL HORN OF THE CAT SPINAL CORD. F.J. Alvarez*, D. Harrington and R.E.W. Fyffe. Dept of Anatomy, Wright State University, Dayton, OH, 45435.

Primary afferent synapses are believed to use excitatory amino acids (EAA) as their principal "fast" neurotransmitters. There is potential for great functional variability at EAA-synapses because of the diversity of EAA-receptor subtypes that can be expressed, but details of this diversity are largely unknown for individual synapses. A promising approach is to use antibodies (Abs) against different subunits to detect their presence at primary afferent synapses using electron microscopy immunocytochemical techniques. Here, we used Abs directed against epitopes found in the GLUR2 and 3 subunits of the AMPA receptor (Chemicon) and in the NMDAR1 subunit of the NMDA receptor (Pharmigen) to detect these subunits in the superficial laminae of the cat spinal cord. The Abs labeled many postsynaptic sites and also the cytoplasm of cell somas and dendrites. All three kinds of known central glomerular types (presumed primary afferent origin) were associated with postsynaptic immunoreactivity (ir) to GLUR2/3 and NMDAR1. Interestingly, within a single glomerular terminal less than half of the synapses were immunolabeled, although many of the postsynaptic profiles displayed cytoplasmic ir. Our results provide immunocytochemical proof supporting the assertion that most classes of primary afferents arborizing throughout laminae I to III use AMPA and NMDA receptors to elicit postsynaptic effects, but intriguingly the Abs did not universally label all primary afferent synapses despite our efforts to increase detection sensitivity to a maximum. It remains to be determined if these results are due to the presence of different subunit types, subtype isoforms, variable conformations, or posttranslational epitope masking (either in vivo or during fixation) at different synapses established by a single primary afferent terminal.

643.12

SUPPRESSION OF RESPONSE OF CUTANEOUS C- AND A-FIBER NOCICEPTORS IN THE MONKEY TO REPEATED MECHANICAL STIMULI DIFFERS. R.M.Slugg*, R.A.Meyer, and J.N.Campbell. Dept of Neurosurgery and The Applied Physics Lab, Johns Hopkins University, Baltimore, MD 21205.

A pronounced decrease in response has been reported for C-fiber nociceptors when identical heat stimuli are repeated at interstimulus intervals less than 5 min. In this study, we investigated whether a similar decrement in response occurs following repeated presentations of a mechanical stimulus. Standard teased fiber techniques were used to record from single primary afferent nociceptors innervating hairy skin in the anesthetized monkey. Force-controlled mechanical stimuli (10, 20, or 40 gm; 3 s) were delivered via a 400 µm diameter cylindrical probe to the receptive field using a recently developed mechanical stimulator (Slugg et al, Soc. Neurosci. Abstr. 18:326). The conditioning and test stimuli were identical and were delivered at interstimulus intervals (ISIs) of 300, 150, 60, 30 and 15 s. A 10 min stimulus free interval separated every conditioning-test pair. For ISI less than 30 s, the response of the C-fiber nociceptors to the test stimulus was significantly lower than the response to the conditioning stimulus, ranging from 81 ± 3% at the 150 s ISI to 57 ± 4% at the 15 s ISI (n=23). In contrast, significant suppression of the A-fiber response was only observed for ISIs of 30 s or less (77 ± 4% at 15 s; n=11). These results indicate that stimulus interaction effects are prominent in the response of C-fiber nociceptors to mechanical and heat stimuli, and suggest that C- and A-fiber nociceptors differ with regard to transduction of mechanical stimuli. Supported by NS14447 and NS09260.

643.13

CAPSAICIN PRODUCES SECONDARY HYPERALGESIA TO MECHANICAL BUT NOT HEAT STIMULI Z.Ali, R.A.Meyer*, S. M. Meleka, and J.N.Campbell, Dept. Neurosurgery, School of Medicine; and Applied Physics Lab., Johns Hopkins Univ., Balto., MD 21287.

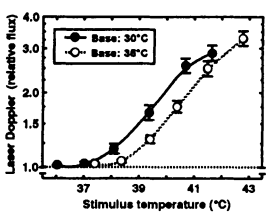
Hyperalgesia to mechanical but not heat stimuli occurs in the uninjured skin surrounding a burn to the glabrous hand (Raja et al., Brain, 1984). In the present study, we investigated whether a similar dichotomy occurs when secondary hyperalgesia is produced by capsaicin injected into the hairy skin. Heat and mechanical testing was done twice before, and 20 and 60 min after an intradermal injection of capsaicin (50µg in 10µl) to the volar forearm of 12 volunteers. A 9 bar Von Frey filament was used to map the zone of hyperalgesia to punctate mechanical stimuli after the capsaicin injection. Our laser thermal stimulator was used for heat testing at multiple sites inside and outside the mechanical hyperalgesic zone plus the site of capsaicin injection. Subjects pressed a key to indicate heat pain threshold to a ramped (1°C/s) heat stimulus (39 to 49°C). Subjects used a visual analog scale to rate pain to intense heat (44, 46 & 48°C; 1 or 2s duration). Sixty minutes after the capsaicin injection, pronounced secondary hyperalgesia to mechanical stimuli was present, but heat pain thresholds (44.7 ± 0.4°C inside; 44.5 ± 0.3°C outside; ± SEM) and normalized suprathreshold pain ratings (85 ± 10 inside; 79 ± 11 outside) were not significantly altered. However, in all subjects, heat pain threshold was significantly higher ($P < 0.01$) and suprathreshold ratings were significantly lower (30 ± 9) at the capsaicin injection site. Similar results were obtained in 5 other subjects tested with contact heat. These results suggest that secondary hyperalgesia in the hairy skin is characterized by hyperalgesia to mechanical but not to heat stimuli. Supported by NIH (NS-14447).

643.14

NOCICEPTOR ACTIVATION IS ESSENTIAL FOR AXON-REFLEX FLARE FOLLOWING MILD HEAT STIMULI. R.-D. Treede, W. Magerl (SPON:ENA*) Inst. Physiol. & Pathophysiol., Johannes-Gutenberg University, D-55099 Mainz, FRG.

Noxious stimulation induces a spreading erythema (flare) in human skin, which is supposed to depend on an axon reflex of cutaneous nociceptors.

To investigate the concordance of flare response and pain perception we applied stepped heat stimuli of different base temperatures (30 or 35°C) to the forearms of human volunteers using a peltier thermode (TSA 2001). Flare responses were measured with a laser Doppler flowmeter at 8, 19 and 30 mm distance. The pain magnitude was rated on a visual analogue scale.



With stimulus duration of 2s, the probability curves for pain and flare were nearly identical, with an average threshold of 40°C. Using different base temperatures, the magnitude of flare was related to the final temperature, not the size of the temperature step (see Fig.). A pure warm-fiber stimulus (5°C step from 30 to 35°C) was ineffective.

Flare thresholds in human skin thus coincide with the activation thresholds of heat-sensitive C-nociceptors in monkey hairy skin (Treede et al., Soc Neurosci Abstr 16, 1990, 416).

643.15

CUTANEOUS COLD STIMULI IN THE RAT: DETERMINATION OF BEHAVIOURAL NOCICEPTIVE THRESHOLDS. H. Bester, A. Dugray and J-P Rivot, (Spon: European Neuroscience Association*), INSERM U.161, 2 rue d'Alésia, 75014 Paris, France.

After habituating rats (mean weight 230g, n=60) to the experimental conditions (2 sessions per week for 3 weeks), a thermal stimulus was applied to their right hind paw by immersion in a thermostated bath (30% alcohol). Ten different temperatures (T°) were used from -20 to +25°C (5°C step). On each 5 subsequent test-days (9h00 to 13h00) 12 rats were randomly selected and exposed to one T° also chosen at random from the ten, each rat being stimulated only once (6 rats/T°). Latencies of paw withdrawal (PWL) were measured and immersion prolonged to 30s, unless vocalization occurred, in which case the stimulation ended, and vocalization latency (VL) was noted. In addition, during a 12 mn post-stimulation observation period, the occurence of nociceptive behaviours (NB: licking, shaking, guarding, lamening) and their duration (ND) were noted.

The relationship between PWL and T° appeared to be an exponential function: $PWL = 5.00504 \times (1.07049)^{T°}$. For T°>0°C, PWL encoded the applied T°, the threshold being situated between 20°C (1/6 rats over the cut-off) and 25°C (3/6 over the cut-off). For T°≤0°C, PWLs did not differ from one another (mean PWL=2.7s). Only for these T°, some of the rats displayed NB and/or vocalization. VL and NB thresholds were -10°C and -15°C respectively. At -20°C, VL was 8.5s; (n=4/6), 19s at -15°C (n=4/6) and 20s at -10°C (n=3/6). All rats displaying NB did not vocalize, and reciprocally. Rats which vocalized displayed a longer ND (mean 75s, 8 rats) than those which did not (mean 9s, 7 rats), although the duration of the stimulation in vocalizing rats was much shorter than the 30s cut-off. This suggests that NB is likely to be related to the sensation of pain, rather than to the duration of the stimulation, even when intense.

In conclusion, behavioral responses to cold innocuous and noxious stimulation in the awake rat can be described with at least 4 parameters: the PWL for 0<T°<25°C; the VL (threshold -10°C), NB and ND for T°≤0°C. In view of the lack of literature on this subject, our results have implications for studies using nociceptive cold stimulation paradigms.

643.16

RESPONSES OF RAT C-FIBER NOCICEPTORS TO NOXIOUS COLD. K.C. Kajander[1*], B.J. Allen[2], B.L. Allard[2], C. Courteix[2] and D.A. Simone[2]. Depts. of [1]Oral Science & Cell Biology & Neuroanatomy and [2]Neurosci. Res. in Psychiatry, Univ. of Minnesota, Minneapolis, MN 55455.

Most C-fiber cutaneous nociceptors are polymodal and are excited by mechanical, heat and chemical stimuli. Few studies, however, have quantitatively examined responses of these nociceptors to noxious cold. It has been reported, using stimuli above 0° C, that a relatively small proportion of nociceptors are excited by noxious cold. We have reported that all Aδ nociceptors in rats are excited by noxious cold and most had response thresholds below 0° C. In the present study, cold stimuli were used to characterize responses of C-fiber nociceptors to a wide range of stimulus temperatures. Electrophysiological recordings were made from the saphenous nerve of anesthetized rats. Nociceptors were searched for by mildly pinching the skin. Once a nociceptor was identified and its receptive field mapped, conduction velocity, mechanical threshold and responses to thermal stimuli were determined. A total of 12 C nociceptors were studied. Mechanical thresholds (von Frey monofilaments) ranged from 0.63-43.2 mN. 8 of 12 nociceptors were excited by noxious heat and had a mean threshold of 44.3° ±1.73° C. All nociceptors were excited by noxious cold. The mean response threshold was 2.8° ±4.01° C, and thresholds ranged from 30° to -14° C. Approximately 50% of the nociceptors studied (5 of 12) had response thresholds at or above 0° C, while 7 of 12 were excited with stimulus temperatures below 0° C. Responses evoked by cold stimuli usually increased with stimulus intensity (lower stimulus temperatures).

It is concluded that like Aδ "mechanonociceptors", C-fiber "mechanonociceptors" are excited by noxious cold stimuli. Responses of Aδ and C nociceptors to cold differ in that a greater proportion of C nociceptors are excited by stimulus temperatures above 0° C (approximately 50% of C nociceptors; 10% of Aδ nociceptors). Supported by NIH grants NS29567 and NS31223.

643.17

TEMPORAL CHARACTERISTICS OF ORAL TRIGEMINAL ACID SENSITIVITY. Bruce P. Bryant* and Paul A. Moore, Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104

Single unit studies of oral trigeminal neurons have been conducted to ascertain what types of trigeminal neurons are likely to contribute to acid irritation and pain. Acid sensitive neurons were found that were solely sensitive to acid although most also responded to cooling. Conduction velocities of acid sensitive neurons were found to be distributed bimodally, corresponding to C- and A-delta fiber types, which functionally corresponded to polymodal, and cold/cool/acid sensitive neurons. Responses of acid-sensitive neurons could be classified into three basic types 1) simple excitation, followed by rapid adaptation, 2) excitation followed by adaptation to sub-spontaneous activity, and 3) non-adapting excitatory responses of long onset latency (5-10 secs). These three types of responses correspond to thermal/acid (response type #1 and 2) and acid only (response type #3) type neurons. To examine whether acid stimulation exhibited the same phenomena of sensitization and desensitization as capsaicin, neural recordings were obtained from fibers during repetitive lingual exposure to 150 mM pentanoic acid. Responses to both acid and cool/cold stimuli were suppressed in most classes of neurons that were initially sensitive to acid. However, hot-sensitivity was induced in a previously cold/cool/acid sensitive neuron and acid sensitivity was reversibly induced in 2 previously silent neurons. Earlier studies of plasma extravasation implicated vascular processes in capsaicin-induced suppression of responses to acid. Under the same conditions that produced adaptation of responses to acid, no evidence of plasma extravasation was observed. Multiple lines of evidence suggest that acid stimuli cause modulation of sensitivity directly at transduction processes and by conduction block. The sustained noxious sensations of acidic stimuli may result from non-adapting acid sensitive neurons as well as by input from relatively fast adapting acid sensitive neurons that is sustained by central or spinal processes.

643.18

ACTIVITY-DEPENDENT VARIATIONS IN CONDUCTION VELOCITY OF C-FIBERS OF RAT SCIATIC NERVE H.C. Shin*, Y.L. Lee, H.Y. Kwon, H.J. Park & S.A. Raymond. Dept. Physiol., Coll. Med., Hallym Univ., Chunchon, Korea, #200-702, Dept. of Anesthesia, Brigham & Women's Hospital, Harvard Med. Sch., MA 02115, USA.

Activity-dependent changes in fiber excitability and conduction velocity are ubiquitous following both natural or electrical stimulation of myelinated axons of peripheral and central nervous system. Activity-dependent changes in excitability are strong and long-lasting and have been implicated in the encoding of discharge trains, and modulation of impulse conduction at regions of low conduction safety such as axonal branches. C-fibers are the most numerous axons in the sciatic nerve, and are highly heterogeneous, biochemically as well as physiologically. Previously, we have demonstrated different profiles of the activity-dependent threshold changes in sciatic C-fibers (Shin & Raymond, 1991, Neurosci. Letts. 129: 242-246). In this study, changes of the conduction velocity (CV) and subsequent conduction block were characterized following impulse activity (0.5, 1, 5, 10, 25, 50 Hz, 1 min) in single C-fibers (n=49, range of resting CVs: 0.25-1.72 m/sec, at 0.5 Hz, 0.12-0.8 mA, 150-570 μA) of rat sciatic nerves. C-fibers which had same resting conduction velocities often exhibited quite different profiles of the activity-dependent latency change and/or conduction block following impulses. Although C-fibers showed only small (4.19±0.6%) decrease of CV at the lowest activity rate (1 Hz), they exhibited much extensive decrease of CV at higher activity rates (5 Hz: 26.61±2.4%, 10 Hz: 36.09±3.8%, 25 Hz: 39.34±10.0%, 1 Hz vs. others: $p < 0.01$). The frequency of conduction block also increased dramatically as the activity rate rose (5 Hz: 16.3%, 10 Hz: 51.0% Hz, 25 Hz: 89.8%) and most of the fibers were blocked at 50 Hz (95.9%). Eleven of 49 C-fibers showed intermittant conduction block before the occurrence of the complete block, whereas 38 of 49 fibers exhibited direct conduction block without showing intermittant firing activities. These results imply underlying variation among C-fibers in the activity-dependent excitability changes, especially in the buildup and recovery of the hypoexcitable phases. (This study has been supported by KOSEF grant 931-0700-026-2).

643.19

CENTRAL PROJECTIONS OF NERVES INNERVATING THE RABBIT MAXILLARY SINUS LOCALIZED USING WHEAT GERM AGGLUTININ-HORSE RADISH PEROXIDASE (WGA-HRP). A.K. Roche* and K.C. Kajander. Depts. of Pharmacology, Oral Science, and Cell Biology and Neuroanatomy, and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

The purpose of this study was to localize the central projections of nerves innervating the rabbit maxillary sinus.

We used adult, male, New-Zealand White rabbits (2.5-3.5 kgs). After the onset of anesthesia, the left maxillary sinus was opened by drilling through the bony wall of the nasal dorsum. To prevent spread of the WGA-HRP to adjacent nasal areas, the maxillary ostium was closed using a cotton plug and cyanoacrylate ester. Crystals of WGA-HRP (2 mgs) were placed into the sinus, and the sinus was left open for two hours. The opening in the nasal dorsum was then closed using dental resin; the skin incision was sutured, and the animal was allowed to recover. Animals were sacrificed five days later for histochemical localization of retrogradely and transganglionically transported WGA-HRP. Tetramethylbenzidine was used as the chromogen.

More than 50 labeled cell bodies were seen in the ipsilateral trigeminal ganglion. Only 1-3 labeled cell bodies were observed in the contralateral trigeminal ganglion. Dense, particulate, terminal-like labeling was observed only in the ipsilateral spinal trigeminal nucleus. No labeling was present at other levels of the neuraxis (C_2-caudal midbrain).

This study appears to be the first to trace the central terminals of nerves innervating the maxillary sinus. We believe these results will help us perform future studies, which will evaluate alterations that occur in the central nervous system following sinus inflammation.

644.1

RESPONSES OF VENTRAL POSTEROMEDIAL THALAMIC NUCLEUS NEURONS AFTER CHRONIC CONSTRICTION INJURY TO THE RAT'S INFRAORBITAL NERVE. B.P.Vos, M.Gautron, J.M.Benoist, G.Guilbaud*
INSERM U161, 2 rue d'Alésia 75014 Paris.

Chronic constriction injury to the infraorbital nerve (IoN-CCI) produces behavioral changes which suggest the presence of trigeminal neuropathic pain (Vos et al., '94, J. Neurosci.). In the present study, single unit recordings were made in the Ventral Posteromedial Thalamic Nucleus (VPM) of rats (n=30) with a unilateral IoN-CCI, once they showed hyperresponsiveness to mechanical stimulation of the affected IoN territory (PO12-15). Extracellular recordings (glass micropipettes filled with NaCl and pontamine) were made under moderate gaseous anesthesia. To date, 125 VPM-units have been recorded: 87 in the VPM contralateral to the nerve injury (VPMc), 43 in the ipsilateral VPM (VPMi). All 43 VPMi-units had identifiable receptive fields (RFs): 26 (60%) had IoN RFs, 4 (9%) mandibular RFs, 2 (4%) intraoral RFs and 11 (25%) non-facial RFs. Of those with IoN RFs, 15 (57%) responded to stimulation of a single vibrissae (60% rapidly adapting, 26% slowly adapting, 13% follicle pinch), 7 (27%) to guard hair deflection, 3 (11%) to gentle skin indentation and 1 (4%) to noxious pinch. Of 87 VPMc-units, 60 (69%) had IoN RFs, 1 (1%) an intraoral RF, 15 (17%) non-facial RFs and for 11 (13%) no RF could be found. Of those with IoN RFs, 10 (17%) responded to vibrissal stimulation, 28 (46%) to mechanical skin stimulation, and 22 (36%) to deep pressure at the level of the Io-foramen, zygomatic arch or rostral eye corner. The 10 VPMc vibrissal units responded only to follicle pinch and were all multi-vibrissal. Of 28 VPMc skin units, 3 (11%) had small RFs, 13 (46%) wide or discontinuous RFs, and 12 (28%) combined RFs (skin, multiple vibrissae and/or guard hair); of these units 11 (39%) were activated by gentle skin indentation, 17 (61%) by pin prick, pinch or deep pressure. Spontaneous discharge rates of IoN-units in VPMc (X=3.7Hz) were not different from those in VPMi (X=2.9Hz). In the majority of VPMc units, responses to mechanical stimulation (10-15sec) outlasted the stimulus; with repeated stimuli (ISI:150sec) VPMc cells showed periods of sustained discharges (30-924sec).

These data suggest that IoN-CCI has important functional consequences at the level of the VPM which may be involved in the behavioral changes that were used as parameters of trigeminal neuropathic pain.

644.2

IMMUNOHISTOCHEMICAL IDENTIFICATION OF A PUTATIVE SPECIFIC PAIN AND TEMPERATURE RELAY IN HUMAN THALAMUS. A.D. (Bud) Craig*, E.-T. Zhang, and A. Blomqvist. Div. of Neurobiol., Barrow Neurol. Inst., Phoenix, AZ, and Dept. of Cell Biol., Fac. of Health Sci., Linköping, Sweden.

Anatomical work using PHA-L in the macaque demonstrated that dense, topographic lamina I spino- and trigemino-thalamic terminations are located in a cytoarchitectonically distinguishable region termed the posterior part of the ventral medial n. (VMpo) (SN 18:385, '92). Physiological examination showed that VMpo contains a topographically organized concentration of nociceptive-specific and thermoreceptive-specific neurons (SN 19:1073, '93). Since VMpo is coextensive with a zone of dense calbindin-positive fibers (SN 18:385, '92), we have used calbindin immunoreactivity to examine the human thalamus.

Blocks of diencephalic tissue, obtained with appropriate permissions from normal autopsy material, were fixed by immersion in 4% paraformaldehyde for 2-3 wks. Serial 50 µm frozen sections were cut in the frontal, transverse, sagittal or horizontal plane and separate 1-in-3 series were processed for calbindin 28kD immunoreactivity (Sigma monoclonal antibody; DAB rxn) or stained with thionin. Endogenous peroxidatic activity was suppressed in some cases by pre-incubation in methanol/peroxide.

A dense, compact region of fibers with strong calbindin immunoreactivity is located in the posterior, inferior lateral thalamus in the region previously called the suprageniculate/posterior complex. It has nearly identical neighborhood relationships with other calbindin-positive structures as VMpo in the macaque, and it has similar cytoarchitectonic characteristics.

We propose that this structure is homologous to VMpo in the macaque and is the specific pain and temperature relay first postulated by Head and Holmes in 1911. Its location is consistent with clinical lesion, stimulation and recording findings relevant to pain and temperature sensibility. (Supported by NS 25616 and the Swedish Medical Research Council)

644.3

BILATERAL VB THALAMOTOMY DOES NOT REDUCE PAIN IN THE FORMALIN TEST

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It has long been proposed that ventrobasal (VB) thalamic neurons are primarily responsible for mediating central responses to noxious peripheral stimulation. Although this may be true for brief noxious stimulation, it is not the case for persistent supra-threshold stimulation. Previous studies in our laboratory have indicated that while lidocaine blockade of intralaminar thalamic nuclei significantly attenuates formalin-induced pain behavior, it does not diminish formalin pain responses when injected into VB nuclei. In the present experiment, Long-Evans rats received large bilateral electrolytic lesions 10 days prior to testing. Each animal was injected with 20 µl of 2.5% formalin acetate (1% formaldehyde) in the plantar surface of one hindpaw and was rated for pain behaviors for 50 min. The behavioral responses of thalamotomized rats were not significantly different from the responses of unoperated control animals. Implications of these results are discussed. Supported by NSERC grant A7896.

644.4

NEURONS IN THE HUMAN THALAMIC PRINCIPAL SENSORY NUCLEUS RESPOND TO PAINFUL MECHANICAL STIMULI. F.A.Lenz*, R.H.Gracely[1], L.H.Rowland, P.M.Dougherty. Depts. of Neurosurg & Neurosci, Johns Hopkins, Baltimore,MD, 21287-7713 and NAB-NIDR-NIH[1].

The human thalamic substrate of mechanical pain and hyperalgesia has been uncertain since cells responsive to painful mechanical stimuli have not been described previously. We now report results of microelectrode recording prior to thalamic procedures for treatment of chronic neuropathic pain (n=3) or movement disorders (n=9). Thermal stimuli were probes at room temperature, 43°C and 51°C or 53°C while mechanical stimuli included a camel hair brush, a large arterial clip and a small arterial clip. The small clip and the highest temperature stimuli evoked pain sensations rated at 3-8/10 on a visual analog scale; the other stimuli were non-painful. The area of Vc was divided into the region where the majority of cells responded to innocuous somatic stimuli (core region) and the region posterior and inferior to the core region (posteroinferior region). Testing with the series of somatic stimuli was carried out on 21 of 87 cells recorded in the core region and 21 of 54 cells in the posteroinferior region. Seven cells demonstrated a clear increase in firing across the mechanical series from brush to small clip but not across the thermal series. All of these cells were located in the core of Vc. The graded response of these cells to mechanical stimuli extending into the painful range suggests that they may signal acute pain evoked by mechanical stimuli and may participate in mechanical hyperalgesia. Support: Lilly Corp, NIH (NS28598, K08-NS1384).

644.5

SII HAS THE MOST ROBUST RESPONSE OF THE MULTIPLE CORTICAL AREAS ACTIVATED DURING PAINFUL THERMAL STIMULI IN HUMANS, USING MULTI-SLICE FUNCTIONAL MRI, P.A. Gelnar[1], N.M. Szeverenyi[2], A.V. Apkarian[1]*, Depts. Neurosurgery[1] and Radiology[2], SUNY HSC; Syracuse, NY.

Cortical areas involved in human pain perception remain controversial in spite of a number of imaging studies performed on the topic. We studied cortical responses to an experimentally induced painful stimulus in normal subjects utilizing functional magnetic resonance imaging (fMRI).

A 1.5 Tesla MRI system with a within slice pixel size of 1.6 X 1.6 mm, a single surface coil, and an echo planar acquisition sequence were used to image changes in activity in the middle third of the cerebral cortex contralateral to experimentally applied stimuli. In a single imaging session multiple repetitions of noxious heat (45-48°C for 50 s, control = 40°C), motor (sequential apposition of D2-4 to D1, control = rest), and mechanical vibratory stimuli (attached to D1, control = rest) were presented to the subject. Eight 6.0 mm slices were imaged during 6 stimulus-control cycles in each session. Five images/slice were obtained during stimulus or control. T-maps were superimposed on high resolution MR images.

Noxious heat stimulation resulted in significant (t-map clusters greater than 5 pixels with $p<0.05$) activation in the primary (SI) and secondary (SII) somatosensory cortices, primary motor (MI) cortex, and posterior area 24. The largest increase in activity occurred in SII where 3.5% increases in intensity over baseline were noted. The activity in MI was larger than that in SI. For the motor task, significant activation was noted in MI (6% increases), premotor cortex, posterior area 24, SI, and SII. Mechanical vibratory stimulation resulted in increases in activity in SI and SII (2.5% increase).

These results show that multiple cortical areas are activated in response to thermal painful stimulation, including the motor cortex. In addition, the somatotopy of pain perception corresponds with those observed for mechanoception and motor tasks.

644.6

USING PSYCHOPHYSICAL RATINGS TO MAP THE HUMAN BRAIN: REGRESSION OF REGIONAL CEREBRAL BLOOD FLOW (rCBF) TO TONIC PAIN PERCEPTION. G.H. Duncan, C. Morin, R.C. Coghill, A. Evans, K.J. Worsley, M.C. Bushnell*. Univ. Montréal and Montreal Neurol. Inst., Montréal, Canada.

Functional imaging studies in man show that phasic noxious stimuli reliably evoke increased CBF in several brain regions. However, studies using tonic noxious stimuli show less consistent results for reasons which remain unclear. In the present study we tried to maximize detection of tonic-pain-related rCBF changes by using a regression analysis which weights experimental conditions according to subjects' perceptions, rather than to the physical characteristics of the stimuli.

Positron emission tomography (PET) was used to measure rCBF following bolus injections of $H_2{}^{15}O$ in 11 normal males during 6 conditions (70-s immersion of the index finger in 35°,39°,45°,46°,48°, or 49°C circulating water). A regression analysis was performed across conditions using subjects' psychophysical ratings to model a monotonic function between perceived pain and rCBF. A directed search of cerebral regions of interest, predicted from studies of phasic pain, revealed significant trends of increasing rCBF with pain perception in the contralateral sensorimotor cortex (SM), secondary somatosensory cortex (SII), rostral insula (RI), anterior cingulate (AC) and thalamus ($t's>3.0$). Ipsilateral SM, SII and RI showed no trend towards activation. A global search of the entire intracerebral volume revealed additional regions of high correlation in areas involved in motor processing such as red nucleus, basal ganglia and cerebellum ($t's>4.0$, significance threshold adjusted for the larger search area.) These data suggest that although tonic pain may be less robust in evoking rCBF changes, more sensitive analysis techniques can reveal effects of such stimuli in regions activated by phasic pain stimuli.

Supported by Canadian MRC and FRSQ.

644.7

SPATIAL DISTRIBUTION OF CHRONIC PAIN-INDUCED BLOOD FLOW CHANGES IN THE HUMAN BRAIN. R.C. Coghill*, K.F. Berman[a], R.H. Gracely, M. Max, M. Byas-Smith, G.J. Bennett, and M.J. Iadarola.
Neurobiology & Anesthesiology Branch, NIDR, and [a]Clinical Brain Disorders Branch, NIMH, NIH, Bethesda, MD 20892

Functional brain imaging may provide one tool by which chronic pain syndromes may be accurately evaluated, yet little data exist on the spatial distribution of brain activity during chronic pain. In order to better characterize possible functional changes during chronic pain, five patients were recruited for functional brain imaging studies. Four patients suffered from post-traumatic chronic pain and secondary hyperalgesia afflicting one of their limbs and one patient suffered from post-herpetic neuralgia in the trigeminal distribution. All subjects exhibited ongoing pain in the absence of overt somatic stimulation. Cerebral blood flow (CBF) was used as an index of functional activity and was measured by 60 s positron emission tomography (PET) scans following bolus intravenous injection of ^{15}O H_2O. Subjects underwent multiple PET scans during rest and during allodynia elicited by low intensity mechanical stimulation. Preliminary analyses reveal that these patients demonstrate statistically reliable and robust asymmetries in thalamic blood flow during both rest and allodynia (ANOVA, $p < 0.0005$). During both conditions, thalamic CBF contralateral to the afflicted side was consistently **lower** than that of the ipsilateral thalamus. At this point, the neural correlate of this asymmetric blood flow remains unclear. These findings, however, are consistent with similar results obtained from cancer patients with unilateral pain (Di Piero, et al., *Pain*, 46:9, 1991) and indicate that asymmetry in thalamic blood flow is a salient feature of different types of chronic pain.

644.8

TEMPORO-SPATIAL DYNAMICS OF HUMAN FOREBRAIN ACTIVITY DURING NOXIOUS HEAT STIMULATION. K.L.Casey*, S.Minoshima, R.A.Koeppe, J.Weeder, and T.J.Morrow. Depts. of Neurology and Div.of Nuclear Medicine, Univ.Michigan, and VAMC, Ann Arbor, Mi. 48105.

Positron emission tomography (PET) with intravenous injection of $H_2{}^{15}O$ was used to detect changes in regional cerebral blood flow (rCBF) while normal, right-handed, awake humans (N=14, ages 18-42) received repetitive 5sec heat pulses to the left forearm. Stimulus intensity was 40°C (rated warm) or 50°C (rated painful) on alternate scans and was constant throughout the 1 min duration of each of 8 scans. Four scans began at the onset of stimulation (early phase) and 4 began 15sec after stimulation onset (late phase). After normalizing each subject's image set to global CBF, stereotaxically standardized subtraction PET images were analyzed statistically voxel-by-voxel and by volumes of a priori interest ($p< 0.05$ adjusted for multiple comparisons). An ANOVA showed a significant ($p=0.002$) effect of phase. In the early phase, significant pain-specific increases in rCBF were seen in the contralateral thalamus, ponto-mesencephalic junction, and S1, anterior cingulate, and supplementary motor cortices. In the late phase, activity appeared in the contralateral lenticular nucleus, S1, S2, anterior cingulate, insula, and premotor cortices. Ipsilateral activity appeared in the insula, thalamus, and cerebellum. A magnitude estimation study of 8 additional subjects showed a significant ($p<0.009$) increase in perceived intensity during identical repetitive 50°C stimuli. A graphical reconstruction method also showed an increase of perceived unpleasantness ($p<0.024$). These results suggest an underlying perceptual correlate of the changes in the temporo-spatial pattern of forebrain rCBF during repetitive noxious heat stimulation.

644.9

CENTRAL REPRESENTATION OF CHRONIC NEUROPATHIC PAIN STUDIED BY POSITRON EMISSION TOMOGRAPHY (PET). J.C. Hsieh[1,4], M. Belfrage[2], P. Hansson[3], S. Stone-Elander[1], E. Kinnman[3]* and M. Ingvar[1]. [1]Dept. Clin. Neurosci., [2]Pain Unit, Dept. Anesthesiol. and Intensive Care, [3]Neurogenic Pain Unit, Dept. Rehabilit. Med., Karolinska, Hospital/ Karolinska Institute, 17176, Stockholm, Sweden; [4]Pain Unit, Dept. Anesthesiol., Veterans General Hospital-Taipei, 11217 Taipei, Taiwan, Republic of China.

PET studies of phasic and tonic experimental pain using regional cerebral blood flow (rCBF) as the index for neuronal activity have demonstrated activation of primary and secondary somatosensory areas (SI, SII), anterior cingulate cortex (ACA) and thalamus. We investigated the cerebral representation of chronic ongoing pain in patients with mononeuropathy (MNP). Seven patients (42-53 yrs) with MNP in the lower extremity (4 in the right, 3 in the left) were recruited. Three PET image sets were obtained with intravenous injection of [^{15}O]butanol (35-40 mCi) in the patient's habitual painful state and following a successful regional lidocaine block (3 mg/kg) in the painfree state, respectively. Pain intensity was assessed before each measurement of rCBF. The images were reformatted to a common anatomical representation and subsequently normalized to the global mean. The images of the painful and painfree states were subtracted pairwise within the subject and averaged according to the side of MNP. The determination of significant change was based on the local Z-maximum. Significant increase in rCBF was observed, in the painful state, in the right ACA (Brodmann 24) regardless of the side of MNP. Other activated regions included insula, association cortices and cerebellum. Reduced rCBF was recorded in the contralateral thalamus. Unaltered rCBF was observed in the SI and SII. The present results suggest a right hemispheric lateralization of ACA (Brodmann 24) as well as a reduced thalamic activity in chronic pain perception. Experimentally induced pain may contain a more expressed component in the cerebral response (SI, SII) pertaining to the localization of the painful event as well as intensity coding. [Supported by Swedish MRC (8276,9847)]

644.10

REGIONAL CEREBRAL BLOOD FLOW (rCBF) CHANGES DURING PAIN: AN ANIMAL MODEL T. J. Morrow*[125], P.J. Danneman[3], K. A. Frey[14] and K.L. Casey[125], Depts. of Neurology[1], Physiology[2], Lab. Animal Medicine[3], Nuclear. Medicine[4], University of Michigan and VAMC[5], Ann Arbor, MI 48105.
BACKGROUND: Recent studies of regional cerebral blood flow (rCBF) in humans using positron emission tomography (PET) have suggested new ideas regarding which CNS structures participate in the perception of pain in man. To examine the effects of CNS lesions and pharmacological manipulations on rCBF during pain , an animal model analogous to PET must be developed. The goal of this study was to evaluate the use of a positron emitting radiopharmaceutical, ^{99m}Tc-HMPAO combined with standard autoradiographic methods as a potential tool for in vivo imaging of CNS structures that participate in the perception and/or modulation of pain. **METHODS**: Under halothane a 24ga catheters were placed in the tail veins of Sprague Dawley rats. Subjects were placed in a soft restraint jacket and recovered from anesthesia for 45 minutes. 2.5% formalin (0.05 ml) injected into the hindfoot served as the noxious stimulus. After 2 minutes stimulation, 10 mCi of 99mTc-HMPAO was injected through the catheter.. Five minutes later the rats were sacrificed and the brain removed. Twenty micron frozen sections were cut and mounted. Standard autoradiographic images of the brain sections were then analyzed for relative optical density. Ipsi- and contralateral ROI's (regions of interest) were sampled separately from several brain regions, and statistically analyzed (t-test) for differences. **RESULTS**: This technique showed similar rCBF changes as seen in human PET studies, e.g. side to side differences in sensorimotor cortex (31.48% diff, $p <.001$) and cingulate cortex. (17.58% diff, $p<0.05$). **CONCLUSION**: Measurement of rCBF promises to be an exciting new tool for examining changes in CNS activation patterns during pain perception in the awake animal brain.

644.11

CHEMICAL LESION IN THE NEOSTRIATUM INHIBITS AUTOTOMY BEHAVIOR IN RATS. N.E. Saadé*, S.A. Shbeir, S.F. Atweh and S.J. Jabbur. Fac. of Med., American University of Beirut, Beirut, Lebanon.

Recent studies have shown that the basal ganglia receive a nociceptive input (J. Neurophysiol., 1993, 69:1890) and play a role in pain relief produced by electroanalgesia (Pain, 1985, 23:83). The aim of this work is to investigate the possible role of the neostriatum in the modulation of deafferentation pain in rats.

The latency and frequency of autotomy (AT) following leg denervation were compared between a control group (n=6), a basal ganglia lesioned group, in which kainic acid (0.5 µl, 0.01M) was stereotaxically injected in the neostriatum one week prior to the leg denervation (n=14), and sham group in which scalp overlying the skull was sectioned and sutured one week prior to the leg denervation (n=6).

All rats in the control and sham groups exhibited autotomy (AT) with a time onsets of 7.8±2.8 days and 10.3 ±3.0 days, respectively. Only 5 out of the 14 (35.7%) neostriatum-lesioned rats exhibited AT which was also significantly delayed in onset to 24.4±6.7 days. These results suggest that the neostriatum may play a role either in processing sensory input or in the ability to locate and orient the AT towards the part of the body originating the pain sensation.
(Supported by grants from the Diana Tamari Sabbagh Fund and the University Research Board).

644.12

POSTNATAL TUNING OF CUTANEOUS NOCICEPTIVE INPUT TO A SPINAL MOTOR SYSTEM
HOLMBERG*, H. AND SCHOUENBORG, J.
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Detailed information about the development of nociceptive systems is neccessary for understanding the mechanisms by which the adult organization of these systems is accomplished. Also, such information is needed for adequate interpretation of childrens responses to potentially noxious stimuli. We have now examined the postnatal development of the hind limb withdrawal reflex (HWR) evoked by noxious thermal stimulation (CO_2-laser pulses) in decerebrated, spinalized rats. Behavioral studies in neonatal rats showed stereotyped, often misdirected reflex withdrawals, indicating immature properties of the HWR system at this age. EMG-recordings in rats examined at postnatal day (PND)0-3 revealed chaotic receptive fields (RFs), typically covering the whole plantar surface, small response amplitudes and great response variability. With increasing age a decrease in RF size and an increase of response amplitudes were observed in mm. extensor digitorum longus, peronei and biceps posterior, whereas responses evoked in gastrocnemius-soleus decreased with age and were absent after PND 21. At PND 21-25 the RFs of the muscles studied were similar to their counterparts in adult rats, although the detailed distribution of sensitivity still differed somewhat from the adult. According to our data the final tuning of cutaneous RFs of the nociceptive withdrawal reflexes occurs after the third week of life. We suggest that an experience dependent process is involved in this tuning.

645.1

CHOLINERGIC INPUTS ONTO DIRECTIONAL GANGLION CELLS. C. Brandon*, Dept. Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

In the rabbit retina, ON-Center, Directionally-Selective neurons (ON-DSGC's) project to the accessory optic system of the midbrain. The directional component of their receptive fields appears to be generated via GABAergic input, and modulated by cholinergic input. The dendrites of ON-DSGC's intermingle with the processes of starburst amacrines (Brandon, SN '93); the present work examines the synaptic connections between these two cell types.

ON-DSGC's were labeled by retrograde transport from the medial terminal nucleus, and individual labeled cells were injected with Lucifer Yellow *in vitro*. The tissue was stained immunocytochemically for both Lucifer and choline acetyltranferase (ChAT), and tangential sections through the inner plexiform layer were examined by EM.

By light microscopy, ON-DSGC dendrites were completely embedded within the plexus of starburst amacrine dendrites. By EM, ON-DSGC dendrites were surrounded by an extremely dense plexus of ChAT-IR processes; they received many direct synaptic inputs from these processes, which covered virtually their entire surface area. The bulk of the remaining input was from very long, *en passant* synapses from *non*-ChAT-IR amacrine processes; these had a very low electron density.

Identified ON-DSGC's therefore receive dense, monosynaptic cholinergic input. The large, non-cholinergic synapses, because of their size and placement, seem ideally placed to veto the massive, excitatory, cholinergic input that impinges on large dendritic branches of the ON-DSGC.

Supported by NIH/BRSG Grant S07 RR05366-28.

645.2

THE EFFICACY OF SEROTONINGERIC AGENTS VARIES WITH ADAPTATIONAL STATE IN RABBIT RETINA.
X.T. Jin, and W.J. Brunken*. Department of Biology, Boston College, Chestnut Hill, MA 02167

We have been studying the role of the serotoninergic amacrine cells in the rabbit's retina. Our approach integrates electrophysiological, anatomical and molecular techniques. Previously, we have shown that antagonists at both $5HT_2$ and $5HT_3$ receptors inhibit On responses and enhance Off responses in all classes of ganglion cell in dark adapted retinae. Agonists for $5HT_3$ receptors have the opposite effect: they enhance On responses and depress Off responses.

We report here on our most recent studies. We have compared the effects of $5HT_3$ drugs on the light-evoked responses of single ganglion cells under both light- and dark-adapted conditions. During light-adapted states, neither $5HT_3$ agonists nor antagonists significantly affected light-evoked ganglion cell responses. In clear contradistinction, under scotopic conditions $5HT_3$ drugs had the same effects as described previously (summarized above).

Thus, these data exclude the possibility that serotoninergic drug effects are pharmacological artifacts. Specifically, since the effectiveness of these agents is dependent on the adaptational state of the retina, it is highly unlikely that: 1, the serotoninergic agents are acting directly on ganglion cells; 2, spare or "vestigial" serotonin receptors can account for the physiological results; 3, blood borne contamination of exogenous serotonin contributes to our observations. On the other hand, these data strongly support the hypothesis that the serotoninergic system specifically modulates signals in the rod pathway and that serotoninergic function plays a role in the network processes of dark-adaptation.

Supported by Grant EY 06776 to WJB.

645.3

REGULATION OF GLUTAMATERGIC TERMINALS IN THE INNER PLEXIFORM LAYER OF THE TIGER SALAMANDER RETINA. D. Henderson*, W. Yu, and R. F. Miller. Department of Physiology, Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Using a retinal slice preparation, we examined the effects of different neurotransmitter agonists/antagonists on evoked synaptic currents in inner retinal neurons of the tiger salamander. Synaptic currents were evoked by light or by a pulse of hyperosmotic sucrose applied to the IPL. Synaptic currents were monitored through whole cell voltage clamp recordings from neurons in the ganglion cell layer. In the presence of picrotoxin and strychnine, $GABA_B$ agonists reduced sucrose evoked glutamatergic synaptic transmission in approximately half of the cells studied, and in some cases rendered the synaptic currents more transient. In almost all cells studied $GABA_B$ agonists inhibited light evoked synaptic transmission. The results of this study are consistent with the idea that $GABA_B$ receptors inhibit transmitter release from glutamatergic terminals in the IPL. The results of this study also suggest that local hyperosmotic stimulation of transmitter release from terminals in the IPL can be used to study presynaptic regulation of transmitter release.

(Work supported by NIH Grant: EY-00844 to RFM).

645.4

VOLTAGE-CLAMP AND CURRENT-CLAMP RECORDING FROM RETINAL GANGLION CELLS. G.M. Ratto*, L. Lambardi, S. Bisti, L. Cervetto and L.M. Chalupa@. Ist. di Neurofisiologia CNR Pisa, Italy and @Center for Neuroscience, University of California, Davis CA.

The electrical properties of retinal ganglion cells (GCs) were analysed in rat retinal slices using the patch clamp technique in the whole cell configuration. The cells were identified morphologically by filling them with diffusable dyes.

Two classes of GCs were identified on the basis of the characteristics of their potassium current. In one class, the K^+ current had two components: a transient rapidly activating current and a slowly inactivating current. In some cells the slow component lasted for several hundred ms. without appreciable inactivation. In the second class of cells the transient current was absent.

Current clamp recordings also provided evidence for two distinct classes of cells. In one group of GCs the onset of a steady depolarisation caused by outwardly injected current evoked a single large spike, occasionally followed by a few spikes of decreasing amplitude. In these cells no sustained discharge was observed upon injection of constant current. In the second group of GCs current injection evoked a sustained discharge whose frequency and amplitude increased with the extend of depolarisation. At the largest levels of depolarisation these cells produced transient discharges superimposed on a gradually increasing depolarisation which followed a time course similar to the closure of the slowly inactivating potassium current. The sustained cells displayed spontaneous activity, with spike frequency varying with relation to the baseline potential.

Supported by grants from NATO and CNR (bilateral project Italy-USA) and a NIH Fogarty International Fellowship (L.M.C.).

645.5

MODULATION OF HIGH -THRESHOLD CALCIUM CURRENT BY METABOTROPIC GLUTAMATE RECEPTOR IN GANGLION CELLS OF *XENOPUS* RETINA A. Akopian[1*] and P. Witkovsky [1,2], Depts. Ophthalmology[1] and Physiology & Biophysics,[1,2] New York University Medical Center, New York, N.Y. 10016

Presumed ganglion cells of the *Xenopus* retina were studied in short term culture by the whole-cell version of the patch clamp technique.The studied neurons were identified by their characteristic current-voltage relation. In a cocktail of TEA (20 mM); 4AP(5 mM); TTX(1μM) and Ba(20 mM) as a charge carrier,we observed both low voltage-activated (LVA) transient and high voltage-activated (HVA) Ca currents. The HVA current was blocked by nifedipine(10μM) and enhanced by dopamine (50 μM). LVA current was blocked by Ni (20 μM) but was not affected by nifedipine. The metabotropic glutamate receptor agonist t-ACPD (200μM) reduced the amplitude of the HVA Ca current by 46 ±2.6% (n=7). The inhibition by ACPD increased to peak within 20 s and was completely reversed within 15s by a Ringer wash.

The involvement of G-protein in the inhibitory effect of ACPD was studied by including GDPβS (300μM) in the patch pipette, resulting in a significant reduction in ACPD-induced inhibition. In contrast, the inhibitory effect of ACPD on Ba current was irreversible when GTPγS (200μM) was added to the patch pipette. In addition, internal application of GTPγS by itself reduced only the HVA component of the Ba current. These results indicate that activation of G- protein-coupled metabotropic receptors on ganglion cells by glutamate can lead to the modulation of voltage-gated Ca current. Supported by NIH grant EY03570 to P.W..

645.6

MICROGLIAL AND MACROPHAGIC RESPONSE IN RAT RETINA FOLLOWING OPTIC NERVE SECTION. S.C. Sharma*, E.Garcia-Valenzuela. Dept. of Cell Biology, New York Medical College, Valhalla, NY 10595.

Cellular debris, following retrograde degeneration of retinal ganglion cells (RGC), has been shown to be cleared by resident microglial cells in the retina (Thanos,1992). Here we show that blood derived macrophages also have a distinct role in the removal of RGCs' debris. The phagocytic process was studied using double labeling with antibodies against macrophages and phagocytic cells (ED-1 and OX-42) together with retrograde tracing of RGCs after application of either a hydrosoluble dye (Fast blue) or a membrane bound dye (Di I). It was found that while resident microglia from different retinal layers take care of most of the debris, macrophages invade the retina primarily in the nerve fiber layer and upper RGC layer. Macrophages are probably dedicated to engulf axonal debris since unlike microglia, they stain only with di I and not fast blue. Temporal and topographic aspects of these phenomena were also studied.

645.7

BLOCKAGE OF VOLTAGE DEPENDENT SODIUM CHANNELS PREVENTS DENDRITIC COMPETITION IN THE CAT RETINA. S. Deplano[2], C. Gargini[1], G.M. Ratto[1] and S. Bisti*[1],. [1]Istituto di Neurofisiologia C.N.R., Pisa, I-56127 and [2]Istituto di Anatomia Comparata, Università di Genova, Genova, I-16100.

A small lesion at the vitreal surface of the retina produces degeneration of ganglion cells whose axons are severed and leaves a small region in the retina depleted of ganglion cells. When the lesion is performed at an early stage during postnatal development the ganglion cells at the border of the depleted area show an abnormal elongation of the dendritic trees toward the bare area. A possible explanation for this is that dendrites compete either for synaptic space or chemical factors. Alternatively one may suppose that the electrical activity plays a role in the regulation of dendritic arborization as it happens in the segregation of retinal afferents at the LGN level. In the present study we combined a small retinal lesion with the block of the regenerative electrical activity by intraocular administration of TTX. The treatment was continued for the whole critical period. The LGNs were then injected with HRP and the retinae were wholemounted and reacted. The ganglion cell population at the border of the depleted zone was then analyzed. It is seen that neurons near the border present a symmetrical dendritic arborization with few exceptions located in correspondence with the lesion. These results suggest that the electrical activity plays a role in shaping the dendritic arborization during the development of ganglion cells.

645.8

SELECTIVE BLOCKADE OF MÜLLER CELL GLUTAMINE IN CAT RETINA CAUSES OCULAR DOMINANCE SHIFTS IN VISUAL CORTEX. S.R. Robinson, K.M. Lee, M.G.P. Rosa, L.M. Schmid and D.I. Vaney*.
Vision, Touch, and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Australia, 4072.

Inhibition of the enzyme glutamine synthetase in Müller cells by methionine sulfoximine (MSO), causes a complete loss of glutamate immunoreactivity in retinal ganglion and bipolar cells[1]. The present study investigates changes in response properties of neurons in cat visual cortex following intraocular MSO injection. Four adult cats were anaesthetised with thiopentone sodium (4mg/kg.hr IV) and N_2O/O_2 (70:30), while pancuronium bromide (Pavulon 0.15mg/kg.hr IV) was administered to minimise eye movements. The right eye was injected with 0.1ml of MSO at a dosage equivalent to 2mM. Multiple electrophysiological responses were recorded from visual cortical areas V1 and V2 over a period of 24 hours. Within eight hours of MSO administration, histograms for ocular dominance exhibited a shift towards the uninjected eye. This shift was statistically significant (Mann-Whitney U; $p < 0.01$) in both hemispheres, and in both V1 and V2; the shift being greatest in V2. The cats were subsequently euthanased with sodium pentobarbital (60mg/kg; IV) and their retinae processed for immunocytochemistry. The retinae were found to be devoid of glutamine, while the ganglion cells and bipolar cells were depleted of glutamate. These data lead us to conclude that Müller glial cells are an essential component of glutamatergic neurotransmission in the primary visual pathway.

[1]D.V. Pow, S.R. Robinson and D. Noone (1993) Proc. Aust. Neurosci. Soc., 4: 118.

645.9

IN VIVO TRANSFECTION OF RETINAL GANGLION CELLS IN THE RAT. E. Garcia-Valenzuela, S.C. Sharma, A.B. Drakontides*. Dept. of Cell Biology, New York Medical College, Valhalla, NY 10595.

Following injury, most central neurons in mammals are unable to regenerate, even after the manipulations of their cellular and molecular microenvironment. Modifying their intracellular functions through gene transfer might be necessary for complete restoration of their anatomy. Here we describe successful transfection of axotomized retinal ganglion cells by immediate administration of plasmid at their cut ends. Two different plasmids were used, containing either the SV40 promoter linked to the luciferase gene, or the CMV promoter linked to the lacZ gene. Assays for the expression of both reporter genes demonstrated that a significant proportion of ganglion cells express them successfully 3 and 6 days after retrograde transport. Such an approach might be useful in studies of neuronal molecular functions in vivo, and as an experimental therapeutic strategy.

645.10

DEMONSTRATION OF SUCLASSES OF RAT RETINAL GANGLION CELLS EXPRESSING MESSENGER RNAS OF γ-AMINOBUTYRIC $ACID_A$ RECEPTOR α1 SUBUNIT AND L-GLUTAMATE DECARBOXYLASE

J. Kosaka, E. Moriit, S. Nomurat and Y. Fukuda*
Dept. of Physiology and Dept. of Pathologyt, Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

We investigated the localization of the mRNAs of γ-Aminobutyric $Acid_A$ receptor α1 subunit ($GABA_A\alpha1$) and L-Glutamate Decarboxylase (GAD) among subclasses of RGCs by non-radioactive *in situ* hybridization.

Adult male Wistar rats were used after overdose injection of pentobarbital. cRNA probes of $GABA_A\alpha1$ and GAD were labeled with Digoxigenin-11-UDP (Boehringer) and hybridized with vertical sections of retina. Soma diameter measurement was performed for all cells with postive signals in the ganglion cell layer (GCL).

$GABA_A\alpha1$ and GAD mRNAs were detected in the inner nuclear layer and the GCL. In this work, we classified the cells with soma diameter larger than 13μm as RGCs (Perry, 1981., Beale and Osborne, 1982.) . Since siganl positive cells for $GABA_A\alpha1$ mRNA distributed within 5-22μm in diameter, RGCs including α-cells (≥17μm) expressed $GABA_A\alpha1$ mRNA. Soma diameter of cells expressing GAD mRNA distrbuted within 6-16μm in diameter. We interpreted to indicate that small (S) and medium-sized (M) RGCs but no α-cells expressed GAD mRNA.

645.11

CHARACTERIZATION OF RETINAL GANGLION CELL TYPES BY QUANTITATIVE ANALYSIS OF DENDRITIC MORPHOLOGY IN THE RAT. A.Aschoff, B.A.Sabel*. Inst. Med. Psychol., Univ. Magdeburg, Germany.

Soma and dendritic field size of retinal ganglion cells (RGC) are not sufficient criteria to seperate clearly the cell types described by Perry (1979) and Dreher et al (1985). We therefore quantified dendritic morphology of RGC to allow unequivocal classification of different RGC types. 50 RGC, identified by retrograde labelling, were injected intracellularly with lucifer yellow and morphometrically analyzed. As previously described, we were able to classify three cells types by soma and dendritic field size. These criteria are not sufficient because many cells cannot be identified as a particular cell type. For example, type I neurons, with soma area ranging from 200-500 μm^2, can clearly be distinguished from type II neurons (50 and 150 μm^2). Type III neurons however, have similar soma sizes than type II neurons. We therefore tried to find additional criteria for RGC classification using quantitative analysis of dendritic morphology. These include: dendritic spread, dendritic complexicity at growing distance from the soma, or segment lenght and segment ordering.

Three cell types can be clearly characterized by average segment length (ASL), which is shortest in type II cells, intermediate in type I cells, and longest in type III cells. In type II dendrites the ASL is similar in all segment orders (15-20 μm), regardless of centrifugal or centripedal order. In type III dendrites the ASL is 32 μm in all branching orders up to the 13th centrifugal order, thereafter shorter. Especially in type III dendrites, the 1. and 2. order segments of the centripetal order (end segments) are very long (40-50 μm). In type I dendrites the segments of the 1. centrifugal order are relatively short (25 μm), the segments of the 2. to the 7. order very long (40 μm) and thereafter extremely short (10-20 μm). By combining ASL, segment order, dendritic field size and soma size all three cell types can be classified unequivocally. There is indication of further subgroups of cells types in all three cell groups.

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645.12

CHARACTERIZATION OF THE RABBIT RETINAL GANGLION CELLS THAT PROJECT TO THE SUPRACHIASMATIC NUCLEI. D.S. Tjepkes, F.R. Amthor and D.C. Tucker*. Dept. of Psychology and NRC, Univ. of Alabama at Birmingham, Birmingham, Alabama 35294.

The retina has previously been shown to have a projection to the suprachiasmatic nuclei (SCN). SCN neurons have been shown to have a maintained firing rate that either has a sustained increase or decrease in its firing rate in response to increased illumination (Groos and Mason, *J.Comp.Phys.*, 1980; Meijer *et.al.*, *Brain Res.*, 1986). The goal of this research is to characterize the retinal ganglion cells that mediate these responses, which are likely to play a role in the photic entrainment of circadian rhythms.

Fluorescently labelled latex beads were stereotaxically injected into the SCN of rabbits. After a recovery period the retinas containing retrogradely labelled ganglion cells were isolated and mounted in a fluorescence microscope and superfused with oxygenated Ames medium. The activity of the labelled cells was examined with extracellular electrodes while visual stimuli were projected through the microscope condenser. The cell was then intracellularly impaled with a pipette electrode and injected with HRP and processed to allow morphological examination.

The first cells labelled exhibit a sustained rate of firing that tonically increases with an increase in general illumination, with no sign of an antagonistic surround. These cells responded poorly to small flashing spots. These responses are consistent with a role of luminance coding. The cell's morphology is characterized by small somata and very fine dendrites that are bistratified.

Labelled cells were located in the inferior and superior retina with the densest labelling among the bundles of fibers just inferior to the myelinated fiber band.

This research was supported by NEI grant EY05070.

645.13

DISTRIBUTION AND COVERAGE OF BETA CELLS IN THE CAT RETINA. J.J. Stein, S.A. Johnson and D.M. Berson*. Dept. Neuroscience, Brown University, Providence, RI 02912.

We have estimated the distribution of beta cells in the cat retina by mapping the densities of medium sized (non-alpha) cells labeled by retrograde tracer deposits largely or completely confined to the geniculate A-layers. We visualized the dendritic fields of selected cells by intracellular staining *in vitro*. In the nasal retina, beta cells exhibited a well-developed visual streak and, except centrally, accounted for about 40-50% of ganglion cells. However, the incidence of beta cells increased substantially in the central retina (to about 60-70% of all ganglion cells; see Neurosci. Abstr. 19:529, '93) and throughout much of the temporal retina (to about 70%). Though beta-cell densities were lower temporally than nasally, the distribution was far more symmetrical about the line of nasotemporal division than the distribution of all ganglion cells. These results are consistent with our evidence that colliculopetal W-cells (which account for the vast majority of non-beta cells) exhibit a complementary distribution: they make up about half of the ganglion cells in the mid- and far-peripheral nasal retina, including the visual streak, but are underrepresented in the area centralis and temporal retina (where they make up, respectively, about 33% and 25% of all ganglion cells). Dendritic-field areas of beta cells systematically reflected local beta-cell density. For example, in the nasal retina (eccentricity: 30°), areas were 4-fold smaller within than outside the visual streak. Coverage factor (beta-cell density X dendritic-field area) remained constant everywhere, whether central or peripheral, nasal or temporal, on or off the visual streak (mean = 3.9; SD = 0.76; N=203). We suggest that the beta-cell system shows greater binocular balance and is more specialized for fine sampling of central visual fields than other cell classes, and has enhanced functional capacity along the horizon.

Supported by NIH EY06108.

645.14

THE SPECTRAL SENSITIVITY OF GANGLION CELLS IN THE MOUSE RETINA VARIES ALONG THE INFERIOR-SUPERIOR AXIS. E. R. Soucy, E. Wu and M. Meister*. Dept. of Cellular and Molecular Biology, Harvard University, Cambridge, MA 02138.

We are interested in the nature of the information encoded in the spike trains of ganglion cells. Using a multi-electrode array, we recorded visual responses from the population of mouse retinal ganglion cells. The response properties of each cell were determined by computing the reverse-correlation function of its spike train to a pseudo-random stimulus. This allowed us to measure the spatial, temporal, and spectral components of an individual ganglion cell's receptive field. Initial characterization of mouse retinal ganglion cells revealed a relationship between spectral sensitivity and retinal location, with cells in the inferior region displaying greater sensitivity to short wavelengths relative to cells in the superior region of the retina. Szel *et al.* (J. Comp. Neurol., 325, 327-342) report a topographic separation of two classes of photoreceptors in the mouse retina: short wavelength cones localized predominantly to the inferior retina and medium wavelength cones localized exclusively to the superior retina. It appears that this distribution of cone densities results in a gradient of spectral sensitivity among ganglion cells. By comparing the ganglion cell response spectra to the photoreceptor absorption spectra, we are currently evaluating the respective contribution of each photoreceptor class.

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645.15

TWO REMARKS ON CODING IN THE OPTIC NERVE OF THE FROG. A.C. Grant*, G.A. Pratt and J.Y. Lettvin. Dept. of Biomedical Engineering, Rutgers Univ., Piscataway, NJ 08855, and Lab for Computer Science, MIT, Cambridge, MA 02139.

We studied pulse interval coding on two types of retinal ganglion cell (RGC) in the frog, Rana pipiens, by recording extracellularly from single optic nerve fibers with metal-filled microelectrodes. Records were taken from color sensitive fibers (RGC Class V) and "dimming detectors" (RGC Class IV).

With regard to the color fibers, we noted strikingly different pulse interval patterns on a single fiber in response to light stimuli of two different wavelengths, despite approximately equal average firing frequencies in each response. These data are consistent with a pulse pattern rather than pulse frequency coding for color.

The dimming detectors had a sensitivity to step changes in light intensity of less than 0.01 decade, and a sensitivity to continuous changes in intensity of less than 6 decades per hour. The evoked response to step decrements in light intensity was logarithmically related to the magnitude of the step, and was independent of background illumination over a range of at least two decades.

645.16

INITIAL PHASE OF ROD RESPONSES, GANGLION CELL FIRING AND BRIGHTNESS SENSATIONS CORRESPOND UNDER DIFFERENT BACKGROUND CONDITIONS. K Djupsund*[1], K Donner[1], N Fyhrqvist[1] and T Hariyama[1,2], [1]Dept Zoology, FIN-00014 Univ Helsinki, Finland and [2]GSIS, Tohoku Univ, Sendai 980, Japan.

The rods are the first filter in the visual sensory chain and ganglion cells (GC) the last retinal processing step before visual information enters the optic nerve and higher processing. Therefore one might in these nerve cells find common constraints and guidelines for processes mainly studied in psychophysics.

We have compared on one hand brightness sensation and adaptation data and on the other hand the timescale of the rise of intracellularly recorded rod responses and extracellularly recorded GC response frequencies in the frog *Rana temporaria* to flash/step stimuli.

We show that if brightness sensations are determined by the initial rise of the photoreceptor signal, this can account for the intensity-brightness sensation relationship under different backgrounds. The rise of rod responses accelerated with a power of -0.14--0.2 over sub-saturating background and stimulus ranges, which corresponded to the rate of GC responses.

VISUAL CORTEX: STRIATE VI

646.1

CHOLINERGIC DEAFFERENTATION OF THE VISUAL CORTEX BY INTRACORTICAL INFUSIONS OF 192 IgG-SAPORIN IN RATS: EFFECTS ON VISUAL DISCRIMINATION AND VISUAL ATTENTION. M. Sarter*, L.A. Holley and M. Matell. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

The visual cortex, as all cortex, is innervated by the cholinergic neurons situated in the basal forebrain. The functions of the cholinergic afferents of this cortex have remained unclear. Furthermore, the role of the cholinergic cell loss in the visual dysfunctions of patients with senile dementia is unknown. Rats were trained to discriminate between simultaneously presented pairs of visual stimuli flashing at 5 Hz versus 4.17, 3.75, 2.5, 1.67, or 1.25 Hz. Cholinergic inputs to the visual cortex were selectively lesioned by infusions of the immunotoxin 192 IgG-saporin into this area (0.01 μg/0.8 μl/hemisphere). Control animals received either infusions of the immunotoxin into the frontoparietal cortex or infusions of vehicle into the visual cortex. Lesions performed after the animals learned the task did not robustly affect performance. Lesioned placed before the acquisition of the task resulted in a transient impairment in the rate of acquisition. The role of cholinergic afferents of the visual cortex in the animals' ability to detect visual signals presented for 25, 50, or 500 msec and to discriminate these signals from non-signal events was also examined. These experiments represent a first step toward the determination of the functions of cholinergic inputs to the visual cortex.

646.2

STRUCTURE-FUNCTION CORRELATION OF SINGLE NEURONS IN THE MONKEY VISUAL CORTEX, *IN VITRO*. J. Zhu* and D. J. Uhlrich. Neuroscience Training Program and Department of Anatomy, University of Wisconsin Medical School, Madison, WI 53706.

In rodents, distinct physiological characteristics of cortical cells, *in vitro*, are correlated with morphological features (Connors and Gutnick, TINS, 13:99, 1990). We used the whole-cell recording technique with biocytin-filled electrodes to examine this relationship in the macaque primary visual cortex. Cells were grouped based on their intrinsic response properties. Regular-spiking cells were correlated with pyramidal (n=20) or stellate cell (n=3) morphology. The duration of the action potentials of stellate cells was shorter than that of pyramidal cells. An intrinsically bursting cell, found in layer V, was a spiny, pyramidal-like cell. Fast-spiking neurons (n=4) had smooth and beaded dendrites and exhibited less spike-frequency adaptation than regular-spiking cells. Transient-spiking neurons (n=3), which responded to long duration, depolarizing current injections with only 1 or 2 spikes, were correlated to another cell type with beaded, smooth dendrites. The cell types also differed in their axonal projection pattern. Other properties were similar across cell types: the mean resting potential of all neurons was -73 mV (after adjustment of the junction potential), the median input resistance was roughly 200 mega-ohm, and most cells showed a strong non-linear I-V relationship. These results indicate that the interaction of distinct intrinsic membrane properties and synaptic organization underlies the sculpting of sensory responses in the visual cortex.

Supported by EY06610 and RR00167.

646.3

NEUROCHEMICAL EFFECTS OF MONOCULAR APHAKIA IN SUPRAGRANULAR LAYERS OF V1 OF THE ADULT MACAQUE. S.S. Leclerc* and R.K. Carder. Zanvyl Krieger Mind/Brain Institute, Johns Hopkins Univ., Baltimore, MD 21218.

Unlike monocular enucleation or intravitreal TTX injections, monocular aphakia results in a chronic defocusing without eliminating retinal output. Loss of neural activity in one retina, as with these other forms of monocular deprivation, leads to chemical changes in V1. In the present study, cytochrome oxidase (CO) histochemistry and calbindin immunocytochemistry were used to determine if chronic defocusing without elimination of retinal input can lead to similar neurochemical changes in the supragranular layers of V1. CO and calbindin staining are normally complementary, with CO staining occupying puffs along the centers of both sets of ocular dominance columns, and calbindin staining occupying the surrounding CO-poor interpuffs. After 3-12 weeks of monocular aphakia, changes in CO histochemistry and calbindin immunocytochemistry are apparent. CO-rich puffs at the centers of normal-eye columns fuse to form stripes while CO-rich puffs at the centers of deprived-eye columns become pale and shrunken. Calbindin immunostaining, normally intense in the CO-poor interpuffs and weak in the CO-rich puffs, is similarly affected. Alternating dark and lightly stained stripes are centered respectively on the stripes of CO-rich puffs of the normal-eye columns and on the shrunken CO puffs of the deprived-eye columns, suggesting a novel induction of calbindin immunostaining in the CO-rich puffs of the normal-eye columns. These results are similar to those found in TTX-injected monkeys indicating that aphakic amblyopia also produces neurochemical changes across compartments and ocular dominance columns.

Supported by EY06344 and FRSQ.

646.4

INTEGRATIVE PHYSIOLOGY OF PYRAMIDAL CELLS IN TURTLE VISUAL CORTEX: I. GENICULOCORTICAL SYNAPSES. P. S. Ulinski*, M. Calef and M. Fowler. Depts. of Organismal Biology and Anatomy and of Psychology, University of Chicago, Chicago, IL 60637.

Attempts at understanding the functional relationships between cortical microcircuitry and receptive field generation have been hampered by the technical difficulty of studying the biophysics and synaptic physiology of cortical neurons with *in vivo* preparations. We have circumvented this difficulty by using an *in vitro* preparation of the eyes and brain of freshwater turtles, allowing us to record intracellularly from cortical neurons while presenting natural visual stimuli to the eye cup. Results from intracellular recordings have been combined with anatomic data and with compartmental models of cortical neurons and of neural circuits implemented with the *Nodus* software package. We investigated the responses of pyramidal cells in the visual cortex to 1 sec, 660 nm light flashes with intensities varying from 0 to 1500 photons/cm^2/sec, finding that threshold light intensities produced monosynaptic EPSPs. Shape parameter analysis indicated that EPSPs recorded at the soma are usually composite EPSPs. Simulations showed these composite EPSPs could result from asynchronous activation of geniculate boutons on several dendrites of the cell, consistent with earlier results from our lab using electrical stimulation of geniculate afferents.

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646.5

ONCOGENE EXPRESSION REVEALS OCULAR DOMINANCE COLUMNS IN ADULT CAT VISUAL CORTEX. L. Arckens[1,2], G. Wouters[1,2], W. Vanduffel[2]*, E. Vandenbussche[2], G. A. Orban[2] and F. Vandesande[1]. [1]Lab. Neuroendocr. & Immunol. Biotechnol., Zoological Institute, KULeuven, [2]Lab. Neuro- and Psychophysiol., KULeuven, B-3000 Leuven, Belgium.

Immunocytochemical methods were employed to determine whether manipulation of visual input can induce changes at the protein level of two oncogenes, c-fos and zif-268, in the primary visual cortex of the adult cat. Under normal visual conditions zif-268 was expressed at high basal levels in layers II, III, IVB and VI of area 17 and 18, while c-fos was hardly detectable. Monocular deprivation drastically influenced the expression of both oncogenes. Already after one hour of monocular visual stimulation, Fos and zif-268 positive nuclei were distributed in a columnar fashion. Using longer monocular deprived cats and cytochrome oxidase histochemistry, we demonstrated that the immunocytochemically detected columns are ocular dominance columns corresponding to the stimulated eye. From immunocytochemical double stainings we know that both oncogenes are expressed in the nuclei of non-GABA-ergic neurons.

Supported by IUAP Vision & Memory.

646.6

RESIDUAL VISION AFTER STRIATE CORTEX DAMAGE IN MONKEYS: STUDIES OF MOTION SENSITIVITY. T. Moore*, H.R. Rodman, A.B. Repp, C.G. Gross. Department of Psychology, Princeton University, Princeton, NJ 08544.

Residual visual capacity in humans with damage to striate cortex has been reported to include sensitivity to presence and direction of motion (e.g., Perenin, 1991, NeuroRep. 2:397). We investigated the ability of three monkeys with large unilateral striate lesions to process motion in the affected portion of the contralateral visual field. Lesions were made at 5 weeks of age in two animals and in adulthood in the third. Stimuli were small (5 deg. diameter) gaussian-filtered fields of dynamic random dots (dot size 0.1-0.5 deg.) presented on a video monitor. Moving stimuli contained 98% correlated motion. A go/no-go paradigm employing a saccadic eye movement response was used to test the ability of each monkey to discriminate 1) upward from downward motion at speeds of 4 and 20 deg./sec., 2) upward motion from motion noise (0% correlated motion) and 3) upward motion from a static field of random dots. All animals quickly learned all discriminations in the intact hemifield. In the impaired hemifield, however, they failed to perform above chance on all tasks except the discrimination of moving vs. static dots. The results indicate that preserved visual capacity after striate cortex damage in monkeys does not include at least some types of motion sensitivity, i.e., the integration of local motion signals present in dynamic random dot displays.

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646.7

EFFECTS OF LONG-RANGE CONNECTIONS ON GAIN CONTROL IN AN EMERGENT MODEL OF VISUAL CORTICAL ORIENTATION SELECTIVITY. D. Somers*, S.B. Nelson, and M. Sur. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

We previously constructed a model visual cortical circuit with short-range ($< 300\mu$) intracortical excitatory and inhibitory connections which yielded emergently sharp orientation selectivity (Somers et al '93). The model predicts a small contribution to orientation tuning from direct inhibitory inputs to single cells, but a substantial contribution from the distributed inhibitory inputs to a cortical column. Intracortical excitation selectively amplifies responses to preferred stimuli, and inhibition primarily regulates the gain of excitatory feedback. Here we demonstrate that cortical gain also depends on stimulus contrast: the gain rises to boost mild suprathreshold stimuli and drops off as stimulus strength increases. This mechanism yields cortical contrast response functions that rise more rapidly and saturate more quickly than do their geniculate inputs.

We have added to our model two forms of longer range cortical connections: very sparse inhibitory inputs from oblique or cross orientations ($300 - 600\mu$) and more extensive excitatory inputs from iso-orientations ($\geq 1mm$). Long range inhibitory connections comprise less than 20% of inhibitory and less than 5% of all model synapses. Although long-range inhibitory connections are not required to achieve sharp orientation tuning, these connections permit the circuit to maintain sharp orientation selectivity and contrast gain control across the full range of suprathreshold stimulus contrasts (Skottun et al. '87). As in the short-range model, inhibition is strongest at the preferred orientation and has predominately distributed rather than direct effects on tuning. Adding long-range excitatory connections produces biphasic effects that depend on stimulus context. Strong iso-orientation surround stimuli can up- or down-modulate the responses to stimuli within the classical receptive field; responses to subthreshold or weak center stimuli are amplified, while those to strong center stimuli are reduced.

Supported by McDonnell-Pew, MH10671, EY06363, and EY07023.

646.8

STUDIES OF HUMAN VISION COMBINING MEG AND ANATOMICAL AND FUNCTIONAL MRI J.S. George*, C.J. Aine, J.C. Mosher, H.A. Schlitt, C.C. Wood[1], J.D. Lewine and J.A. Sanders[2]. 1 Los Alamos National Laboratory, 2 Albuquerque Federal Regional Medical Center. Integrated analyses of human anatomical and functional measurements offer a powerful paradigm for human brain mapping. MEG and EEG provide excellent temporal resolution of neural population dynamics. MRI provides excellent spatial resolution of head and brain anatomy and an alternative measure of functional activation. These methodologies constrain and complement each other and can thereby improve our interpretation of functional neural organization. In these experiments, MEG data (3x37 channels) and fMRI data (6 slices, 8 or 10mm thick, with 2 mm in-plane resolution) were elicited by a patterned stimulus in the lower right visual field. T1 weighted 3-D volumetric MRI data (128 slices 1.5 or 2mm thick, with 1mm in-plane resolution) were acquired during the same session. The geometry of cortex and of major conductivity interfaces within the head was defined by segmentation of MRI data using semiautomatic image analysis procedures in *MRIview*, a software package developed in our laboratory. Cortical edge voxels were identified and cortical normal vectors were calculated for edge voxels using a 3-D convolution over the 26 nearest neighbors. 35-50,000 voxels were required to define the cortical surface at 2 mm isotropic resolution. Conductivity boundaries were defined by shrink-wrapping an icosahedron-based mesh over the surfaces. Electric potential was evaluated at node points using a boundary integral calculation. Magnetic fields at sensors were calculated by evaluating an integral over all node potential values. As a first estimate of MEG sources, the MUSIC metric was evaluated for voxels under the sensor array, assuming locations and orientations derived from anatomical MRI. In agreement with previous dipole-based analyses of MEG data and with fMRI data, at least least three sources are evident in these images, a primary response (V1/V2) and bilateral sources near the parietal/occipital border. As a second step, distributed current sources were defined based on clusters of active voxels identified in fMRI maps. Component dipole sources were assigned locations and strength based on fMRI and orientations based on anatomical MRI. Such geometrically complex composite sources can be treated as single sources for temporal analyses based on MEG.

646.9

PAN II: A TWO LAYER NEURAL NETWORK MODEL OF LAYER IV IN PRIMARY VISUAL CORTEX F.W.Grasso* BUMP, MBL Woods Hole, MA 02543

Previous simulation results demonstrated that Primitive Acquisition Networks (PANs) were capable of self-organizing distinct representations of both experienced and novel input sets by storing repeated features contained in the training set in their plastic connections. In the earlier studies model network elements in the PAN layer shared properties of spatial and temporal summation as well as orientation selectivity with 'simple' cells in primary visual cortex. In the model, orientation selectivity developed in a systematic 'columnar' arrangement across the layer when the net was trained with digitized images of natural forest scenes. In recent simulations a new layer of processing elements has been added which receives as input the output of the PAN layer and a parallel copy of the PAN layer input. The elements in this second layer share properties with 'complex' cells in the primary visual cortex: they are not significantly activated by points of light smaller than their receptive fields but are activated by and selective in their responses to larger patterned inputs presented within their receptive fields. Elements in the second layer preserve the orientation selectivity and spatial frequency selectivity of their corresponding PAN elements together the two layers form a 'column' with a depth of two cells. In both model layers the various selectivities of the elements arise from the joint effects of excitatory afferent input and intrinsic lateral inhibitory connections. In the first (PAN) layer there is greater dependence on the afferent input while in the second layer evolved selectivities depend more on intrinsic connections. These results suggest the model embodies a plausible mechanism by which useful representations of the visual environment my be constructed from experience of that environment while providing an explanation for origin of major observable properties of neural networks and neurons in primary visual cortex.

646.10

INTERMODULATION COMPONENTS IN THE STEADY-STATE VEP AS INDICES OF FIRST HARMONIC BINOCULAR PROCESSING. S. Suter*, P.S. Suter, D.T. Perrier, and J.A. Fox. Vision Laboratory, Psychology Dept., California State Univ., Bakersfield, CA 93311.

The steady-state visual-evoked potential (VEP) to an ordinary pattern-reversal grating contains a robust second harmonic (2H), but little or no fundamental (1H) activity. Cortical 1H processing is of considerable importance, however, because only 1H is clearly linked to neural substrates carrying spatial phase information, which is necessary for the binocular processes of fusion and stereopsis. A stimulus incorporating several simultaneous modulation rates (e.g. F1, F2) enables inferences about cortical processing of 1Hs from nonlinear intermodulation (IM) components--the sum and difference of the 1Hs (e.g. F1-F2, F1+F2). Steady-state VEPs were recorded (Oz to right ear) from 17 stereonormal (RanDot E) adults. Stimuli were 0.86 c/deg gratings subtending 10 deg presented for 60-sec trials. Stimuli contained up to three simultaneous modulation rates (F1=6.1, F2=8.5, F3=12.8 Hz) with monocular, binocular or dichoptic conditions achieved using liquid crystal lenses synched to the 256/sec monitor frame rate. Fourier analysis extracted amplitudes at frequencies of interest. Monocular IM components, but not monocular 2Hs, were significantly reduced when a different temporal frequency was added to the other eye. Therefore, in these conditions, 1Hs, but not 2Hs, seem to index binocular processing. Nonlinear models of visual processing are discussed in relation to these findings. The Dorothea Haus Ross Foundation.

646.11

LAMINAR SPECIFICITY IN THE RELAY OF MAGNOCELLULAR AND PARVOCELLULAR STREAMS TO THE SUPERFICIAL AND DEEP LAYERS OF MACAQUE STRIATE CORTEX.
W.M. Usrey* and D. Fitzpatrick, Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Magnocellular and parvocellular neurons in the lateral geniculate nucleus terminate primarily in layers 4Cα and 4Cβ of striate cortex respectively. In order to examine the relay of information from these layers to the more superficial and deep layers, we made *in vivo*, intracellular injections of biocytin into spiny neurons in layers 4Cβ, 4Cα and 4B of macaque striate cortex. Our results show that spiny stellate neurons in layer 4Cβ give rise to axons that arborize in layers 4A and 3B. In contrast, spiny stellate neurons in layer 4Cα only occasionally send axons above layer 4A. Rather, layer 4Cα neurons extend long distance, horizontal axons that arborize almost exclusively within layers 4Cα and 4B. The relay of activity in the magnocellular stream to the superficial layers appears to depend primarily on neurons in layer 4B: Both spiny stellate and pyramidal neurons in 4B give rise to axons that teminate in the superficial cortical layers. However, unlike the neurons in 4Cβ, those in 4B have axons that pass through 4A and 3B to terminate in layers 3A, 2 and 1. These results suggest that laminar stratification may play an important role in the integration of magnocellular and parvocellular information within layers 2 and 3.

The projections of neurons in layers 4Cβ, 4Cα and 4B are also selective with respect to their targets within the deep layers of striate cortex. Layer 4B neurons give rise to axons that terminate primarily in layer 5, while layer 4Cβ and 4Cα neurons give rise to axons that terminate almost exclusively in layer 6. Within layer 6, the terminals from 4Cβ and 4Cα neurons are restricted to different sublaminar divisions of the layer. Terminals from 4Cβ neurons are found restricted to the upper half of layer 6 while terminals from 4Cα neurons are found restricted to the lower half of layer 6. These divisions of layer 6 correspond to those previously identified as receiving restricted inputs from the parvocellular and magnocellular layers of the LGN respectively. Supported by EY06821 and EY06661.

646.12

EXPANDED REPRESENTATION OF THE FOVEA IN BOTH THE MAGNOCELLULAR AND PARVOCELLULAR SUBDIVISIONS OF THE RETINOCORTICAL PROJECTION OF THE MACAQUE. P. Azzopardi & A. Cowey (SPON: European Brain and Behaviour Society), Dept. of Exptl. Psychology, University of Oxford, Oxford OX1 3UD, UK.

A well known feature of the retinocortical projection is that in angular terms the fovea is overrepresented in the striate cortex. Recent evidence suggests that more cortex is allocated to the representation of the most central retina than expected from the distribution of retinal ganglion cells alone (Azzopardi & Cowey 1993 Nature, 361, 719-721). We have now extended these findings to the parvocellular and magnocellular subdivisions of the retinocortical projection.

A retrograde, transneuronal tracer (WGA-HRP) from cortex to retina was used to relate precisely regions of the striate cortex with corresponding regions of the retina in three macaque monkeys. This enabled us to evaluate the amount of cortex per ganglion cell projecting in the central few degrees of the visual field (mean 8.6°, range 5.7° - 11.9°) and the amount of cortex per ganglion cell in the whole of the projection. The ratio of these two quantities is called the expansion ratio (ER). Overall, ERs ranged between 2.13 and 3.02 (mean 2.71), and the fact they are greater than 1.0 shows that the representation of the central field is expanded between retina and cortex. In the magnocellular subdivision, ERs ranged from 2.6 to 3.62 (mean 3.11), and in the parvocellular subdivision they ranged from 2.09 to 3.00 (mean 2.67). Thus, the central fields of both subdivisons are expanded.

This result eliminates the most trivial explanation of central expansion in the retinocortical projection, which is that it could arise simply because the two (parvocellular and magnocellular) retinal maps, represented by populations of ganglion cells with different distributions, are expanded to different extents in order to preserve the topographic co-registration of the inputs to the striate cortex. It suggests that the expansion subserves some visual function.

646.13

DIFFERENTIAL DISTRIBUTION OF GLUTAMATERGIC SYNAPSES BETWEEN CYTOCHROME OXIDASE (CO)-RICH PUFFS AND CO-POOR INTERPUFFS IN PRIMARY VISUAL CORTEX OF MONKEYS. F. Nie, R. Curtis* and M. Wong-Riley, Dept of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

Glutamate is a major excitatory neurotransmitter in many regions of the brain. Our previous study indicated that puffs contained more asymmetric synapses (presumed excitatory) than interpuffs. In the present study, a double labeling technique combining cytochrome oxidase with glutamate was used to determine whether the distribution of glutamate-positive synapses is correlated with a difference of CO activity between puffs and interpuffs at the EM level. Our results showed that almost all glutamate-positive synapses originated from asymmetric axonal terminals. Quantitative analysis demonstrated that there were more glutamate-positive synapses in puffs than in interpuffs: 21.33±1.5 in puffs versus 14.53±1.2 in interpuffs within 100 um^2 of neuropil ($P<0.005$). Furthermore, glutamate-positive axonal terminals in puffs contained a higher proportion of darkly CO-reactive mitochondria than those in interpuffs, indicating that they might be more active in puffs. In addition, glutamate-positive axosomatic synapses selectively targeted GABAergic neurons, which contained more darkly reactive mitochondria. Thus, the present study suggest that glutamatergic synapses play an important role in excitatory transmission within puffs and interpuffs. Glutamate may be the neurochemical basis for higher metabolic demands related to greater depolarizing activity in puffs than in interpuffs. (Supported by NIH grants EY05439 and NS18122).

646.14

ANATOMICALLY BASED NEURAL NETWORK MODEL FOR GENERATION OF ORIENTATION SPECIFICITY IN MONKEY PRIMARY VISUAL CORTEX (V1).
Q. Wu*, J.S. Lund and P.Hadingham, Inst. of Ophthalmology, Univ. of London, London EC1V 9EL and Dept. of Computer Science, Univ. of Western Australia

To understand the correlation between structure and function in primary visual cortex (V1) of primates, we are attempting to develop neural network models which are closely based on anatomical observations and able to simulate physiological results. Our previous modeling study of the primary thalamic recipient layer 4C in V1 suggests that spiny stellate neurons in layer 4C accomplish weighted summation of distinct input sets from LGN through dendritic overlap to produce a physiological gradient of contrast sensitivity and receptive field size. We hypothesize that the strategy of combining distinct input sets to produce a gradient is quite ubiquitous in cortical information processing and may also apply to the issue of generation of orientation specificity in V1. Human psychophysical studies demonstrate that there is a meridional anisotropy (or oblique effect) for discriminating horizontal and vertical orientations versus oblique ones, and that the latency associated with horizontal and vertical orientations is shorter than for other orientations. Based on this evidence, we propose that orientation specificity in primate V1 is generated in two stages: the first stage (in mid 4C) is the generation of two tuning functions centered around horizontal and vertical orientations, and the second stage uses these functions to produce a continuum of all possible orientations. In our model we incorporate the lateral spread of axon collaterals from spiny stellate neurons in mid 4C as the anatomical substrate for producing the two orthogonal orientations in the first stage, and lateral dendritic overlap of spiny stellate and pyramidal cells in layers upper 4Cα and 2&3 for combining these two orthogonal orientations to produce a full continuum of orientation specificities in the second stage. Compared to the Hubel and Wiesel model of generation of orientation specificity, our model is more economical in terms of neuronal wiring and has anatomical, physiological and psychophysical support. Supported by MRC G9203679N and NIH-EYI 10021.

646.15

MONOCLONAL ANTIBODY CAT-305 LABELS GENICULOCORTICAL RECIPIENT LAYERS IN PRIMARY VISUAL CORTEX OF CAT, MONKEY AND FERRET S. Hockfield*, C. Blakemore† and P.C. Kind, Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510 and †Univ. Lab. of Phys., Oxford, UK

Monoclonal antibody Cat-305 labels cortical layer IV of areas 17 and 18 in the cat (Cerebral Cortex, 1994). This restricted localization suggested that the Cat-305 protein may be associated with the presynaptic terminals of axons originating in the dorsal lateral geniculate nucleus (dLGN). To address this possibility we have now examined Cat-305 staining in the cerebral cortex of macaque monkey and ferret, and in the cortex of cats following lesions in the dLGN.

As in the cat, Cat-305 staining is also restricted to primary visual cortex in the adult ferret and monkey. In the ferret, immunoreactivity appears as a dense band of label through layer IV in areas 17 and 18. In macaques, Cat-305 labels two bands, both of which are restricted to striate cortex. Laminar analysis demonstrates that the more superficial band of staining demarcates layer IVa and the deeper band layer IVc, corresponding to the laminar termination of geniculocortical afferents.

To determine whether the Cat-305 antigen is associated with or dependent on presynaptic elements in layer IV, unilateral ibotenic acid lesions were made in the dLGN of adult cats. Three weeks following the lesion, Cat-305 immunoreactivity was markedly reduced in the cortex ipsilateral to the lesion compared to the contralateral, non-lesioned hemisphere and to normal controls. Staining for monoclonal antibody Cat-301 and GFAP in layer IV were not affected by the lesion, indicating that there was little or no degeneration of cortical neurons. The decrease in Cat-305 immunoreactivity in cortical layer IV after dLGN lesions suggests that the loss of the Cat-305 antigen is a result of degeneration of the presynaptic terminals of geniculocortical afferents. (Supported by EY06511, MRC, UK, and Oxford McDonnell-Pew Centre for Cognitive Neuroscience)

VISUAL PSYCHOPHYSICS AND BEHAVIOR II

647.1

ASSESSMENT OF VISUAL ABILITIES IN ALZHEIMER'S DISEASE AND MENTAL RETARDATION. S.A. Shimp* & S. Oross III. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Alzheimer's patients exhibit visual losses including elevated contrast sensitivity thresholds, blue-yellow color deficiencies, and elevated thresholds for detecting coherent motion in kinematograms. To assess the degree to which profiles of visual losses may differentiate between clinical populations, we have begun comparing the performance of Alzheimer's patients to three groups of adults with mental retardation: unspecified etiology, young (<35 years) Down Syndrome, and old (>35 years) Down syndrome. Measurements of visual acuity, contrast sensitivity, color perception, and detection of motion coherence were obtained. Detection of motion coherence was assessed using a 2AFC task in which subjects had to determine which one of two simultaneously presented kinematograms contained coherent motion (signal strength varied between 1 to 100%). The other kinematogram contained only random noise. Relative to standards obtained with control subjects, all groups exhibited elevated visual acuity, contrast sensitivity and motion coherence detection thresholds. Variations in these threshold elevations suggest that profiles of visual losses may differentiate between the groups. Discussion concerning the construction and use of a battery of visual tasks for assessing the neurological status of various clinical populations will be advanced.

Supported by HD29556

647.2

SPATIAL SUMMATION ACROSS THE VERTICAL MERIDIAN AFTER COMPLETE OR PARTIAL HEMISPHERECTOMY. F. Tomaiuolo^, A. Ptito*, T. Paus and M. Ptito. Istituto di Fisiologia Umana, Universita' di Verona, Verona, Italy^. Montreal Neurological Institute, McGill University, Montreal H3A 2B4, Canada.

Three patients with right hemispherectomy, one with right-sided removal of the temporo-parietal-occipital cortex and four normal control subjects were tested for spatial summation in the visual field across the vertical meridian. The double stimulus paradigm described by Tassinari et. al (1984) and Marzi et al (1986) was used. Monocular eye fixation was monitored with a pupil/corneal reflection tracking system and all subjects used their right eye. Single or pairs of flashes (LEDs) were randomly presented during 10 msec at 10° or 30° to the left or to the right of the fixation point, either within or across hemifieds. Subjects had to respond as fast as possible to the stimuli by a keypress. As expected, control subjects reacted more quickly to double stimuli than to single ones. Although the patients never responded to stimuli in the blind field, they were significantly faster when two flashes were displayed in both hemifield rather than when a single flash was presented in the intact hemifield only. This summation effect across hemifields in subjects in whom a whole cerebral hemisphere has been removed or deafferented supports the possibility that alternate visual structures (e.g. superior colliculi) contribute to residual vision in the blind field. Supported by NSERC OGPNIN 012.

647.3

PROCESSING PROFILES AT THE RETINAL VERTICAL MIDLINE OF A CALLOSOTOMY PATIENT. R Fendrich*, CM Wessinger, MS Gazzaniga. Center. for Neuroscience, UC, Davis 95616.

Previously, we reported that a callosotomy patient could not compare small outline shapes presented 15' from the retinal vertical midline with comparison shapes in the opposing visual field. This foveal splitting argued against the attribution of macular sparing to a zone of nasotemporal overlap. We now report evidence that a narrow zone of overlap does occur at the human retinal vertical midline, but is limited in its ability to convey visual information to the cerebral hemisphere contralateral to each hemiretina. Pairs of 2°x 2° square wave gratings ranging from 1 to 8 cpd were displayed to a callosotomy patient with one grating in each visual field. The subject judged whether the gratings had the same orientation. Retinal stabilization insured the grating patches remained properly lateralized. When the gratings were presented for 200 msec *or* their medial edges were 2° from the retinal vertical midline, the subject performed at or near chance. When presentations lasted 2 seconds *and* the grating medial edges were 1° from the midline, above chance performance was obtained with the 1, 2 and 4 cpd gratings. Performance degraded when both gratings were displaced 2° upward from the horizontal meridian, but was unaffected or improved by a like downward displacement. Remarkably, accuracy rates improved when the gratings were offset vertically away from each other (one 2° up, one 2° down). Supported by NIH/NINDS P01 NS17778-12 & PHS NS31443-01

647.4

Residual Vision in The Blind Field after Partial or Complete Hemispherectomy. C.M. Wessinger*[1], R. Fendrich[1], A. Ptito[2], and M.S. Gazzaniga.[1]

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[2]Montreal Neurological Institute, Montreal H3A 2B4, Canada.

One patient with left functional hemispherectomy (JB) and one with right-sided removal of the temporo-parietal-occipital cortex (SE) were tested for residual vision in their blind field. Using image stabilization procedures, we identified a zone of residual vision along the vertical meridian in each patient. The lateral edge of this zone was generally within 3.5° of the vertical meridian, although in both patients we found a local area of detection beyond 3.5°. The region of residual vision was not identical in the two patients. In JB it was confined to the superior quadrant; in SE it was present in both superior and inferior quadrants. Each patient could detect stimuli and correctly perform shape discriminations within the zone of residual vision, but not outside of it. In contrast, neither patient was able to name complex line drawings presented within the zone.

Acuity profile assessments in each patient's seeing field argue against the attribution of these abilities to eccentric fixation. Given the reported complete absence or deafferentation of a hemisphere contralateral to the residual vision, alternate structures would be mediating this residual vision. The superior colliculi and/or the remaining cerebral hemisphere could be candidate structures.

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647.5

FURTHER RESULTS FROM A TRAINING OF BRIGHTNESS PERCEPTION AND REACTION TIMES IN HOMONYMOUS HEMIANOPIA. L. Dornheim, F. Schmielau*. Inst.for Medical Psychology, Med. Univ. of Luebeck,Luebeck Germany, GER 23538.

Visual field (VF)defects in patients with lesions of the post- chiasmatic visual system demonstrate a reduction of perceived brightness at the border of the residual VF. Dornheim and Schmielau (1994) showed that visual functions can be improved by a rehabilitation training using a magnitude estimation technique of apparent brightness. Now further results are demonstrated for a female patient(age 73 yrs.) who suffered from an incomplete homonymous hemianopia of the right VF caused by stroke.Monocular scaling of threshold and super threshold stimuli and a specific training of light-dark discrimination(70 therapy sessions of 45 min. each) by a computerized perimeter within the lower right quadrant caused a VF enlargement, a reduction of reaction times and threshold as well as an increase of perceived brightness within the trained area and an increase of visual acuity. The authors explain the improvements by recovery of visual cortex neurons due to repetetive stimulation during selective attention.

647.6

VISUAL EVOKED POTENTIALS (VEP) TO MOVING SINE-WAVE GRATING AT EQUILUMINANCE. Doris Braun*,Sect. Visual Science, University Eye Clinic, Röntgenweg 11, D-72076 Tübingen, Germany

Cavanagh et al. (1984) reported that at equiluminance perceived slowing of sine-wave gratings with low spatial frequency is proportional greatest for slow drift rates (at or below 1 deg/sec).

The effect of chromatic contrast was investigated with motion-onset VEPs recorded in three subjects in response to chromatic and luminance sine-wave gratings moving at 1 and 8 deg/sec across a 19 in colour monitor screen. For each subject the point of equiluminance was defined by equalizing the red and green luminance of a checkerboard pattern flickering at 12 Hz. Chromatic gratings (1.6 cpd) were generated by superimposing red and green sinusoidal gratings 180 deg out of phase. While mean luminance was held constant (24 and 15 cd/m2), luminance of red and green was modulated around equiluminance. For comparison gratings with different luminance contrasts were also tested

4 channel EEG-recording equipment amplified cortical potentials recorded bipolarly against the ear lobe by gold plated cup electrodes placed over the occipital scull, 3 cm above, and 3 and 5 cm left or right of the mid-sagittal. Potentials of 100 sweeps were separately band-pass filtered (1-80 Hz) for each channel and digitized at 250 Hz. To avoid motion adaptation a 8% duty cycle (200 msec motion & 2500 msec stationary phase) was used.

A motion-related negative component as described by Kuba & Kubova (1992) was found in the VEPs of all subjects even at equiluminance. It had with a negative peak latency of about 180 msec for 8 deg/sec and over 200 msec for 1 deg/sec. At equiluminance the duration of the negative component increased and the peak latency shifted by about 10 msec for 8 deg/sec and up to 40 msec for 1 deg/sec compared to gratings with higher chromatic or luminance contrast.

657.7

DYSPHONEIDETIC DYSLEXICS DEMONSTRATE A MAGNOCELLULAR PATHWAY DEFECT. W.H. Ridder,III, E. Borsting, M. Cooper, B. McNeel, and P. Simmons*. Southern California College of Optometry, Fullerton, CA., 92631.

A review of the literature reveals that approximately 75% of dyslexics have a processing deficit in the magnocellular pathway (Lovegrove et al., 1990). In addition, a recent report demonstrated that the dyseidetic dyslexic subtype, which comprises 10 to 30% of the dyslexic population, does not have an abnormal magnocellular pathway (Ridder et al., 1993). These results suggest that the other two subtypes of dyslexia (dysphonetic and dysphoneidetic) should have a magnocellular pathway deficit. The purpose of this study was to determine if dysphoneidetic dyslexics exhibit a magnocellular pathway defect. Five dysphoneidetic dyslexics and five age and sex matched, normal controls were examined. All subjects had normal intelligence and educational opportunities, no ocular disease, sensory impairments, overt psychological problems, or systemic pathology. The presence of dyslexia was determined with the Adult Dyslexia Test. Contrast sensitivity functions were determined with vertically oriented sine wave gratings (0.5, 1.0, 2.0, 4.0, 8.0, and 12.0 c/deg drifting at 1.0 and 10.0 Hz) and for a full field flickering stimulus (5, 10, 15, 20 and 25 Hz) by employing a temporal, 2 alternative, forced-choice technique. Unpaired t-tests demonstrated significant differences existed between the dysphoneidetic dyslexics and the normal controls for the data obtained at low spatial and high temporal frequencies. These results suggest that dysphoneidetic dyslexics have a magnocellular pathway defect.

647.8

SPATIAL FREQUENCY TUNED MECHANISMS OF THE RED-GREEN CHANNEL ESTIMATED BY OBLIQUE MASKING. R.L.P. VIMAL[1] and R. PANDEY*[1,2], New England College of Optometry, Boston, MA 02115

Purpose. Last year, we reported that threshold elevation (TE) curves for the Red-Green (R-G) Channel fell into 5 empirical groups (ARVO '93).The curves were broader at 11 years of age (OSA '93) than were those of adults. Our purpose was to (i) measure the TE curves at 12 years of age with the same subject, and (ii) to extract the spatial frequency (SF) tuned mechanisms for all 3 observers. **Methods.** Threshold contrasts were measured with oblique masking and the method of constant stimuli using a color monitor system. We used the STEPT optimization procedure (Chandler, QCPE, 1976) to estimate the mechanisms. **Results.** We found that (i) TE curves of the boy were narrower at 12 years of age than those at 11 years, and were closer to those of the adults, (ii) his contrast sensitivities were still lower than those of adults, (iii) as the number of SF tuned mechanisms was increased from 1 to 8, the correlations between the data and the best fit and the multiple-R^2 increased and the sum of the square of the relative error [(data-fit)/max (data, fit)] per data point decreased, (iv) a minimum of 6 SF tuned mechanisms [one lowpass to satisfy the chromatic contrast sensitivity function (CCSF), and 5 bandpass (peak SFs: 0.125, 0.5, 2, 4 and 8 cpd) to satisfy the 5 groups of the bandpass TE curves] appeared to achieve a reasonable fit, and (v) a large number of mechanisms helped to bring the best fit amplitude and bandwidth of the TE curves closer to the data by a small amount. **Conclusions.** We conclude that (i) TE curves of the R-G channel for the 12 year old boy were close to adult levels but, the amplitude of his CCSF was still developing, (ii) a minimum of 6 SF tuned mechanisms for the R-G channel were necessary to simultaneously satisfy the CCSF and TE data. [Supported by NEI, NIH grant R01 EY09511-03].

647.9

THE EFFECT OF RETINAL ECCENTRICITY ON IMPLICIT AND EXPLICIT JUDGEMENTS OF OBJECT WIDTH. K.J. Murphy*and M.A. Goodale. University of Western Ontario, London, Ontario, Canada N6A 5C2.

We report here evidence to suggest visual sensitivity in normal subjects can vary as a function of response mode. Subjects estimated the width of five different rectangular objects presented at various retinal eccentricities. Three different modes of responding were used. In one block of trials, subjects were asked to indicate the width of the object by stating which of the five different blocks (numbered 1 to 5) it was. In another block of trials, subjects indicated how wide they thought the block was by opening their forefinger and thumb to the same width. In addition to these two explicit judgements of object width, another more implicit judgement was obtained by simply requiring subjects to reach out and grasp the object using a precision grip (with forefinger and thumb). Based on previous work, it was expected that grip aperture would be strongly correlated with object width. In this case and in the previous manual estimation condition, the opening between the finger and thumb was measured using standard opto-electronic recording (WATSMART). The effect of retinal eccentricity on size estimation varied as a function of the response used. When verbal responses were required, size estimates decreased as a function of retinal eccentricity; when manual estimates were required, there was little change. When subjects were required to grasp the object, their grip aperture increased with eccentricity. In short, increasing retinal eccentricity had different effects on judgements of object width, depending upon the response mode. This finding lends further support to the proposal that visual perception and the visual control of action depend on different transformations of visual information (Goodale, *Curr Opin Neurobiol 3:* 1993). This research was supported by a grant from MRCC to MAG.

647.10

INFLUENCE OF NON VISUAL CUES ON BILATERAL SYMMETRY DETECTION: THE EFFECT OF PROLONGED WEIGHTLESSNESS. G. Leone[1], M. Lipshits[2], V. Gurfrinkel[2] and J. R. Duhamel[1*]. 1 Laboratoire de Physiologie de la Perception et de l'Action, CNRS Collège de France, Paris, France; 2 Institute for Problems of Information Transmission, Russian Academy of Sciences, Moscow, Russia;

One of the most striking properties of bilateral symmetry detection is the spatial anisotropy in the performance of the visual system. Thus, the shapes of error rates or response times (RT) as a function of symmetry axis orientation show a clear oblique effect, i.e there is superior performance for a retinally vertical/horizontal orientation of symmetry axis than for oblique orientations. This oblique effect is assumed to be a basic property of the visual system. We investigated, with eight cosmonauts, if performance in symmetry detection could be altered by a prolonged exposure to weightlessness (between 4 days to 7 months). The cosmonauts were maintained in a body restraint system, facing a computer screen where 2D polygons were displayed during 50 ms. The subjects were asked to press the right button if shape was symmetrical or the left one if asymmetrical. We compared RTs and error rates for in-flight versus ground tests.

The results indicate an overall increase in RT (and a decrease in accuracy) in weightlessness that can be related to an increase in computational load associated specifically with the visual symmetry detection task. We found also that the significant advantage of a vertical symmetry axis over an horizontal one vanished in microgravity. Nevertheless, some individual differences appeared. In a ground control experiment, naive subjects performed the experiment in an upright posture and in a horizontal supine posture. The results again indicated a trend towards a decreasing vertical orientation advantage in the latter condition. Our results suggest that the processing of low level visual information integrates also non-visual information. We suggest that otolithics inputs could dynamically modify the properties of some orientation selective cells in the visual cortex.

647.11

VISUAL REPRESENTATIONS FOR CLASSIFYING 3D OBJECTS.
Hiroshi Ando* and Satoshi Suzuki.
ATR Human Information Processing Research Labs., Kyoto, Japan.

There have been increasing interests in how the brain represents visual information for object recognition. Physiological experiments have shown that IT neurons may represent local features or prototypes of learned objects (Fujita et al., *Nature*, 360, 343, 1992; Logothetis, et al., *Neurosci. Abstr.*, 1993). Psychophysical studies have indicated that the visual system may extract view-specific prototypes of 3D objects in *identification* tasks where human subjects differentiate the target object from distractors (Edelman & Bülthoff, *Vision Research*, 32, 2385, 1992). This research examines through psychophysical and computational investigations whether the visual system may extract global prototypes or local distinctive information in 3D object *classification* tasks.

In the psychophysical experiments, subjects were trained to classify 2D views of a fixed set of wire-frame 3D objects. The results show that some views yield higher error rates. To investigate what representations are extracted, we examined three network models, i.e., the MLP (multi-layer perceptron) network which can extract local linear features, the GRBF (generalized radial basis function) network which extracts global prototypes, and an extension of HBF (hyper basis function) network that allows to emphasize various local templates. The simulations on the same objects used for the psychophysics show that with a small number of hidden units the MLP and HBF networks perform better than the GRBF network. The results suggest that it is useful to extract local distinctive information that is stable over different view directions, such as acute angles formed by successive segments. Analyses of errors reveal that some subjects made errors at particular views similar to the views these networks fail, suggesting that the visual system may extract local distinctive information for classifying 3D objects.

647.12

MAGNETIC AND ELECTRIC OSCILLATORY RESPONSES TO FLASH STIMULATION: EFFECTS OF LUMINANCE AND ECCENTRICITY. L. Lopez[1]* M. Peresson[1], A. Pasquarelli[2], G.L. Romani[1], W.G. Sannita[3,4] [1]ITAB, University of. Chieti, Italy, [2]IESS-CNR, Rome Italy, [3]DISM, Center for Cerebral Neurophysiology University of Genova, Italy, [4]Dept. Psychiatry SUNY, Stony Brook, NY USA,.

Flash stimulation elicits fast frequency (100-120 Hz) oscillatory responses, taht can be recorded electrically from retinal and scalp locations. Magnetic recordings in the occipital regions have suggested that oscillatory responses recorded electrically at scalp locations are originated in the cortex. In this study we wanted to further characterize the origin and possible functional role of these responses.

We measured electric and magnetic responses to full-field and to various spots that progressively varied in area and eccentricity from point of fixation (from 5º to 30º), alternatively in the upper left and right quadrants of visual field. Eight healthy volunteers were studied. Stimulation was delivered monocularly, to the left eye with a Grass PS22 (10 s duration, 0.5 Hz, 3.5 cd/m^2 (at full-field) 90 dB masking white noise) The stimuli could be delivered without moving the subject. One position, covering an area of 250 mm^2 over the left occipital lobe, was chosen as a standard for magnetic recording.

Electric and magnetic oscillatory responses during full-field stimulation, were obtained. When changing the stimulus to eccentric and smaller spots, the retinal oscillatory potentials tended to disappear, together with ERG, while the cortical oscillatory responses could still be detected with features that varied as a function of eccentricity and luminance. Such findings would indicate that the mechanisms underlying the generation of oscillatory responses in the cortex are partly independent of the retinal oscillatory potentials and that a magnification occurs in the visual system.

647.13

DYSOXIA AND DYSBARIC CONDITIONS AFFECTS RED/GREEN COLOR VISION. Nico Schellart[1,3] and Ad van der Kley[2] (SPON: Europ. Neurosci. Ass.). [1]Lab. Med. Physics, [2]Dept. Surg., Univ. Amsterdam, [3]Dutch Found. Diving Res., Amsterdam. The Netherlands NL 1105-AZ.

It is known that acute and semi-acute hypobaric conditions reduce the (relative) sensitivity to green light, which lasts for at least 3 days. To what extent red/green color vision is affected under acute normobaric hyperoxia and hypoxia, hyperbaric conditions, and chronically increasing hypobaric conditions has not been reported before. The red-green balance-flicker (16 Hz) fusion point was measured psychophysically for normal subjects with the portable OSCAR color vision tester.

The red/green (R/G) modulation sensitivity ratio is enhanced by normobaric acute hypoxia (10-13 kPa O_2, 0-11% increase, 3 subjects, Sa_{O2} 58-83%) and reduced by normobaric hyperoxia (60-100 kPa O_2, 2-10% decrease, 6 subjects). The effects are maximal after 20 min of exposure. Exertion (>30 min 150 W) also results in an enhanced (2-9%) R/G ratio. Hyperbaric air pressures, examined up to 520 kPa, reduce less the ratio. An hypothetical explanation of this lesser reduction is an opposite effect of N_2 narcosis (and hypercapnia) at air pressures ≥ 300 kPa. Above 100 kPa O_2 the effect of O_2 saturates (5 subjects). This may partly be due to hypercapnia since normobaric CO_2 mixtures (2 and 4 kPa) resulted in an increased sensitivity ratio (2 subjects). Color discrimination, except for acute hypoxia, was hardly affected. Surprisingly, long-term hypobaric conditions (2 weeks trekking from 800 and 5400 m altitude with altitude adaptation, 2 subjects) resulted in a 4% decrease of the R/G ratio superimposed at the diurnal rhythm.

647.14

Color Sensations in Honeybees? Werner Backhaus*, Institut für Neurobiologie, c/o Institut für Biophysik, Freie Universität Berlin, Thielallee 63, 14195 Berlin, Federal Republic of Germany.

Neuronal color coding and color choice behavior of honeybees is very well described by the color theory for the bee (Backhaus, 1991, Vis. Res. **31**, 1381; 1992, **32**, 1425). The results of ordinary color training experiments are described by the color theory on the basis of electrical excitations of two color opponent coding neurons. The results of double color training experiments, in which two color stimuli were trained simultaneously, cannot be explained exclusively on the basis of the electrical properties of neurons. The choices rather appear to be related to color sensations (Backhaus & Kratzsch, 1993, Proc. 21st Göttingen Neurobiol. Conf., **39**, New York: Thieme).

A quantitative model is presented which describes color sensations in bees to consist of unique-colors, as in humans (Hering, 1905, Leipzig). Since bees ignore brightness differences in color training experiments, only five unique colors are needed. The amounts of unique-colors are assumed to be linearly related to the electrical excitation values of the two color opponent coding neurons. In double color training experiments, honeybees appear indeed to learn the unique-colors which the two trained colors have in common and to choose the stimuli in the tests according to the amounts of these unique-colors. Quantitative predictions for further experiments are derived from the model for further testing the hypothesis about the existence of unique-color sensations in the honeybee.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: REFLEX FUNCTION I

648.1

A STUDY OF CUTANEOUS INHIBITORY INPUT TO A SPINAL NOCICEPTIVE MOTOR SYSTEM
H.-R. Weng* and J. Schouenborg. Department of Physiology and Biophysics, University of Lund, Sölvegatan 19, S-22362, Lund, Sweden.

The nociceptive withdrawal reflex system has been extensively studied from both motor and sensory aspects. A "modular" organization of this system has recently been described, each "module" acting on a single or a few synergistic muscles (Schouenborg et al, News in Physiological Sciences, in press, 1994). Each "module" receives excitatory input from the skin area withdrawn by its effector muscle(s). We have now studied the cutaneous inhibitory input to this motor system in the decerebrate spinal rat (N=15).

Reflex responses in single hindlimb muscles evoked by sustained noxious pinch (2N, 1mm^2) were recorded using electromyographic techniques. Inhibitory receptive fields of mm.peroneus longus and brevis, extensor digitorum longus, tibialis anterior and biceps posterior were mapped using mechanical pinch (up to 2.5N, 1 mm^2) and CO_2-laser thermal stimulation (50-300 mJ, 3 mm diameter).

For each muscle, the reflex responses could be inhibited by mechanical and thermal stimulation of the skin area which is moved towards the cutaneous stimulation on contraction of the muscle. Noxious stimuli produced stronger inhibitory effects than innocuous stimuli, indicating a nociceptive inhibitory input.

In conclusion, the spatial organization of the excitatory and inhibitory receptive fields of withdrawal reflexes to single muscles reflects the movement pattern caused by the muscles. Both the excitatory and inhibitory actions on this system protect the body from injury.

648.2

ESTIMATED NET SYNAPTIC POTENTIAL IN HUMAN MOTONEURONES.
K.S. Türker* and H.B. Cheng, Department of Physiology, University of Adelaide, S.A.5005,Australia

Although recording single motor unit activity from human muscles is technically simple, quantifying reflex responses has not been easy. The most commonly used method for quantifying the reflex response has been the peri-stimulus time histogram (PSTH) and its cumulative sum (CUSUM) which illustrates the change in the firing probability of the single motor unit in response to a stimulus. According to this method, an increase in the firing probability relative to the pre-stimulus period indicates excitation and a reduction represents inhibition. However, it has been demonstrated that the stimulus may induce synchronous activity of the unit and this may induce several peaks in the histogram many of which are not due to the stimulus-induced extra activity in the motoneurone. These peaks are, in fact, due to resumption of normal discharge activity from a fixed point of time "the autocorrelation effect". The frequency of firing of motor units, however, avoids these synchronous activity-induced false peaks and troughs. Furthermore, the frequency of firing of a motoneurone integrates the excitatory and inhibitory effects and represents the net effect of the stimulus on the membrane potential. An increase in the frequency of discharge of a motoneurone indicates that the stimulation has a net excitatory effect, and vice versa. Therefore, in order to estimate the net synaptic potential induced by the low-threshold stimulation of a mixed nerve and the oral mucosa the raw frequency values that belong to individual trials of a reflex experiment are superimposed, averaged and normalized to the afterhyperpolarization of 10 mV. This procedure brings out the estimated net synaptic potential (ENSP) that is initiated in the motoneurone by the activation of the afferent system. Normalization procedure allows the comparison of such responses between experiments.

648.3

REFLEX RESPONSES TO LOW-INTENSITY STIMULATION OF THE SURAL, TIBIAL AND PERONEAL NERVES DURING HUMAN WALKING. B.M.H. Van Wezel, F.A.M. Ottenhoff and J. Duysens*. Dept of Medical Physics & Biophysics, University of Nijmegen, 6500 HB Nijmegen, The Netherlands.

The skin of the foot is innervated by 3 nerves, namely the sural, tibial and peroneal nerve. Most studies on the reflex responses to stimulation of these nerves during human locomotion have concentrated on only one of these nerves. The question arises whether the reflexes, and their phase-dependent modulation during locomotion, show differences for these different nerves. The purpose of the present study was to compare the reflex responses to stimulation of these 3 nerves in the same subjects, while walking on a treadmill.

Both facilitatory and suppressive reflex responses with a latency of about 80 ms were observed following stimulation at 2 times perception threshold of all 3 nerves. In general, the responses for the 3 nerves are of the same order of magnitude. The pattern of the responses can be different, however. For example, the responses in biceps femoris (BF) were generally facilitatory during the whole step cycle, whereas responses in BF to tibial nerve stimulation at end swing were often suppressive. In tibialis anterior suppressions at end swing were observed in the response pattern for all three nerves. During the stance phase, however, a clear difference was found: generally no responses were found for sural and peroneal nerve stimulation, but large facilitatory responses were often found during mid to end stance for tibial nerve stimulation.

In conclusion, the low-threshold afferents from the 3 nerves, innervating the skin of the foot, have both excitatory and inhibitory pathways to various leg muscles. The phase-dependent reflex modulation can be different, presumably by adjusting the balance of activity in these opposite paths. This suggests the existence of differences in the reflex pathways for the different nerves, which can be controlled separately during the course of the step cycle.

648.4

DOES THE NERVE BRANCH PATTERN REFLECT A RE-MODELING WITHIN THE MOTONEURON POOL - MUSCLE COMPLEX OF OLDER RATS? **S. Vanden Noven*, N.K. Latham and M.H. Partidas. Sch. of Physical & Occupational Therapy, McGill Univ., Montreal, QC H3G 1Y5.**

In this laboratory, the medial gastrocnemius (MG) motoneuron pool-muscle complex in rat (Sprague-Dawley; Fischer 344) is being used in various morphological and electrophysiological studies in order to further our understanding of the mechanisms underlying the function of aged motoneurons. The two extra-muscular nerve branches innervating the MG muscle in the Sprague-Dawley rat subserve a physiological and histo-chemical compartmentalization (Vanden Noven et al., 1994). The afferent contribution to the two MG nerve branches has also been found to differ between rats of different ages (Vanden Noven, 1993). In this study, dorsal root volleys (Group I stimulus strength) and the MG muscle nerve branch pattern was compared between young adult (n=5) and older animals (19-23 mo; n=5). Neural potentials, evoked by stimulation of the L4-L6 dorsal roots, were recorded from each of the two nerve branches. The MG muscle nerve branch pattern was more complex and the topographic distribution of DR afferents entering the spinal cord was disrupted in the older age group. Taken together with other data on motoneuron and muscle properties, results suggest that the MG complex undergoes a re-organization with age. **(Supported by NSERC Canada).**

648.5

EFFECTS OF THE SEROTONIN-2 AGONIST DOI ON S VS. F MOTONEURONS *IN VIVO*. R.H. Lee, J.F. Miller, W.Z. Rymer and C.J. Heckman*. Physiology, Northwestern Univ. Med. Sch. and VA, Lakeside Hospital, Chicago IL, 60611.

The orderly recruitment of motor units is largely due to systematic differences in the threshold currents (rheobases) of slow twitch (S) vs. fast twitch (F) motoneurons (MNs). Serotonin (5HT) agonists can greatly reduce rheobases and our goal was to investigate the relative amount of this decrease in rheobase in S vs. F MNs.

DOI, a 5HT-2 agonist, has long lasting actions *in vivo* (>2 hrs; see the companion poster by Miller et al.). The intrinsic properties of two populations of triceps surae MNs in the decerebrate cat were compared, one in control conditions and one following i.v. administration of DOI (0.5-1 mg/kg). The preparation was spinalized at T10 to eliminate the effects of descending 5HT tracts.

The average rheobase of the DOI population (8.2 nA; range: -8 to 21 nA; n=62) was significantly less (t-test, $p < 0.005$) than that of the control population (12.8 nA; range: 0 to 32 nA, n=42). DOI MNs with negative rheobases (n=12) had sustained rhythmic firing in the absence of any injected current. Negative rheobase MNs had slow conduction velocities (CVs) and were probably type S. However, the slopes of the regression lines for the rheobase-CV relations were not significantly different in the two populations, indicating that the effect of DOI was approximately the same in both S and F MNs. These data suggest that 5HT input can enhance the excitability of the motoneuron pool without altering its recruitment order.

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648.6

EFFECTS OF THE SEROTONIN AGONIST DOI ON FORCES AND STRETCH REFLEXES OF THE DECEREBRATE CAT. J. F. Miller*, K.D. Paul, W.Z. Rymer and C.J. Heckman. Physiology, Northwestern Univ. Med. Sch. and VA, Lakeside Hospital, Chicago IL, 60611.

5-HT-2 agonists produce subthreshold depolarizations of spinal motoneurons. Such actions should substantially increase the input-output gain of the motoneuron pool. We therefore investigated the effect of the 5HT 2A/2C agonist DOI on the reflex output of the triceps surae muscles in the precollicular decerebrate cat preparation.

The stretch reflex and background force of extensors are greatly enhanced in the decerebrate cat. Intrathecal administration of DOI (1-5 µmol, cumulative) further increased stretch reflex amplitude by 30-50%. Even more striking changes in background tension generation were observed. For example, soleus background force typically increased 2-4 fold after DOI, and in one experiment exceeded 20 N, which is near the maximal tetanic force for this muscle. Similar results were seen following i.v. administration of DOI (0.5-1 mg/kg).

The duration of DOI's actions by either route typically exceeded 120 min. Ketanserin (0.5-1 mg/kg i.v.) transiently reversed the effects of DOI for 30-45 min. In one experiment, the spinal cord was transected at T10, resulting in a great reduction in background force and stretch reflex amplitude. DOI (1 mg/kg i.v.) restored force and reflexes to their pre-spinalized level. These results show that serotonergic synaptic input can greatly enhance the output of the motoneuron pool.

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648.7

DISYNAPTIC AUTOGENIC AND HETEROGENIC EXCITATION FROM GROUP I AFFERENTS IN HINDLIMB EXTENSOR MOTONEURONS DURING FICTIVE LOCOMOTION. M.J. Angel*, P. Guertin, I. Jiménez[1] & D.A. McCrea. Dept. of Physiology, U. of Manitoba, Winnipeg, CANADA, R3E 0W3 and CIEA-IPN[1], MEXICO.

Stimulation of plantaris group I afferents during the extensor phase of fictive locomotion evokes disynaptic EPSPs in medial gastrocnemius motoneurons, thus replacing the inhibitory potentials evoked at rest (McCrea et al., Congr. Int. Union Physiol. Sci. 1993). The present investigation examines the distribution of disynaptic EPSPs from ankle extensor group I afferents to hindlimb extensor motoneurons during MLR-evoked fictive locomotion in the cat. During extension but not flexion, stimulation of ankle extensor nerves (single shocks, 1.5 times threshold) produced EPSPs in ankle and hip extensor motoneurons and in the bifunctional PBSt motoneurons (hip extensor + knee flexor). Autogenic excitation was also observed in ankle and hip extensor motoneurons during extension. The latencies of the EPSPs (1.1 - 1.9ms) suggest the opening of a disynaptic pathway from group I afferents to extensors during extension. The presence of group I autogenic and heterogenic, non-monosynaptic excitation is a functional reorganization of ankle extensor group I reflexes during locomotion. The switch from inhibition at rest to excitation during locomotion indicates recruitment of a previously undisclosed population of excitatory group I interneurons. Activation of these interneurons by group I afferents during overground locomotion would excite hindlimb extensor motoneurons and act as a positive feedback system enhancing centrally programmed extension. *Suppported by MRC & The Rick Hansen Legacy Fund .*

648.8

BOTH DISYNAPTIC AND LONGER LATENCY INTERNEURONAL PATHWAYS MEDIATE EXTENSION ENHANCEMENT EVOKED BY GROUP I MUSCLE AFFERENTS DURING FICTIVE LOCOMOTION. P. Guertin*, M.J. Angel, I. Jiménez[1] and D.A. McCrea. Depts of Physiology, U of Manitoba, Canada and CIEA-IPN[1], Mexico.

Trains of group I stimuli to ankle extensor nerves increase the amplitude and duration of extensor electroneurogram locomotor bursts (Guertin et al. Soc. Neurosci. 19:61.15, 1993). The present study examined the synaptic effects of group I stimulation intracellularly in motoneurons during MLR-evoked fictive locomotion. Plantaris nerve stimulation (1.5 times threshold; 20 pulses; 200Hz) during extension increased the amplitude and duration of hindlimb extensor motoneuron locomotor drive potentials (LDPs). The increase in LDP amplitude began within 10 ms after the first shock in the train and often continued well beyond the duration of the stimulus train. Accompanying the LDP enhancement, and depending on the preparation, were distinct disynaptic excitatory or other post-synaptic potentials following each stimulus pulse. In most cases, the depolarization produced by the LDP enhancement was larger than the disynaptic depolarizations. Since LDP enhancement occured in the presence or absence of short latency EPSPs, multiple interneuronal pathways mediate group I reflex effects during fictive locomotion. Both disynaptic and longer latency group I excitation would increase motoneuron recruitment and enhance locomotor extensor bursts. These studies further support the hypothesis that ankle extensor group I afferents are part of a positive feedback system during locomotion that shapes the amplitude and duration of centrally programmed extensor bursts. *Supported by the MRC and the Rick Hansen Legacy Fund.*

648.9

STIMULATION OF THE GROUP I EXTENSOR AFFERENTS CAN PROLONG THE STANCE PHASE IN WALKING CATS. P.J. Whelan*, G.W. Hiebert, & K.G. Pearson. Dept. of Physiol., Univ. of Alberta, CANADA.

Group Ib afferents from extensor muscles exhibit a reflex reversal during locomotion that leads to excitation of the extensor motoneuronal pool. This excitation can prolong and enhance the extensor bursts during fictive locomotion. A question that arises is whether these excitatory actions are functionally relevant in a moving animal that is receiving phasic afferent inflow from many sources.

To address this issue, extensor nerves in the hindlimbs of decerebrate cats were stimulated, while the animals walked spontaneously on a treadmill. The ankle (Lg-Sol & Pl) and knee (Vl & Vi) extensor nerves were dissected, cut, and enclosed in stimulation cuffs. Stimulation of each nerve with trains (400-1500 ms duration, 200 Hz, 1.8 X T) during the extensor portion of the step cycle resulted in a prolongation of the extensor burst. Flexor burst initiation was delayed during stimulation but not always to the end of the stimulus volley. Stimulation during early flexion resulted in premature termination of the flexor burst and a delayed excitation of the extensors, followed by a reinitiation of flexion coincident with stimulus offset. When the stimulation intensity was reduced to sub threshold levels for each nerve, it was possible to stimulate two nerves simultaneously and elicit an increase in the extensor duration. This nonlinear summation suggests that regulation of the stance phase be partly regulated by group I afferents from many extensor muscles converging onto a common interneuronal pool.

Since the observed effects are similar to those attributed to group Ib afferents in reduced preparations, it is concluded that a reduction in force feedback from Ib extensor afferents is necessary for the initiation of the swing phase in a walking animal.

648.10

POSITIVE FEEDBACK OF MUSCLE CONTRACTION IN CAT PERONEAL MOTONEURONS J.-F. Perrier, N. Kouchtir, D. Zytnicki and L. Jami* URA CNRS 1448, UFR des Saints-Pères, 75270 Paris, France.

Motoneurons supplying peroneal muscles were recorded in anesthetized cats during sustained isometric contractions of peroneus brevis (PB) produced by stimulation of cut ventral root filaments. In contrast with the declining inhibition previously observed in triceps surae motoneurons during contractions of gastrocnemius medialis (1) the afferent input generated by PB contractions elicited excitatory potentials.(EPSPs) in peroneal motoneurons. These EPSPs were ascribed to spindle afferents because 1) their amplitudes increased when ventral root stimulation strength was raised to recruit γ axons; 2) with stimulation under γ threshold, PB contraction still excited peroneal motoneurons and evidence was obtained that activation of spindles by ß-axons accounted for this excitation. The lack of contraction-induced inhibition in peroneal motoneurons and the prevalence of contraction-induced excitation raise the possibility that, in contrast to the usual effects of tendon organ afferents, Ib fibers from PB might excite peroneal motoneurons. The fact that electrical stimulation of group I afferents in PB nerve only exceptionally evoked inhibition in peroneal motoneurons would appear compatible with this hypothesis. Further, stimulation of cutaneous afferents, known to facilitate transmission in Ib pathways, only exceptionally revealed a contraction-induced inhibition. The functional significance of a positive feedback of PB contraction on peroneal motoneurons is probably related to the complex biomechanical operations of peroneal muscles in preservation of foot contact with support.

(1) Zytnicki et al. (1990) *J. Neurophysiol.* **64**:1380-1389.

648.11

AUTOGENIC (IB) INHIBITION IN SITTING AND WALKING IN HUMANS. M.J. Stephens*, & J.F. Yang. Dept. of Physical Therapy and Div. of Neuroscience, University of Alberta, Edmonton, Canada, T6G 2G4

Recently, it has been shown that autogenic inhibition arising from group Ib afferents in extensor muscles is largely eliminated during walking in reduced cat preparations (Conway et al. 1987 Exp Brain Res 68:643, Pearson & Collins 1993 J Neurophysiol 70:1009). Instead, a longer latency autogenic excitation is seen. The purpose of this study was to determine whether this change is also apparent in humans. Putative Ib responses can be elicited by conditioning the soleus H-reflex with a stimulus to the medial gastrocnemius nerve at or just below motor threshold (e.g., Pierrot-Deseilligny et al. 1979 Brain Res 166:176). The response was observed both in quiet sitting and in the stance phase of walking. The inhibition during sitting, observed by others, was reproduced. Preliminary results indicate this inhibition was either reduced or eliminated in walking. Sometimes, this was replaced by excitation. Hence, qualitatively, the results support those obtained from the cat, but quantitatively, the effect was considerably more modest in the normal human.

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648.12

WALKING AND TONIC CONTRACTIONS WITHOUT ACTIVITY IN γ-MOTONEURONS IN CLONIDINE AND NALOXONE TREATED CATS. D.J. Bennett*, S.J. De Serres & R.B. Stein. Div. of Neuroscience, Univ. of Alberta, Edmonton, Canada, T6G 2S2.

Recordings in both awake chronic cats and acute cats show that activity of γ-motoneurons can occur without α-motoneuron activity (Prochazka, 1989, Prog. Neurobiol. 33:281-307). The γ-motoneurons have a lower threshold for recruitment, so γ-motoneurons are often activated tonically with only phasic α activity. We present evidence here that just the opposite recruitment can occur in the soleus muscle of decerebrate cats treated with the noradrenergic agonist clonidine (50 μg/kg) and the opioid antagonist naloxone (25 μg/kg). In this preparation α-motoneurons are often recruited without apparent activation of γ-motoneurons. The γ drive was inferred from recordings of muscle spindle group Ia and II afferents. The rate of firing in these afferents dropped markedly during a contraction elicited by a crossed-extensor stimulus, indicating that the extrafusal muscle contraction had unloaded muscle spindles. When evidence for γ drive was seen, it was weak and of a γ static type, as indicated by the slight increase in Ia firing and decrease in sensitivity to stretch (4 Hz sinusoid, 0.25 mm). A similar finding of unloading of muscle spindles due to lack of γ drive was found in the same cats during locomotion produced after spinalization. Thus, both phasic and tonic contractions can occur without corresponding activity in γ-motoneurons.

This research was supported by MRC and AHFMR of Canada.

648.13

THE EFFECTS OF A TONIC INCREASE OF PRESYNAPTIC INHIBITION OF MUSCLE SPINDLE AFFERENTS ON THE STRETCH REFLEX PARAMETERS OF THE CAT. C. CAPADAY* Centre de Recherche en Neurobiologie, Université Laval, Québec City, (Qué), Canada, G1J 1Z4.

Experiments were done in cats decerebrated at the precollicular postmammillary level, or intercollicularly, to determine how a tonic increase of presynaptic inhibition of the intraspinal terminals of muscle spindle afferents changes the mechanical properties of the soleus stretch reflex (s.r.). Baclofen, a specific GABAb receptor agonist, was injected i.v. (1mg/Kg) so as to induce a tonic increase of presynaptic inhibition. The effects of Baclofen on the stiffness and threshold of the s.r. were determined from plots of stiffness vs. background force and force vs. length (length-tension plot), respectively. Baclofen, at these doses, had no effect on the excitation-contraction coupling properties of muscle, or on the intrinsic stiffness-force relation. Changes of the soleus background force, required to obtain the stiffness vs. force plots, were produced by stimulation of the contralateral common peroneal nerve or the posterior tibial nerve and occasionally by electrical stimulation in the area of the red nucleus. The stiffness of the s.r. as a function of the background force level was determined by stretching the muscle with a square pulse of 1-2mm amplitude and 200-300ms duration. The length-tension relation of the soleus stretch reflex was determined by stretching the muscle 12-17 mm at a constant rate of 1-2mm/s. Baclofen produced a significant decrease (40% or more) of the s.r. stiffness within 10-15 mins of injection as determined from the stiffness-force plots. The length-tension plots revealed that the decrease of s.r. stiffness was always accompanied by an increase of the s.r. threshold (typically 3-4mm). Therefore, the s.r. threshold is not an independent variable, it depends on the level of presynaptic inhibition on the muscle spindle afferent terminals. It is concluded that it may be possible for the CNS to adaptively modify the s.r. stiffness via presynaptic inhibition of the intraspinal terminals of muscle afferents. However, any such change of s.r. stiffness will be accompanied by a change of the s.r. threshold. Supported by MRC of Canada.

648.14

HETEROGENIC REFLEXES AMONG MUSCLES OF THE CAT'S FORELIMB. D.A. Backus, B.J. Silverstein, and T.R. Nichols*. Dept. of Physiology, Emory University, Atlanta, Ga 30322.

Previous results have shown that muscles that provide weight-bearing and postural stabilization in the cat hindlimb are linked by powerful force-dependent and inhibitory reflexes, including reciprocal inhibition, Ib inhibition, and long latency inhibition (Nichols, 1989, J. Physiol.: 463-477). Initial studies in decerebrate cats show that all 3 types are also observed in the muscles of the elbow and wrist of the cat forelimb. In addition, the forelimb musculature demonstrates similar reflex organization whether activated via peripheral stimulation or brainstem stimulation. Elbow flexor muscles appear to be linked by mutual inhibition, with latencies ranging from 20 to 70 ms. In addition, Extensor Digitorum Communis receives excitatory input from Extensor Digitorum Longus, which is consistent with findings regarding Ia afferents between these muscles. These findings suggest that reflex patterns exhibited in cat hindlimb muscles may also exist in forelimb muscles. Supported by NS20855 to TRN.

649.1

THE INNERVATION OF THE DORSAL AND VENTRAL HORNS OF THE RAT SPINAL CORD BY AXONS DESCENDING FROM THE LOCUS COERULEUS/SUBCOERULEUS COMPLEX AND A5 CELL GROUP M. Antal*, E. Polgár and Á. Cs. Berki. Department of Anatomy, University Medical School, Debrecen, H-4012 Hungary.

The spinal projections of noradrenergic brainstem nuclei that influence complex sensory and motor activities in the spinal cord were studied in the rat by using the anterograde neural tracer Phaseolus vulgaris leucoagglutinin (PHA-L). After injecting PHA-L unilaterally into the nucleus coeruleus, nucleus subcoeruleus and A5 cell group, labelled fibers and terminals were detected at cervical, thoracic as well as lumbar segments of the spinal cord. Most of the terminals (60-80%) from all three nuclei were found in the ventral horn (laminae VII-IX). The superficial dorsal horn (laminae I-IV) received a relatively sparse innervation from the locus coeruleus and A5 cell group. The subcoeruleus complex, however, projected more intensively to the dorsal horn. More than 30% of the terminals arising from the subcoeruleus complex were revealed in laminae I-II at the level of thoracic and lumbar segments on both sides of the spinal cord.

To study the postsynaptic targets of these descending fibers sections were stained for both PHA-L and calbindin-D28k (CaB), a calcium-binding protein that have been reported to be markers of certain subsets of spinal neurons including stalked cells and supraspinally projecting neurons in the dorsal horn and Renshaw-cells in the ventral horn. All of the aforementioned types of CaB-immunoreactive neurons were found to receive contacts from fibers descending from the investigated noradrenergic brainstem nuclei. Synaptic contacts of terminals in laminae I-II and laminae VII-IX as well as GABA and glycine immunoreactivities of their postsynaptic targets were also investigated in a correlative electron microscopic study.

649.2

PUDENDAL MOTONEURON AXON COLLATERALS AS REVEALED BY INTRACELLULAR INJECTION OF TETRAMETHYLRHODAMINE-DEXTRAN. S.J. Shefchyk*[1], P.A. Carr[1], M.-C. Perreault[1], I. Jimenez[2] & B. Segura[3]. Depts. Physiol.; Univ. of Manitoba[1], Canada, R3E 0W3, CIEA-IPN[2], and Iztacala, UNAM[3], Mexico.

Previous reports have described some of the anatomical characteristics of sacral motoneurons innervating the external urethral and anal sphincters in cat (Beattie & Bresnahan, Neurosci. Lett. 1990, 111:69; Thor et al., J. Comp. Neurol. 1989, 288:263). The present study uses intracellular injection of the fluorescent dye tetramethylrhodamine-dextran (TMR-D) into individual external urethral or anal sphincter motoneurons in the cat to determine if these motoneurons have axon collaterals. In chloralose anaesthetized male cats, single pudendal motoneurons were impaled with glass microelectrodes containing 2% TMR-D (3000 MW) in 0.9% saline. The cells were then injected with dye using 27 to 69 nA x min. of depolarizing current. Following a minimal post-injection interval of two hours, cats were perfused with an aldehyde-based fixative and the lumbosacral spinal cord removed for post-fixation and subsequent cryoprotection. The tissue was then sectioned at 30 µm and examined using standard epifluorescence microscopy. Five of the eight urethral and anal pudendal motoneurons examined had axon collaterals which branched from the primary axon near the ventral spinal white matter. This anatomical evidence suggests that some type of recurrent system from these motoneurons exists in cat, perhaps similar to those observed in rat (W. Collins III, pers. comm.). No physiological evidence has been found for the presence of recurrent inhibitory connections to cat pudendal motoneurons (Jankowska et al., J. Physiol. 1978, 285:425; Mackel, J. Physiol. 1979, 294:105) such as described for hindlimb motoneurons. Both the target neurons and the functional significance of these collaterals have yet to be determined. Funded by the Medical Research Council of Canada.

649.3

DORSAL BORDER PERIAQUEDUCTAL GRAY NEURONS PROJECT TO THE SPINAL CORD IN THE CAT. L. J. Mouton* L. Kerstens and G. Holstege. Dept. Anatomy and Embryology, Faculty of Medicine, Rijksuniversiteit Groningen, The Netherlands.

The mesencephalic periaqueductal gray (PAG) plays an important role in many different functions, such as the regulation of pain, cardiovascular control, vocalization and micturition. In the previous tracing study in the cat (Mouton and Holstege, 1994) HRP injections in the upper thoracic and cervical spinal cord resulted in many strongly labeled neurons in the ventrolateral PAG, but also a few faintly labeled small neurons were found at the border between the dorsal PAG and the adjacent intercollicular zone. Nothing is known about the pathway or the function of these small dorsal border PAG-spinal neurons. The purpose of the present retrograde and anterograde tracing study was to exactly describe this dorsal border PAG-spinal pathway.

In nine cats WGA-HRP was injected in the spinal cord, each at a different spinal level. Prior to the injection a hemisection was made rostral to the injection site. In order to identify and count the retrogradely labeled PAG neurons every fourth 40 µm transverse section of the brainstem was incubated using the TMB method. In an anterograde tracing study WGA-HRP injections were made in the area which contained the small dorsal PAG-spinal neurons.

Results show that there exist several hundreds of relatively small neurons at the dorsal border of the subtentorial PAG projecting to the cervical and thoracic spinal cord. Only a few dorsal border PAG neurons seem to project to the lower lumbar cord, while almost one hundred neurons project to the sacral spinal cord. The neurons project ipsilaterally to the area immediately surrounding the central canal, but not corresponding to lamina X. Further electronmicroscopic studies are needed to more preceisely reveal the nature of this pathway.

649.4

DIRECT PROJECTIONS FROM THE NUCLEUS RETROAMBIGUUS TO HINDLIMB MOTONEURONS; A COMBINED ELECTRON- AND LIGHTMICROSCOPIC STUDY IN THE CAT Veronique G.J.M. VanderHorst*, Henk DeWeerd and Gert Holstege Department of Anatomy, University of Groningen, The Netherlands

The nucleus retroambiguus (NRA) is a compact group of interneurons in the caudal medullary lateral tegmental field. It receives a strong and specific projection from the periaqueductal gray (PAG). NRA interneurons are known to project to motoneurons innervating the pharynx, larynx, intercostal, and abdominal wall muscles. These PAG-NRA-motoneuron projections are involved in vocalization, an example of specific emotional behavior. Apart from the projections related to sound production, the NRA also appears to project directly to specific groups of L4-S2 motoneurons innervating iliopsoas, adductor, hamstring, and proximal tail muscles. This combined light- and electronmicroscopic study was done to obtain direct evidence for monosynaptic projections from the NRA to the lumbosacral motoneurons. To anterogradely label NRA axons, in four cases injections of wheat germ agglutinin horseradish peroxidase (WGA-HRP) were made in the NRA. In two of these cases plain HRP was injected in hamstring muscles to retrogradely label motoneurons receiving projections from the NRA. After a three day survival time, the animals were perfused and lumbosacral segments were cut on a vibratome into 60 µm sections. All sections were processed for either electron- or lightmicroscopy using TMB as chromagen. The results show that NRA terminals form asymmetrical contacts on proximal dendrites of lumbosacral hamstring motoneurons. The terminals contain mainly spherical vesicles, which implicates the direct NRA-motoneuron projections to be excitatory. The direct NRA-hamstring motoneuron projection might be part of a PAG-NRA-lumbosacral motoneuronal pathway, possibly involved in mating behavior (called lordosis in females).

649.5

EFFERENT CONNECTIONS OF THE DEEP MESENCEPHALIC NUCLEUS: A PHA-L STUDY IN THE RAT. M. GOTO, M. COSTA, A. PERACOLI AND S.J. SHAMMAH-LAGNADO*. Dept. of Physiol. & Biophys., Inst. Biomed. Sci., Univ. of São Paulo, SP, 05508-900, Brazil.

The deep mesencephalic nucleus (DP) is an extensive territory included in the mesencephalic reticular formation. In 32 rats small deposits of *Phaseolus vulgaris* leucoagglutinin (PHA-L) were placed in the DP. Striking differences were noted in the projections of different DP regions to the thalamus, zona incerta, superior colliculus, accessory oculomotor nuclei, periaqueductal gray substance, reticular formation, precerebellar and cranial motor nuclei. Thus, the reticular and central lateral thalamic nuclei, ventrolateral geniculate, intergeniculate and subgeniculate nuclei, zona incerta, anterior pretectal nucleus and superior colliculus (superficial layers) are targeted mainly by ventral DP districts (Olszewski and Baxter's ('54) nucleus subcuneiformis); the central medial, paracentral and mediodorsal (ventrolateral sector) thalamic nuclei, superior colliculus (intermediate and deep layers) and periaqueductal gray, chiefly by dorsal DP districts; the rostral interstitial nucleus of the medial longitudinal fascicle, medial accessory oculomotor nucleus and inferior olive, primarily by medial DP districts; and whereas the rostrodorsal DP innervates the parvicellular reticular formation, the remaining DP districts terminate mainly in the magnocellular ventromedial reticular formation.

These hodological data stress the heterogeneous character of the DP and provide an anatomical foundation for its participation in arousal, visuomotor functions and rhythmic jaw movements.
(Supported by FAPESP grant 91/0450-5)

649.6

PHA-L ANALYSIS OF ASCENDING PROJECTIONS FROM THE MEDIAN RAPHE NUCLEUS IN THE RAT. William J. Fortin* and Robert P. Vertes, Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431.

The median raphe nucleus (MR) is directly involved in the desynchronization of the hippocampal EEG. The present study was carried out primarily to examine MR projections to the hippocampus (HP) and associated structures as well as to the neocortex. The projections of the MR have not previously been examined with the PHA-L technique. Detailed analysis was carried out on 16 rats with single injections correctly placed in the MR.

At the level of the brainstem, MR was shown to project heavily to the lateral dorsal tegmental n., pedunculopontine tegmental n. (PPT), central gray, interpeduncular n., ventral part of n. pontis oralis/B9 area and the ventral tegmental n. Light terminal labeling was seen in the substantia nigra, pars compacta. Within the forebrain, the supramammillary n. (SUM), medial mammillary body, lateral habenula, posterior hypothalamus, central lateral n. of the thalamus, vertical limb of the diagonal band and the medial septum (MS/DB) were densely labeled. There was also light labeling in the dorso-lateral septum. The MR also projected heavily to the HP, mainly to the stratum lacunosum-moleculare of Ammon's horn and to the granule cell layer of the dentate gyrus. Light to moderate labeling was observed in the entorhinal cortex, n. accumbens, cingulate cortex and frontal cortex.

These findings indicate that MR influences the HP directly as well as indirectly through intervening nuclei including the PPT, SUM and MS/DB complex, each of which is known to influence the hippocampal EEG.

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649.7

ANATOMICAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF MASSETERIC EFFERENT NEURONS OF CELL GROUP K IN THE RABBIT.
M. Saad, C.G. Widmer*, S. Lepage, D. Umbriaco, K.G Westberg, R. Dubuc and J.P. Lund. Centre de recherche en sciences neurologiques, Univ. de Montréal, Montréal, Canada H3C 3J7; Dept. of Oral Surgery, Univ. of Florida, Gainsville, Dép. de Kinanthropologie, Univ. du Québec à Montréal.

It has been shown that efferent neurons supplying the masseter and digastric muscles of the rabbit are located in two brain stem nuclei: the trigeminal motor nucleus (mot V) and Cell group k (group k), a series of three cell columns ventrolateral to mot V. We have now conducted two series of experiments to characterize the masseteric efferent neurons of group k. In the first, retrograde labelling with fluorescent dyes (fluorogold and fastblue) was used. The dyes were applied to the central ends of cut branches of the masseteric nerve and animals were killed after 8 days. We found that the nerve branches to superficial, intermediate and deep layers of the muscle are all represented in group k, and that the efferent neurons to these branches are intermingled. As a first step towards understanding the function of group k neurons, their immunoreactivity to choline acetyltransferase (ChAT), a specific marker for cholinergic neurons was tested. Animals were anesthetized and perfused. After fixation, serial vibratome coronal sections of the brain tissue were cut at 50μm. Free floating sections were treated with a ChAT monoclonal antibody (Code AB8), using the avidin-biotin peroxidase method. Like mot V, the vast majority of cells in group k were immunostained and were of the same size and shape as retrogradely labelled neurons. This suggests that neurons of group k are cholinergic and are likely to be motoneurons. However, group k neurons are fusiform and smaller in size (mean diameter=31μm) than mot V cells, which are round or ovoid (mean diameter= 44μm). This suggests that Cell group k neurons supplying the masseter muscle could be gamma motoneurons; but this is contradicted by the fact that the digastric muscle, which contains no muscle spindles in the rabbit, is also innervated by this nucleus.

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649.8

THE BRAINSTEM IMAGE OF A PRIMATE HAND NERVE. X-F Wu*, S.K. Rasey, and J.T. Wall. Department of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The primate hand is innervated by the ulnar, median, and radial nerves. Inputs from each nerve dominantly activate band or patch-like aggregates of neurons in the cortical area 3b map of the hand. The shapes and extents of these aggregates are similar in different normal monkeys. To begin study of the ascending anatomical substrates that underlie these aggregates, horseradish peroxidase conjugates were injected into the ulnar nerve near the wrist, and three dimensional reconstructions of labeled terminations were made from serial sections through the brainstem. Brainstem label was restricted to divisions of the cuneate nuclei. Dense label was distributed in a continuous manner across the rostral, central (pars rotunda and triangularis), and caudal divisions of the main cuneate nucleus. Sparse label sometimes extended to the border region between the main and external cuneate nuclei. Within the pars rotunda, labeled terminations were consistently dense in medial and mediodorsal locations, with less dense fringes extending laterally and ventrally. The observed pattern of label was similar in different monkeys. The peripheral receptors of ulnar nerve sensory fibers innervate a continuous territory on the ulnar side of the hand. The central terminals of ulnar nerve fibers, in turn, are consistently shaped as a continuous, rostrocaudally elongated substrate in the main cuneate nucleus. It is suggested that the shape and extent of this substrate contributes to the shape and extent of the bandlike ulnar nerve dominance aggregate in the hand cortex. Supported by NS21105.

649.9

POPULATIONS OF MYELINATED NERVE FIBRES IN THE DORSAL AND VENTRAL ROOTS OF THE LUMBOSACRAL ENLARGEMENT IN THE OPOSSUM, *MONODELPHIS DOMESTICA*.
H. Leblond and T. Cabana*. Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Canada, H3C 3J7.

A light microscopic study of the dorsal and ventral roots at the lumbosacral enlargement of the spinal cord was made in the opossum in order to establish the total number of myelinated fibres and their size distribution. Dorsal and ventral roots were removed from deeply anesthetized animals and were immersed overnight in 2% glutaraldehyde - 2.5% paraformaldehyde in cacodylate buffer (pH 7.3). The tissue was rinsed, postfixed for 4 hours with 1% osmium, rinsed again, dehydrated in graded ethanol and embedded in Spurr Mixture. Semithin (1μm) sections were cut from the proximal part of the roots, stained with toluidine blue and dried. They were photographed so as to obtain a montage of the whole root section. An average of 809 ventral root fibers are myelinated. They can be divided in two populations: small fibres having a mean diameter of 6±2μm (42%), and large fibres having a mean diameter of 18±4μm (58%), myelin sheaths included. An average of 2450 dorsal root fibres are myelinated. They are distributed in three populations: a population of small and poorly myelinated fibres with a mean diameter of 4±2μm (38%), a second population of fibers with mean diameter of 12±4μm (51%), and a third population of large caliber fibres with a mean diameter of 20±3μm (11%). These data will serve as an endpoint to a developmental study of myelogenesis of the spinal cord in the *Monodelphis* opossum. (Supported by NSERC.)

649.10

THE PROJECTION OF PHYSIOLOGICALLY IDENTIFIED JAW-MUSCLE SPINDLE AFFERENTS TO THE CAUDAL BRAINSTEM USING INTRACELLULAR BIOTINAMIDE. R. Wong*, P. Luo and D. Dessem*, Dept. Physiol., U. Maryland Dental Sch., Baltimore, 21201

Jaw-muscle spindle afferents were physiologically identified and intracellularly stained with biotinamide in rats. Rostrally, these axons projected to the trigeminal motor nucleus, supratrigeminal region, mesencephalic trigeminal nucleus and dorsomedial part of the principal sensory nucleus as reported in previous studies. Additional, previously undescribed jaw-muscle spindle projections to the caudal brainstem were also found. Axon collaterals with boutons emerged from the tract of Probst and projected to the dorsomedial part of spinal subnuclei oralis (Vodm) and interpolaris (Vidm), the parvicellular reticular formation (PCRt) of the caudal brainstem, laminae IV and V of the spinal trigeminal subnucleus caudalis (Vc) and the dorsal division of the medullary reticular nucleus (MdD). Labeled axon collaterals in the caudal Vodm formed a dense reticular distribution. Labeled spindle boutons in Vodm formed predominately axo-dendritic synapses. Some of these boutons received presynaptic inputs from unlabeled boutons containing type 'S' and 'F' vesicles. In Vidm, labeled collaterals and boutons were densely clustered into glomerular-like structures which surrounded individual or small groups of neurons. Labeled boutons in Vi made axo-dendritic, axo-somatic and axo-axonic synapses and received synaptic contacts from unlabeled boutons containing either type 'S', 'F' or dense core vesicles. Axon collaterals in Vc were much sparser and simpler than in Vidm. Labeled boutons mainly formed synaptic contacts with large diameter dendrites in the neuropil of Vc. Synaptic convergences between labeled boutons and multiple unlabeled boutons were often encountered contacting the same dendrite in Vc. Supported by NIH DE10132.

649.11

ENCODING OF LIMB GEOMETRY BY SPINOCEREBELLAR NEURONS.
G. Bosco and R.E. Poppele*, Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

Only in the recent past has attention been devoted to the integrative features of second order sensory neurons. The dorsal spinocerebellar tract (DSCT) represents a good model to investigate this process in a sensory-motor system because recent evidence suggests that it may convey to the cerebellum a complex signal related to parameters of movement for the whole limb, in particular, the direction of limb displacement (Bosco & Poppele, J.Neurophysiol., 1993, 70: 863). It has also been suggested that a directional coding by this population of cells, constitutes a reference frame which may relate to limb geometry, namely its length and the orientation of the limb axis.

We explored this possibility by recording from DSCT neurons following perturbations of the hindlimb in four directions (up, down, forward and back) from two initial positions characterized by different limb geometries. The result was that directional preferences were more closely related to the orientation of the limb axis than to the extrinsic vertical. In fact, the mean population vectors, computed by applying Raleigh statistics to the distribution of the cells' preferred directions, differed in the two experimental conditions by 15.75°±5.88 SD, which corresponded to the difference in limb axis orientations. This finding suggested a rotation of the mean population vectors consistent with the tilting of the limb axis. Indeed translating the mean vectors' directions from extrinsic cartesian coordinates to limb axis coordinates reduced the phase difference to 5°±6.2 SD.
(Supported by N.I.H. grant NS 21143).

649.12

BRAINSTEM DISTRIBUTION OF *C-FOS* IMMUNOREACTIVITY FOLLOWING REPETITIVE COUGHING IN DECEREBRATE CATS.
C. Gestreau, F. Portillo, M. Yousfi[1], J.J. Puizillout[1], A.L. Bianchi* & L. Grélot, URA CNRS 1832, St Jérôme, 13397 Marseille Cedex 20; (1) CNRS-LNB, 13402 Marseille Cedex 20, France (Spon: ENA)

Using the expression of the immediate early gene *c-fos* as a marker of neuronal activation, we compared in the brainstem of cats the pattern of distribution of cells exhibiting a *Fos*-like immunoreactivity (FLI) in response to repetitive coughing (i.e stimulated animals) to that observed in shamed operated animals. In decerebrate, paralyzed and ventilated cats, 100 to 250 coughing episodes were elicited by stimulation (1-3 V, 0.4 ms, 2-4 Hz, 45 min) of superior laryngeal nerves (SLN), applied 4 hours after surgery was completed. Coughing was monitored on the phrenic and ilio-hypogastric neurograms. Brainstem tissues were immunoprocessed using an antibody recognizing both the *fos* and *fos*-related nuclear antigens, and the avidin-biotin peroxidase complex. In the brainstem of stimulated cats, intense FLI was observed in various subnuclei (i.e. commissural, lateral, ventrolateral, medial and dorso-medial) of the nucleus tractus solitarii, the ambigual and retrofacial nuclei, the ventral aspect of the medial accessory inferior olive, the retrotrapezoid nucleus, the inferior central (raphe) nucleus and the locus caeruleus. In these regions only very weak or none FLI was evidenced in control cats. FLI in the medullary reticular formation and in the pontine lateral and medial parabrachial nuclei was only augmented in experimental cats.

649.13

RETICULOSPINAL NEURONS AND THEIR SPINAL PROJECTIONS IN LAMPREYS: A STUDY USING RETROGRADE TRACERS. N. Bussières* and R. Dubuc. Dép. de kinanthropologie, UQAM H3C 3P8 and Centre de recherche en sciences neurologiques, Université de Montréal H3C 3J7 Canada.

As in other vertebrates, lamprey reticulospinal (RS) neurons play an important role in the supraspinal control of locomotion. In the present study, retrograde tracers were used to study the organization of RS lamprey neurons within the different reticular nuclei and to characterize their spinal projections in adult *Ichtyomyzon unicuspis*. Cobalt-lysine was applied *in vitro* to the rostral end of the spinal cord (n=30), or applied iontophoretically to label descending cells projecting to specific spinal cord tracts (n=10). Another tracer, horseradish peroxidase (HRP), was applied at the 12th, 40th, 57th and 88th spinal cord segments and transported *in vivo* in order to determine the length of the axonal projections of RS cells (n=28). Retrogradelly labelled cells were counted from serial sections on the best specimen for each injection site and their soma was measured. 3-D computer reconstructions were made of each reticular nucleus. A total of 1299 RS neurons were counted on one side. The posterior rhombencephalic reticular nucleus (**PRRN**) contains the largest number of RS cells (845), 21% (177) of which project to the contralateral spinal cord and are located mainly in the caudal end of the nucleus. The middle rhombencephalic reticular nucleus (**MRRN**) contains fewer RS neurons (339), 18% (60) of which project contralaterally and are found predominantly in the lateral basal plate. Of the 90 RS neurons counted in the anterior rhombencephalic reticular nucleus (**ARRN**), 27% (24) project to the contralateral spinal cord. They are intermingled with cells projecting to the ipsilateral side. Finally, the mesencephalic reticular nucleus (**MRN**) contains 102 neurons, projecting their axon exclusively to the ipsilateral spinal cord. Iontophoretic application of cobalt-lysine to the lateral tract on one side labelled, on both sides, a higher proportion of cells in the PRRN (635) than in the other nuclei (MRRN=91, ARRN=1, MRN=1). Spinal cord injections at the 12th segment labelled 716 RS cells, with a steep (63%) decrease in the number of small (less than 20 μm) RS cells counted as compared to injections in the 2nd spinal segment. Injections at the 40th (first dorsal fin), 57th (second dorsal fin) and 88th segments (tail) labelled a gradually decreasing number of neurons with only 10% (138) of all RS cells projecting to the level of the tail and distributed predominantly in the PRRN (96) and the MRRN (33). Results from this study reveal a higher number of RS cells than previously reported, with a noticeable (20%) component of contralaterally projecting cells. (Supported by MRC, FCAR, FRSQ and NCE for neural regeneration and recovery).

649.14

THREE-DIMENSIONAL DENDRITIC ANALYSIS OF RAT PHRENIC MOTONEURONS DURING POSTNATAL GROWTH. K.G. Smithson*, Y.S. Prakash and G.C. Sieck. Departments of Anesthesiology and Physiology, Mayo Foundation, Rochester, MN 55905.

The rapid growth of diaphragm muscle fibers between postnatal day 21 (D21) and adulthood may also be reflected by growth of phrenic motoneurons and their dendritic arborization. Concurrent growth of the spinal cord may also induce changes at the dendritic level, in order to properly integrate descending synaptic input. In the present study, qualitative and quantitative features of rat phrenic motoneuron dendritic morphology were examined in D21 and adult animals. Choleratoxin B-fragment was used to retrogradely label phrenic motoneurons. Computer-assisted three-dimensional neuromorphometry was used to statistically summarize dendritic branching patterns. While these analyses broadly confirmed previous qualitative observations on rat phrenic motoneurons, several new features were highlighted. For example, contralateral projections of dendrites were more frequent in D21 animals than in adults. Dendritic diameter was smaller in D21 compared to adults. Total dendritic lengths were shorter at D21, and showed less variability compared to adults. Branching angles of D21 dendrites were also greater compared to adults, suggesting a re-orientation of dendrites during growth. This was also reflected by smaller receptive areas of motoneurons at D21. Receptive areas at D21 also showed smaller variance compared to adults, indicating a differential growth of motoneurons. However, when normalized for spinal cord growth, receptive areas were not different between the two ages. We conclude that these morphological changes may contribute to changes in motoneuron recruitment during growth. (Supported by NIH grants HL37680, HL34817 and GM08288.)

649.15

PROJECTIONS FROM THE RAT SPINAL TRIGEMINAL SUBNUCLEUS ORALIS TO THE SPINAL CORD STUDIED WITH THE PHA-L METHOD. R. Dallel, P. Raboisson, J. Pajot, C. Aigouy, and A. Woda. (SPON: European Neuroscience Association*). *Lab. de Physiologie Oro-faciale, Faculty of Dentistry 63000 Clermont-Ferrand (France).*

Subnucleus oralis (Sp5O), the rostral subdivision of the spinal trigeminal nucleus has been reported to project to the spinal cord in the rat, but the precise locus of termination of this descending pathway is unknow. This location was examined using the anterogradely transported Phaseolus Vulgaris Leucoagglutinin (PHA-L).

Male Sprague-Dawley rats were anaesthetised with chloral hydrate and PHA-L was electrophoretically injected into either the ventrolateral or the dorsomedial region of the Sp5O. Two weeks later, the rats were perfused transcardially, the brain and the spinal cord were removed and cut in serial sections which were reacted with PHA-L antibody and ABC complex.

Spinal projections from the *ventrolateral part of Sp5O* were found in the ventral horn (lamina IX) of the whole cervical cord, bilaterally but with a clear contralateral dominance. The highest density of labelling was at the C2-C4 level. Projections to the dorsal horn were only ipsilateral. The labelling (lamina V) was of medium density at the C1 level and progressively decreased up to the C4 level. Projections from the *dorso-medial part of Sp5O* to the ventral horn (lamina IX especially) were of lower density than after injection into the ventrolateral part of Sp5O. Labelling was bilateral with ipsilateral dominance and only observed from C1 to C4 level. Labelled terminals were also noted in the ipsilateral dorsal horn (lamina III-V) especially at the C1 level. No labelled terminal was observed in the lower thoracic and lumbar cord.

Our findings are consistent with the hypothesis that Sp5O is involved in the integration of head and neck motor functions. However, observation that Sp5O projects to the dorsal horn suggest that this nucleus could also contribute to modulation of incoming sensory information at the spinal level.

649.16

BRAINSTEM PROJECTIONS TO THE SUPRAMAMMILLARY NUCLEUS AND MEDIAL MAMMILLARY BODY: A WGA-HRP TRACING STUDY. J.S. Thinschmidt,[1] A.M. Crane,[2] K.S. Panickar*,[1] and R.P. Vertes.[2] (1) Dept. of Neuroscience, Univ. of Florida College of Medicine, Gainesville, FL 32610 and (2) Ctr. for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431.

We have recently shown that a subset of cells of the supramammillary nucleus (SUM) and mammillary body (MB) discharge synchronously with the hippocampal theta rhythm (Kocsis and Vertes, *J. Neurosci.*, in press). Recent findings indicate that the theta-related cells of SUM may control the frequency of the theta rhythm (Kirk and McNaughton, *Hippocampus* 3:517, 1993). With the exception of a single recent report by Hayakawa et al. (*Anat. Embryol.* 188:139, 1993), afferents to SUM have not been examined. The present report examined brainstem afferents to the SUM as well as to the medial MB in the rat.

Under sodium pentobarbital anesthesia, single pressure injections of WGA-HRP (25-30 nl of 3% WGA-HRP) were made into the SUM (10 rats) and MB (5 rats). The HRP reaction procedure was the same as described previously (Vertes, *Neuroscience* 24:907, 1988). Injections in either site gave rise to labeled cells that were essentially restricted to the upper brainstem (pons and midbrain). In the caudal pons, labeled cells (with SUM or MB injections) were mainly seen within nuclei imbedded in the pontine gray including nucleus incertus, the laterodorsal tegmental nucleus, Barrington's nucleus and the locus coeruleus. At the rostral pons-caudal midbrain, labeled cells were present in the dorsal and median raphe nuclei, pontomesencephalic gray, and the dorsolateral pons (lateral parabrachial-pedunculopontine region). The MB cases, but not the SUM cases, gave rise to labeled cells in the ventral tegmental nucleus and anterior tegmental nucleus (of Gudden). The results indicate some common brainstem inputs to SUM and medial MB. Some of the brainstem inputs to SUM may regulate SUM in its modulation of the hippocampal EEG. Supported by NIMH grant MH45075.

649.17

GAP JUNCTIONS ON SOMATA, DENDRITES AND CHEMICAL SYNAPSES OF MAPPED NEURONS IN RAT SPINAL CORD J. E. Rash*, R. K. Dillman, L. R. Whalen, P. B. Guthrie, D. W. Wheeler, and H. S. Duffy. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523

"Grid-mapped" freeze-fracture was modified to allow mapping of gap junctions (GJs) to individual neurons in histological slices of adult rat spinal cord. After freeze-fracture replication, each sample was immobilized in a Lexan film on a gold "Finder" grid, thawed, and the replica plus attached tissue was mapped in three dimensions by confocal microscopy. To date, we have used this technique to identify a total of thirty-six neuronal GJs on twelve mapped motor neurons and interneurons in Rexed Laminae VI, VII, VIII, and IX. GJs were found on neuron somata, on basal, proximal, and distal dendrites, and on "synaptic plateaus" of chemical synapses. Most neuronal GJs contained fewer than 50 connexons (range = 6-800 connexons). GJs were present at an overall density of approximately 1/30 μm^2 of neuronal plasma membrane, implying that the average neuron in rat ventral horn contains several hundred to several thousand GJs. Since most neuronal GJs are less than 0.06 μm in diameter, they were too small to be detected by previous thin section TEM methods. Likewise, because most GJs on spinal cord neurons are small, they contain few antibody binding sites, and thus, would not be detectable by conventional immunocytochemical staining techniques. Grid-mapped freeze fracture has permitted identification and precise mapping of GJs on neurons in adult rat spinal cord.

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649.18

THE PROJECTION OF BULBOSPONGIOSUS MOTONEURON AXON COLLATERALS IN THE RAT. M. Gonzalez* and W.F. Collins, III. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Bulbospongiosus (BS) motoneurons (MNs) are bilaterally activated during penile reflexes (e.g., penile cup formation). To investigate the possible involvement of axon collaterals, we have examined the projection of BS MN axon collaterals and their relationship to dendritic processes from ipsilateral and contralateral BS MNs.

In male Sprague-Dawley rats (300-450 g), anesthetized with ketamine + xylazine and prepared for in vivo intracellular recording, BS MNs were penetrated with glass micropipettes filled with biocytin (2% in 1.75M KCl) and identified by antidromic activation. Biocytin was then injected intracellularly using brief pulses of pressure (10-60 psi). In each rat 1-4 BS MNs were filled, or biocytin was dumped into the dorsomedial (DM) nucleus. The rats were allowed to survive 1-2 hr after the last injection. The biocytin was visualized in transverse spinal cord sections using standard ABC and nickel-enhanced diaminobenzidine (DAB) reactions. In a number of experiments, BS MNs were retrogradely labeled with subunit B of cholera toxin (CTB; 1 µl; 0.5% in H_2O) injected into the BS muscles 2-3 d prior to the electrophysiological experiment and visualized immunohistochemically using DAB.

BS MN axons coursed 300-400 µm from the DM nucleus in the medial dendritic bundle before making a ventral turn to exit the spinal cord. Of 21 BS MNs labeled with biocytin, 3 (14%) were found to possess a single axon collateral. In each case, the axon collateral emerged at the point where the axon began its ventral turn and projected dorsally 10-50 µm before exhibiting a limited arborization (10-30 boutons) in the region of the ipsilateral medial BS MN dendritic bundle.

Since the medial dendritic bundle contains dendrites from both ipsilateral and contralateral BS MNs, axon collaterals terminating within the medial dendritic bundle could provide excitatory inputs to both ipsilateral and contralateral BS MNs. However, the limited frequency and projection of BS MN axon collaterals suggest that they play a minimal role, if any, in the bilateral activation of BS MNs.

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649.19

IDENTIFICATION OF FACIAL MUSCLE INTERNEURONS USING PSEUDORABIES VIRUS. R. Fay* & R. Norgren, Neuroscience Program & Department of Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

The facial, lingual, and masticatory muscles all participate in a variety of functions including ingestion, respiration, swallowing, and communication. We previously identified the interneurons that are involved in the control of the masticatory and tongue musculature. The present experiments complete the series by identifying the sources of brainstem afferent input to the facial motor nucleus (Mo 7) in Sprague-Dawley rats. The facial muscles are of particular interest because of their role in emotional expression. The posterior digastric was exposed and the virus injected directly into the belly of the muscle. The buccinator, platysma, and zygomatic muscles are arranged in thin sheets just beneath the skin. In order to label them, the virus was injected subcutaneously. The animals were allowed to survive for up to 90 hours. Prior to viral injection, the superior cervical ganglia were removed bilaterally. Injection volumes ranged from 1 to 12 µl at an average titre of $5x10^8$ pfu/ml. Primary infections were restricted to the Mo 7 in a predictable myotopic pattern. Virally infected neurons appeared in nuclei known to project monosynaptically to Mo 7, including the central tegmental field, supratrigeminal, Kolliker-Fuse, parvocellular reticular formation, and the dorsal region of the spinal trigeminal nucleus caudalis. At 90 hours post-infection, tertiary (and possibly higher) infections were present in neurons in the pedunculopontine, laterodorsal tegmental, gigantocellular, lateral paragigantocellular, rostroventrolateral, and paratrigeminal nuclei. Labeled neurons also were found surrounding the nucleus ambiguus and primarily within the ipsilateral nucleus of the solitary tract. The trigeminal ganglia and trigeminal motor nucleus were devoid of PrV infected cells in all animals. Supported by PHS grants DC 00240 and MH 00653.

649.20

VESTIBULAR AND CEREBELLAR PROJECTIONS TO THE GRACILE AND CUNEATE NUCLEI IN RATS AND CATS. S.J. Jabbur*, H. Jourdi, W. Takash, M.Z.M. Ibrahim, S.F. Atweh, and N.E. Saadé. Fac. of Med., Am. University of Beirut, Beirut, Lebanon.

The roles of the vestibular nuclei (VN) and the cerebellum in motor functions including muscle tone and activities in 3-dimensional space are well established. During the past 25 years, various motor functions have also been ascribed to the dorsal column nuclei (DCN). These include serial motor acts (Exp. Brain Res., 1973, 18:165) and anticipatory motor behavior (Exp. Neurol., 1974, 42:274). In the present study, we searched for direct projections from the VN and the cerebellum to the DCN.

The DCN of anesthetized cats (n=6) and rats (n=7) were injected with horseradish peroxidase (which was strictly limited to the nuclei), either free or coupled with wheat-germ agglutinin. After 24-48 hr, the animals were reanesthetized and transcardially perfused. The brainstem and cerebellum were then removed and sectioned. In addition to other well known projecting areas to the DCN, labeled neurons were also observed bilaterally in the inferior and medial VN and the fastigial nuclei of the cerebellum. Such projections could be part of reciprocal loops between the VN, cerebellum and DCN and could serve as neuroanatomical substrate for the motor functions ascribed to the DCN. (Supported by grants from the Diana Tamari Sabbagh Fund the Lebanese Nat. Res. Council and the University Research Board).

SPINAL CORD AND BRAINSTEM: TRANSMITTER LOCALIZATION

650.1

SUPRASPINAL CONTROL OF CERVICAL SPINAL CORD MOTONEURONAL DENDRITE BUNDLES IN THE RAT. W.J.Anderson* and G.Bennett. Neurobiology Lab, Indiana Univ. Medical center, Terre Haute, IN 47809

Our laboratory has previously described motoneuronal columns in the cervical spinal cord of the rat into discrete dendrite bundles with continuity with the brain stem and thoracic cord. This organization identified a midline, medial (which included the phrenic nucleus) and a lateral column (internal and external). These dendrite bundles in the rat have electrotonic junctions that are dendrodendritic, dendrosomatic, and somatosomatic. There are interconnections between these columns which are referred to as microbundles. This study will present data that this organization of dendrites acts as a substrate for descending supraspinal axons which include serotonin, norepinephrine and thyrotrophic hormone. Utilizing double immunocytochemical techniques, utilizing choline acetyltransferase, tyrosine hydroxylase, thyrotrophic releasing factor, and serotonin with different substrates from Chemicon Corporation, we have demonstrated that specific supraspinal axons specifically innervate motoneuronal columns with pericellular contacts, distribute along dendrite bundles in the longitudinal plane in a specific manner, and finally interact with microbundles which interact with the different columns. The distribution of each transmitter varies in it's distribution to the various bundles. We hypothesize that this supraspinal control has specific control over different columns, and yet can influence all to varying degrees through their microbundle distribution.

650.2

CO-LOCALIZATION OF ACETYLCHOLINE AND GLUTAMATE IN NEURONS AT THE FELINE MESOPONTINE JUNCTION J.-P. Wu, Y.Y. Lai*, J.S. Kuo, C.Y. Chai, and J.M. Siegel. Dept Med Res, Taichung Vet Gen Hosp, Taiwan; Dept Psychiat, UCLA and VAMC, Sepulveda, CA 91343.

Both cholinergic and glutamatergic neurons have been identified in the laterodorsal tegmental (LDT) and pedunculopontine (PPN) nuclei. Both cholinergic and glutamatergic neurons in LDT and PPN project to the pontine inhibitory area, an area that is involved in the control of REM sleep atonia. The present study was designed to clarify if neurons in LDT and PPN co-contain Ach and glutamate, using choline acetyltransferase (ChAT) and glutamate immunohistochemical techniques. Three cats were perfused with saline followed with 3% paraformaldehyde and 0.25% glutaraldehyde in phosphate buffer solution, pH 7.4. The tissue was cut at 50 µ and alternate sections were processed with the following 1) ChAT 2) glutamate and 3) ChAT and glutamate. We found that neurons in both LDT and PPN co-contain glutamate and acetylcholine. However, the percentage of cholinergic neurons co-containing both transmitters was higher in PPN than in LDT. 78% of cholinergic neurons in PPN were double labeled with glutamate, while only 44% of neurons in LDT contained both transmitters. We suggest that the neurons in LDT and PPN that co-contain Ach and glutamate may play an important role in REM sleep control.

650.3

COLOCALIZATION OF SUBSTANCE P IN SEROTONERGIC AFFERENTS TO THE HYPOGLOSSAL NUCLEUS. **S. Manaker* and J.N. Henry, Center for Sleep and Respiratory Neurobiology and Pulmonary and Critical Care Division, Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104.**

The serotonergic (5HT) innervation of the hypoglossal nucleus (Mo12) originates from the caudal raphe nuclei (CRN). Non-5HT neurons in these nuclei also project to the Mo12. Substance P (SP) neurons are present in the CRN, often colocalized with 5HT. We sought to determine the presence of SP in non-5HT afferents to the Mo12. Rhodamine microspheres (Rh; 100nl) were injected into the Mo12 of anesthetized rats, who survived 6-9 days. Two days before sacrifice, rats were reanesthetized and injected intraventricularly with 100 µg colchicine. Following perfusion and fixation, sections (32 µm were cut through the entire brainstem. In cases with the Rh injection site restricted to the Mo12, every seventh or tenth section was processed for dual immunofluorescence for 5HT (rabbit anti-5HT, 1:2000; AMCA-conjugated goat anti-rabbit, 1:50) and SP (rat monoclonal anti-SP, 1:200; FITC-conjugated goat anti-rat, 1:100). Retrograde labeling by Rh was observed in all sources of Mo12 afferents, while 5HT or SP immunofluorescence was present in all major groupings of 5HT or SP somata, respectively. Within the medial tegmental field, most SP neurons also contained 5HT. Of the neuronal afferents projecting to the Mo12, most (>75%) of the SP immunoreactive neurons also contained 5HT, and these triple-labeled cells were present mainly within the CRN. Very few (<10%) of the non-5HT afferents to the Mo12 contained SP alone. These observations suggest that the SP projections to the Mo12 are a subset of the 5HT projections to the Mo12, and that SP neurons account for a very small proportion of the non-5HT afferents to the Mo12. (Supported by SCOR HL-42236).

650.4

A STUDY OF THE TRANSMITTER SYSTEMS MEDIATING TECTORETICULAR INPUTS IN LAMPREYS. I.C. Zompa* and R.Dubuc. Dép. de kinanthropologie, Université du Québec à Montréal, H3C 3P8 and CRSN, Université de Montréal, H3C 3J7, Canada.

It has been shown by Ullén et al. (Behav.Brain Res. 54:107-110, 1993) that illumination of a lateral eye in lampreys induced a turning movement away from the light source. Anatomical studies revealed that the retina projects to the contralateral tectum (Kennedy & Rubenson, J.Comp.Neurol. 171:465-480, 1977). However, little is known on the interactions between visual inputs and motor systems in lampreys. In a previous study, we identified populations of neurones on either side of mesencephalic tectum, that project to the middle (MRRN) and to the posterior (PRRN) rhombencephalic reticular nuclei of lampreys. These nuclei contain reticulospinal (RS) neurones which are known to play a key role in descending motor control. We have also shown that microstimulation of the tectal cell populations, in the *in vitro* isolated brainstem-spinal cord preparation, evoked mixed excitatory and inhibitory responses in RS neurones. This study was aimed at characterizing these tectoreticular inputs further. Large Müller cells of the MRRN received strong excitatory inputs, while some RS neurones in the caudal PRRN received predominantly inhibitory inputs. To identify the transmitter systems involved, CNQX (10 µM) was added to the perfusate, and both the excitatory and inhibitory inputs were abolished, suggesting that AMPA receptors were involved in mediating these responses and that inhibition is di- or oligosynaptic. Strychnine (5µM) completely abolished the inhibitory responses, indicating that glycine is the inhibitory transmitter. Perfusing the preparation with Mg^{2+}-free Ringer's, increased the duration of the excitation and this was suppressed by 100 µM AP5 or when normal Mg^{2+} concentrations were restored. NMDA receptors are thus present in this pathway. In conclusion, we have shown that excitatory and inhibitory inputs from tectum to reticulospinal neurones, are similar to those reported in cats (Peterson et al., Exp. Brain Res, 21:19-44, 1974). Moreover, this study indicates that, within tectoreticular pathways of lampreys, excitation is mediated by excitatory amino acids and inhibition by glycine. Funded by MRC Canada, FCAR and FRSQ Québec.

650.5

SYNAPTIC INTERACTIONS OF SUBSTANCE P IMMUNOREACTVE (SP) NERVE TERMINALS WITH HYPOGLOSSAL (N.XII) MOTONEURONS WHICH INNERVATE THE INTRINSIC MUSCLES OF THE TONGUE: AN ELECTRON MICROSCOPIC DUAL LABELING STUDY. P.J. Gatti[1], T.A. Johnson[2], M. Shirahata[3] and V.J. Massari[1], Depts. of Pharmacology[1] and Veterinary Services[2], Howard Univ. Coll. Med., Washington, D.C. 20059, and [3] Div. Physiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205.

The intrinsic muscles of the tongue play important roles in speech and respiration. Little is known of the central mechanisms regulating N.XII motoneurons. To elucidate which afferent neurotransmitters modulate functionally associated subgroups of N.XII motoneurons, we have employed an electron microscopic dual labeling technique. Cholera toxin-horseradish peroxidase (CTB-HRP) or unconjugated HRP was injected into the intrinsic tongue muscles of the cat. The animals were sacrificed 48 hr later and sections were processed histochemically for the visualization of HRP using tetramethylbenzidine as the chromogen. Retrogradely labeled neurons were found predominantly ipsilaterally in the intermediate and ventromedial subdivisions of N.XII particularly at the level of the rostral pole of the area postrema. These motoneurons showed abundant cytoplasm and organelles, and round nuclei. Sections were processed for the simultaneous immunocytochemical localization of SP using diaminobenzidine as the chromogen. SP immunoreactivity was found in nerve terminals primarily associated with large dense core vesicles. Some of these terminals made synaptic contact with proximal dendrites and perikarya of labeled N.XII motoneurons. These data indicate that SP afferents modulate the activity of N.XII motoneurons which control the function of intrinsic tongue muscles. Supported by NHLBI 44922, & Howard Univ. Grad. Sch. Collab. Core Progr.

650.6

DESCRIPTION AND ANATOMICAL TRACING OF PROJECTIONS OF THE B9 SEROTONIN-CONTAINING NUCLEUS: 5-HT IMMUNOSTAINING AND PHA-L ANTEROGRADE TRACING. R.P. Vertes* and A.M. Crane. Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431.

The present study examined the distribution of 5-HT containing cells in the ventral pontomesencephalic tegmentum (commonly referred to as the B9 area) as well as the projections of this cell group. The brains of 15 rats were immunohistochemically-reacted for the presence of 5-HT in cells and fibers according to a modified procedure of Arita et al. (Exp. Brain Res. 95:100, 1993). The projections of B9 were examined in a separate group of 20 rats using PHA-L (Vertes, J. Comp. Neurol. 326:595, 1992). 5-HT-containing cells of B9 distribute over a widespread region of the pontomesencephalic tegmentum from the level of the mid-pons rostrally to approximately the level of the red nucleus. They are most densely concentrated directly above the medial lemniscus and further rostrally immediately lateral to the interpeduncular nucleus. As in the cat (Wiklund et al., J. Comp. Neurol. 203:613, 1981), there are more 5-HT-containing cells in B9 than in any other raphe group, with the possible exception of the dorsal raphe. In the brainstem, B9 was found to project heavily throughout the extent of the reticular formation (medulla through midbrain), the ponto-mesencephalic central gray, the parabrachial-pedunculopontine region, midline raphe groups, the laterodorsal tegmental nucleus, and parts of the tectum and pretectum. In the forebrain, B9 projects densely to the lateral hypothalamus, the zona incerta, and the substantia nigra, as well as lightly to moderately to the striatum, amygdala, and prefrontal cortex. Few labeled fibers were seen in the septum or hippocampus. Overall, B9 sends considerably stronger projections to the brainstem than to the forebrain. The present results indicate that B9 is an overlooked source of relatively prominent 5-HT projections to several structures of the brainstem and forebrain. Supported by NIMH grant MH45075.

650.7

DIRECT SEROTONERGIC INPUT TO DORSAL SPINO-CEREBELLAR TRACT NEURONS IN THE CAT. J.C. Pearson, D.E. Dewey, F.J. Alvarez, D. Harrington, M.J. Sedivec* and R.E.W. Fyffe. Dept of Anatomy, Wright State Univ, Dayton, Ohio 45435, and * Dept of Biology, Appalachian State Univ, Boone, N.C. 28608

Serotonergic systems exert important modulatory effects on sensory neurons and motoneurons in the mammalian spinal cord. These effects, which may be inhibitory or facilitatory, are mediated by synapses and receptors distributed over the surface of the target neurons. We were interested to determine if an important sensory feedback pathway providing proprioceptive information to the cerebellum, the dorsal spinocerebellar tract (DSCT), received direct serotonergic input and to what extent such input was distributed over the proximal versus distal dendrites of DSCT cells. Identified DSCT cells were intracellularly stained with horseradish peroxidase, and 5-HT immunoreactive varicosities in contact with the cells were revealed by immunohistochemistry using an antibody raised in guinea pigs against keyhole limpet hemocyanin-conjugated 5-HT. At the light microscopic level, 5-HT contacts were observed on the soma and all regions of the extensive dendrites of stained DSCT cells. However, the density of 5-HT contacts was much lower than previously observed on spinal motoneurons, suggesting that the DSCT is not subject to particularly powerful descending serotonergic influences, thereby allowing the system to faithfully and rapidly relay its information regardless of activity in other systems. Supported by NIH grant NS25547.

650.8

IMMUNOHISTOCHEMICAL ANALYSIS OF SPINAL NEUROTRANSMITTER SYSTEMS AND RECEPTORS IN CATS WITH SPINAL CORD TRANSECTION. R.E.W. Fyffe, F.J. Alvarez, J.A. Hodgson, R.R.Roy, V.R. Edgerton, J.C. Pearson*, D.E. Dewey, D.A. Harrington and J.V. Priestley. Dept of Anatomy, Wright State Univ. Dayton, OH 45435, Dept of Physiological Sciences, UCLA, CA 90024, and Dept of Physiology, UMDS, St Thomas's Campus, London SE1 7EH, UK.

Spinalized cats exhibit variable degrees of weight bearing and locomotor ability in their hindlimbs, and these parameters can be differentially affected by training and certain pharmacological manipulations. To determine if the variability between animals is correlated with different patterns of neurochemical reorganization we studied the distribution of immunocytochemical markers for a variety of neurotransmitters and receptors involved in descending and segmental control of motor function, in cats that had undergone different training paradigms and exhibited different types of motor behavior following transection at T12-T13. Unlike controls, immunoreactivity for serotonin, tyrosine hydroxylase, and thyrotropin releasing hormone was completely absent in all cats in segments below the lesion site, indicating that the transections were complete in all cases. Interestingly, CGRP immunoreactivity was decreased in the ventral horn, suggesting some direct or indirect supraspinal influence. A similar result was observed for substance P labelling. There was no obvious rearrangement or expansion of markers for likely segmental transmitters (e.g. GAD, glycine receptors) to occupy sites vacated by the descending systems. It appears that the difference in locomotor capability among cats is the result of more subtle changes in circuitry or transmitter/receptor expression in spinal neurons. Supported in part by NIH grants NS16333 and NS25547.

650.9

SOMATIC MEMBRANE COVERING BY GLYCINERGIC TERMINALS AND GLYCINE RECEPTORS OF α-MOTONEURONS AND RENSHAW CELLS IN CAT SPINAL CORD. D. A. Harrington, F. J. Alvarez, and R.E.W. Fyffe*. Department of Anatomy, Wright State University, Dayton OH 45435.

Glycine is a major inhibitory neurotransmitter in the ventral horn of the spinal cord, exerting powerful effects over neural elements involved in motor behavior. Here we evaluated quantitatively various synaptic parameters related to glycinergic terminals over the cell somas of α-motoneurons (α-MN) and Renshaw cells (RC). We assessed glycine receptor presence using electron microscopy pre-embedding immunocytochemistry with antibodies directed against gephyrin, a protein associated with postsynaptic glycine receptor clusters. α-MNs were identified by their size, location and characteristic synaptology. RCs were identified by their location in ventral lamina VII and their distinctive gephyrin-immunoreactivity (geph-ir). Overall synaptic covering was similar in α-MN and RCs (45-65% of the available somatic membrane), with geph-ir terminals being the most abundant type on both RCs and α-MNs. But, while terminals with geph-ir synapses constitute about 90% of the synaptic covering in RCs, they constitute only 40-45% in α-MNs. An average of 87% of all terminals that contacted RC somas displayed geph-ir while this value was 49% for α-MNs. All geph-ir terminals were of the F type, and had similar ultrastructural features on RCs and α-MNs. The percentage of apposition area occupied by postsynaptic geph-ir was larger in RC somas: 36-40%, compared to 15-20% on α-MNs. The extension of postsynaptic geph-ir was almost identical to the extension of the opposed presynaptic active zone. In conclusion, while glycinergic control is very substantial over both neuronal types, it is much more abundant over the cell somas of RCs. Individual glycinergic synapses on RC somas have the potential to elicit larger postsynaptic effects since their active zones are larger and the postsynaptic receptor regions are usually more intensely immunoreactive than those on α-MN somas. Supported by NIH grant NS25547.

651.1

SEROTONIN (5-HT) REDUCES AN AFTERHYPERPOLARIZATION IN NEONATAL RAT MOTONEURONS THROUGH INHIBITION OF N- AND P-TYPE CALCIUM CHANNELS. D.A. Bayliss*, M. Umemiya and A.J. Berger. Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195

We have shown that 5-HT increases input-output gain of neonatal rat hypoglossal motoneurons (HMs) via decreased amplitude of Ca^{2+}-dependent afterhyperpolarization (AHP) (*Neurosci.Lett.* **143**: 164, 1992). To determine if the decreased AHP is mediated by decreased Ca^{2+} influx, we tested the effect of 5-HT on Ca^{2+} currents in visualized HMs using a thin-slice preparation of rat brainstem. 5-HT inhibited high-voltage-activated (HVA) Ca^{2+} currents (measured at 0 mV from a holding potential of -70 mV), by >10% in ~70% of HMs tested (n=99); in responsive neurones, 5-HT decreased HVA current by 22%. Low-voltage-activated currents, evoked during steps to between -30 and -40 mV from hyperpolarized potentials, were unaffected by 5-HT (n=7). The 5-HT_{1A} receptor mediated inhibition of both the AHP and HVA current. Thus the effect of 5-HT on the AHP was mimicked by 8-OH-DPAT; HVA current inhibition by 5-HT was mimicked by 5-CT and 8-OH-DPAT, but not by TFMPP, and was blocked by the 5-HT_{1A} antagonist, NAN-190, but not by ketanserin, a 5-HT_2 antagonist. 5-HT inhibited both N-type (ω-Conotoxin GVIA-sensitive; 3 μM) and P-type (ω-Aga IVA-sensitive; 0.2 μM) Ca^{2+} channels in neonatal HMs; 5-HT had no effect on the residual current remaining after application of both toxins. Effects of 5-HT on HVA current were irreversible, and subsequent applications of 5-HT were occluded when GTPγS was substituted for GTP in the pipette solution. In addition, inhibition of Ca^{2+} current was partially relieved following large depolarizing prepulses, indicating that inhibition of Ca^{2+} channels by 5-HT is mediated by G proteins, perhaps directly. Therefore, inhibition of Ca^{2+} current by 5-HT is responsible, at least in part, for the 5-HT-induced decrease in AHP in neonatal HMs and, in this way, 5-HT enhances the excitability of HMs. (Supported by NS14857 and Francis Families Foundation).

651.2

AT LEAST TWO DISTINCT IONIC MECHANISMS UNDERLIE THE RESPONSE OF HYPOGLOSSAL MOTONEURONS (HMs) TO NOREPINEPHRINE. M. A. Parkis*, D. A. Bayliss, and A. J. Berger. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195

The *in vitro* response of HMs to norepinephrine (NE) mimics their response to thyrotropin releasing hormone (TRH), i.e. depolarization accompanied by increased input resistance (R_N) recorded in current-clamp, or development of an inward current with decreased input conductance in voltage-clamp (*J. Neurophys.* 68:1733, 1992, *Soc. Neurosci. Abstr.* 18:512, 1992). The NE current, like the TRH current, reverses near -100 mV. In addition, the effects of NE on HMs are occluded by TRH in the perfusing artificial cerebrospinal fluid (ACSF, *Soc. Neurosci. Abstr.*, 19:988, 1993), suggesting that these two neuromodulators share ionic mechanisms of action in HMs.

Since the TRH-mediated increase in R_N is occluded by substitution of barium (Ba^{2+}) for calcium in the ACSF, while the depolarization is not, we tested whether the same held true for the response of HMs to NE. We found that the increase in HM R_N elicited by NE was reduced from 56% in control to only 3% in Ba^{2+}-ACSF, despite the cells' continued ability to exhibit a depolarizing response (n = 7). After return to Ba^{2+}-free ACSF, HMs again showed increased R_N in response to NE.

To investigate the ionic mechanism responsible for the Ba^{2+}-resistant depolarizing response, we substituted choline chloride for sodium chloride in the ACSF. Within ten minutes of switching into a Ba^{2+}- and choline-substituted ACSF, HMs no longer depolarized in response to NE (n = 4). After longer periods of time the response became a hyperpolarization. In choline-substituted ACSF without Ba^{2+}, a depolarization with increased R_N could still be elicited by NE, but recovery from the depolarization was prolonged, the potential taking longer to return to baseline (n=3).

We conclude that NE depolarizes HMs by at least two ionic mechanisms: reduction of a Ba^{2+}-sensitive resting K^+ conductance, and activation of a Ba^{2+}-resistant cationic current carried predominantly by sodium ions. (Supported by HL - 49657).

651.3

ADENOSINE MODULATES EXCITATORY SYNAPTIC TRANSMISSION TO RAT HYPOGLOSSAL MOTONEURONS. M.C. Bellingham* and A.J. Berger, Dept. Physiol. & Biophys., Univ. Washington, Seattle, WA 98195.

Adenosinergic modulation of excitatory inputs may be especially significant in hypoxic responses of HMs, as extracellular CNS adenosine levels rise many-fold under these circumstances. To investigate this modulation, intracellular recordings were made from adult rat hypoglossal motoneurons (HMs; n = 25) in brainstem slices. Electrical stimulation lateral to the hypoglossal motor nucleus evoked short-latency (0.6-1.4 ms) excitatory postsynaptic potentials (EPSPs). These EPSPs were markedly suppressed or abolished by bath application of kynurenic acid (1 mM; n = 7), showing that they were glutamatergic. Bath application of adenosine receptor agonists 2-chloro-N^6-cyclopentyladenosine (CCPA, 100 nM) or 2-chloroadenosine (2-CA, 1 μM) significantly reduced EPSP amplitude to 42 ± 5% (n = 6) and 76 ± 7% (n = 6) of control, respectively. The adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.1-1 μM) significantly increased the EPSP amplitude to 124 ± 14% of control (n = 9), and blocked EPSP reduction by bath or local application of CCPA or 2-CA (n = 7). CCPA, 2-CA and DPCPX did not significantly alter HM input resistance or membrane potential. Repeated local application of ω-conotoxin (ω-CgTx, 1 μM), which caused maximal reduction of the medium afterhyperpolarization that followed action potentials evoked by brief current pulses, also reduced EPSP amplitude to 68 % of control (n = 2), but failed to occlude EPSP amplitude decrease in response to bath application of 2-CA (10 μM; reduction to 50% of the remaining EPSP after ω-CgTx, cf. reduction to 61% of control values before ω-CgTx). These data indicate that excitatory glutamatergic inputs to rat HMs are modulated by adenosine A_1 receptors, most probably at a presynaptic site. Modulation of presynaptic N-type calcium channels does not appear to be essential for the mechanisms by which presynaptic A_1 receptors reduce excitatory synaptic transmission to HMs. (Supported by NS 14857, HL 49657).

651.4

PRESYNAPTIC INHIBITION BY SEROTONIN OF GLYCINERGIC INHIBITORY POSTSYNAPTIC CURRENTS (IPSCs) IN RAT MOTONEURONS. M. Umemiya* and A.J. Berger, Dept. Physiology and Biophysics, University of Washington, Seattle, WA 98115.

Serotonin (5-HT) plays an important role in the neuronal function of synaptic modulation. Using a thin-slice preparation, we found that 5-HT presynaptically inhibited unitary glycinergic IPSCs evoked by extracellular stimulation of interneurons and recorded in motoneurons of rat brainstem. One possible mechanism for presynaptic inhibition is K^+ channel activation and/or voltage-activated Ca^{2+} channel inhibition at the presynaptic terminal. In this regard, the 5-HT_{1A} receptor activated inwardly rectifying K^+ channels and inhibited voltage-activated Ca^{2+} channels recorded in somata of presynaptic interneurons. However, the 5-HT_{1B} receptor was primarily responsible for inhibition of evoked glycinergic IPSCs, whereas 5-HT_{1A} receptor activation inhibited IPSCs by only 24%. In addition, 5-HT_{1B} receptor activation at the presynaptic terminal reduced the frequency of spontaneous miniature IPSCs (mIPSCs) without changing the mIPSCs amplitude distribution in the presence of TTX. These results indicate that activation of inwardly rectifying K^+ channels and the inhibition of voltage-activated Ca^{2+} channels by 5-HT_{1A} receptor activation is not a main pathway for presynaptic inhibition by 5-HT. Modulation of the transmitter release mechanism through 5-HT_{1B} receptor activation may be a dominant pathway for presynaptic inhibition by 5-HT. (Supported by NS 14857).

651.5

THYROTROPIN-RELEASING HORMONE (TRH) DEPOLARIZES RESPIRATORY RELATED NEURONS IN A THICK SLICE PREPARATION OF THE NEWBORN MOUSE BRAINSTEM. J.C. Rekling*, J. Champagnat and M. Denavit-Saubié. C.N.R.S., Institut Alfred Fessard, 91198 Gif-sur-Yvette, France.

A thick (1.5-2.0 mm) slice preparation of the 1-6 day old mouse brainstem was developed. TRH (1-5 μM) increased the frequency of respiratory related discharges recorded from hypoglossal roots by a factor of three. Whole cell patch recordings from anatomically identified hypoglossal motoneurons showed that the neurons were depolarized by TRH and the respiratory bursts of EPSPs were increased in duration, as were the discharges on the hypoglossal nerve. In the ventrolateral part of the preparation a subset of inspiratory related neurons were depolarized by TRH, others were unaffected. Expiratory related neurons (hyperpolarized during the nerve discharge) were also depolarized by TRH.

It is concluded that TRH increases the respiratory rhythm of the newborn mouse by an action at the level of the brainstem caudal to the facial nucleus. The results suggest that the increase in respiratory frequency induced by TRH may result from depolarization of a subset of ventral respiratory neurons, either by a direct postsynaptic action or by excitation of other modulatory neurons present in the preparation. (*Supported by an EEC grant and The Augustinus Foundation*)

651.6

EXCITATORY AMINO ACIDS AND GABA ANTAGONISTS POTENTIATE THE EFFECT OF CARBACHOL IN PONTINE RETICULAR FORMATION - CATALEPSY AS THE BEHAVIORAL MEASURE. Z. Elazar* and A. Berchansky, Depart. of Physiology & Pharmacology, Sackler School of Medicine, Tel-Aviv University, Israel.

Microinjections of different amounts of carbachol into the pontine reticular formation (PRF) in rats could induce REM sleep, catalepsy, or epilepsy. The intensity of catalepsy, the object of this study, is high for 5-15 min and decreases to low levels 30 min after the injection. The excitatory amino acids (EAA) L- glutamate, N-methyl-D-aspartate, kainate and quisqualate injected in PRF in picomol doses (0.2 μl) did not by themselves induce catalepsy or induced only mild catalepsy. When injected 15 min before the injection of 1 μg/0.2 μl carbachol in the same PRF site, unilaterally, they greatly potentiated the effect of carbachol: the intensity of catalepsy increased up to more than ten fold and the duration several times. Picrotoxin (30-40 pmol/0.2 μl) injected in the same PRF site 15 min before the carbachol similarly potentiated the intensity and duration of catalepsy. Slightly higher doses of EAA or picrotoxin reduced or prevented the cataleptogenic effect of carbachol. These results suggest the importance of the interactions between the cholinergic mechanisms in PRF and EAA and GABA.

651.7

DESCENDING SEROTONERGIC CONTROL OF CROSSED GROUP II INHIBITION IN THE SPINAL CORD OF THE CAT. N.C. Aggelopoulos[1], R.W. Clarke[2] and S.A. Edgley[1]. [1]Department of Anatomy, University of Cambridge and Department of Physiology and [2]Environmental Science, University of Nottingham, United Kingdom. SPON: Brain Research Association.

Stimulation of hindlimb group II muscle afferents inhibits many contralateral hindlimb motoneurons. This crossed inhibitory reflex depends on a descending system as it disappears on section of the spinal cord. We have tested the hypothesis that crossed inhibition is dependent on serotonergic transmission.

Experiments were carried out in four cats under chloralose general anesthesia. Intracellular recordings were obtained from hindlimb motoneurons. Electrical stimulation of the contralateral quadriceps nerve at a strength sufficient to activate group II afferents (5T) elicited IPSPs in the majority (44/46 : 96%) of gastrocnemius/soleus and hamstring motoneurons. The incidence of IPSPs was reduced after spinal transection at the thoracic level (9/40 : 22%) and the IPSPs were smaller. After spinalization, intravenous administration of (+/-)-8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, Research Biochemicals), a selective agonist of $5HT_{1A}$ receptors (dose 0.11-1.21 mg/kg), restored the crossed inhibition of many motoneurons (41/48: 85%) within 5-15 min. The effect of 8-OH-DPAT was antagonized with a latency of 10-20 minutes by the selective $5HT_{1A}$ antagonist (+)-WAY-100135 (a gift of Wyeth Research UK) injected intravenously at a dose of 0.7-3.7 mg/kg, which markedly reduced the incidence of IPSPs in motoneurons (10/45 : 22%).

The simplest explanation of these results is that a descending pathway acting via $5HT_{1A}$ receptors permits crossed group II inhibition to operate.

Supported by the Wellcome Trust and MRC (UK).

651.8

NITRIC OXIDE INHIBITION CAUSES STATE-DEPENDENT DEPRESSION OF ACETYLCHOLINE (ACh) RELEASE IN THE MEDIAL PONTINE RETICULAR FORMATION (mPRF). T.O. Leonard*, L. Becker and R. Lydic. Dept. Anesthesia, Penn. State Univ., College of Med., Hershey PA 17033.

NADPH-diaphorase staining is thought to reveal neurons which use nitric oxide as a neuromodulator (J. Comp. Neurology 324:410, 1993). Cholinergic neurons of the pedunculopontine tegmental nucleus (PPT) projecting to the mPRF are NADPH-positive, and electrical stimulation of PPT neurons increases mPRF ACh release (Am. J. Physiol. 264:R544, 1993). Therefore the present study is testing the hypothesis that inhibition of nitric oxide synthase with N^G-nitro-L-arginine (NLA) will result in state-dependent reduction of mPRF ACh release. A cat was chronically implanted with electrodes for monitoring sleep and wakefulness. For each experiment, a microdialysis probe was stereotaxically placed in the mPRF for simultaneous recovery of ACh and delivery of either Ringers solution or 10 mM NLA in Ringers. Ten min dialysate samples (n) were collected during waking (n=46), non-rapid eye-movement (NREM) sleep (n=48), and REM sleep (n=16). Samples were analyzed for pmol of ACh using HPLC. ACh levels (mean±SD) during waking with NLA dialysis (0.16±0.11) were significantly less (t=3.27, df=44, p=0.002) than ACh levels with Ringers alone (0.24±0.07). Likewise, during NREM sleep ACh levels with NLA dialysis (0.12±0.11) were less (t=3.29, df=46, p=0.002) than ACh levels with Ringers (0.20±0.05). During REM sleep, however, there was no significant difference (p=0.777) in ACh levels between NLA dialysis (0.34±0.29) and dialysis with Ringers (0.38±0.12). These data suggest nitric oxide may modulate state-dependent cholinergic neurotransmission within the mPRF. *Support: Department of Anesthesia, HL-40881 (RL).*

651.9

SEROTONIN AND NOREPINEPHRINE MODULATE EXCITATORY AMINO ACID RECEPTOR CURRENTS IN ACUTELY ISOLATED RAT VENTRAL HORN NEURONS. S.C. MacDonald, S. Hochman, and L.M. Jordan*, Dept. of Physiology, University of Manitoba, Winnipeg, MB, R3E 0W3

It has previously been reported that serotonin (5-HT), and norepinephrine (NE) and N-methyl-D-aspartate (NMDA) are involved in locomotor-like acitivity in the neonatal rat preparation.[1] We hypothesize that neuromodulation of excitatory synaptic transmission by monoamines plays a role in the generation and control of locomotion. Our goal is to examine the mechanisms of this modulation at a single cell level. We examined the effects of two monoamines, 5-HT and NE, on excitatory amino acid (EAA) currents in the ventral horn. Whole-cell voltage clamp recordings were obtained from acutely dissociated neurons from the area of the spinal cord where locomotor-related neurons are found. Local perfusion of NMDA and kainate evoked inward currents. These currents increased or decreased in the presence of either 5-HT or NE. Within single neurons, 5-HT and NE exhibited common actions for a tested EAA receptor subtype, whether facilitatory or inhibitory. Kainate currents were examined in 38 cells and were depressed in 26% of cells and potentiated in 45%. There was a mean current increase of 77.9±64.5% and 66.9±40.3% and decrease of 37.9±17.6% and 21.9±5.2% in the presence of 5-HT and NE respectively. Similarily , out of 28 cells examined with NMDA, 18% of cells were potentiated and 54% were depressed. Mean increases in current were 27.3±22.2% and 33.8± 17.2% and decreases 36.6±11% and 16.9±11.4% for 5-HT and NE respectively. Preliminary results indicate that 5-HT1a and α_2 noradrenergic receptors contribute to the observed effects. These results suggest that 5-HT and NE can modulate excitatory synaptic transmission at the level of the post-synaptic receptor. Supported by MRC. S.C.M. is a Network of Centres of Excellence Trainee. [1](Cazalets,et al.,Neuroscience Lett., 1990)

651.10

SEROTONIN INCREASES HYPOGLOSSAL (XII) REFLEX RESPONSE TO UPPER AIRWAY NEGATIVE PRESSURE. M.A. Douse* and D.P. White, Dept. of Medicine, UCHSC, and Respiratory Care, VAMC, Denver, CO 80220.

Serotonin (5HT) is known to increase XII motoneuron activity levels, yet nothing is known concerning 5HT modulation of XII reflex responses. We determined the effects of 5HT and methysergide (broad 5HT antagonist) microinjection into the XII motor nucleus (200-500 nl; pH=7.2-7.4) on XII whole nerve response to upper airway negative pressures (Paw) in 4 decerebrate, vagotomized, paralyzed and artificially ventilated cats. Results to date indicate control negative Paw tests resulted in graded increases in the reflex response (n=4; Table 1) measured as the inspiratory peak height of XII integrated neural activity (time constant=100ms) in arbitrary units (AU) normalized to pre-test control. Microinjection of 0.5 mM 5HT resulted in an increase in peak inspiratory integrated XII activity at each Paw (cf. to pre-test control), but a decrease in the slope of the response curve from 2.76 to 0.81 AU/cm H_2O (n=2; Table 1). Prior microinjection of 1.0 mM methysergide resulted in a decrease in XII activity at each Paw and a decrease in the response curve slope to 0.88 (n=2; Table 1). Subsequent microinjection of 0.5 mM 5HT had no further effect. These preliminary results indicate exogenous and endogenous 5HT increases the peak XII reflex response to upper airway negative pressures and suggest that state dependent decreases in 5HT may be important in the loss of this reflex during sleep. This may have important implications in the development of obstructive sleep apnea. (NIH HL-48531).

Table 1 - XII Reflex Responses to Paw (Arbitrary Units)

Paw (cm H_2O)	0	-5	-10	-15	-20
Control	100	98.1	119.5	136.2	150.0
5HT	175.6	178.9	180.5	181.8	194.5
Methysergide	44.3	30.9	37.1	44.4	59.6

651.11

FAILURE OF INTRATHECAL NIMODIPINE TO INCREASE SPINAL CORD BLOOD FLOW. H. Imamura and C.H. Tator*. Division of Neurosurgery and Playfair Neuroscience Unit, Toronto Western Division, The Toronto Hospital, University of Toronto, Toronto, Ontario M5T 2S8, Canada

We have demonstrated that intravenously administered calcium channel antagonist nimodipine produced an increase of spinal cord blood flow (SCBF) in normal and injured rats. However, in the injured rats, due to the hypotension caused by both cord trauma and the vasodilator effects of nimodipine it was necessary to counteract the hypotension and maintain the posttraumatic arterial blood pressure. Nimodipine has high lipid solubility and penetrates well into the central nervous system, so the present study was performed to investigate whether intrathecally infused nimodipine could increase the SCBF in normal rats. Male Wister rats anesthetized by alpha-chloralose and urethane had a laminectomy from C1 to T1 and a silastic tube was inserted into the subarachnoid space via the atlantooccipital membrane to the C6 level. We administered intrathecal nimodipine (0.05 mg/kg; n=5, 0.2 mg/kg; n=5) and placebo (n=5), and measured SCBF at C7/T1 with the hydrogen clearance method before infusion, during infusion and 30 minutes after infusion of the drug. Neither 0.05 nor 0.2 mg/kg of nimodipine increased SCBF during infusion (df=2,12; F=0.35; P>0.05) or after infusion (df=2,12; F=1.31; P>0.05). Although 0.2 mg/kg nimodipine caused a 26.4% decrease in blood pressure at the end of infusion (p=0.006), it recovered to 85.2% of preinfusion value at the end of the experiment (1 hour after infusion, p=0.001). These results show that intrathecal nimodipine at these doses cannot increase spinal cord blood flow.

652.1

DOPAMINERGIC MODULATION OF THE SWIM NETWORK AND SPINAL NEURONS IN LAMPREY. C.P. Kemnitz, J.T. Buchanan', W. Merlau, & I.V. Batueva. Department of Biology, Marquette University, Milwaukee, WI 53233.

We have previously shown that dopamine is present in cell bodies and processes in the lamprey spinal cord and that dopamine modulates fictive swimming (*Neurosci. Lett.*,166:23-26,1994). To further investigate the actions of dopamine, we examined the effects of dopaminergic agents at several levels: 1) in intact swimming lamprey, 2) on network, cellular, and synaptic properties in the isolated spinal cord, and 3) on ionic currents in isolated spinal neurons. The experiments were done on adult sea lampreys (*Petromyzon marinus*) and adult silver lampreys (*Ichthyomyzon unicuspis*).

Injection of the dopamine agonist apomorphine into intact adult sea lampreys decreased the cycle period of swimming, an effect similar to that of low concentrations (<10μM) of dopamine during fictive swimming. The D1 dopamine receptor agonist SKF-38393 decreased cycle period during fictive swimming, while the D2 agonist 3-PPP had no effect. Dopamine reduced the late after-spike hyperpolarization in motoneurons (MN), primary sensory dorsal cells (DC), stretch receptor edge cells (EC), and giant interneurons (GI) and increased the firing frequencies of MNs, ECs, and GIs to current injection. Calcium action potentials in MNs, DCs, and GIs were reduced by dopamine, suggesting that reduced Ca^{++} influx leads to decreased activation of the Ca^{++}-activated K^+ current responsible for the late after-spike hyperpolarization. Poly- and monosynaptic inhibitory postsynaptic potentials in motoneurons were also reduced by dopamine. Whole-cell patch clamp studies of isolated dorsal cells indicate that dopamine reduces high-voltage activated calcium currents.

These results suggest that dopamine may modulate fictive swimming in lamprey via a D1 receptor by altering calcium influx, leading to changes in both the firing properties and synaptic strengths of spinal neurons. (Supported by NIH NS-28369, TW00245, and MH-49581)

652.2

PHOTO-ABLATION OF COMMISSURAL INTERNEURONS ALTERS FICTIVE SWIMMING IN LAMPREYS. J.T. Buchanan, D.R. McPherson*, & T.R. Strauss. Department of Biology, Marquette University, Milwaukee, WI 53233.

We used photo-ablation to test the involvement of commissural interneurons in fictive swimming in the isolated spinal cord. Spinal interneurons were retrogradely labeled by applying a mixture of fluorescein- and eosin-dextran amines to a transverse hemisection of the midbody spinal cord of adult sea lampreys (*Petromyzon marinus*). After 1 to 5 weeks of survival, fictive swimming was induced in 8-segment lengths of isolated spinal cord with N-methyl-DL-aspartate. An argon laser beam with a diameter of one-half the width of the spinal cord was moved along the spinal cord contralateral to the dye application site, allowing four minutes per location.

A few minutes after laser onset, an asymmetry began to develop in ventral root burst proportions. On the illuminated side (contralateral to dye application site), burst duration as a proportion of cycle period decreased from 0.41±0.06 (±S.D.) to 0.25± 0.06, while on the non-illuminated side it increased to 0.61±0.09 (n=5). The changes in burst proportion were observed in spinal cord preparations both caudal and rostral to the dye application site. Cycle period increased about 35% with illumination. Phase coupling of left-right burst midpoints was not altered, nor was rostral-caudal coupling. In two cases, all rhythmicity appeared to be lost, leaving continuous firing of ventral roots on the non-illuminated side and weak, irregular spiking on the illuminated side. Control cords without tracer did not show these effects of laser illumination. Intracellular recordings of retrogradely-labeled neurons demonstrated that illumination caused depolarization, action potential broadening, and blockage of axonal propagation.

The results suggest that commissural interneurons play a role in the production of fictive swimming. One interpretation, supported by computer modeling, is that the outputs of crossed inhibitory cells were decreased by the laser, disinhibiting the opposite side. The resulting imbalance in the reciprocal inhibitory network produces an asymmetry in the burst proportions. (Supported by NIH NS-28369 & NS-09111)

652.3

INTERSEGMENTAL COORDINATION AFTER CHRONIC AND ACUTE LESIONS IN LAMPREY SPINAL CORD ASSESSED DURING FICTIVE SWIMMING. L. Guan, T. Kiemel, D. Liao and A.H.Cohen*. Department of Zoology, University of Maryland, College Park, MD 20742.

Previously, we reported the use of correlational analysis in the isolated lamprey spinal cord to demonstrate that during fictive swimming there is strong intersegmental coupling. We define strong coupling in terms of the speed with which the bursting returns to its regular frequency after external perturbations. In this analysis we compare cross-correlation of periods and auto-correlations of delay between pairs of segments. High cross- correlation of periods and low auto-correlation of delays indicated strong coupling. We comapred the results to simple models of coupled non-linear oscillators to illustrate the plausibility of the conclusion.

Here we extend the method to spinal cords previously either acutely or chronically lesioned. Lesions were of either the lateral or the medial fiber tracts or the entire spinal cord. At the time of testing, in partially lesioned animals acute lesions of the formerly spared fibers were often added to the chronic lesion to examine the function of regenerated fibers in the absence of the unlesioned fibers. Chronically lesioned cords were compared to acutely lesioned healthy cords. The analysis in the acutely lesioned spinal cords revealed moderate to strong coupling strength of either medial or lateral tract coordinating fibers. However, there was little evidence that the regenerated fibers were capable of strong or moderate strength coupling even after 10 months of recovery. Only in the presence of spared fibers was any strong coupling found. In only two cases was there even a hint of moderate strength coupling of regenerated fibers with this method of analysis. Thus, the regeneration, at best, seemed to restore only weak or perhaps occasionally moderate strength coupling among the segments. Supported by NIMH MH44809 and NIH NS16803 to AHC; DL was supported by the Howard Hughes Medical Institute.

652.4

ROLE OF RETICULOSPINAL NEURONS IN LOCOMOTOR CONTROL IN THE LAMPREY: INVESTIGATION USING A NEURAL NETWORK MODEL. R. Jung* and A. H. Cohen. Department of Zoology, University of Maryland, College Park, MD 20742.

Neurons in the spinal cord of the lamprey form the central pattern generator(CPG) for swimming. These CPG neurons and the motoneurons(MN) receive input from reticulospinal neurons (RN). During fictive locomotion induced in in-vitro spinal cord preparations some of the RN are phasically active. They are believed to be part of a spino-reticulo-spinal loop. We investigated the role of reticular and spinal neuron interaction on locomotor control in a neural network model by examining (a) the change in ascending-descending coupling between the RN and spinal cord neurons (b) the effect of brief perturbing stimuli to an RN during different phases of its cycle. In the model each neuron pool was represented by a single neuron which received tonic input as well as weighted synaptic input from other connecting neurons. In the absence of input from RN the CPG and MN outputs were rhythmic with phase relationships similar to those observed experimentally. (a) With the RN connected the MN cycle period (MN_pd) is dependent on the ascending-descending coupling gain ratio (GNratio). For GNratio < 1, MN_pd decreased as descending gain (dscgn) increased ultimately resulting in a tonic output. For GNratio > 1 and low dscgn, increasing the ascending gain (ascgn) increased MN_pd and at very high ascgn the output became unstable. For high dscgn however, the GNratio must not only be > 1 but also above a threshold level in order to elicit rhythmic output from the CPG and MNs. (b) The effect of perturbing stimuli (20 msec pulse) to the RN on MN output was strength and phase dependent. Stimuli of low strengths had negligible affect on the MN cycle. Stronger stimuli caused obvious phase resetting; stimuli applied early in the cycle increased MN_pd (longer burst), while those late in the cycle decreased MN_pd. These simulation results are consistent with a role for the RNs both in generating the basic locomotor rhythm and in sensory integration in the intact lamprey. Supported by NRSA NS09462 to RJ and NIMH MH44809 to AHC.

652.5

CONTROL OF VENTRAL ROOT BURST PROPERTIES DURING FICTIVE SWIMMING IN THE LAMPREY SPINAL CORD. W. L. Miller and K. A. Sigvardt*. Dept. of Neurology, University of California, Davis, CA, 95616

The main tasks of the lamprey spinal central pattern generator (CPG) for locomotion are to produce bursts of activity in the segmental motor nerves that result in an appropriately sized contraction of the segmental muscles, and to maintain stable, coordinated activity among all segments, over a large range of cycle frequencies. These functions most likely involve both local and long-distance interactions among spinal interneurons, but the details of these interactions are not yet understood. To investigate the relationship between the segmental and coordination functions, we analyzed ventral root burst characteristics from data collected in "split bath" experiments using *in vitro* preparations of the lamprey spinal cord (50 segments) in which the concentration of excitatory amino acid (EAA) bathing the rostral and caudal halves of the cord could be controlled separately. Simultaneous recordings were made from six extracellular electrodes placed at regular intervals along the cord. The analyzed trials comprised a four-fold range of cycle periods, and a three-fold differential range of EAA concentrations in the rostral and caudal bathing solutions. Parameters of interest in the present study include burst duration, burst proportion (burst duration/cycle period), mean amplitude, integrated amplitude, time-to-peak and the variance in these parameters. Several of these values are calculated using both burst recognition and time series approaches. Burst proportion did not change relative to controls over the full range of cycle periods investigated, and rostral burst proportions remained equal to caudal burst proportions when the EAA concentrations were unequal. By contrast, a previous analysis of the same experimental data showed that rostral phase lags decrease relative to controls while caudal phase lags remain unchanged when the EAA concentration is higher in the caudal compartment (Sigvardt et al., Neurosci. Abst. 17:122 (1991); Sigvardt and Williams, in preparation).

652.6

EFFECTS OF APAMIN ON THE FICTIVE GILL AND LUNG VENTILATION IN TADPOLE, *RANA CATESBEIANA*, BRAINSTEM *IN VITRO*. G.-S. Liao, R.J. Galante, A.P. Fishman, L. Kubin and A.I. Pack*. Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA 19104.

Small conductance, Ca^{2+}-dependent potassium channels (sK_{Ca}) are involved in regulation of afterhyperpolarization of neurons and affect their repetitive firing properties. This may be particularly important in the generation of rhythmic behaviors. The *in vitro* brainstem of tadpoles generates two respiratory rhythms corresponding to lung and gill rhythms of an intact specimen (Galante *et al.*, Soc. Neurosci. Abstr. 18:125, 1992). The neural output changes with the development. Thus, it offers an attractive model with which to study mechanisms underlying rhythmic behaviors and their development. In order to begin to determine the role of sK_{Ca} in the fictive gill and lung ventilation, we recorded neural activities from the facial nerve in brainstems of tadpoles at intermediate developmental stages (VII-XI) while superfusing the preparation with 5 different concentrations of apamin (0.1-2.5 μM). Apamin increased the amplitude of gill-related activity in a reversible and dose-dependent fashion (at the highest concentration: 290%±86(SD) of the control; n=5). In contrast, changes in the amplitude of lung-related bursts were minor and did not show a dose-dependence. In the time domain, apamin had no effect on the frequency of gill bursts, while it increased the duration of lung bursts in a dose-dependent manner (from 1.5s±0.3 in control, to 2.3s±0.5 at the highest concentration; n=5). Thus, apamin, without disrupting either of the rhythms, had differential effects on gill and lung activities in that only the amplitude of the former and primarily the burst duration of the latter were affected. Because both rhythms are found in most facial motoneurons (Liao *et al*, Soc. Neurosci. Abstr. 19:558, 1993), the effects of apamin must occur at premotoneuronal, including pattern generator, levels. These effects have to be studied in younger and older tadpoles to assess the possible changes in the role of sK_{Ca} with development. (Supported by HL-07713 and HL-49486)

652.7

SIMULTANEOUS ACTIVATION OF BOTH MAUTHNER AXONS CAN RESET THE FICTIVE SWIMMING RHYTHM IN GOLDFISH. K.R Svoboda* and J.R. Fetcho. Dept. Neurobiology and Behavior. SUNY at Stony Brook, NY 11794.

A single action potential elicited in one of the two Mauthner axons (M-axon) while a fish is "fictively" swimming can dramatically reset the swimming rhythm. We are trying to identify cellular elements within the M-cell network that may subserve this resetting phenomenon. Firing both M-axons simultaneously results in cranial motor output, but no spinal motor output because the spinal commissural inhibitory interneurons in the M-cell network block firing of motoneurons and excitatory interneurons. We fired both M-axons simultaneously during bouts of fictive swimming to assess whether the resetting of the swimming rhythm could occur in the absence of spinal motor output from the escape network. Fictive swimming was elicited by stimulation of the midbrain of decerebrate, paralyzed goldfish and the motor pattern was monitored by recording extracellularly from branches of ventral roots. A single intracellularly elicited action potential was initiated in each M-axon simultaneously (within 0.1ms of each other) during bouts of fictive swimming. Simultaneous firing of both M-axons could reset the swimming rhythm as indicated by a shift in the midpoints of swimming bursts post-axon stimulation relative to when they would be expected to occur based upon the pre-stimulation burst pattern. This result indicates that the resetting of the swimming rhythm can occur in the absence of spinal motor output from the Mauthner cells and suggests that the commissural inhibitory interneurons in the M-cell network contribute to the resetting. Support: NIH NS26539 (JRF).

652.8

CONFOCAL IMAGING OF CALCIUM DYNAMICS IN SPINAL NEURONS IN THE INTACT ZEBRAFISH. D.M. O'Malley* and J.R. Fetcho. Dept. Neurobiology & Behavior, Howard Hughes Medical Institute, SUNY at Stony Brook, NY 11794.

Calcium green dextran (CGD) was used to monitor activity in the spinal cord of larval zebrafish. CGD (10k, Molec. Probes) was injected into muscle or spinal cord. After periods ranging from 16 hours to 9 days, the fish were curarized, placed in a glass-bottomed petri dish on the stage of an inverted Zeiss IM 35 microscope, and imaged with a Biorad MRC 600 confocal microscope. The CGD labeling revealed sufficient details of axonal and dendritic morphology to individually identify cells as sensory neurons (Rohon-Beard cells), primary or secondary motoneurons, or spinal interneurons (Cid cells, COPA cells). These cells showed fluorescence increases of up to 150% after electrical stimulation of the body surface or elicitation of an escape reflex. Increasing the duration of electrical stimulation produced a graded fluorescence increase, consistent with an accumulation of intracellular calcium. Fluorescence increases were not observed in surrounding, unlabeled tissue. Spatial resolution was optimized by closing the confocal aperture and fish were stimulated with a head tap during linescans. Fluorescence rose most markedly and rapidly at the rim of the nerve cell; signals in the interior rose more slowly and were attenuated in amplitude, indicating that calcium was diffusing from the plasma membrane to the cell interior. Also, the decay of fluorescence signals paralleled the decay of calcium signals observed in cultured neurons loaded with CGD via patch pipette. These results are consistent with earlier studies performed on isolated chick spinal cord, where neurons were loaded by a similar approach (O'Donovan et al., 1993). Instances of clear nuclear labeling confirmed that much of the CGD was not bound in organelles. This approach permits imaging of subcellular calcium dynamics in the zebrafish CNS. Because mutant lines of zebrafish have been created for genetic and developmental studies, this approach can be used to evaluate the effects of such mutations on neural activity/calcium homeostasis. Supported by HHMI, NS09113 & NS26539.

652.9

CONFOCAL IMAGING OF RESPONSES IN POPULATIONS OF IDENTIFIED MOTONEURONS DURING ESCAPE BEHAVIORS OF INTACT ZEBRAFISH. J.R. Fetcho* and D.M. O'Malley. Dept. Neurobiology and Behavior and Howard Hughes Medical Institute. SUNY at Stony Brook, NY 11794.

One of the major problems in studies of vertebrate neural networks is the difficulty in simultaneously monitoring the activity in populations of identified neurons. We took advantage of the transparency of larval zebrafish and used confocal microscopy and a calcium indicator to look into intact living fish and observe, with millisecond temporal resolution, activity-related changes in populations of individually identifiable neurons. Pools of primary and secondary motoneurons were backfilled with calcium green dextran and their fluorescence was monitored during escapes elicited by a contralateral tap of the head. Fluorescence increases in motoneurons were well correlated with escapes detected by an electrode grid beneath the fish. In every trial (n=30) from six fish in which 2 or 3 primary motoneurons were monitored, we found that when one of the motoneurons responded, they all did. In addition, whenever segmental groups of primary and secondary motoneurons were monitored (41 trials from 9 fish), all of the secondary motoneurons responded in conjunction with the primary ones. The relative timing of the responses in different motoneurons was examined by rapidly scanning a single line through the cells. These line scans indicated that some motoneurons responded within 6 to 14 milliseconds of one another. Although all motoneurons contralateral to a head tap responded, differential responses of motoneurons were evident in pools ipsilateral to the head tap and in responses to electrical stimulation. The synchronous response of the motoneurons to a contralateral head tap may account for the massive activation of the axial musculature characteristic of the C bend of the escape. This optical approach should allow studies of the functional role of populations of neurons throughout the spinal cord and brain of normal and mutant lines of zebrafish. (Supported by NIH NS26539 (JRF), NS09113 (DMO); a Sloan Foundation Fellowship (JRF) and the Howard Hughes Medical Institute)

652.10

MECHANISMS CONTRIBUTING TO ROSTROCAUDAL PHASE LAGS IN SPINAL LOCOMOTOR NETWORKS IN LARVAL LAMPREY. A. Hagevik* and A.D. McClellan Div. of Biol. Sciences, Univ. of Missouri, Columbia, MO 65211

The mechanisms that contribute to rostrocaudal phase lags in larval lamprey (*Petromyzon marinus*) were investigated in partitioned *in vitro* brain/spinal cord preparations and by computer modeling. In *in vitro* preparations, spinal locomotor activity was elicited by chemical microstimulation in brainstem locomotor areas.

Short distance coupling. Strychnine applied to the rostral spinal cord (n=11) converted left-right alternation to synchronous bursting in the rostral cord as well as in the caudal spinal cord (Neurosci. Abst. 19:349, 1993). Strychnine applied to the caudal spinal cord resulted in synchronous activity in the caudal cord (n=7) but had less effect on the left-right phasing of the locomotor activity in the rostral cord. These results suggest that ipsilateral excitatory coupling is stronger in the descending direction. Computer modeling confirmed the biological results and suggested that a dominant descending coupling contibutes to rostrocaudal phase lags.

Oscillator frequency gradient and long distance coupling. Cycle times of locomotor activity were examined in sections of the spinal cord when low-calcium Ringer's solution was applied to the rest of the cord. Cycle times of the isolated rostral cord were similar to cycle times in the entire cord (n=7), but did appear to depend on the length of the rostral segment and the intrinsic cycle time. Cycle times of the isolated caudal cord were mostly longer than the overall cycle times (6 of 7). Low-calcium Ringer's solution applied to the middle spinal cord resulted in a reduction in the phase lag between the rostral and caudal cord compared to control.

One interpretation of these results is that a frequency gradient along the cord contributes to rostrocaudal phase lags, and that long-distance coupling is symmetrical or perhaps stronger in the ascending direction which, by itself, would tend to reduce intersegmental phase lags. We are currently conducting experiments and performing computer modeling to investigate the combined effects of short distance coupling, long distance coupling, and oscillator frequency gradient on rostrocaudal phase lags.

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652.11

PEPTIDERGIC MODULATION OF THE SPINAL NETWORK FOR LOCOMOTION IN LAMPREY BY NEUROTENSIN AND SOMATOSTATIN. J.-Y. BARTHE and S. GRILLNER*. Department of Neuroscience, the Nobel Institute for Neurophysiology, Karolinska Institute, S-17177 STOCKHOLM - SWEDEN.

Spinal neurones containing the neuropeptides somatostatin (SS) and neurotensin (NT) occur in lamprey. To gain insight into the ionic mechanisms and the functional role of these two peptides the effects of somatostatin and neurotensin were studied both at the cellular and at the network level. Fictive locomotion was elicited by bath application of the glutamatergic agonist NMDA (50-150μM) and monitored by recordings from ventral roots. When bath applied, both NT and SS (10^{-8} - 10^{-6} M) reversibly slowed the NMDA-induced rhythmic activity by 5-25%. Furthermore, SS induced a reduction of the burst proportion (burst duration/cycle period), whereas NT did not alter the burst proportion. In some instances the swimming activity became irregular with higher doses of NT.

NT induced a depolarization (4.4±0.5 mV) of motoneurons and interneurons which remained after blockade of voltage sensitive sodium channels with TTX and after removal of calcium. The fast and slow phases of the afterhyperpolarization were not affected.

SS induced a hyperpolarization of spinal neurons and a decreased their firing frequency in response to depolarizing current pulses. SS also reduced the firing of both interneurons and motoneurons recorded during fictive locomotion. It also induced a slowing of TTX-resistant, NMDA-induced membrane potential rhythmic oscillations.

This study demonstrates that NT and SS modulate spinal neurons by two different mechanisms, which both lead to a slowing of the swimming activity but with a differential control of the burst parameters.

652.12

A CONTINUOUS NETWORK MODEL OF THE LAMPREY SWIMMING RHYTHM GENERATOR - INTERSEGMENTAL COORDINATION. J. Hellgren[1,2], T. Wadden[2]*, A. Lansner[2] and S. Grillner[1]. [1]Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden. [2]Department of Numerical Analysis and Computing Science, Royal Institute of Technology, S-100 44 Stockholm, Sweden.

In the lamprey, a simple eel-like vertebrate, swimming is produced by bursts of activity alternating between the left and right sides, with a frequency from 0.25 to 10 Hz in combination with a constant phase lag between consecutive segments resulting in a wave travelling down the body pushing the animal forward through the water. The swimming rhythm generating network has previously been modelled as a chain of coupled oscillators, due to its ability to produce fictive swimming with only short sections of in vitro spinal cord. Here we introduce a continuous network model, using populations of neurons and synaptic connectivity spread rostrocaudally along the spinal cord. Excitatory interneurons and caudally projecting crossed inhibitory interneurons are included in the network. The neurons are modelled according to a Hodgkin-Huxley type formalism. Excitatory synapses extend seven segments both rostrally and caudally with strengths decreasing over distance. The same connection strategy is used for crossed inhibitory synapses with the addition that they extend 20 segments caudally. A network consisting of 2600 neurons and over 700,000 synapses has been simulated using a powerful workstation. This network model produces stable forward swimming over a wide range of frequencies and can exhibit backward swimming by increasing excitability to the caudal segments. In order to further analyze the detailed contribution of different elements in the network we have studied different reduced networks. This has provided a deeper understanding of the different mechanisms underlying intersegmental locomotion in undulatory swimming.

652.13

VISUALIZATION OF SLICES OF THE TURTLE BRAINSTEM BY SECTIONING THROUGH ITS THREE-DIMENSIONAL RECONSTRUCTION. L. He*, R. Sarrafizadeh, E. Mugnaini†, and J. C. Houk. Department of Physiology, Northwestern University Medical School, Chicago, Illinois 60611-3008; †Laboratory of Neuromorphology, University of Connecticut, Storrs, Connecticut 06269-4154.

Three-dimensional reconstruction of the turtle rubro-cerebellar network was recently generated from anatomical data which were obtained from serial sections prepared for *in vitro* neurobiotin and biocytin tracer studies and immunocytochemistry. In the present study, our goal was to visualize slices of the turtle brainstem by sectioning through its three-dimensional reconstruction to identify optimal sectioning schemes of brainstem slices for *in vitro* electrophysiological studies.

Brain slice preparations have been frequently used for investigations of neuronal microcircuits, cellular properties, and synaptic plasticity. One of the difficulties in preparing brain slices is to establish optimal thicknesses and orientations with which the slices are to be cut. During the rendering of our reconstruction, two parallel clipping planes were defined in the object coordinates. Only those graphics primitives that fell in between this pair of clipping planes were rendered. In this way, a virtual brain slice was cut out of the three-dimensional reconstruction. By adjusting the offset of and the distance between the clipping planes and the viewing angles of the reconstruction, the thickness and orientation of the virtual brain slice could be changed to include cell groups and fiber tracts of interest and thus preserve converging inputs for analysis of excitatory and inhibitory synaptic processes. The rendering of the brainstem and ventricle surfaces in such brain slice provides landmarks which guide the preparation of slices for actual experiments.

Several interesting slices of the turtle brainstem obtained with this technique have inspired new potential experimental designs in our turtle premotor network studies. The same technique can be applied to three-dimensional reconstruction of other parts of the central nervous system to facilitate the experimental design of brain slice preparations.

652.14

FICTIVE HINDLIMB MOTOR PATTERNS EVOKED BY APPLICATION OF GLUTAMATE AGONISTS TO THE TURTLE SPINAL CORD. S.N. Currie*, Dept. Neuroscience, Univ. of California, Riverside, CA 92521.

In turtles, it has been shown that both NMDA and non-NMDA type glutamate receptors contribute to cutaneous sensory processing in the scratch reflex pathway (Currie and Stein, 1990). In the present study, we tested the ability of NMDA (20-100 μM) and AMPA (2-10 μM) to evoke fictive hindlimb motor output when applied exogenously to restricted regions of the turtle spinal cord. Drugs were applied onto the exposed dorsal surface of 2-3 adjacent spinal cord segments near and within the anterior hindlimb enlargement. These segments receive cutaneous afferents from the pocket scratch and flexion reflex receptive fields (Mortin and Stein, 1990) and contain both hindlimb motor neurons and key elements of the scratch motor pattern generator (Ruigrok and Crowe, 1984; Mortin and Stein, 1989). Motor output was recorded unilaterally from 3-5 hindlimb muscle nerves. Both NMDA and AMPA elicited coordinated bursting motor discharge in all recorded hindlimb nerves. These chemically evoked motor patterns exhibit rhythmic alternation between hip flexor (VP-HP) and hip extensor (HR-KF) motor neuron activity; the timing of knee extensor activity within the hip flexor-extensor cycle is similar to that of sensory-evoked pocket scratch motor patterns. These chemically evoked motor patterns interact strongly with cutaneous-evoked reflexes. Stimulation of flexion reflex on the ipsilateral or contralateral side produces phase-dependent resets of the chemically evoked rhythm. Stimulation within a scratch receptive field can reset the rhythm and in some cases, increase burst frequency for several cycles. Supported by NSF Grant IBN-9308804 to S.N.C.

652.15

THE ROLE OF GLYCINE- AND $GABA_A$ -MEDIATED INHIBITION IN INTERLIMB COORDINATION IN THE ISOLATED MAMMALIAN SPINAL CORD. E. S. Simon* and A. Lev-Tov, Laboratory of Neural Control, NIH, NINDS, Bethesda MD 20892 and Dept. of Anatomy,The Hebrew University Medical School, Jerusalem, Israel

The effects of the glycine and $GABA_A$ receptor antagonists, strychnine and bicuculline, on neurochemically-induced locomotor rhythms were studied in the isolated spinal cord of the neonatal rat (P2-P6), using wide-band electrotonic recordings from ventral roots (VRs). Bath application of strychnine (1-2 μM) or bicuculline (2-10 μM) to otherwise untreated spinal cord preparations induced bilaterally-synchronous paroxysmal bursts in homologous lumbar VRs. These paroxysmal bursts were abolished by addition of the NMDA receptor blocker APV (10-20 μM) or the non-NMDA antagonist CNQX (1-5 μM) to the bath. In contrast, bath application of serotonin (5HT, 25-40 μM) and NMDA (2-5 μM) induced alternating discharges in homologous VRs, interpreted to be a locomotor rhythm. Addition of the above concentrations of APV or CNQX slowed but did not abolish the alternating rhythm. Alternating VR discharges were abolished and replaced by paroxysmal synchronous VR bursting after addition of the above concentration of strychnine or bicuculline, as might be expected if the alternating rhythm depends on a half-center system with mutual inhibition. However, the alternating rhythm persisted when APV (20 μM) was added to the bath prior to application of either inhibitory antagonist. Washout of APV returned the activity to bilaterally-synchronous paroxysmal bursting. This result suggests that the neural control of interlimb coordination in the isolated spinal cord may be more complex than a simple half-center model involving only mutual inhibition.

652.16

THE EFFECTS OF INHIBITORY AGONISTS AND ANTAGONISTS ON THE OCCURRENCE AND PATTERN OF SPONTANEOUS ACTIVITY IN THE CHICK EMBRYO SPINAL CORD. N. Chub and M. J. O'Donovan* Lab.of Neural Control, NINDS, NIH, Bethesda, MD 20892.

The isolated spinal cord of the chick embryo generates spontaneous episodes of rhythmic motor activity. During each cycle sartorius (SART) motoneurons experience a pause in their firing during peak femorotibialis (FEM) activity. We have investigated the role of inhibitory agonists and antagonists the genesis and phasing of this activity. Bath application of bicuculline (10-100μM), picrotoxin (1μM), GABA (10-500μM), isoguvacine (5μM) or strychnine (20-50μM) suppressed spontaneously occurring motor activity, although electrically evoked episodes could still be obtained.

The SART pause was blocked following local application of bicuculline (1mM, 20sec) or picrotoxin (100μM, 20sec) and was depressed by strychnine (1 mM, 20sec) over SART motoneurons (see also Sernagor and O'Donovan, 1991 *Soc.Neurosci.Abstr.* 17:120). Paradoxically, however, the pause persisted in the presence of bath applied bicuculline and strychnine (20-50μM) and a pause appeared in FEM discharge. Under these conditions local application of the NMDA antagonist AP-5 (500μM, 20 s) or the non-NMDA antagonist CNQX (500μM, 20 sec) completely abolished inhibition in SART and FEM motoneurons in a reversible manner. Neither AP-5 nor CNQX had this effect in normal Tyrode's solution although CNQX could depress the SART pause. Our results indicate that inhibitory mechanisms may participate in the regulation of spontaneous activity although we cannot exclude the possibility that inhibitory antagonists act non-specifically. They also indicate a clear role for synaptic inhibition in the phasing of motoneuron activity and raise the possibility that, under certain circumstances, glutamate may be functionally inhibitory during development.

652.17

DUAL PATTERNS OF LOCOMOTOR ACTIVITY INDUCED BY 5-HT AND DOPAMINE IN THE *IN VITRO* NEONATAL RAT. O. Kiehn* and O. Kjaerulff. Sect. of Neurophysiology, Dept. of Medical Physiology, Univ. of Copenhagen, Copenhagen 2200, Denmark.

From invertebrate studies it is known that different transmitters can produce distinct motor output patterns by re-wiring a common network or intermixing adjacent networks (Harris-Warrick and Marder, *Ann. Rev. Neurosci.* **14**, 39; 1991). To what an extent the same is true for the spinal locomotor networks in mammals is at present unknown. As a first approach to answer that question we have performed a detailed analysis of dopamine (DA) and 5-HT-induced EMG patterns in the *in vitro* hindlimb-attached spinal cord preparation from neonatal rats aged 0 to 4 days.

5-HT induced a regular fast rhythm while DA evoked a slow irregular rhythm. Furthermore, the two transmitters elicited fundamentally different overall locomotor patterns. Thus, in 5-HT there was a dominant flexor activity in the vastus lateralis/medialis and rectus femoris muscles, while the same muscles shifted to be mainly active in the extensor phase during DA-induced locomotion. Similarly, biceps femoris and semitendinosus activity could shift from extensor activity in 5-HT towards flexor activity in DA. Other hindlimb muscles maintained similar phasing independent on the transmitter. During upstart of rhythmic activity uncoupling and phase-drift of muscle activity was sometimes noted, indicating that the locomotor networks are composed of a mosaic of unit burst generators.

Our results show that the spinal locomotor networks in mammals can have a high degree of flexibility, and we suggest that the networks are reconfigured by extrinsic modulation.

652.18

The role of NMDA and non-NMDA receptors in locomotor neural networks of embryonic spinal cords. §Tabak, J., §Prime, L., #Ritter, A., #O'Donovan, M. °Murphey, C.R. ¤Danel-Moore, L. and §°Moore, L. E.* §C.N.R.S. & ¤INSERM U391, Univ.Rennes I, Rennes, Fr., #Lab. of Neural Control., NIH, Bethesda, Md. °Dept. Physiol.& Biophy.,UTMB, Galveston,Tx,

Locomotion Neural Networks of Xenopus and chick embryonic spinal cords were compared at different developmental stages. Single cell neuronal models were developed from frequency domain data explicitly incorporating the dendritic structures present in spinal neurons. The neuronal models were based on the biophysical properties of individual neurons measured in both systems. The simulation neural network model of Roberts& Tunstall(Eur.J.Neurosci.2:11;1990) was extended to incorporate both NMDA and non-NMDA receptors distributed on dendritic cables, providing a more robust pattern generating system. Locomotor activity, induced by pulses of current applied to the skin, was recorded with extracellular electrodes from ventral roots of curarized, spinalized Xenopus embryos. Locomotor activity was abolished after developmental stage 38 by 100 μM APV, however at stage 28, there were no or minimal effects. At stage 28, 5 μM CNQX minimized or abolished locomotor responses and at later stages the duration of activity was shortened. Locomotion was easily evoked at stage 38 in nominally zero Mg, however, 2.5 mM Mg shortened the duration of activity, and 5 mM often led to total blockage. At stage 42, 2.5 mM Mg generally blocked locomotion. At stage 28, 2.5 mM Mg had no clear effect on locomotion. These results support the hypothesis that during the initial stages of embryonic development, locomotion neural networks utilize principly non-NMDA excitatory receptors, however, during development NMDA receptors become more prevalent in conjunction with the appearance of complex dendritic trees. In addition, probes for mapping glutamate receptor distributions on dendritic structures were developed using PCR methods to select highly conserved regions from membrane spanning regions of Xenopus and lamprey. NIMH(MH45796), CNRS& INSERM.

652.19

RHYTHMIC OUTPUT OF EMBRYONIC SPINAL NETWORKS IN CULTURE IS INDUCED BY DISINHIBITION BUT NOT BY INCREASED EXCITATION J. Streit*, Institute of Physiology, Bühlplatz 5 3012 Bern, Switzerland

Simple locomotor patterns are based on rhythmic output of local spinal networks. In order to investigate the minimal structure and the formation of such networks, the spontaneous output of cultured slices of the embryonic rat spinal cord was studied. This was done by coculturing the slices with skeletal muscle fibres and recording the patterns of spontaneous muscle contractions using a simple optical device. Neuron-driven muscle contractions were distinguished from autocontractions of the muscle fibres by their typical patterns and by pharmacological tools.

Roughly 20 % of the cocultures showed spontaneous neuron-driven muscle contractions, most of them with random patterns. In all of these cultures rhythmic patterns of muscle contractions were induced by classical pharmacological disinhibition of the spinal networks (strychnine, bicuculline or both). Antagonists at GABA B receptors or substances interfering with cholinergic, catecholaminergic or peptidergic pathways had no effect on rhythmic activty patterns, suggesting that these pathways were not critical for rhythmogenesis.

On the other hand rhythmic activity was not evoked by NMDA, glutatmate or the glutamate uptake blocker dihydrokainate. NMDA in the absence of magnesium increased the rate of spontaneous activity without inducing rhythmic activity. Spontaneous activity completely ceased in presence of the glutamate antagonist CNQX.

These findings suggest that rhythmic output patterns arise in glutamatergic spinal networks and that they are induced by reducing inhibitory transmission within these networks but not by increasing excitatory transmission.

Supported by SNF and Roche Foundation

652.20

NMDA-INDUCED OSCILLATIONS IN SPINAL MOTONEURONS BECOME MASKED BY INHIBITION DURING DEVELOPMENT M.-S. Rioult-Pedotti*. Department of Biology, Yale University, New Haven, CT 06511

Responses were recorded from ventral roots (VR) in isolated hemisected frog and tadpole spinal cords (SC). In frog SCs intracellular recordings were simultaneously made from identified lumbar MNs. Tadpole swimming was recorded with a video camera. Application of NMDA (50 μM) evoked periodic bursting of VR activity in tadpoled but not in frogs (5-200 μM). However, after strychnine (20-30 μM) application, synchronized periodic bursts were also initiated in the frog VRs. These persisted after strychnine removal and were coincident with periodic oscillations measured intracellularly in the motoneurons (MN). Thus, this periodic bursting behavior reflects a periodic oscillation of MN membrane potential. In the adult frog this behavior is initiated only after removal of inhibition. The periodic (~1Hz) tadpole VR bursts occur at the same frequency as the tadpole tail moves during swimming. Oscillations in the frog were much slower (>10s/cycle). Oscillations of tadpole and frog MNs have the same properties: they require the presence of physiological Mg++ concentration (1mM), they are blocked by APV (100 μM) but not affected by CNQX (10-15 μM), and they are resistent to TTX (3μM).

These data provide evidence for the existence of a central pattern generator that is active in the tadpole and becomes inhibited during metamorphosis as the locomotor behavior changes from rhythmic to episodic.

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INVERTEBRATE MOTOR FUNCTION

653.1

ACTIVITIES OF TARSAL AFFERENTS IN FREELY WALKING COCKROACHES. G. S. Larsen, S. F. Frazier and S. N. Zill*. Dept. of Anat., Cell and Neurobiology, Marshall Univ. Sch. Med, Huntington, WV 25755.

Sensory inputs from the distal segments of legs have been shown to modulate walking in many animals. We have recorded activities of sense organs of the distal (tarsal) segments of the cockroach hind leg in restrained and freely walking animals. In restrained preparations, neurographic wires implanted adjacent to sensory nerves in the first tarsal segment recorded afferents responding to the position and movements of the distal tarsal joint. We have morphologically identified a chordotonal organ proximal to the joint and ablation studies strongly suggest that it is the source of these activities. Recordings in freely walking animals show peak activities at the onset and termination of the stance phase. These discharges occur at the time that the claw is engaged and then disengaged when walking or climbing over rough surfaces. We have also shown that electrical stimulation of afferents through the recording electrodes reflexly excites the tibial flexor muscle. We propose that the discharge of the organ signals disengagement of the claw from the substrate, reflexly exciting the flexor muscle at the initiation of swing.

Support by ONR grant N00014-93-1-0088 and the Whitehall Foundation.

653.2

KINEMATIC AND EMG ANALYSIS OF THE FAST AND SLOW RUNNING IN THE COCKROACH. J. T. Watson* and R. E. Ritzmann. Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

As part of a project to implement biological principles into the design of legged robots, we are combining high speed video motion analysis of leg movements with EMG recordings from leg muscles in cockroaches. We have recorded from the depressor and levator coxa and the extensor and flexor tibia muscles in the hind and middle legs at various running speeds.

Slow walking (1-3 Hz) is characterized by long bursts of graded slow motor activity. As expected, the kinematics of joint movement follow the neuromuscular activity closely. During fast running (> 10Hz) distinct changes occur. Slow motor activity increases in frequency and fast motor neurons are recruited. However, muscle potentials associated with fast motor neurons are not recorded on each cycle. They tend to be recruited more regularly at the beginning of runs than in cycles occurring near the end. The principle difference in joint kinematics associated with fast motor activity is a more abrupt transition from stance to swing.

The temporal relationships between various motor activities are consistent within a single animal. These include latency from fast depressor to fast levator of the coxa and latency from fast depressor to fast extensor of the tibia. Although the fast depressor activity occurs at or near the beginning of coxal depression, fast levator activation tends to occur much earlier in the step cycle than expected; i.e. during the stance phase.

We are beginning to make similar observations on the middle legs. Our preliminary findings indicate that although the kinematics of the middle legs are very different from the hind legs, the pattern of electrical activity does not reflect these differences. We believe that this paradox may reflect the influence of passive forces and leg morphology upon leg movements.

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653.3

CONTRIBUTIONS OF PERIPHERAL PROPERTIES TO INSECT AND ROBOT LOCOMOTION. R.D. Beer*, W.J. Marx, G.M. Nelson, K.S. Espenschied, R.D. Quinn, J.T. Watson, R.E. Ritzmann, H.J. Chiel. Depts. of Computer Eng. and Science, Biology, Mechanical and Aerospace Eng., and Biomedical Eng., Case Western Reserve University, Cleveland, OH 44106.

In order to better understand the role of peripheral properties in cockroach walking, we have been developing biomechanical models of the insect. We have constructed a passive musculoskeletal model of the femur-tibia (FT) joint of the metathoracic leg. This model suggests that passive properties (i.e. passive muscle tension and damping about the FT joint) play an important role in this joint during the swing phase of walking. Indeed, the initial swing movement can be generated entirely by passive forces. The FT joint model is currently being extended to incorporate active properties. In addition, we have developed a full body dynamic model of the free walking insect with 30 articulated degrees of freedom. This model is being used to estimate joint torques and ground reaction forces from kinematic data.

We have also constructed a 6 legged robot with 18 active degrees of freedom. The purpose of this robot is to investigate the application of biological control principles to robotics. A generalization of leg coordination mechanisms derived from stick insect walking are used to control the robot's locomotion. Muscle-like control of the joints is achieved using a scheme based on the equilibrium point hypothesis. Force feedback permits the robot to comply to uneven surfaces. An implementation of the elevator reflex allows the robot to step over obstacles. The robot can walk in a continuum of gaits, turn and negotiate irregular terrain.

Supported by ONR grant N00014-90-J-1545.

653.4

CHANGES IN A PROPRIOCEPTIVE PATHWAY DURING MATURATION OF LOCOMOTOR RHYTHMICITY. J.R. Gray* & R.M. Robertson, Dept. Biology, Queen's University, Kingston, Ontario, Canada, K7L-3N6.

The flight system of the locust is a well established model for studying the role of proprioceptive input in motor pattern generation. Gradual hemimetabolous development of this insect makes it ideal for examining maturation of preexisting circuitry as well as potential changes in afferent input.

During maturation of the flight system the wingbeat frequency increases from 13 Hz at 1-2 days after imaginal ecdysis to approximately 23 Hz 14 days later. We examined the morphology of a single cell proprioceptor, the wing hinge stretch receptor, and the response of flight interneurons to stretch receptor activity during maturation.

Preliminary results indicate that during maturation the central arborization of the forewing stretch receptor axon displays heteromorphic growth. Further, within the mesothoracic branch there is an increase in the number of axonal swellings and the branch grows positively allometrically relative to the ganglion. Artificial excitation of the stretch receptor with patterns of stimuli that correspond to increased afferent activity at different stages of maturation produced increased activity of postsynaptic flight interneurons.

These results indicate that changes in the stretch receptor may alter its effect on the operation of the flight motor and suggest that proprioception influences, in part, motor pattern development.

653.5

PHASIC MODULATION OF WING HINGE MECHANICS BY THE FIRST BASALAR MUSCLE OF THE BLOW FLY *CALLIPHORA*. M.S. Tu* and M.H. Dickinson. Department of Organismal Biology and Anatomy, University of Chicago, 1025 E. 57th ST. Chicago IL 60637.

Among the 17 direct steering muscles in flies, the First Basalar (B1) has been implicated as the muscle most likely to control the timing of ventral wing pronation (the ventral flip). Because of the potentially large aerodynamic forces associated with the ventral flip, rapid and precise modulation of the B1 output may be crucial for flight control. In the blowfly *Calliphora vicina*, the B1 fires an action potential and undergoes a cycle of length oscillation each and every wing beat at 150 Hz. Under conditions simulating flight we previously found that the B1 may function as a spring, with properties of dynamic stiffness and mechanical energy dissipation under neural control. Changes in the dynamic stiffness of the B1 could control the extent of oscillations of the basalar apodeme, which forms the mechanical linkage of the B1 to the wing hinge.

To test the hypothesis that rapid phase changes in B1 activation can phasically modulate oscillations of the basalar apodeme, we measured B1 muscle length changes while making extracellular recordings of B1 action potentials. By measuring the deflection of a laser beam reflected from a small mirror mounted on the external portion of the basalar apodeme, we could non-invasively measure oscillations of the basalar apodeme and the corresponding length changes of the B1 during tethered flight.

We found that changes in the interval between action potentials corresponded to increases in the maximum extent of stretch in the B1 and the extent of posterior deflection of the basalar apodeme. Changes in the amplitude of basalar oscillations occurred within one cycle of changes in action potential timing. This rapid mechanical response suggests that the B1 can modulate wing kinematics on a cycle by cycle basis, even though wing beat frequency stimulation of the B1 under isometric conditions causes nearly complete tetanus.

653.6

THE ROLE OF HALTERE AFFERENTS IN THE ACTIVITY OF A STEERING MUSCLE IN THE BLOWFLY, *CALLIPHORA VICINA*. A. Fayyazuddin*, W. P. Chan, C. E. Jordan and M. H. Dickinson. Department of Organismal Biology & Anatomy, Univ. of Chicago, Chicago, IL 60637.

The large direct synchronous basalar muscles in the blowfly are active during turning maneuvers, and are thought to control the steering motions of the wings. One of these muscles, the first basalar (B1), is unique in that it fires one spike each wing beat at a precise phase within the stroke cycle. We have been investigating whether the campaniform fields at the base of the haltere provide the wing beat-synchronous feedback required for this sharp phase tuning of the B1 during flight. Both mechanical oscillation of the haltere and electrical stimulation of the haltere nerve cause the B1 motor neuron (MNB1) to fire a phase-locked action potential. Furthermore, MNB1 can follow stimulation frequencies up to and exceeding the normal wing beat frequency (150 Hz), suggesting that the halteres may be sufficient in establishing the phase-locked firing pattern of B1 during flight. We have begun to characterize the physiology of the connections between MNB1 and haltere afferents by recording intracellularly from the motor neuron while mechanically stimulating identified fields of campaniform sensilla on the base of the haltere. Stimulation of two large dorsal fields, (dF2 and dF3), produce short-latency (<1ms) EPSPs in MNB1, Stimulation of the third dorsal field (dF1) has no effect on MNB1 activity. Campaniform neurons from both dF2 and dF3, visualized with biotinylated dextran, project to the meso-thoracic neuropile near the site of origin of the MNB1 axon. In contrast, neurons originating from dF1 terminate in a different region, consistent with their failure to elicit a short-latency physiological response in MNB1. In other invertebrates, the strength of sensory-motor connections can be modulated by neuro-transmitters such as octopamine (OA). In the blowfly, OA also enhances the synaptic efficacy of the connections between the haltere and MNB1.

653.7

THE STRUCTURE OF THE FLUID WAKE GENERATED BY A FLYING FRUIT FLY, *DROSOPHILA MELANOGASTER*. M. H. Dickinson*. Dept. of Organismal Biology & Anatomy, Univ. of Chicago, Chicago, IL 60637.

The flight behavior of insects has been an important model system for the study of rhythm generation, sensory-motor integration, and motor output. However, the net result of these neural processes - the generation of flight forces by the wing - is poorly understood. In an attempt to characterize the aerodynamics of flight behavior in flies, I have visualized the flow structure generated by a tethered flying *Drosophila*. Flow was visualized using *Lycopodium* spores illuminated with laser sheets (670 nm) and imaged using a cooled CCD camera. The capture of images could be synchronized to the wing beat cycle following fixed delay allowing the generation of phase-reconstruction movies showing the change in flow throughout the wing stroke.

The flow visualizations reveal that *Drosophila* generate an inverted single heart-shaped vortex tube during each wing stroke. The dorsal portion of each vortex ring is produced gradually from the shedding of tip vorticity throughout the downstroke. During the ventral flip, the quick supination of the wings at the start of the upstroke, the ventral arms of the vortex ring are rapidly shed, merging distally with the dorsal portion of the ring, and attaching medially to the thorax. It is at this time that the vortex ring detaches completely from the wings and is shed into the wake. In contrast, no vortex structures are generated during the upstroke. The vortex rings generated by successive downstrokes coalesce to form a vortex tube directed posteriorly, roughly normal to the wing stroke plane. The fluid velocity within the central region of the vortex tube is high, instantaneously reaching values nearly ten times that of the free stream velocity.

The rapid shedding of vorticity during the ventral flip correlates with a large transient in the production of lift measured using laser interferometry. The timing and position of the ventral flip shedding may provide an important means for the control of steering forces during flight.

653.8

THE ROLE OF THE FRONTAL GANGLION IN ADULTS OF THE MOTH *MANDUCA SEXTA*. C.I. Miles* and R. Booker. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

As part of an ongoing study of metamorphosis of the frontal ganglion (FG) of *Manduca*, we have examined its function in the adult moth. We have found that the FG innervates the muscles of the moth's cibarial pump. These include dilators which expand the pump chamber, as well as compressors which reduce it. The cibarial pump is used during feeding to bring nectar through the proboscis into the foregut. We have recorded the motor program for the cibarial pump muscles during feeding. Its primary component consists of a relatively long burst of dilator activity immediately followed by a brief burst of the pump compressors. The cibarial pump also plays a critical role in the expansion of the moth's wings following its emergence from the pupal cuticle. During wing expansion, the cibarial pump is activated and the animal swallows air, using a motor pattern that is similar to that exhibited during feeding. Air- swallowing is apparently required for expanding both the wings and the abdomen. Animals from which the FG was removed at the onset of metamorphosis develop normally into adults and successfully emerge from the pupal cuticle, but they fail expand their wings and their abdomens are flattened. Feedback for turning off air- swallowing apparently requires connections between the FG and brain, as animals in which these were bilaterally cut at the end of the larval stage expanded their wings normally but exhibited distended or burst abdomens by 24 hrs after adult emergence.

653.9

MOTOR NEURONS INVOLVED IN A REFLEX CIRCUIT EXHIBIT STAGE-SPECIFIC DIFFERENCES IN THEIR PROPERTIES. J.L. Casagrand*, and R.B. Levine, Univ. of Arizona, ARL Div. of Neurobiology, Tuscon, AZ 85718.

In *M.sexta* a mechanosensory reflex circuit produces distinct behaviors in larvae and pupae. Many elements in the pupal reflex circuit are retained from the larval stage. However, the activity that is evoked in the motor neurons by sensory nerve stimulation is quite different. We previously reported (Casagrand and Levine, 1993) a difference in the firing properties of motor neurons involved in this reflex at the two stages due to positive current injected into the soma. Pupal neurons exhibited a higher threshold for action potential initiation and a lower level of excitability. These differences were consistent with the changes in physiological responses observed.

We wished to investigate whether these data reflected actual differences in the spike generating regions, or were the result of subtle changes in morphology (Levine and Truman, 1985) that were affecting events recorded at the soma. We thus looked at a number of properties of the neurons in response to injected current. We observed no differences in input resistance, resting potential, or amplitude and rise time of action potentials. We also looked at the threshold for action potentials during responses evoked by synaptic activity (sensory nerve stimulation). As with the injected current experiments, pupal neurons exhibited a higher threshold, contrary to what one would expect if these differences were due to changes in passive properties. These data thus suggest that the differences in action potential threshold and excitability are not due to changes in passive properties of the motor neurons. Thus, it is likely that changes in the active properties of these motor neurons contribute to the altered reflex. Supported by NIH fellowship NS 09249 to J.L.C.

653.10

DISTRIBUTED MOTOR ACTIVITY IN A SEGMENTALLY RESTRICTED REFLEX. W.C. Lemon* and R.B. Levine. Div. of Neurobiology, University of Arizona, Tucson, AZ 85721.

Stimulation of sensory neurons innervating hairs in the gin traps on the abdomen of pupal *Manduca sexta* evokes a rapid bending of the abdomen that is restricted to three posterior segments. Electrical stimulation of the gin trap sensory nerve in an isolated abdominal nerve cord evokes characteristic motor neuron activity in every abdominal segment. To determine if the segmentally distributed motor activity also occurred in intact animals and how it contributed to the segmentally restricted reflex movement, mechanical stimulation of the sensory hairs in intact animals was used to evoke reflex responses that were recorded as electromyograms synchronized with video recordings of the behavior. Reflex behaviors were compared with the underlying patterns of motor activity to determine if there was activity in many segments when the movement was restricted to one, two, or three segments. Coordinated muscle activity was evoked throughout the abdomen in response to stimulation of any of the three gin traps, even when movement was restricted to one segment. Electromyograms of gin trap muscle activity correlated well with the intracellular and extracellular recordings of motor neuron activity obtained from isolated abdominal nerve cords and semi-intact preparations. These findings show that the neural circuit underlying the bending reflex is distributed throughout the abdominal nerve cord. This network generates a complex yet coordinated motor pattern with muscular activity in every abdominal segment whose concerted action produces a deceptively simple and localized bending reflex. Supported by NIH fellowship NS 07309 to W.C.L.

653.11

MODIFICATIONS OF THORACIC LEG MOTOR ACTIVITY DURING POSTEMBRYONIC DEVELOPMENT IN *MANDUCA SEXTA*. R. M. Johnston* and R. B. Levine. Div. of Neurobiology, University of Arizona, Tucson, AZ 85721

At the end of larval life in *Manduca sexta*, the thoracic legs and muscular system are replaced by new adult legs that differ dramatically in both form and behavior. Kinematic and electromyographic data show that during larval crawling, the left and right prothoracic legs move synchronously. In contrast, during adult walking the prothoracic legs alternate. Many if not all of the thoracic leg motor neurons present in the adult stage are retained from the preceding larval stage (Kent and Levine 1988). To follow the transitions between these coordination patterns, we quantified the overt behavior and electromyograms of the thoracic legs in developing adults.

Leg movements in the proximal segments precede movements in the more distal leg segments during adult development. In the beginning final third of adult development (stage 14), synchronous, phasic muscular activity in femoral levators and depressors is present even though there are no observable movements of the femur. Two days later (stage 16), both synchronous and alternating patterns of leg movement and corresponding muscular activity are seen. At this stage in development, the frequency and temporal patterns of leg motor output are similar to those seen in adult moths. These data will help us determine if the changes in thoracic legs patterns are due to a whole-scale restructuring of the underlying circuit or if the changes reflect modulation of a pre-existing larval circuit. Supported by NIH fellowship NS 09387 to R.M.J. and an NIH grant NS 24822 to R.B.L.

653.12

A SUPERFAST TRAP-JAW REFLEX IN ANTS

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Ants of the ponerine genus Odontomachus employ a trap-jaw mechanism to instantaneously close their mandibles to catch prey or to defend themselves. When particular mechanosensory trigger hairs, located on the inner edge of the mandibles, are touched by a prey object, the mandible strike is released in less than 0.5 ms (Gronenberg et al. 1993; *Science 262:561-563*). Photoelectric scanning reveals that this reflex may take as little as 4 ms and is thus probably the fastest reflex yet described for any animal. This speed is based on a catapult-like catch mechanism in the mandible joint that keeps the extended mandibles open during contraction of the powerful closer muscle, thus cocking the mandibles. A small specialized trigger muscle unlocks the catch, instantaneously releasing the stored energy to accelerate the mandible. This trigger muscle is activated only a few milliseconds in advance of the strike. It is composed of specialized tubular muscle fibers the central canal of which contains the cell nuclei and mitochondria. The trigger muscle is supplied by two unusually large motor neurons which are enclosed in a glial sheath. Together with the giant afferents originating from the trigger hairs (Gronenberg and Tautz 1994; **J. Comp. Physiol. 174: 49-60**), the entire reflex system is composed of 2 sensory and 2 motor giant neurons on either side. The giant neurons are most likely monosynaptically coupled, which further decreases the latency of the reflex. **Supported by DFG (Gr 933/3-2)**

653.13

PERIPHERAL MODULATION OF A CENTRAL SYNAPSE BETWEEN IDENTIFIED MOTORNEURONS IN THE METATHORACIC GANGLION OF THE LOCUST. T. Jellema & W.J. Heitler*. Gatty Marine Lab., St. Andrews University, Fife, Scotland KY16 8LB.

The fast extensor tibiae (FETi) motorneuron of the hind leg of locusts, which innervates the muscle providing the propulsive force for the jump and kick, also establishes excitatory, monosynaptic, connections with several flexor tibiae (FITi) motorneurons. These central synaptic connections, which are apparently unique among insect motorneurons, contribute to the co-activation of the FETi and FITi motorneurons during the preparation for a kick or jump. Simultaneous intracellular recordings from the FETi and FITi motorneurons, while evoking ortho- or antidromic FETi spikes, reveal that the amplitude and duration of the central PSPs in the FITi motorneurons are modulated by leg position: during leg flexion the PSPs are larger than during extension. This is in part due to sensory feedback of tension in the extensor muscle summing with the central FETi-FITi synaptic component. However, when extensor tension is abolished by dye-mediated laser photo-axotomy of FETi, the amplitude of the EPSP is still modulated by leg position. The photo-axotomy technique allows specific de-innervation of the extensor muscle, without effecting any of the other structures, and the innervation can swiftly be restored via an electronic "axonal bypass" from the FETi soma to the extensor muscle. In the locust, hind leg position is mainly monitored by the chordotonal organ located in the distal femur. Manipulation (alternately stretching and relaxing) of the tendon of this sense organ, while keeping the leg in fixed position, accounts for most of the PSP amplitude modulation. A relatively enhanced PSP in the FITi with leg flexion makes sense since leg flexion is a prerequisite to go into the co-activation phase. The site and mechanism of the modulation (e.g. pre-synaptic FETi spike amplitude, input resistance of the FITi, presynaptic modulation of the central synapse itself) is currently being investigated.

653.14

NEURAL ELEMENTS OF GRASSHOPPERS SUPPORTING SEXUALLY DIMORPHIC BEHAVIOR. K.J. Thompson* and J.L. Roosevelt. Dept. of Biology, Agnes Scott College, Decatur, GA 30030.

In a continuing study of the neural basis of oviposition behavior in grasshoppers, motor neurons (MNs) and dorsal unpaired median neurons (DUMs) of the terminal ganglia of males have been identified by backfilling lateral nerves with $CoCl_2$. This information is being compared to information already available for females. Two hypotheses for how the nervous system produces sexual dimorphism in behavior are being tested: *1)male-deficient hypothesis*, which predicts that the male nervous system is missing female-specific ovipositor elements (males do not lay eggs), and *2)male-respecified hypothesis*, which predicts that the male contains neurons homologous to ovipositor neurons but involved in other functions. Four clusters of ovipositor MNs have previously been identified in females. Two of these clusters of MNs have axons in different branches of the 8th tergal nerve of the terminal abdominal ganglion. One cluster, the protractors, is a group of 3 MNs with cell bodies contralateral to the filled nerve and the second cluster, the closers, is a group of 3 MNs with cell bodies in the next anterior ganglion. Backfills of the tergal nerve in males produced a similar pattern of MNs, in terms of numbers of cells, cell body positions, and distinctive axonal trajectories. A single DUM neuron, located in the terminal abdominal ganglion, fills from the tergal nerve in both sexes. Thus, for two of the four groups of efferent neurons that have been identified, limited gender differences in MN and DUM complement have been found, despite differences in male and female behavior, and differences in the size and shape of the terminal ganglion. Supported by Whitehall grant #AF93-01.

653.15

DYNAMICS OF A POSTURAL REFLEX IN HERMIT CRAB STUDIED WITH SINUSOID AND NOISE VIBRATION. W.D. Chapple*. Dept. Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

A systems model of a phasic stretch reflex, important in the postural support of its shell by the hermit crab, *Pagurus pollicarus*, has recently been developed from experiments using simultaneous recording of muscle force, length, and spike frequencies of pairs of motoneurons during ramp stretch and release of the right fourth segment of the abdominal ventral superficial muscles. (Chapple 1993). A prediction of this model is that the reflex is important primarily in increasing abdominal stiffness in response to sustained rather than transient stimulation. Sinusoidal and bandpass limited noise lasting for 20 seconds was used to vibrate the muscle. Reflex activation of the motoneurons occurred at thresholds of 0.8% of optimum lengths and frequencies above 2.5 Hz. After a transient peak, frequency declined to a steady state level that lasted for the duration of stimulation. This reflex activation of the motoneurons increased muscle stiffness as stimulus amplitude increased, but did not alter its dynamics. The form of the transfer function that best approximated these dynamics was that of a high pass filter with a lower cutoff frequency of 10 Hz, similar to that observed in the isolated, electrically stimulated muscle. The model's prediction that this reflex could increase muscle stiffness during sustained vibration was confirmed. However, in this isolated abdominal preparation, the reflex did not compensate for variations in muscle stiffness with stimulus amplitude, and, thus, did not regulate muscle stiffness.

653.16

DEVELOPMENT OF STOMATOGASTRIC MEDIATED MOTOR PROGRAMS IN THE AMERICAN LOBSTER. Kari Lavalli, Joseph Ayers*, Marine Science Center, Northeastern Univ., East Point, Nahant, MA 01908

Feeding and stomatogastric mediated behavior are composed three *phases* in all larval stages: an ingestion phase a relatively quiescent phase and a periodic grinding phase. We have demonstrated three behavioral *stages* of development of stomatogastric behavior. The first stage is limited to Stage I and II larvae and consists of: a) a fast peristaltic component which occurs only during ingestion; b) a slow "cardiac sac" rhythm which begins several minutes after the cessation of feeding and continues on until the gut is empty. This rhythm is the only one present for the first hour post-feeding and may serve to mix ingested food with digestive juices. It then alternates with c) the "gastric" rhythm resulting from a constriction of the lateral walls of the cardiac sac and a forceful anterior movement of the entire cardiac sac. No separate pyloric rhythm is noted in either Stage I or II larvae.

The second behavioral stage is observed only in Stage III larvae and adds a medial gastric component due to elaboration and participation of the medial tooth.

The third behavioral stage is first observed in Stage IV postlarvae and demonstrates an excellent replica of the adult patterns with several forms of modulation. Here, separate movements of the cardiac sac, gastric mill and pylorus are present. After Stage IV have been able to identify different modes of operation of the gastric mill, particularly that of "cut and grind" and "squeeze." In addition several modes of modulation of the movements are present in all three stages. *Supported by NSF Grant IBN-9121224.*

653.17

ENHANCEMENT OF NEUROMUSCULAR EFFICACY BY FLRF PEPTIDES IN THE CRAB, *Cancer borealis*. J.C.Jorge-Rivera* and E.Marder. Ctr. for Complex Systems, Brandeis Univ., Waltham, MA 02254

Modulators play a major role in re-shaping the motor rhythms produced by Central Pattern Generators (CPGs). The same modulators can also alter neuromuscular interactions. Recent work has shown that SDRN- and TNRN- FLRFamide produce dramatic changes of the gastric and pyloric motor patterns of the stomatogastric ganglion (STG) of *Cancer borealis* (Weimann et al, 1993). These include: enhancement of the pyloric rhythm frequency, activation of pyloric and gastric rhythms in quiescent preparations, and activation of oscillatory properties in the Dorsal Gastric (DG) neuron. We now use neuromuscular preparations to determine if FLRFamide peptides also modulate neuromuscular junctions (NMJs).

We found that both SDRN- and TNRN-$FLRF_{NH2}$ increase nerve-evoked Excitatory Junctional Potentials (EJP's) on DG-innervated muscles, gastric muscle 4b,c. Nerve-evoked contractions are also enhanced by these peptides in the same muscle: $TNRNFLRF_{NH2}$ produces a 200% increase and $SDRNFLRF_{NH2}$ a 90% increase in contraction. These effects are dose-dependent over the range 10^{-10} to 10^{-7}M. Pyloric (p2, p7), and cardiopyloric valve (CPV4) muscles are also modulated by these peptides.

Our results indicate that in addition to modulating the motor rhythms, FLRFamide peptides increase the efficacy of neuromuscular interactions. Modulation of the NMJ by itself will amplify incoming inputs, or will ensure that changes in the motor rhythms will be translated into changes in motor outputs. This might have different behavioral consequences depending on the physiological context where modulation of the NMJ takes place.

Supported by NS17813.

653.18

TEMPERATURE SENSITIVITY OF THE GIANT MOTOR SYNAPSE AND TAILFLIP BEHAVIOR IN CRAYFISH. M.F. Burgess*, F. Issa, D.H. Edwards and W.J. Heitler. Gatty Marine Laboratory, School of Biological and Medical Sciences, University of St. Andrews, Fife, KY16 8LB, Scotland, and Department of Biology, Georgia State University, Atlanta, GA 30302-4010.

The rectifying electrical giant motor synapse (GMS) between the giant fibers (GFs) and motor giant motor neuron (MoG) has previously been shown to have an extreme temperature sensitivity. The large difference between the Q_{10} of the GMS (near 11) and that of the active membrane in the GFs and MoG (presumably near 3) prompted us to examine whether effective transmission would fail at temperatures away from room temperature and so prevent GF-mediated tailflips. We found that transmission was reliable at cold temperatures down to 8°C as both the presynaptic (GF) and postsynaptic (MoG) spikes and the MoG EPSP broadened considerably. Transmission failed at warm temperatures, usually between 25° C and 30° C, as the duration of the presynaptic spike shortened. Compartmental models of the circuit indicate that transmission fails at high temperatures when the GF spike is too brief to inject enough depolarizing current into the MoG to reach threshold. Transmission is maintained in the cold because presynaptic spike broadening compensates for the slowing of the GMS conductance change. GF stimulation in freely behaving animals evokes tailflips that fail between 25° C and 30° C, and are gradually reduced in amplitude between 15° and 5° C. Recordings in an isolated abdomen indicate that tailflip failure at high temperatures results from transmission failure at the GMS, whereas tailflip reduction in the cold appears to result from slowing of neuromuscular transmission, excitation-contraction coupling, or contraction of the fast flexor muscle that produces the tailflip.

653.19

STATIC- AND DYNAMIC-RADULA/ODONTOPHORE KINEMATIC MODELS OF THE BUCCAL MASS OF *APLYSIA CALIFORNICA*. R.F. Drushel,*[1] H.J. Chiel,[1,3] and P.E. Crago,[2] Departments of Biology,[1] Biomedical Engineering,[2] and Neuroscience,[3] Case Western Reserve University, Cleveland, Ohio 44106 U.S.A.

A computer kinematic model of the buccal mass of *Aplysia californica* (Neustadter, 1992) was revised to more accurately reflect the true shapes and dimensions of buccal mass components *in vivo*. The Neustadter model represents the radula/odontophore as a sphere, the I1/I3 muscles as 6 toruses, and the I2 muscle as a posterior band connecting the dorsal and ventral aspects of the first I1/I3 torus; a ventral hinge attaches the I1/I3 and I2 elements to the odontophore. Protraction/retraction is accomplished by forward/backward rotation of the sphere, and the I1/I3 toruses expand/contract isovolumetrically to conform to the protruding surface of the sphere.

Buccal mass shapes from transilluminated feeding slugs and from models were classified as rest, protraction, or retraction. Actual measurements of the shapes were used to develop plots of eccentricity *vs.* ellipticity in a "shape space". *In vivo*, shapes classified as rest/protraction/retraction cluster together in distinct regions of the shape space plots. However, the Neustadter model generated shapes only roughly equivalent to those observed *in vivo*, and the shape space plots did not match. To improve the model, two different odontophore shapes (#1 and #2) derived from dissection were substituted for the sphere of the Neustadter model. These shapes were asymmetric in mid-sagittal section, though assumed to be circular in cross section when in contact with the I1/I3 toruses. The model was rewritten to account for arbitrary, asymmetric shapes. Shape #1 gave accurate buccal mass retraction shapes, but poor rest/protraction shapes. Shape #2 gave accurate rest/protraction shapes, but poor retraction shapes. In both cases, the shape space plots, while improved compared to the Neustadter model, did not match those from *in vivo*.

Since no single odontophore shape gives correct buccal mass shapes over the entire range, we hypothesize that the odontophore changes shape dynamically as it moves. We are currently analyzing videotapes of transilluminated feeding slugs to generate a gallery of possible odontophore shapes, and are modifying the model to allow the I1/I3 elements to be fit to arbitrary, non-circular cross sections. NIH PPG HL-25830-11A1.

653.20

BIOMECHANICS OF APLYSIA I2 MUSCLE. S.-N. Yu, P. E. Crago*, and H. J. Chiel Dept. of Biomedical Engineering and Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106

The buccal muscle I2 is a smooth muscle which plays an important role in the feeding behavior of the sea hare, *Aplysia californica*. In this study, we examined both isometric properties and contractile dynamics of this muscle. The whole I2 muscle was isolated and the activation was applied by electrically stimulating the radula nerve with a suction electrode. The isometric force-frequency relationship had a frequency threshold of about 6 Hz and rose to a saturating force of about 150 mN at 15-20 Hz. The steady state force was approximately linearly related to the firing period.

We studied contractile dynamics by superimposing isovelocity movements on the plateau of isometric contractions during constant stimulation. During isovelocity shortening, the tension always fell below the isometric value and continued to decrease throughout the period of shortening. However, during lengthening, the response showed a quick rise of tension, termed short range stiffness, followed by an abrupt yield, when the force developed more slowly. The higher the velocity, the more the active force gained in lengthening and lost in shortening. The force-velocity relationship also depended on stimulation frequency. However, after normalization to the isometric force value, the responses were very similar. The similarity was more consistent in shortening than in lengthening. The shape of the responses to isovelocity length changes were very similar to those reported for cat soleus.

We modeled the contractile dynamics of this kind of smooth muscle by a 4-state latch bridge model. The model responses to lengthening and shortening were similar to those of the I2 muscle.

This work was supported by NIH PPG HL-25830-11A1.

653.21

SEROTONIN FACILITATES NEUROMUSCULAR TRANSMISSION IN PARAPODIA OF *APLYSIA BRASILIANA* BY BROADENING MOTOR NEURON ACTION POTENTIALS J. E. Blankenship* and P. J. Laurienti. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77555-0843

Identified cholinergic motor neurons (MNs) and serotonergic modulatory neurons (POP cells) in the pedal ganglion of *A. brasiliana* innervate peripheral parapodial muscle and fire rhythmically during swim locomotion. POP-cell firing increases the amplitude of MN-induced excitatory junctional potentials (EJPs) in muscle fibers and increases their contraction amplitude and relaxation rates. This study was designed to determine whether part of the facilitatory effect of serotonin (5-HT) is due to presynaptic actions by measuring its effect on the action potential half-width of MNs using standard intracellular electrophysiological methods. Motor neurons were penetrated with microelectrodes and action potentials were recorded in four experimental conditions: control artificial sea water (ASW), 50mM tetraethyl-ammonium (TEA), 50mM TEA + 5 uM 5-HT, and after washout with ASW. Spike half-widths were measured as the time taken for the spike to repolarize from its peak back to pre-spike baseline. In 11 experiments, in ASW the average spike half-width was 3.6 ms; the average MN half-width increased significantly ($p<.001$) by 39% to 4.9 mS in TEA. The addition of 5-HT increased the spike half-width to 6.7 mS, a significant ($p<.005$) 35% increase when compared to the TEA spike. Following washout, the average MN spike half-width returned to control values. These results demonstrate that 5-HT, in the presence of TEA, can increase MN action-potential width and can account, at least in part due to increased transmitter release, for the increase in the amplitude of parapodial muscle EJPs and contractions during POP cell firing. Currently, experiments are being performed to identify an effective 5-HT antagonist which is able to block the spike-widening effect of 5-HT.

653.22

A POPULATION OF CEREBRAL INTERNEURONS MEDIATE THE COORDINATION OF VASCULAR RESPONSES INVOLVING FEEDING BEHAVIOR OF *APLYSIA*. Y.Xin, K.R.Weiss and I.Kupfermann*, Center for Neurobiology and Behavior, Columbia University, 722 W. 168 St. New York, NY 10032 and Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

A population of neurons (CC neurons) were identified in the cerebral ganglion C cluster. They were found to affect various parts of the body wall as well as cardiovascular system. Each of these CC neurons produces mono or polysynaptic effects on various cerebral, pedal, plural and abdominal ganglion neurons. The CC neurons receive synaptic inputs when the lip is touched with food or during buccal mass movements. They have mutually excitatory or inhibitory interactions with the feeding command-like neuron C-PR. CC5, which excites the ipsilateral pedal artery shortening neuron (PAS), is excited by touch of the ipsilateral lip or during buccal mass retraction. CC7, which monosynaptically excites abdominal vasoconstrictor neurons (LBvc cells), is excited during buccal mass protraction and inhibited by a brief touch of the lip. CC6, which monosynaptically inhibits the ipsilateral pedal artery shortening neuron, could be excited by firing of the C-PR and is inhibited by touch to the ipsilateral lip. CC4, which excites a neuron that shortens the buccal artery, is excited during buccal mass retraction and by a touch to the lip. CC3, which monosynaptically excites abdominal heart excitor neurons (RBHE cells), increases its firing rate when the lip is touched with food. CC2, monosynaptically excites the abdominal L2 neurons, while firing of CC1 contracts the pericardium. Hyperpolarization of specific CC neurons revealed that they mediate all or most of the synaptic effects of buccal mass movements and lip stimulation on cardiovascular motor neurons. Our results indicate that a small population of the cerebral interneurons orchestrates a complex pattern of cardiovascular responses during feeding behavior.

653.23

STRETCH-ACTIVATION OF THE ACCESSORY RADULA OPENER MUSCLES OF *APLYSIA*.

C.G.Evans*,E.C. Cropper, S.C. Rosen, I. Kupfermann and K.R. Weiss. Mt. Sinai Schl. Med., N.Y., N.Y., 10029.

The accessory radula opener musculature of *Aplysia* is comprised of seven strap-like muscles, (three pairs and one single), named I7, I8, I9, and I10, innervated by a common cholinergic buccal motor neuron, B48. The muscles originate from a muscular sheath covering the collostyle at the base of the radula sac and become stretched as the accessory radula closer muscles contract. Even when denervated, the muscles will contract rhythmically if subjected to a constant stretch. Intracellular recordings from muscle fibers of I7 reveal that stretching the muscle induces a depolarization which can cause a slow increase in tension followed by discrete contractions accompanying muscle fiber spikes. The stretch-activated depolarization appears to be sodium-dependent since it is reversibly abolished in sodium-free artificial seawater. Nifedipine, at concentrations of 10^{-6}M and 10^{-5}M, can abolish the spikes but not the stretch-activated depolarization.The stretch-activated depolarization may augment the depolarization of the muscle fibers by the motor neuron elicited excitatory junction potentials to promote a greater contraction. Indeed we found that motor neuron-driven contractions of I7 initiated from a constant length decreased as the load increased, but if the length was permitted to increase, the amplitude of contraction increased. The stretch-activated depolarization may represent a peripheral mechanism to ensure that the muscle is optimally responsive to the mechanical demands of the system.

653.24

THE WHOLE-BODY SHORTENING REFLEX OF THE LEECH: MOTOR PATTERN, SENSORY BASIS, AND INTERNEURONAL PATHWAYS.

B. K. Shaw* and W. B. Kristan, Jr. Dept. of Biology, Univ. of California at San Diego, La Jolla, CA 92093-0357.

The leech whole-body shortening reflex consists of a rapid, synchronous contraction of the whole body elicited by a strong mechanical stimulus to the anterior of the animal. We have studied this reflex in semi-intact and isolated nerve cord preparations. The motor pattern consists of an activation of excitatory motor neurons innervating dorsal and ventral longitudinal muscles (DE and VE respectively), as well as the L cell, a motor neuron innervating both dorsal and ventral longitudinal muscles. The motor pattern has phasic and tonic components: the L cell is transiently activated, while cell 3 (a representative DE) shows a more sustained activation.

Stimuli that produce shortening activate the T (touch), P (pressure), and sometimes the N (nociceptive) types of mechanosensory neuron. Stimulating single sensory neurons did not cause the behavior; it appears that a critical number must be activated to yield shortening.

The S cell network, which makes up a 'fast conducting pathway' running through the leech nerve cord, is active during shortening and accounts for the shortest-latency excitation of the L cell. However, the S cell network is not sufficient by itself to account for the reflex: firing the S cells at physiological rates with an intracellular electrode causes just a weak excitation of L cells and little to no behavior. Recordings from the nerve cord connective during shortening reveal fast-conducting spikes besides those of the S cell; these spikes are correlated with motor neuron PSPs. This suggests that the S cell network and other pathways operate in parallel to produce the reflex. Supported by an NSF Predoctoral Fellowship (BKS), NIMH training grant (BKS) and NIMH research grant MH44396 (WBK).

653.25

MAPPING MOTOR NEURON ACTIVITY TO OVERT BEHAVIOUR IN THE LEECH: A BIOMECHANICAL MODEL OF THE LEECH HYDROSKELETON.

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The leech has proved a good system in which to determine how neuronal elements generate patterned motor neuron activity. However, little is known as to how these neuronal patterns coordinate the behaviour of the animal as a whole. We have undertaken a study to determine how motor neuron activity interacts with the biomechanical properties of the leech hydroskeleton to produce overt behaviours.

A model was developed to predict the shape of a leech for a given spatial pattern of motor neuron activity. The model was based on the following experimental data: the dimensions of the leech at behavioural extremes, the passive length/tension properties of the muscles in the body wall and the active tension produced by activating single identified motor neurons at different muscle lengths and firing frequencies. We based the model on the assumption that (a) the volume of each segment remains constant and (b) when at a steady-state the shape of the leech is such that the potential energy of the whole body is minimized. The potential energy for the whole body was calculated as the sum of that for each muscle, which in turn was calculated as the integral of the corresponding active and passive tension over the length of the muscle. A key test of the model has been a comparison between predicted and observed pressures recorded in the gut during the extremes of four very different behaviours: crawling, swimming, shortening and feeding.

The model as it stands provides new insight into the biomechanical properties of hydroskeletons and how these properties may be adapted for behaviour. We intend to make the model more realistic by including neuronal, muscle and fluid dynamics. Supported by NSF Research Grant IBN9222039.

MUSCLE: FIBER TYPES AND ENDPLATES

654.1

THE ROLE OF THYROID HORMONE IN FAST MUSCLE FIBER-TYPE-SPECIFIC EXPRESSION OF A TROPONIN I\LACZ CHIMERIC TRANSGENE

H.L. Bradshaw* and K.E.M. Hastings. Molecular Genetics Laboratory, Montreal Neurological Institute, Montreal, P.Q. H3A 2B1

We are interested in the expression and regulation of fiber-type-specific contractile protein isoforms in developing fast skeletal muscle fibers. This current study focuses on the early post-natal expression of a TnIfast\LacZ chimeric transgene that is driven by an avian TnIfast promoter. Unlike the endogenous TnIfast gene, this chimeric transgene is differentially expressed in the fast muscle fibers of adult mouse, ie. IIB >> IIA. In the neonatal transgenic mouse, this differential expression is not observed at birth, but emerges during the first week of postnatal life.

These results suggest that transcriptional mechanisms present in IIB fibers may be distinct from those operating in IIA fibers. Because its concentration peaks during the first post-natal week, thyroid hormone may subserve such a mechanism and act to direct the high level TnIfast\LacZ expression seen in IIB fibers.To investigate the role of thyroid hormone in the post-natal emergence of the differential expression pattern, muscle sections from hypothyroid (induced by 5-propyl-2-thiouracil and iodine-deficient chow) and untreated-control neonates will be compared and analyzed using quantitative beta-galactosidase histochemistry.

654.2

DISTRIBUTION OF MYOSIN HEAVY CHAIN-BASED INTRAFUSAL FIBER TYPES IN SMALL SPINDLES OF CHICKEN LEG MUSCLES AND THEIR DEVELOPMENTAL SIGNIFICANCE.

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The myosin heavy chain (MHC) composition of intrafusal fibers in postnatal chicken leg muscle spindles containing from 1-4 intrafusal fibers was examined in sections incubated with monoclonal antibodies against slow or fast MHC. In these muscles receptors with fewer than five intrafusal fibers make up a substantial portion of the spindle population. The small receptors are presumably normal spindles, except that they acquired fewer than the average number of seven intrafusal fibers. Oligofibral postnatal spindles present an alternate approach for determining the order of appearance of intrafusal fiber types, which in embryonic tissue is difficult to assess. The single fiber in postnatal monofibral spindles almost always had a fast MHC profile. In duofibral spindles there was a 60:40 fast/slow split. Spindles with three intrafusal fibers typically presented two fast and one slow fiber, while in quadrofibral spindles the number of fast and slow intrafusal fibers was nearly equally divided. These data support the earlier finding that the proportion of slow intrafusal fibers increases gradually from the onset of spindle development until the time when 5-6 intrafusal fibers have been formed (Cell Tiss Res, 274:383, 1993). The constant fiber type composition of small postnatal spindles also suggests that the first four fibers in a spindle arise in a fast-slow-fast-slow sequence, provided there is no significant postgenesis MHC transformation.

654.3

NEURAL CONTROL OF MYOSIN HEAVY CHAIN EXPRESSION IN REGENERATED RAT MUSCLE SPINDLES. Jun Wang and J.M. Walro*, Dept. of Anatomy, NE Ohio Univ. Coll. of Med., Rootstown OH 44272.

Neural regulation of slow-tonic (ST), α-cardiac (α-c), 2A and 2B myosin heavy chains (MHC) was investigated in rat muscle spindles which regenerated in the presence of sensory innervation only (DE), motor innervation only (DA), and in the absence of all innervation (DN group). Either dorsal root ganglia L3-6 were removed (N=9), ventral roots L3-6 were severed (N=10) or sciatic n. was severed (N=5) in young adult female rats. Three days later, a nerve-intact orthotopic graft was performed on the ipsilateral extensor digitorum longus (EDL) muscle. Grafts were excised after 30 days, frozen, cut into 10 μm sections, reacted with monoclonal antibodies specific for each of the four MHC isoforms or reacted for histochemical demonstration of motor (non-specific esterase) or sensory (NADH-TR) innervation. No fibers in DN grafts expressed ST, α-c, 2A, or 2B MHC. No fibers in DA grafts expressed ST or α-c, although 75% of encapsulated fibers expressed either 2A or 2B uniformly along the length of the fiber. In contrast, 13% of fibers in DE grafts expressed ST. However, only 1% of fibers expressed α-c and no fibers expressed 2A or 2B in DE grafts. Collectively these data suggest that in the absence of sensory innervation, fibers formed within the basal lamina tubes of intrafusal fibers cease to express "spindle-specific" MHCs such as ST and resemble extrafusal fibers in their patterns of MHC expression. Supported by a Research Challenge grant from the O.B.O.R.

654.4

AGE-RELATED INCREASE IN MUSCLE FIBER "TYPE GROUPING" IN THE HUMAN THYROARYTENOID MUSCLE: A DESIGN-BASED STEREOLOGICAL STUDY. L.T. Malmgren*, T. Uno and P.J. Fisher. Dept. Otolaryngology, S.U.N.Y. Health Sci. Ctr., Syracuse, NY 13210.

In many human muscles age-related partial denervation leads to muscle fiber "type grouping", a departure from the normal tendency toward random spatial distribution of muscle fiber types. Techniques for quantifying muscle fiber "type grouping" have generally been based on two-dimensional models that are not applicable to geometrically complex muscles such as the thyroarytenoid. In the present study, a design-based (free of assumption of a geometrical model) second order, stereological technique (Kroustrup et al., J. Microsc. 149:135) has been used to quantify age-related muscle fiber "type grouping" in the human thyroarytenoid muscle.

In humans the thyroarytenoid muscle plays crucial roles in protecting the airway and in vocalization, and age-related changes in this motor system are of concern. Samples were obtained from male and female autopsy cases (49-97 years; n=20). The results indicate a significant (P=.032) age-related increase in the tendency for muscle fibers to be nonrandomly grouped in clusters with fibers of the same type (based on expression or lack of expression of fast myosin isoform). Since this phenomenon is the result of reinnervation of denervated fibers by collateral sprouting of a common axon, the muscle fibers in these clusters tend to alter their myosin isoform expression in response to the influence of the new motor neuron. This result therefore indicates an age-related partial denervation followed by reinnervation in the human thyroarytenoid muscle, which is consistent with a hypothesis of age-related motor neuron cell death in this system. (Supported by NIH grant AG0918603)

654.5

NEUROMUSCULAR JUNCTION MORPHOLOGY DURING FAST-TO-SLOW MUSCLE TRANSFORMATION. T.Somasekhar*, R.H. Nordlander and P.J.Reiser, Dept. Oral Biology, Ohio State Univ., Columbus, OH 43210.

Chronic low frequency stimulation results in transformation of muscle from fast- to slow-twitch phenotype. We examined the morphology of neuromuscular junctions (NMJs) in two fast-twitch rabbit muscles, extensor digitorum longus (EDL) and tibialis anterior (TA), after 3 weeks of chronic electrical stimulation at 10Hz. These muscles were stained with rhod-amine-alpha-bungarotoxin and their NMJs analyzed with Meta-Morph software. Stimulated TA muscles showed significant reductions in mean NMJ area (30%), length (15%) and width (19%) compared to unstimulated contralateral controls (n = 2 rabbits). Changes in NMJ morphology were more profound in TA than in EDL. Mean NMJ area in normal slow soleus muscle was similar to control EDL but was significantly smaller (22%) than control TA. A similar range of NMJ configurations were seen in both controls and stimulated muscles. These observations show that stimulation induces marked changes in NMJ size. Work is underway to determine whether NMJs simply shrink in size or selectively retract branches during fiber transformation. Supported by NIH AR39652 and NS18773.

654.6

IMMUNOREACTIVITY FOR THE NMDA-RECEPTOR, N-ACETYL-ASPARTYLGLUTAMATE AND NAALADASE AT THE RAT NEUROMUSCULAR JUNCTION. U. V. Berger* and J. T. Coyle, Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Boston, MA 02129.

The neuropeptide N-acetylaspartylglutamate (NAAG) is co-localized to a number of different neurotransmitter systems in mammalian central nervous system including the ventral horn cholinergic motoneurons. NAAG is released upon depolarization and catabolized by the extracellular enzyme NAALADase to N-acetylaspartate and glutamate. Furthermore, NAAG displays partial agonist activity at NMDAR1-type glutamate receptors. The physiological function of NAAG is still uncertain, but may include a role as a neuromodulator or as a glutamate precursor. In the peripheral nervous system, NAAG-like immunoreactivity (LI) is present in axons of sensory and motor nerves while NAALADase is expressed by non-myelinating Schwann cells. The present study investigates the presence of NAAG, NAALADase and NMDAR1-receptors at the rat neuromuscular junction. Three days after sciatic nerve ligation, axonal NAAG-LI was increased proximal to the ligation site, indicating that NAAG is transported in sciatic nerve axons. In diaphragm, NAAG-LI was observed in the terminals of phrenic nerve while NAALADase-LI was present in the basal lamina that surrounds neuromuscular junctions and perisynaptic Schwann cells. NMDAR1-LI was observed in the endplate region of diaphragm using immunocytochemistry and immunoblotting. The NMDAR1-LI was closely associated with alpha-bungarotoxin binding to nicotinic acetylcholine receptors, suggesting that NMDA receptors are present postsynaptically on muscle cells. The presence of NAAG in motor nerve endings corroborates other recent findings of glutamate-like immunoreactivities in rat motor neurons and terminals. These results suggest that NAAG, or glutamate cleaved from NAAG by NAALADase, may be involved in vertebrate neuromuscular transmission.

654.7

GROWTH-RELATED ADAPTATIONS OF ENDPLATES ON DIAPHRAGM MUSCLE FIBERS: A 3D STUDY USING CONFOCAL MICROSCOPY. Y.S. Prakash*, K.G. Smithson and G.C. Sieck. Departments of Anesthesiology and Physiology, Mayo Foundation, Rochester, MN 55905.

Previous studies have shown 1) differences in endplate size on slow (type I) & fast (type II) muscle fibers, and 2) a correlation between endplate size and muscle fiber size. The differential growth of type I & II fibers during maturation may account for these differences in endplate size. In the present study, the three-dimensional (3D) surface areas of endplates on type I & II muscle fibers of 21-day old (D21) and adult rat diaphragms were examined using confocal microscopy. Motor endplates were labelled with fluorescein α-bungarotoxin, and muscle fiber type was determined using an anti-fast myosin antibody and indirect immunocytochemistry. Surface areas were calculated from computer-generated reconstructions of confocal optical sections. At D21, in spite of no difference in fiber size, surface areas of type I fiber endplates were greater than those of type II fibers. These differences were enhanced in adults, even though adult type II fibers grew disproportionately to type I fibers. In adults, endplate surface area was positively correlated to muscle fiber size, but only within a fiber type. When normalized for fiber diameter, surface areas of type I fiber endplates were still significantly greater than those of type II fiber endplates, in both age groups. In type I fibers, the increase in endplate surface area was disproportionate to fiber growth, whereas in type II fibers, the increase in endplate surface area was proportionate to fiber growth. We conclude that differences in endplate size on type I & II fibers reflect phenotypic differences unrelated to differential fiber growth. These phenotypic differences in endplate size may impact synaptic efficacy. (Supported by NIH grants HL37680, HL34817 and GM08288).

654.8

INFLUENCE OF INACTIVITY ON ENDPLATE SIZE AND NEUROMUSCULAR TRANSMISSION. H.Miyata, W.Z.Zhan, Y.S.Prakash and G.C.Sieck* Departments of Anesthesiology and Physiology, Mayo Foundation, Rochester, MN 55905.

In the rat diaphragm (DIA), we induced inactivity of the right hemidiaphragm by hemisection of the C2 spinal cord (spinal isolation, SI) or by tetrodotoxin (TTX) blockade of phrenic axonal propagation. After 2 wks of inactivity, we examined adaptations of motor endplates on type I and II muscle fibers, as well as susceptibility to neuromuscular transmission failure (NF). Paralysis of the right hemidiaphragm was confirmed by the absence of EMG. NF was assessed *in vitro* by stimulating the phrenic nerve at 40 or 75 Hz in 330 ms trains repeated each sec for 5 min. Direct muscle stimulation was superimposed every 15 sec, and the forces generated by each stimulus mode were compared to estimate NF. Motor endplates were labeled with fluorescein-conjugated α-bungarotoxin and muscle fiber type was determined immunocytochemically using an anti-fast myosin antibody. Double-labeled samples were analyzed using dual channel laser-scanning confocal microscopy to reconstruct 3D images of motor endplates. In CTL, type II fiber endplates were smaller than type I fiber endplates. Type II fiber endplate surface area increased in the TTX and SI diaphragm but type I fiber endplate size was unchanged. As compared to CTL, NF at both 40 and 75 Hz was less pronounced in the SI group but more pronounced in TTX animals. We conclude that endplate adaptations to inactivity are restricted to type II fibers, but do not necessarily reflect associated alterations in neuromuscular transmission. Supported by NIH grants HL34817 and HL37680.

655.1

CIRCADIAN CHANGE OF VIP mRNA EMERGES IN THE SUPRACHIASMATIC NUCLEUS IN CONSTANT DARKNESS FOLLOWING DEPLETION OF SEROTONIN BY PARACHRROROPHENYLALANINE (PCPA) H. Okamura, F. Kawakami*, Y. Tamada, T. Nishiwaki, Y. Ibata, S.-I.T. Inouye. Depts Anatomy and Psychiatry, Kyoto Pref. Univ. Med., Kyoto 602: Lab. Integrative Brain Function, Mitsubishi Kasei Inst. Life Sci., Tokyo 194, Japan

Neuronal activity of the suprachiasmatic nucleus (SCN) is known to be influenced by two major innervations to the SCN: photic stimulus conveyed by the direct retinohypothalamic tract or indirect geniculohypothalamic tract, and the serononergic (5-HT) neurotransmission by ascending forebrain 5-HT tract. In the present study, we examined the time-course of VIP mRNA expressions in the SCN of rats in which the two major neuronal transmissions being eliminated, by using the *in situ* hybridization technique combined with computer assisted image analysis. Male Wistar rats were housed in constant darkness, and the 5-HT input to the SCN was blocked by the three days successive intraperitoneal injections of *p*-chlorophenylalalnine methylester (300 mg/kg, daily), an inhibitor of tryptophan hydroxylase. After 24 hrs of the last injection, VIP mRNA levels in the SCN were asscessed by in situ hybridization. In saline treated controls, VIP mRNA levels were almost constant at any time of the day. In contrast, PCPA treatment induced the rhythm of VIP mRNA with the peak at CT 4 and trough at CT 20. These findings suggested that the removal of photic and 5-HT influence induces VIP mRNA rhythm in the SCN, and indicates that VIP mRNA is controled also by the circadian clock.

655.2

DISTRIBUTION OF NEUROPEPTIDES IN THE HAMSTER SUPRACHIASMATIC NUCLEUS. Kazue Semba*, Hugh D. Piggins, and Benjamin Rusak, Depts. of Anatomy & Neurobiology, and Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The suprachiasmatic nuclei (SCN) of the hypothalamus are the site of the primary circadian pacemaker in mammals. The distributions of various neuropeptides have been described in the rat SCN but have not been as well documented in other mammals such as the hamster. Using mono- and polyclonal antibodies, we examined the distribution of immunoreativity for calcitonin gene-related peptide (CGRP), galanin (GAL), gastrin-releasing peptide (GRP), peptide histidine isoleucine (PHI), substance P (SP), vasoactive intestinal polypeptide (VIP), and vasopressin (VP) in the SCN region of both colchicine treated and untreated Syrian hamsters. Intense labelling of cell bodies, fibres and terminals was observed for GRP, PHI, VIP, and VP within the SCN. In contrast, only sparse labelling of GAL and SP immunoreactive fibres and terminals was seen in the SCN, while more intense labelling was present in adjacent hypothalamic areas. A small number of SP-positive cell bodies were observed in the central SCN, whereas GAL-positive cell bodies were found only in hypothalamic areas bordering the SCN region. A few CGRP-positive fibres and terminals were found in the ventral and lateral aspects of SCN, but no cell bodies stained for CGRP.

In summary, various neuropeptides show different patterns of distribution in the hamster SCN. These data suggest different functions for these peptides in circadian processes.

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655.3

ELECTROPHYSIOLOGICAL EFFECTS OF IONOPHORETICALLY APPLIED SUBSTANCE P ON HAMSTER SUPRACHIASMATIC NUCLEUS NEURONS *IN VITRO*. Hugh D. Piggins*, David J. Cutler, and Benjamin Rusak, Depts. of Anatomy & Neurobiology, and Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J1.

The suprachiasmatic nuclei (SCN) of the hypothalamus function as the primary circadian pacemaker in mammals. The tachykinin substance P (SP) and tachykinin receptors have been identified in the rat SCN, but little is known about the role of SP in circadian processes of other mammals. In this study, we examined the effects of local applications of SP on the extracellularly recorded discharge rate of Syrian hamster SCN neurons maintained in an *in vitro* brain slice preparation. Brain slices (400-500 μm thick) were prepared from hamsters housed under a 14:10 light:dark schedule during the light phase of the cycle. SP (1 mM in 165 mM NaCl) or vehicle were ionophoresed as cations (20-400 nA) from a multibarrel pipette located 50-80 μ m from a recording electrode (containing 2 M NaCl) situated in the SCN. When collapsed across all recording sessions (ZT4-20, where ZT0=lights on), SP increased the firing rates of 57 cells, and suppressed the firing rates of 13 cells of 146 cells tested. In contrast, control ejections of vehicle evoked responses from only 3 of 36 cells tested. A larger percentage of cells responded to SP during the middle of the projected day (62.5%) than during the middle of the projected night (25%). These data indicate that local ejections of SP can alter hamster SCN neuronal activity, particularly during the projected day. This study raises the possibility that local release of SP from intrinsic SCN neurons or from afferent projections may play a role in the entrainment of the circadian pacemaker.

Supported by grants from the US AFOSR, and NSERCof Canada, and an MRC postdoctoral fellowship to HDP.

655.4

COMPARISON OF NEUROTRANSMITTER PEPTIDE mRNAs: TAU MUTANT HAMSTERS EXHIBITING GIANT PHASE SHIFTS TO LIGHT *vs.* WILDTYPE HAMSTERS. K. Scarbrough*, F.W. Turek, NSF Center for Biological Timing, Northwestern Univ, Evanston, IL 60208

Tau mutant hamsters respond to saturating white light pulses presented between CT 11 and CT 16 with giant phase shifts (type 0 resetting) under certain circumstances. These include prolonged time in DD, entrainment to a T cycle of 19 h (Shimomura and Menaker, 1992 SRBR abstract #128) and early in the aging process (Zee *et al.* 1994 SRBR). In our study, after 49 circadian cycles in DD, 8 out of 8 tau mutant hamsters responded to a 1 h light pulse at CT15 with phase shifts averaging 10.19 ± 0.35 h. Among wildtype hamsters the mean phase shift was 1.22 ± 0.34 h and the largest observed was 3.67 h. We determined whether neurotransmitter peptide mRNA levels in the SCN differed between wildtype and tau mutant hamsters exhibiting these divergent responses to light. Wildtype and tau mutant hamsters were killed after 66-71 cycles in DD at either CT4 or CT16. Brains were frozen on dry ice and stored at -80°C until cryostat sections (10 μm) through the suprachiasmatic region were collected. *In situ* hybridization was performed using ^{35}S-labeled oligonucleotide (48mer) probes and clusters of silver grains in the SCN were quantitated under dark field illumination using an image analysis program by Image 1 Inc. Levels of suprachiasmatic somatostatin mRNA tended to be lower among tau mutants compared to wildtypes but this difference did not reach statisitical significance ($p = 0.07$). A comparison of VIP and vasopressin gene expression in these same animals is currently underway. Differences in levels of neuropeptide mRNAs correlating with this unusual response to light by the circadian system of tau mutant hamsters may provide insight into the cellular basis of the mutation. Sponsored by HD 07068, NSF STC 8920162, AG 10870.

655.5

DEVELOPMENT OF GLIA AND VIP-IMMUNOREACTIVE NEURONS IN THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN). G.I. Botchkina* and L.P. Morin, Dept. Psychiatry, Stony Brook University, NY 11794.

Radial glia are thought to guide neurons to their targets in developing brain and to facilitate their compartmental organization. We have studied the developmental relationships between radial glia, astrocytes and VIP-IR neurons in the SCN, site of the mammalian circadian clock.

Vimentin-IR (VIM) radial glial processes are evident at embryonic day 8 (E8), achieving their highest density in the SCN by E13-14. By postnatal day 5 (P5), VIM-IR radial glia in the SCN have gradually disappeared. VIP-IR identifies a set of ventral SCN neurons as early as E13. A VIP-IR fiber plexus is evident in the SCN by E15. It then progressively increases in density while extending dorsally.

The first GFAP-IR appears in glia of the ventral SCN and optic chiasm by E15. GFAP-IR astrocytes are evident by P0 and they progressively increase in number to adulthood. A few VIM-IR astrocytes are present in the SCN at P21. The embryonic hamster circadian clock may be functional at E15. Thus, it is possible that astrocytes may not appear until after embryonic rhythmicity has begun. Supported by NS22168.

655.6

MUSCIMOL REDUCES PHASE DELAYS PRODUCED BY COADMINISTRATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP), PEPTIDE HISTIDINE ISOLEUCINE (PHI), AND GASTRIN RELEASING PEPTIDE (GRP) INTO THE SUPRACHIASMATIC NUCLEUS (SCN). **C.F. Gillespie*, T.O. Babagbemi, K.L. Huhman, H.E. Albers, Lab of Neuroendocrinol. & Behav., Georgia State Univ., Atlanta, GA 30303.**

Coadministration of VIP, PHI, and GRP into the hamster SCN produces large phase delays in activity rhythms. Since GABA has been reported to be found in most neurons of the SCN, the present study examined the phase shifting effects of coadministration of the $GABA_A$ agonist, muscimol, with VIP, PHI, and GRP. Male golden hamsters were implanted with guide cannulae aimed at the SCN region and housed in constant darkness. After a stable free-running rhythm of wheelrunning was established, the hamsters were microinjected at CT 12-14 with a 200nl cocktail consisting of either VIP (50ng in 50nl saline) + PHI (50ng in 50nl saline) + GRP (50ng in 50nl saline) + muscimol (570ng in 50nl saline) and VIP + PHI + GRP + 50nl saline in a counterbalanced order. Microinjection of the cocktail containing muscimol resulted in phase delays (0.23±0.06hr) that were significantly smaller ($p < 0.001$) than those phase delays induced by microinjection of the cocktail not containing muscimol (0.88±0.13hr). These data suggest that GABAergic activity within the SCN may be important in regulating the phase-delaying effects of VIP, PHI, and GRP.

(Supported by NS30022)

655.7

MICROINJECTION OF THE $GABA_B$ ANTAGONIST, PHACLOFEN, INTO THE SUPRACHIASMATIC REGION BLOCKS NEUROPEPTIDE Y-INDUCED PHASE ADVANCES. K. L. Huhman*, T. O. Babagbemi, C. F. Gillespie, H. E. Albers, Lab. of Neuroendocrinol. & Behav., Georgia State Univ., Atlanta, GA 30303.

The primary neurotransmitter in the geniculo-hypothalamic tract (GHT) appears to be neuropeptide Y (NPY). There is also evidence that GABA is important in the functioning of this projection. GABA is colocalized with NPY in some GHT neurons, and NPY and GABAergic fibers synapse on some of the same target neurons in the SCN. Recently, we have shown that microinjection of the $GABA_A$ antagonist, bicuculline, into the SCN completely blocks phase advances induced by microinjection of NPY. To determine whether the $GABA_B$ antagonist, phaclofen, could also block NPY-induced phase advances, male golden hamsters were implanted with an indwelling cannula aimed at the SCN and were housed in individual cages with running wheels in constant darkness. Hamsters were microinjected at CT6 with either NPY (100 ng in 100 nl saline)/ vehicle (50 nl saline), NPY/ phaclofen (3.5 μg in 50 nl saline), or phaclofen/ vehicle (100 nl saline) in a counterbalanced order (injections separated by 5 min). NPY/saline injection induced a phase advance of 1.1 $\pm$ 0.34 hr, however, when NPY was injected with phaclofen, NPY-induced phase advances were blocked (0.02 $\pm$ 0.12 hr). Phaclofen/saline injection caused no phase change (0.07 $\pm$ 0.06 hr). The data indicate that $GABA_B$ activity may be important for phase advances induced by NPY. (Supported by NS30022)

655.8

PEPTIDE PHENOTYPES OF SUPRACHIASMATIC NUCLEUS (SCN) NEURONS IN THE RAT I.C. Speh*, N. Suhan and R.Y. Moore, Depts. of Psychiatry and the Center for Neuroscience, Univ. of Pittsburgh, PA 15261

The SCN is a circadian pacemaker in the mammalian brain. It has two apparent anatomical subdivisions, a dorsomedial (DM-SCN), containing a population of vasopressin-containing (VP+) cells and a ventrolateral (VL-SCN) with vasoactive intestinal polypeptide (VIP+) cells. Our recent data indicate that most if not all SCN cells are GABA containing and cell counts for VP and VIP are 3176 $\pm$ 43 and 2081 $\pm$ 59 respectively (Moore and Speh, 1993). Other peptides have been reported localized in SCN neurons, including, gastrin releasing peptide, (GRP), calretinin (CAR), neurotensin (NT) substance P (SP), and somatostain (SS). In this study cell counts and colocalization immunocytochemistry were used to analyze the distribution of these neuronal elements. Cell counts from the present study for each of these additional phenotypes is as follows; GRP 1140 $\pm$ 30; CAR 1370 $\pm$ 282; NT 283 $\pm$ 10; SP 168 $\pm$ 33; SS 302 $\pm$ 18. Total cell counts for the entire SCN have been reported between 8000 (van den Pol, 1980) and 10,000 (Guldner, 1976). The total of the mean cell counts for the phenotypes reported here, including VP and VIP is 8520. The colocalization studies indicate that GRP and SP are each partially colocalized with VIP and that GRP, NT, SP and VIP are found in the VL-SCN; VP, SS and CAR are found in the DM-SCN; CAR cells also occupy a lateral third subdivision of the SCN. Neither VP, VIP nor any of the other cell peptides reported here are found in this area. Van den Pol and Tsujimoto (1985) report that the cells of the DM-SCN and VL-SCN are densely packed with mean cell diameters of 7.8 μm^2 whereas cells in this lateral group are much more loosely arranged with mean cell diameters of 9.6μm^2. The functional implications of these peptide phenotypes remains to be elucidated. Supported by NS-16304.

NEUROPEPTIDES AND BEHAVIOR: OTHER

656.1

ROLE OF SUBSTANCE P IN MEDIAL AMYGDALOID SUPPRESSION OF PREDATORY ATTACK BEHAVIOR IN THE CAT. Y.C. Han*, M.B. Shaikh and A. Siegel. Laboratory of Limbic System & Behavior, Department of Neurosciences, New Jersey Medical School and Graduate School of Biomedical Sciences, UMDNJ, Newark, New Jersey 07103.

The present study tested the hypotheses that: (1) the medial amygdala (ME) suppresses quiet biting "predatory" attack behavior (QBA), and (2) substance P (SP) is utilized as a neurotransmitter in the pathway from ME mediating suppression of QBA. *Phase I:* stimulating electrodes were implanted into the ME and lateral hypothalamic sites (LH) from which QBA could be elicited by electrical stimulation. Response latencies for QBA were significantly ($p < .05$) increased after dual stimulation of the ME and LH relative to single stimulation of the LH alone. *Phase II:* microinfusions of the NK_1 antagonist, CP, 96,345, (0.05, 0.5 and 2.5 nm/0.25μl) were placed into the medial hypothalamus (MH) because it is known to receive inputs from an SP pathway originating in the ME which, in turn, are relayed via a presumed, short inhibitory projection to LH from MH. Significant dose and time dependent blockade of ME-induced suppression was observed. *Phase III:* microinjections of SP into MH (0.5, 1.0 and 2.0 nm/0.25 μl) resulted in significant ($p < .05$) suppression of LH-elicited QBA, thus mimicking the effects of ME stimulation. These results suggest that ME suppresses QBA by acting through an SP mechanism within MH.
[Supported by the Harry Frank Guggenheim Foundation].

656.2

DIFFERENTIAL EFFECTS OF THE NON-PEPTIDE NEUROTENSIN (NT) ANTAGONIST, SR 48692, ON THE NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF NT AGONISTS. T. A. Pugsley*, H. C. Akunne, S. Z. Whetzel, S. DeMattos, A. Corbin, J. N. Wiley, D. J. Wustrow, L. Wise and T. G. Heffner, Neuroscience Pharmacol., Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105.

This study examines the effects of the non-peptide NT receptor antagonist SR 48692 on some neurochemical and behavioral effects of NT agonists. In in vitro studies, SR 48692, a non-peptide NT receptor antagonist, inhibited the binding of [^{3}H]NT to membrane preparations from 10-day old post-natal mouse brain and from HT 29 cells with K_i values of 3.9 nM and 8.6 nM, respectively, in agreement with previous findings. SR 48692 also antagonized (IC_{50}=159 nM) the NT (50 nM)-induced mobilization of intracellular calcium in HT 29 cells. In rat cerebellar slices, SR 48692 (30-300 nM) blocked in a dose-dependent manner the increase in cyclic GMP levels evoked by NT (100 nM). In vivo, SR 48692 (0.05 and 0.5 mg/kg, i.p.) inhibited the increase in rat brain mesolimbic dopamine turnover (DOPAC/DA ratio) induced by the systemically active NT peptide, EI [(N-Me)Arg-Lys-Pro-Try-*tert*--Leu-Leu] dosed at 2 mg/kg, i.p. 30 min after the SR 48692. No effects on dopamine turnover of either EI or SR 48692 were observed in the striatum. SR 48692 (1, 3 and 10 mg/kg, i.p.) did not antagonize the EI-induced (1 mg/kg, i.p.) decrease in spontaneous locomotor activity (LMA), the decreases in LMA induced by i.c.v. administered NT (0.05 to 0.1 ug/mouse) and did not inhibit the EI (0.1-1.2 mg/kg, s.c)-induced fall in colonic temperature in mice. SR 48692 alone did not alter LMA or colonic temperature. These findings support the hypothesis that a subtype of the NT receptor mediates the locomotor and hypothermic actions of this peptide and that it is different from the NT receptor that is involved in dopamine turnover.

656.3

HYPOTHALAMIC DYNAMIC INSULIN CHANGES IN WISTAR RATS, IN RELATION TO MEALS AND TO THE MACRONUTRIENT COMPOSITION. A MICRODIALYSIS STUDY. S. Nicolaïdis*, K. Gerozissis, M. Orosco, Neurobiologie des Régulations, URA 1860 CNRS, Collège de France, pl M. Berthelot, 75005 Paris, France

The presence of insulin in the brain and its influence on feeding and body weight when centrally infused is now well established. The question of dynamic changes in brain insulin in relation to meals is still unanswered and addressed here.

Immunoreactive insulin was measured by a sensitized radioimmunoassay in microdialysates from the VMH and PVN nuclei during and around scheduled meals in male Wistar rats (Gerozissis et al., Brain Research, 1993, 611, 258).

We indeed observed elevations (+260%) in hypothalamic insulin during the first thirty minutes of one-hour-lasting standard chow meals with a progressive return towards premeal levels. When the rats were accustomed to the scheduled meals, an anticipatory increase in insulin levels (+70% to 125%) was found in the hypothalamus, but not in the plasma, during the thirty minutes preceding the time of the meal. This increase occurred whether the meals were presented or not upon the due time.

When the standard laboratory chow meals were replaced by exclusively fat meals (lard), the rats ingested very large amounts of it but hypothalamic insulin did not rise nor did plasma insulin. This result is not in favor of a role of brain insulin as a signal of satiety per se.

These data show that insulin changes at the hypothalamic level often reflect those in the plasma but with some exceptions that cannot be accounted for by a simple plasma-brain tissue delivery. It can be suggested that the system which delivers insulin to the hypothalamus mainly reflects the beta cells function but it possesses its own way of weighing its potency. Brain insulin might not represent only a satiety signal, as currently proposed.

656.4

OLFACTORY BULBECTOMY ALTERS LEVELS OF GALANIN AND NEUROPEPTIDE Y mRNA IN THE RAT LOCUS COERULEUS
P. V. Holmes*, U. Koprivica, and I. N. Crawley. Section on Behavioral Neuropharmacology, Experimental Therapeutics Branch, NIMH, Bethesda, MD 20892.

The noradrenergic locus coeruleus (LC) contains high levels of galanin (GAL) immunoreactivity and GAL mRNA and moderate levels of neuropeptide Y (NPY) immunoreactivity and NPY mRNA in the rat. LC neurons project to several forebrain areas, including the olfactory bulb. The effects of surgical ablation of the olfactory bulb (OBX) on levels of mRNA for GAL, NPY, and tyrosine hydroxylase (TH) in the LC and other brain regions were examined with quantitative *in situ* hybridization histochemistry. Increases in GAL mRNA levels in the LC were observed two weeks following bilateral OBX. Levels of NPY mRNA were slightly decreased and levels of TH mRNA were unchanged in the LC at this time point. Unilateral OBX increased GAL mRNA levels in the ipsilateral LC, suggesting that the elevation in GAL mRNA was a direct result of axotomy. Behavioral experiments confirmed previous reports of differences in open field activity between rats receiving bilateral OBX and sham-operated controls. Furthermore, alterations in open field activity depended on the aversiveness of the environment. The activity of rats with OBX was increased compared to controls under bright but not dim lighting conditions. Freezing behavior in response to footshock was attenuated in rats with OBX compared to controls. These results suggest that alterations in GAL and NPY mRNA in the LC and other brain regions may underlie the alterations in stress-related behaviors induced by OBX.

656.5

DISTRIBUTION OF NEUROPEPTIDE Y IN THE MALE SYRIAN HAMSTER BRAIN. DB Parfitt* and SW Newman, Dept. of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616

Pharmacological data implicated neuropeptide Y (NPY) in regulation of gonadotropin secretion and mating behavior. While the limbic pathways controlling mating behavior were defined in the male hamster, the pathway's neurotransmitters were not fully characterized. This study examined the distribution of NPY containing cells, fibers, and terminals in the male hamster brain. Brains from colchicine (ICV injection, n=5) and non-colchicine (n=10) treated male hamsters were cut into 40µm coronal sections, and processed for immunocytochemistry using one of two primary antibodies for NPY (Incstar or donation from Dr. Marvin Brown, UCSD). The regional distribution and intensity of neuronal staining was the same in colchicine treated brains using either antibody. However in non-colchicine treated brains, the intensity of neuronal staining was more robust with the Brown antibody than with the Incstar, while the distribution was unchanged. NPY cells were found in the deep layers of cortical structures throughout the telencephalon, including the olfactory bulbs, anterior olfactory nucleus, piriform cortex, and isocortex, as well as in region CA4 of the hippocampal formation. Cells were also distributed throughout the dorsal and ventral striatum. In the extended amygdala, fibers and terminals were prominent in the central amygdaloid nucleus and the bed nucleus of the stria terminalis. Hypothalamic fiber plexuses and terminal fields were found in the medial preoptic area, paraventricular nucleus, suprachiasmatic nucleus and arcuate nucleus. Thus, NPY neurons and fibers distributed in limbic structures that relay chemosensory information to control mating may mediate this essential sensory processing. (Supported by NIH NS-20629, T-32-HD070408).

656.6

EFFECTS OF PARAVENTRICULAR GALANIN AND NEUROPEPTIDE Y ON DOPAMINE AND ACETYLCHOLINE IN THE NUCLEUS ACCUMBENS OF FREELY MOVING RATS. P.V. Rada*, G.P. Mark and B.G. Hoebel. Dept. of Psychology, Princeton Univrsity, Princeton, NJ 08544.

Galanin (GAL) and Neuropeptide Y (NPY) injected into the hypothalamus can induce feeding behavior. Moreover, feeding is known to cause changes in extracellular dopamine (DA) and acetylcholine (ACh) in the nucleus accumbens (NAc) of rats. Therefore, the following experiments used microdialysis to study the effects of GAL and NPY injections into the paraventricular nucleus (PVN) on accumbens DA and ACh. Dialysis probes (2 mm tips) aimed at the NAc were implanted 24 hrs before experimentation and DA or ACh was monitored. During dialysis each rat received counterbalanced injections into the PVN of GAL, NPY or saline. Following the dialysis procedure on separate days the effect of GAL, NPY or saline on feeding was tested. The main result was GAL (300 pmoles) significantly increased DA levels to 152 $\pm$ 18% (n=8) and decreased ACh to 75 $\pm$ 7% (n=7) 60 minutes after the injection. The neurochemical effect was observed exclusively in rats that later showed feeding behavior during testing with GAL. Neither NPY nor saline modified DA or ACh concentrations in the NAc even though NPY induced feeding in 6 of 12 rats. This result demonstrates that hypothalamic GAL activates the mesolimbic dopamine system and suggests that GAL could be involved in the reinforcement of feeding behavior.
Supported by USPHS grant NS 30697.

656.7

DIPSOGENIC DOSES OF ANGIOTENSIN II (AII) DO NOT AFFECT COGNITION, ACETYLCHOLINE (ACh) OR GABA RELEASE IN THE RAT. K. Szczepanski, A. Buxton, S. E. Daniels, P. A. McNeeley, R. M. Johnson*, and D. J. Fontana. Department of Neurosciences, Institute of Pharmacology, Syntex Discovery Research, Palo Alto, CA 94303, U.S.A.

We assessed the behavioral and neurochemical action of renin and angiotensin II (AII) in the rat. We studied the behavioral effects of dipsogenic doses of AII and renin on learning and memory in two cognitive tasks: the Morris Water Maze and Passive Avoidance. AII (0.1 and 1 µg, i.c.v.) did not affect latency to find the platform in the water maze. AII (0.01 and 0.1 µg, i.c.v) and renin (0.1 and 10 µg, i.c.v.) given 30 min before training, and AII (1 µg, i.c.v.) and renin (1 µg, i.c.v.) given 5 min after training, did not affect retention in passive avoidance. These results suggest that while AII and renin are involved in thirst or fluid regulation, they may not be involved in cognition. In neurochemical experiments we used superfusion technology to study the action of AII on K^+-evoked [^{3}H]-ACh release and [^{3}H]-GABA release in slices of hippocampus and entorhinal cortex. Unlike DuP 996 (10 µM) which increased [^{3}H]-ACh release and [^{3}H]-GABA release in hippocampal and entorhinal cortex slices, AII did not affect release in hippocampal slices (1 µM) or entorhinal cortex slices (0.1 µM). These results suggest that AII does not affect ACh or GABA release. The findings from our behavioral and neurochemical studies suggest that either the brain renin-angiotensin system is not involved in cognition or it mediates cognition through a system other than the cholinergic or GABAergic system.

656.8

DISTRIBUTION OF ARGININE VASOTOCIN IN THE EARLY AMPHIBIAN EMBRYO. S.K. Boyd*. Dept. of Biological Sciences, Univ. of Notre Dame, Notre Dame, IN 46556.

The neuropeptide arginine vasotocin (AVT) is widespread throughout the brain of adult amphibians. It is unknown, however, when AVT first appears during development. We used immunocytochemistry to map the distribution of AVT in early embryos of the South African Clawed Frog, *Xenopus laevis*. Staining was done in whole albino embryos or in cryostat sections of embryos, at ages from fertilization onward. AVT-immunoreactivity first appeared at the neural tube stage of development (Nieuwkoop and Faber stage 21). Cells (5-10) were located ventro-medially with strong fiber projections extending laterally. These cells were located at the rostral end of the neural tube, within the region which will form the brain. Scattered cells and fibers were also observed throughout the rest of the neural tube, within presumptive spinal cord regions. Around stage 42, primary brain divisions are evident and the majority of AVT-ir was located in the diencephalon. Fibers were also observed in the developing eye. AVT-immunoreactivity thus appears very early in amphibian nervous system development. The homologous peptides in mammals, vasopressin and oxytocin, do not appear until considerably later in embryonic mammalian development. AVT in the early embryo many influence aspects of neural development, including organization of sensory or motor circuits.

656.9

EXPRESSION OF AMPHIBIAN BRAIN THYROTROPIN-RELEASING HORMONE AND ARGININE VASOTOCIN RECEPTOR GENES IN *Xenopus laevis* OOCYTES. C.L. Mitchell*, J.S. Hosler, H.E. Esch and S.K. Boyd. Dept. of Biological Sciences, Univ. of Notre Dame, Notre Dame, IN 46556.

Thyrotropin-releasing hormone (TRH) and arginine vasotocin (AVT) are neuropeptides found throughout the amphibian brain. Although both have numerous CNS effects, brain receptors for TRH and AVT have not been fully characterized in any non-mammalian vertebrate. We expressed amphibian brain TRH and AVT receptors by injecting *Xenopus* oocytes with brain whole mRNA. Expression of receptors was measured by voltage clamping to monitor current flux across the membrane of oocytes in response to ligand. Injected oocytes were responsive to TRH, TRH free acid, cyclo-(his-pro), [3-MeHis^2]TRH and AVT, as indicated by inward membrane currents. The response was specific, since mRNA-injected cells showed no response to the non-endogenous peptide isotocin, and uninjected or water-injected cells showed no change in current when exposed to any neuropeptides. The TRH response was reversible, dose-dependent, and saturable. Time course of response to maximally effective ligand doses was consistent. Latency (from ligand addition to onset of current flux) and response time (from onset of response to saturation) were characteristic of agonist applied. Time course differences suggest that the amphibian brain expresses more than one subtype of TRH receptor. The V_1 vasopressin receptor antagonist d(CH$_2$)$_5$[Tyr(Me)^2]AVP did not alter response when added to cells already maximally stimulated with AVT. When added alone, however, it produced a gradual current flux which was increased by secondary application of AVT. The mammalian antagonist thus appears to have agonistic effects in this system. We conclude that unique receptors for TRH and AVT are encoded in *Xenopus* brain, and our data represent the first expression of these receptors from any non-mammalian vertebrate.

656.10

V1-RECEPTOR MEDIATED EFFECTS OF VASOTOCIN ON MOTIVATIONAL, MNEMONIC AND AVERSIVE COMPONENTS OF SEXUAL LEARNING IN QUAIL. G. Bernroider* and St. Leutgeb, Institute for Zoology, University of Salzburg, A-5020 Salzburg, Austria.

Numerous studies have uncovered "hormonal constraints" imposed on adaptive specializations of sexual learning (Holloway & Domjan, *J Experim Psychol*, 19:47-55, 1993). Studies in rats have revealed a sexually differentiated, steroid dependent and V1-receptor mediated effect of arginine-vasopressin (AVP), facilitating social recognition by olfactory investigation. Because similar peptidergic substrates can be found in birds (e.g the vastocinergic innervation within the lateral septal area), it was of interest whether visually dominated sexual learning in birds involves comparable neurochemical mechanisms.

Adult male quail were tested for approach behavior to their own mirror image. Arginine vasotocin (AVT, 10ug/kgBW in 0.1ml saline, s.c) inhibited the response to the mirror between 30 min and 100 min posttreatment and the effect was completely antagonized by a concommitant application of the mammalian V1 specific receptor antagonist dPTyr(Me)AVP, 40ug/kgBW. Copulation opportunity following this test did not reveal changes in head grab latency time, indicating undisturbed recognition of females. AVT effects on social proximity to females during five successive days, showed an inhibitory action of AVT to the reinforcing effect of copulatory "award" following visual access. Again this effect could be antagonized by dPTyr(Me)AVP.

A dissociation of peptide treatment from copulation experience for up to 6 hours did not change AVT effects on proximity behavior during the following day. Together with a comparison of training effects after positive reinforcement of social proximity by copulation, the experiments provide evidence for AVT action on motivational rather than mnemonic and aversive components of sexual learning. These effects appear to be mediated by receptors homologue to mammalian vasopressor AVP-V1.

656.11

RESPONDING FOR REWARDING BRAIN STIMULATION FOLLOWING SYSTEMIC INTERLEUKIN-2 TREATMENT. H. Anisman[1], S. Zalcman[2], Z. Merali[2] & L. Kokkinidis[3]* [1]Dept. Psychology, Carleton Univ., Ottawa, Ont. K1S 5B6, [2]Dept Psychology, Univ. of Ottawa, Ottawa, Ont. K1N 9A9, [3]Dept. Psychology, Univ. of Saskatchewan, Saskatoon, Sask. S7N 0W0.

Antigenic challenge influences the turnover and levels of biogenic amines in hypothalamus, as well as in mesolimbic brain regions. In addition to its other functions, the immune system may act in a sensory capacity informing the brain of immunologic challenge, such that immune activation may be interpreted as a stressor. It was suggested that cytokines released from activated immune cells may serve as messengers between the immune and central nervous systems. Indeed, interleukin (IL)-1 and IL-2 both provoke alterations of central neurotransmitters. We report the effects of IL-2 on brain stimulation reward. Using a conditioned discrimination paradigm, rate-current intensity functions were determined for both reward- and nonreward- associated responding for electrical self-stimulation of the medial forebrain bundle at the level of the lateral hypothalamus. Systemic administration of IL-2 (0.5 - 1.0 ug) produced rightward shifts in the rate intensity functions without significantly influencing either maximal rates of operant responding for electrical brain stimulation, or nonreinforced performance levels. Increases in thresholds were evident after both IL-2 doses. It seems that the altered responding for rewarding stimulation could not be ascribed to motoric factors, associative or attentional disturbances, and might reflect anhedonic effects engendered by the cytokine.

656.12

INTERLEUKIN (IL)-1, IL-2 AND IL-6 INDUCE CYTOKINE-SPECIFIC ALTERATIONS OF EXPLORATORY BEHAVIOR. L. Murray, S. Zalcman*, D. M. Nance, D. G. Dyck and A. H. Greenberg. Manitoba Institute of Cell Biology and Dept. of Pathology, University of Manitoba, Winnipeg, Mb. R3E 0V9.

Immunological challenge induces alterations of behavior that are similar to those induced by uncontrollable stressors. Products of an activated immune system, notably IL-1, have also been shown to induce behavioral changes. We previously reported that IL-1ß, IL-2 and IL-6 induced cytokine-specific alterations of central neurotransmitter activity. We presently report that cytokine-specific alterations of exploratory behavior were also evident in male BALB/c mice following peripheral adminstration of these cytokines. Exploration of a novel environment (i.e., locomotion, rearing, digging, grooming) was markedly decreased 15-50 minutes after IL-1ß administration (200 ng, ip). In contrast, IL-2 and IL-6 induced behavioral activating effects. IL-2 (50, 100, 200 or 400 ng, ip) induced dose-dependent increases in time spent in non-ambulatory exploration, number of rears and distance travelled in a novel environment. IL-6 dose-dependently (50, 100, 200 or 400 ng, ip) increased the number of rears, digging episodes and the time spent per episode as well as the number of grooming episodes. Moreover, time-dependent increases in the number of contacts with a novel stimulus and mean-contact time were evident in mice administered IL-2, but not in IL-6-treated animals. Taken together, these data suggest that IL-1, IL-2 and IL-6 induce cytokine-specific behavioral alterations. The data are discussed in terms of the spectrum of behavioral alterations induced by immune-derived products and implications on the characterization of sickness behavior. (Supported by NIMH and MRC of Canada)

656.13

CHANGES IN NPYir AND LHRHir IN COCAINE-, ETHANOL-, OR COCAINE AND ETHANOL-TREATED FEMALE RATS. G.S. Fraley* and C. Ulibarri. Dept of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

Chronic use of cocaine and ethanol (EtOH) has drastic behavioral and physiological effects. Among them are alterations in reproduction and food intake, however, the mechanisms underlying these changes are unknown. Neuropeptide Y (NPY) is a neuromodulator that functions in the central nervous system's control over both food intake and reproduction. It is hypothesized that cocaine and EtOH alter the NPY release to bring about these physiologic changes. To investigate this, female rats were ovariectomized, given steroid replacement therapy, and placed on three binge-type drug treatments of 10 days each to simulate human drug addiction paradigms. Females were randomly assigned to treatment groups of 1) free-fed control (Bio-Serv liquid control diet), 2) EtOH liquid diet (Bio-Serv, EtOH 30% of calories), 3) cocaine (20mg/kg/day), 4) EtOH + cocaine, or 5) nonsteroid lab block fed controls. Rats were weighed and food intake measured daily. All rats were colchicine-treated before being killed by aldehyde perfusion. Brains were collected, sectioned coronally (40 μm) and stained immunocytochemically for NPY or for luteinizing-hormone releasing hormone (LHRH) and c-fos gene product. All drug treatments resulted in significant loss of body weight. However, only EtOH and cocaine treatments significantly reduced food intake. All drug treatments produced a significant increase in NPYir in the arcuate nucleus as compared to controls. Cocaine and cocaine + EtOH treatments produced a significant reduction in LHRHir in the diagonal band. The number of NPYir and LHRHir cells were significantly negatively correlated. Since NPY is a common neuromodulator between food intake and reproductive control, these results suggest NPY may be an important factor in pathologies of human drug addiction. Supported by WSU Program for Research on Alcohol and Drug Abuse to CU and Sigma Xi Grants-in-Aid to GSF.

656.14

ACTH-INDUCED GROOMING IN TWO SUBLINES OF SPRAGUE-DAWLEY RATS WITH HIGH-(HY) AND LOW-YAWNING (LY) SPONTANEOUS FREQUENCY. J.R. Eguibar*, A. Moyaho, H. Herrera, B. Holmgren, and R. Urbá-Holmgren. Centro de Investigaciones en Ciencias Fisiológicas, ICUAP. Apdo. Postal 406. Puebla, Pue. México.

The intracerebroventricular (i.c.v.) injection of ACTH in rodents produces a behavioral syndrome characterized by an increase in the frequency of grooming, yawning, stretching, penile erections and wet-dog shakes. In our laboratory we have two sublines of Sprague-Dawley rats which differ in spontaneous yawning and in their sensitivities to cholinergic- and dopaminergic-induced yawning. Additional observations demonstrated that these animals also differ in the structural organization of grooming sequences. In the present study we analyze the effects that i.c.v. injection of ACTH have on grooming and yawning in these two sublines of rats. The animals were maintained in a controlled dark/light cycle, lights on at 7:00 AM, with free access to water and balanced rodent diet (Purina, Mex), and the experiments were carried out when animals were 3 months old. We implanted stainless steel cannula 23 gauge in the right lateral ventricle. One week after surgery we injected ACTH or saline i.c.v. in a random manner using a Hamilton syringe. The observation, with animals placed in individual cages, lasted 70 min and yawning and grooming rates were monitored according to a continuos sampling method. The i.c.v. injection of 1 μg/μl produced a statistically significant increment in the total time spent in grooming and in the mean duration of grooming bouts in comparison to saline injected rats ($p<0.05$, t test), but not in the number of grooming episodes in both groups of rats. The number of stretches was also significantly increased in both sublines ($p<0.05$, t test). Quite unexpectedly yawning behavior decreased around 50% in both sublines of rats. These results show that at the ACTH dose used grooming behavior prevails in respect to yawning, which might be inhibited by the former, suggesting that different SNC structures mediate these behaviors. Partly supported by Sistema Nacional de Investigadores, México.

656.15

MELATONIN ANXIOLYTIC EFFECTS ARE TIME DEPENDENT. Naranjo-Rodríguez, E., Mendoza, V. and C. Reyes-Vázquez*. Sección de Farmacología, Facultad de Química and Departamento de Fisiología. Facultad de Medicina, UNAM.

Anticonvulsive, sedative and hypnotic effects had been described after melatonin (MEL) systemic administration. Furthermore, biochemical and pharmacological studies had linked the effects of MEL with those produced by anxiolytic drugs such as diazepam and chlorodiazepoxide, suggesting a possible anxiolytic action of MEL. Male Wistar rats were deprived of water for 48 hrs prior to a Vogel's conflict procedure, in which the number of electric shocks delivered trough a drinkometer was computed. The effects of several MEL doses (0.5 to 2.0 mg/kg) and three classical anxiolytic drugs (Diazepam 2 and 5 mg/Kg), Chlorodiazepoxide (5 and 10 mg/Kg) and Buspirone (5 and 10 mg/KG), were tested in this experimental model. Flumazenil, a benzodiazepine receptor antagonist, was administered prior MEL and Diazepam application. Different animal groups were tested at 8:00, 12:00, 16:00 and 20:00 hrs. MEL showed higher anxiolytic effects at 20:00 hrs, and no significant effects at 12:00 hrs. Whilst, Diazepam, Chlorodiazepoxide and Buspirone had similar effects at all the tested hours. The application of Flumazenil reverted Diazepam anxiolysis but had not effect on the anxiolytic actions of MEL. The results showed that MEL exert anxiolytic effects in a time dependent manner with effects not related to benzodiazepine receptors.
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656.16

NITRIC OXIDE MODULATES RETENTION IN THE PORSOLT SWIMMING TEST. D. Jefferys* and J.W. Funder. Baker Medical Research Institute, Melbourne, Victoria, Australia 3181

In the Porsolt swim test intact animals become progressively immobile over a 15 min test period and 24h later are immobile for ~70% of the 5 min retest period. Adrenalectomy (ADRX) blocks retention of the response, with animals remaining immobile for only ~35% of the retest, an effect reversed by Dexamethasone (6 μg) or Ketocyclazocine (1 mg/kg). When the antiglucocorticoid RU38486 is administered to intact rats it is without effect, as is MR2266, a kappa opioid antagonist. In contrast, when RU38486 and MR2266 are simultaneously administered the animals have similar levels of immobility on retest as seen in ADRX. Retention of the immobile response thus can be independently mediated by glucocorticoid or kappa opioid pathways.

In the present studies we show that N-nitro-L-arginine (NAME), a nitric oxide inhibitor dose-dependently blocks retention, with animals remaining immobile for 35-42% on retest at a dose of 50 mg/kg. This effect of NAME is blocked by L-arginine (50 mg/kg), with animals immobile for 77% on retest. NAME is effective given immediately pre-test, but was without effect administered 1h post-test. When ADRX rats were given L-arginine the effect of adrenalectomy was blocked, with animals remaining immobile for 62% on retest. These findings suggest that both glucocorticoid and kappa opioidergic effects on retention are mediated by a final common pathway involving nitric oxide.

656.17

THE CENTRAL ADMINISTRATION OF NITROGLYCERIN INDUCES PENILE ERECTION AND YAWNING IN MALE RATS
M.R. Melis*, R. Stancampiano & A. Argiolas. Bernard B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

The effect of the central administration of nitroglycerin, a potent organic nitrate vasodilator, on penile erection and yawning was studied in male rats. When given intracerebroventricularly (ICV), nitroglycerin (33 - 99 μg) induced penile erection and yawning in a dose-dependent manner. Nitroglycerin induced penile erection and yawning also when injected in the paraventricular nucleus of the hypothalamus, a brain area that plays a key role in the control of these responses induced by agents such as N-methyl-D-aspartate, dopamine agonists and oxytocin. Nitroglycerin-induced penile erection and yawning were prevented by methylene blue (400 μg), $d(CH_2)_5Tyr-(Me)^2-Orn^8$-vasotocin (0.1 μg) but not methemoglobin (100 μg) given ICV. In contrast all these compounds were ineffective when injected in the paraventricular nucleus. Ineffective was also haloperidol (1 mg/kg IP). The results suggest that nitroglycerin induces penile erection and yawning by activating oxytocinergic transmission through the production of NO in the paraventricular nucleus of the hypothalamus.

656.18

AN ENDOGENOUS INCREASE IN CYCLIC GMP IS ASSOCIATED WITH INCREASED EXCITABILITY IN IDENTIFIED NEUROSECRETORY CELLS IN MANDUCA SEXTA. Stephen C. Gammie, John Ewer, and James W. Truman*. Department of Zoology, University of Washington, Seattle WA 98195.

Ecdysis in insects is triggered by the neuropeptide, eclosion hormone (EH). During the course of EH action in the tobacco hornworm, Manduca sexta, a group of neurosecretory cells show a dramatic increase in guanosine 3':5' cyclic monophoshate (cGMP). This increase preceeds the onset of the ecdysis behavior and is sustained at high levels in some of the neurons for over 2 hours. Using intracellular recording techniques we have found that this endogenous increase in cGMP is correlated with a significant lowering of the threshold of these neurons. Also, the somata of these cells show a calcium dominated action potential and this is lengthened during the time of cGMP increase. We found, however, no changes in either input resistance, resting potential, or action potential amplitude to be associated with cGMP expression. Currently, we are injecting cGMP into these neurons prior to their endogenous increase in cGMP to directly test the actions of this cyclic nucleotide on cell excitability. These experiments will be followed by whole cell voltage clamp techniques to determine which ionic currents are affected and how.

656.19

EFFECTS OF PRENATAL STRESS ON DEFENSIVE WITHDRAWAL IN THE RAT. HE Ward, EA Johnson, DL Birkle, MS Cratty, AJ Azzaro*. Depts. of Behavioral Medicine/Psychiatry, Pharmacology/Toxicology, and Neurology, West Virginia University School of Medicine, Morgantown, WV 26506.

Adult, virgin, female Sprague-Dawley rats were bred with adult males. Between gestational day 14 and 21, pregnant females were exposed to the mild stress of daily handling and saline injection (0.1 ml, s.c.). Control (unstressed) dams were not handled except for normal animal care. Behavioral testing in a defensive withdrawal apparatus was performed on male offspring at 60 days of age. There were no significant behavioral differences noted between control and prenatally stressed animals. However, following 2 hr of restraint stress, prenatally stressed offspring had a longer latency to exit from the defensive withdrawal chamber and spent less time in the open field (n=8, $p<0.05$) Specific binding of corticotropin-releasing factor (CRF) was increased by 30% in the amygdala of the prenatally stressed animals (n=8, $p<0.05$). CRF levels and secretion were similarly elevated (Cratty et al, this volume). In the defensive withdrawal paradigm, without an acute stress (restraint), behavioral differences were too subtle to detect. However, it would appear that the animal's ability to adapt to acute stress (restraint) was compromised by prenatal stress and may be a function of prenatal perturbation of developing CRF systems within the amygdala. Supported in part by NIH (2S07RR05433-31 and MH19444) and UHA.

DRUGS OF ABUSE: ALCOHOL AND BENZODIAZEPINES

657.1

ANXIOLYSIS IN MOUSE LINES BRED FOR DIFFERENTIAL SENSITIVITY TO DIAZEPAM: USE OF A CONFLICT PARADIGM. F.E. Lotrich and E.J. Gallaher*. Departments of Medical Psychology and Pharmacology, Oregon Health Sciences University and VA Medical Research Service, Portland, OR 97201.

The Conditioned Suppression of Drinking conflict paradigm (CSD) permits repeated testing of the same animal and provides the ability to measure both anxiolytic and anxiogenic effects. In the current study, CSD was modified for use with mice and then used to delineate some of the effects of diazepam (DZ) in four lines of mice which were selectively bred for DZ sensitivity (generation 10). These lines of mice have been selected for either high performance (DHP-1 and DHP-2) or low performance (DLP-1 and DLP-2) on an accelerating rotarod. Following 40 mg/kg DZ, rotarod performances were: DHP-1 +0.7±0.7 rpm, DHP-2 +2.0±0.9 rpm, DLP-1 -13.7±1.7 rpm, and DLP-2 -10.6±0.9 rpm. With CSD, dose-dependent sedative-like effects on unpunished responding were observed in all four lines (0.3-100 mg/kg; $p<0.0001$); both DLP lines were more sensitive than the DHP lines to these sedative-like effects ($p<0.01$), consistent with the rotarod data. A dose-dependent increase in conflict responding--a measure of anxiolysis--was also found ($p<0.01$). However, no signficant difference was observed between the DLP and DHP lines for these anxiolytic effects of diazepam. DHP mice exhibited anxiolysis at doses below sedative doses while in DLP mice these responses overlap. The results suggest that the effectiveness of BZs as clinical anxiolytics may have a genetic influence. (Supported by VA Medical Research Service and USPHS grant MH10317.)

657.2

COMPARISON OF THE RESPONSE OF 4 HIPPOCAMPAL (H) SITES IN DIAZEPAM (DZ) DEPENDENT RATS TO FLUMAZENIL (F) MICROINJECTIONS. **J.W. Sloan*, E.P. Wala and X. Jing, Dept. of Anesthesiology, College of Medicine, Univ. of Ky., Lexington, Ky., 40536.**

In order to identify sites in the female Sprague-Dawley central nervous system that contribute to the development of DZ dependence, guide cannulae (GC), also serving as recording electrodes, were implanted into the occipital cortex (OCTX), area 2 (mediomedial [MM]) [AP=3.8; RL=2.4; V=8] or OCTX (area 2, lateral [LAT]) [AP=3.8; RL=4.5; V=8]. Electrodes were implanted into the H (AP=3.7; LL=2; V=6 and AP=4.7; RL=2; V=6.2) and frontal CTX (AP=10.7; LL=1; V=10). After recovery, the rats were implanted subcutaneously with 3 silastic capsules filled with 180 mg DZ/cap/week. After the 3rd implant and the attainment of stable plasma levels of DZ and its metabolites, rats were microinjected weekly with F (25 μg in 1 μl of DMSO) or DMSO into the H via GC. EEGs, behavioral (BEH) and precipitated abstinence scores (PAS) were recorded prior to and for 40 min after microinjection. When F was microinjected via GC into area 2, MM of OCTX with chemotrode (CTR) in CA1 of H, V=7.2, (n=5 rats) neither BEH nor PAS were elevated whereas convulsive signs (twitches and jerks), head and body tremors and respiratory rate were increased. When the CTR was lowered into dentate gyrus, V=6.2 (n=4), F produced no significant signs of PA as was the case when F was microinjected via GC in area 2, LAT of OCTX through CTR into H, V=5.8 (n=5), although 1 rat had a clonic convulsion. In contrast, when F was microinjected via same GC through CTR in CA1 of H, V=6.8 (n=8), BEH and PAS were elevated. Convulsive activity was apparent both by observation and EEG in some but not all rats. These data indicate that DZ given chronically produces dependence in some but not all areas of the H and that the CA1 area of the dorsal H is involved. Supported by NIDA Grant DA02195.

657.3

HISTOLOGICAL AND ULTRASTRUCTURAL BRAIN ALTERATIONS OF A MAN CRONICALLY EXPOSED TO HIGH DOSES OF DIAZEPAM. M.C. Márquez-Orozco,* M.E. Pérez-Chavira, M.V. Gazca-Ramírez and A. Márquez-Orozco. Embryology Dept., Medicine School UNAM. México 04510 D.F. and HGZ FPT 2-A, IMSS.

Our purpose was to investigate whether the histological and ultrastructural brain alterations observed in a 55 years-old man, exposed to daily diazepam (DZ) dosis (50/mg) during the last ten years of her life, are similar to those observed in adult mice prenatally treated with DZ. Cerebral and cerebellar cortices and striatum samples were studied with a light and transmission microscopes. The causes of death were not clearly determinated. Pathology autopsy showed generalizated cortical atrophia, without pre-senil or senil dementia and premature aging. Histological and structural sections showed II y III cortical layers disorganized presence of abundant amyloideus bodies, perinuclear vacuolization. Less number of Purkinje cells than in cerebellum of three healty men from the similar age. In all neural cells the heterochomatine was seen in dense clumps scattered in its interior, around the nucleolus and mainly located near the nuclear envelope. The euchromatine, was reduced. Golgi complex, mitochondriae, rough endoplasmic reticulum and synaptic vesicles were disorganized. Electrondense bodys were observed. These alterations are similar those showed in adult mice prenatally exposed to DZ and suggest may be due to chronic exposure to high doses of DZ.

657.4

DIAZEPAM EFFECTS IN THE ULTRASTRUCTURE OF CEREBRAL CORTEX OF FETAL MICE. A. Márquez-Orozco,* M.V. Gazca-Ramírez, R.Andrade-Martínez and M.C. Márquez-Orozco. Embryology Dept., Medicine School, UNAM. México 04510 D.F.

We investigate whether diazepam (DZ) caused ultrastructural alterations in fetal mice cerebral cortex. Three CD-1 strain gestating mice groups were injected sc between the 6th to 17th gestation day, the first group with single daily DZ doses (2.7 mg/kg/bw), the second with 0.9% saline solution (S) and the third was non treated (NT). All were killed with CO_2 atmosphere the 18th day and the fetus removed. The cerebral cortex was fixed and embedded. Fine sections were observed under transmission microscope. In the DZ fetal mice cerebral cortex was evident delay cellular and neuropile differentation. The nuclear density per area and the thickness were greater than in the S and NT fetuses ($p < 0.05$). Heterochromatin was seen in clumps scattered mainly in its interior and near of the nuclear evelope. The rough endoplasmic reticulum showed distended cisternae, the Golgi complex, the mitochondriae and the polyribosomes were more abundant. Such alterations could reveal disruption in cell multiplication and also it could be atributed to DZ inhibition protein synthesis and to the modification of the metabolic pathways mediated by central and peripheral types of benzodiazepine receptors. The results suggest that DZ produce long-lasting ultrastructural alterations in the cerebral cortex.

657.5

SEXUALLY DIMORPHIC ALTERATIONS IN THE BEHAVIOR OF ADULT RAT PROGENY AFTER PRENATAL EXPOSURE TO LORAZEPAM. G. T. Livezey* and C. V. Smith. Dept. Obstetrics & Gynecology, Univ. of Neb. Coll. of Med., Omaha, NE 68198.

This experiment is part 1 of a study of the effects of prenatal exposure to the benzodiazepine (BZ), lorazepam (LZ), on the behavior, EEG and brain-specific receptors of the mature offspring. Twenty adult female Sprague-Dawley rats received 1 mg LZ/ kg S.C. or the equivalent volume of vehicle, once daily, for 28 days prior to breeding, during breeding and throughout their 21-day gestation. The behavior of the offspring of both sexes was evaluated at 3-4 months of age. We used a modified radial arm maze test which incorporates measures from the open field test, hyponeophagia model of anxiety test, Olton's original RAM test and other parameters of our own design. Prenatal exposure to lorazepam produces performance deficits indicating a long-lasting state of hyperarousal or "anxiety" in the male offspring. Conversely, the female offspring prenatally exposed to lorazepam show enhanced performance that may indicate a reduction of "anxiety" relative to controls. Thus, prenatal lorazepam exposure has produced an exaggeration of the normal sex-dependent differences in maze performance and spontaneous behaviors. These results could result from an elevation of sex steroid levels or an alteration of the interaction of sex steroids with the benzodiazepine receptor which enhances the efficacy of circulating steroids.

657.6

LONG-LASTING EFFECTS OF PRENATALLY EXPOSURE TO DIAZEPAM ON SEXUAL BEHAVIOR OF MALE MICE. L.A.I. Hernández-Alvarez,* A. Martínez-Vargas, B. Victoria-Romero, A. Márquez-Orozco and M.C. Márquez-Orozco. Dept. of Embryology. School of Medicine, BUAP and Dept. of Embryology. School of Medicine, UNAM, México 04510 D.F., México.

Effects of prenatal exposure to diazepam (DZ) in adult mice were showed on sexual reproductive behaviors. We assessed the sexual behavior of senile males CD-1 strain mice exposed to DZ during gestation. One group of female mice was s.c. treated with daily DZ doses (2.7 mg/kg/bw) from the 6th to 17th gestation day and a control group received saline sol. On the 27th month of age, the spontaneous male sexual activity to females from the same breeds was tested and videorecorded under red light. Precopulating and copulating activities were evaluated. No difference was found in precopulating behaviors from both groups. During copulating stage, greater sets of mount series with and without penile intromission, as well as falls and interruptions of intravaginal penetration were found in DZ animals. Although ejaculations per animal were unusually in both groups, DZ mice had fewer. Results show a long-lasting effect of prenatal exposure of DZ on sexual behavior.

657.7

BENZODIAZEPINE LIGANDS MODULATE ETHANOL DRINKING IN ALCOHOL-PREFERRING AA RATS. E.R. Korpi*, K. Wegelius, T. Ovaska and A. Honkanen. Biomedical Research Center, Alko Ltd, POB 350, FIN-00101 Helsinki, and Department of Pharmacy, Division of Pharmacology and Toxicology, University of Helsinki, Finland.

The effects of positive allosteric modulators (agonists) at the benzodiazepine site of the $GABA_A$ receptor (midazolam, abecarnil, ZK 91296, bretazenil, and CGS 9895) and those of negative allosteric modulators (inverse agonists, Ro 15-4513 and Ro 19-4603) were studied on voluntary ethanol consumption in selectively-bred alcohol-preferring AA rat line using limited access paradigm. Each drug was tested after IP injections of three different doses using saline injections as a control treatment. The benzodiazepine agonists had only modest effects on ethanol intake, measured 1 and 4 h after the injections, whereas the inverse agonists strongly decreased ethanol consumption. Food consumption during the 4-h session was decreased by all other benzodiazepine agonists except bretazenil, which also significantly increased the ethanol drinking. The inverse agonists had no significant effect on food intake. Picrotoxin-sensitive [^{35}S]TBPS binding was differently modulated by benzodiazepine ligands between the AA rats and alcohol-avoiding ANA rats. [^{3}H]Ro 15-4513 binding to the benzodiazepine sites did not, however, reveal any drastic differences between the lines. In conclusion, the results suggest that the $GABA_A$ receptor may be involved in the regulation of voluntary ethanol drinking in alcohol-preferring AA rats, but do not indicate that these rats would consume ethanol because of its anxiolytic effects in a way that benzodiazepine agonists (full or partial) could substitute for it.

657.8

THE BENZODIAZEPINE (BDZ) PARTIAL AGONIST RO16-6028 (BRETAZENIL) AUGMENTS THE DEPRESSANT EFFECTS OF ETHANOL (EtOH). A.R. Starosta*, G.G. Blakley, H.L. June, and M.J. Lewis. Neurobehavioral Laboratory, Dept. of Psychology, Temple University, Philadelphia, PA 19122 and IUPU Dept. of Psychology, Indianapolis, IN 46202.

RO16-6028 (Bretazenil), a partial BDZ agonist with pronounced clinical anxiolytic properties, has been shown to increase EtOH consumption in EtOH preferring and non-preferring rats and recently in randomly bred rat stocks. The present experiment investigated the effects of RO16-6028 on EtOH-induced changes in open field activity. Male Sprague-Dawley rats were tested for basal locomotor activity for one week in a Coulbourn video activity apparatus at 10 min. intervals for 60 min. Animals were then injected IP with saline and vehicle and either EtOH alone (0.25-1.0 g/kg), RO16-6028 (0.5-2.0 mg/kg) alone or RO16-6028 and EtOH (same respective doses) in combination prior to measurement of horizonal and vertical activity. Both EtOH and RO16-6028 generally decreased activty over the period of testing. RO16-6028 did not effect the early effects of EtOH (0-20 min.), but did increase the depressant effects of EtOH 20-60 min. These data suggest a differential role GABA-BDZ mechanisms in early vs late EtOH effects.

657.9

COMPARISONS OF [^{3}H]Ro15-4513 BINDING IN CEREBELLUM FROM ETHANOL-NAIVE WITHDRAWAL SEIZURE PRONE AND WITHDRAWAL SEIZURE RESISTANT MICE. A. Leslie Morrow*, F. Donelson Smith, and Leslie L. Devaud, UNC Sch. of Medicine, Chapel Hill, NC 27599.

Ethanol withdrawal seizure resistant (WSR) and withdrawal seizure prone (WSP) lines of mice have been selectively bred for investigation of the mechanisms involved in alcohol withdrawal. Ethanol-naive WSP mice are more sensitive than WSR mice to GABAergic chemoconvulsants, suggesting differences in $GABA_A$ receptor function or structure are involved. WSR mice exhibit 50-100% higher levels of $GABA_A$ receptor $\alpha1$, $\alpha6$ and $\beta2$ subunit mRNAs in cerebellum, but not cortex. Ro15-4513 is a $GABA_A$ receptor inverse agonist which labels recognition sites on $\alpha6$ subunits. The present study was undertaken to determine if there are differences in [^{3}H]Ro15-4513 binding characteristics between WSR and WSP mice. Saturation binding analysis was conducted with [^{3}H]Ro15-4513 (0.2-20 nM) using individual cerebella from WSR1, WSR2, WSP1 and WSP2 mice. No differences in binding were observed between the WSR1/2 or the WSP1/2 lines, respectively (n=8). Moreover, no differences in [^{3}H]Ro15-4513 binding were observed in cerebella from ethanol-naive WSR vs. WSP mice. Binding density was 1467± 52 fmol/mg protein in the WSR and 1543 ± 41 fmol/mg protein in WSP cerebella. K_D values were 2.90 ± 0.09 nM and 2.82 ± 0.11 nM for WSR vs. WSP mice, respectively. These data indicate a discrepency between the expression of $GABA_A$ receptor $\alpha6$ subunit mRNAs and [^{3}H]Ro15-4513 recognition sites in these mice. (Supported by AA09013, ES07126 and Pharm. Man. Assoc. Found.).

657.10

DIFFERENTIAL SENSITIVITY OF THE BENZODIAZEPINE INVERSE AGONIST RO19-4603 ON THE LOCOMOTOR DEPRESSANT EFFECTS OF ETHANOL IN ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. H.L. June*, T.L. Greene, M. Lin and J.M. Murphy. *Dept. of Psychology, Inst. of Psyc. Res. and Prg. in Medical Neurobiology, IUPUI, Indianapolis, IN 46202.*

The present study investigated the extent to which possible genetic differences, which are phenotypically expressed as differences in EtOH preference, may influence sensitivity to the BDZ partial inverse agonist, RO19-4603 (R) in modifying low to high doses of EtOH (0.125 -2.5 g/kg). P (N=20) and NP (N=20) rats of the *F2* inbred line were habituated to a photocell apparatus and tested for 10 min sessions during the experimental phase. Animals from each line were randomly assigned to an EtOH only group, or to a R+ EtOH group (n=10/group). Animals in both groups received three daily consecutive EtOH injections. Animals in the R groups, however, were given R (0.15 mg/kg), 5 min prior to the EtOH injection on Day 1 only. EtOH alone caused dose-dependent reduction in motor behaviors on Day 1-3 (50 to 70% of control), with tolerance seen only with the NP rats for the 0.125 g/kg dose on Day 2. R enhanced the suppressant effects of the 0.125 g/kg EtOH dose in the P and NP line on Day 1. R was without effect on Day 1-3 when given before the 1.0 and 1.5 g/kg EtOH dose in the P rats, as well as Day 1 in the NP rats. R antagonized, however, the EtOH suppression on Day 2 and 3 in the NP rats with the 1.0 g/kg dose, as well as Day 1 and 3 with the 1.5 g/kg dose. R was without effect on the 2.5 g/kg dose of EtOH in the P or NP rats or given alone. The results suggest R may induce rapid changes at the $GABA_A$ receptor and mediate the motor-impairing effects of EtOH for 48 hr in the *F2* line NP rat, but not P rat. These effects may be related to the low EtOH preference of the NP rat. *(Supported in part by grants AA08553 and AA07611)*

657.11

THE BENZODIAZEPINE ANTAGONIST CGS-8216 EXERTS PROLONGED AND SELECTIVE ATTENUATION OF ETHANOL INTAKE IN ALCOHOL-PREFERRING (P) RATS. J. Williams, H.L. June, S. Dejaravu, M.J. Lewis*, and J.M. Murphy. *Dept. of Psychology, Inst. of Psychiactric Res. and Program in Medical Neurobiology, Indiana University-Purdue University, Indianapolis, IN 46202 and Dept. of Psychology, Temple Univ., Phila., PA 11513*

The present study investigated dose dependence and time course profiles of CGS 8216 (0.0, 0.5, 1.0, 5.0, 10, or 20 mg/kg), a pyrozoloquinoline BDZ antagonist in attenuating EtOH intake in alcohol-preferring P rats (N =13). Animals were provided a 4 hr daily limited access to a two bottle choice between EtOH (10% v/v) and saccharin (0.0125% g/v) solutions. For the remaining 20 hr, rats were provided unlimited access to water, with food available ad libitum. Acute administration of CGS 8216 (1.0 - 20 mg/kg) dose-dependently reduced EtOH intake to 42% - 79% of controls at the initial 15 min interval of the 4 hr access on Day 1. All doses reduced total consumption for the 4 hr period to 15-60 % of control levels. On Day 2 (24 hr post-drug administration), the 5.0 and 20 mg/kg doses of CGS 8216 reduced EtOH intake to 41% of controls during the initial 15 min interval, while total consumption was reduced to 20-32% of control levels for all doses except the 10 mg/kg dose. Saccharin drinking generally showed no changes at the initial 15 min interval, however, compensatory increases paralleled the decreased EtOH consumption and the total consumption measure for several of the CGS 8216 doses. These results suggest the BDZ component of the $GABA_A$ receptor complex may play a direct role in the reinforcing actions of EtOH. *(Supported in part by grants AA08553 and AA07611).*

657.12

INTERACTIONS OF ETHANOL AND DIAZEPAM ON HIPPOCAMPAL LONG TERM POTENTIATION. J.L. Polan-Curtain*, M.J. Wayner and D.L. Armstrong. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249-0662.

Within a limited range of doses, co-administration of ethanol (EtOH) and diazepam (DZ) produce enhanced anxiolytic effects. These combined effects on long-term potentiation (LTP) in the hippocampus of rats anesthetized with urethane were studied in an attempt to provide an explanation at a more fundamental neuronal level. Male Sprague-Dawley rats received 0.1, 0.5, 0.75 and 1.0 mg/kg DZ i.p. in combination with 0.1, 0.5, 0.75 g and 1.0 g/kg EtOH by gavage, respectively. Drugs were administered 20 min (EtOH) and 15 min (DZ) prior to tetanic stimulation of the medial perforant path which resulted in LTP induction measured in terms of the relative change in slope of the population EPSP as compared to baseline. Effects of both drugs alone and in combination on LTP will be presented. Both drugs depressed LTP induction and significant enhanced interactive effects were observed.

657.13

THE EFFECT OF ANXIOLYTICS ON NOVELTY-INDUCED PLACE PREFERENCE. J.E. Klebaur*, D. Miller, & M.T. Bardo. Department of Psychology, Univ. of KY, Lexington, KY 40506-0044

Previous evidence has shown that rats exposed to one compartment of a place preference apparatus will prefer the novel compartment over the familiar. While this suggests that novelty is rewarding, an alternative interpretation is that rats avoid the familiar compartment because it is associated with stress induced during the inescapable exposure sessions. To test this later possibility, the anxiolytics diazepam (0.1, 0.3, 1.0, 3.0 mg/kg) and gepirone (0.1, 0.3, 1.0 mg/kg) were examined for their ability to alter novelty-induced place preference in rats. As expected, control animals showed a novelty-induced place preference. On test day, this preference was blocked by diazepam, but only at a dose (3 mg/kg) that also decreased locomotor activity. Gepirone failed to alter the preference behavior, even at a dose (1 mg/kg) that decreased locomotor behavior. These experiments indicate that preference for the novel compartment is an indication of the rewarding effects of novelty and not an avoidance of stress associated with the familiar.

658.1

DIFFERENTIAL ANALGESIA IN ALCOHOL-PREFERRING AA RATS AND ALCOHOL-AVOIDING ANA RATS. A. Honkanen[1], T. Ovaska[2], L.M.J. Attila*[1] and E.R. Korpi[2]. [1]Department of Pharmacy, Division of Pharmacology and Toxicology, POB 15, FIN-00014, University of Helsinki, [2]Biomedical Research Center, Alko Ltd. POB 350, FIN-00101 Helsinki, Finland

Brain opioid systems may have a role in the ethanol reward. Our aim was to study possible differences in the sensitivity of the CNS opioid mechanisms of alcohol-preferring (AA) and alcohol-avoiding (ANA) rats by activating the central opioid mechanisms with ethanol, morphine and swimming-induced stress followed by measurement of the analgesia induced by these treatments. Analgesia was measured with the tail-flick (TF) test. The baseline TF-latency was measured 4 times with 5 min intervals before treatments. The mean of the last 3 trials served as baseline (BL). Ethanol (15 %, i.p., 1.0, 1.5 or 2.0 g/kg) or saline was given after the last BL-trial. TF-latency was measured 5, 10, 20 and 30 min after injections. Morphine (MO) was given (s.c) with cumulative dosage (0.5-16 mg/kg) with 20 min intervals. The TF-latency was measured just before each injection. Stress was induced to rats by exposing them to 3-min swimming in 15 °C water. TF-latencies were measured just before and 3, 6 and 9 min after swimming.
TF-latencies increased in the AA rats during 4 BL-trials but not in the ANA rats. No difference was found in the MO or stress induced analgesia between rat lines. The smallest ethanol dose (1.0 g/kg) failed to produce analgesia in either rat line, but 1.5 g/kg of ethanol produced slight analgesia in the AA rats but not in the ANA rats. Both rat lines showed similar analgesia after 2.0 g/kg ethanol. These results suggest that the ANA rats may be more resistant to habituate to repeated nociceptive stimuli than the AA rats. The AA rats appear to be slightly more sensitive to analgesic effect of ethanol than the ANA rats. Whether this effect is due to greater release of endogenous opioids in these rats remains to be studied.

658.2

EFFECTS OF LOW LEVEL HYPERBARIC EXPOSURE ON MORPHINE-INDUCED STIMULATION OF LOCOMOTOR ACTIVITY: DOSE-RESPONSE. R.L. Alkana*, B.L. Jones, M.L. Palomares, D.L. Davies and J. Mørland. Dept. Molec. Pharmacol. & Toxicol., School of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033

Exposure to 12 atmospheres absolute (ATA) helium-oxygen gas mixtures (heliox) antagonizes a broad range of acute and chronic behavioral effects of ethanol. If pressure antagonizes ethanol by blocking or offsetting its initial molecular actions, then the antagonistic effects of pressure should be limited to drugs acting via similar mechanisms. The present study tests this hypothesis by extending preliminary investigations of the effects of hyperbaric exposure versus morphine-induced locomotor activation. Drug-naive C57BL/6J mice were habituated for 60 minutes, injected with saline or morphine (10-32 μmoles/kg) and then exposed to 1 ATA air, 1 ATA heliox or 12 ATA heliox at temperatures which offset helium-induced hypothermia. Morphine induced a dose-dependent stimulation of locomotor activity in air. Exposure to 1 or 12 ATA heliox did not significantly affect morphine induced locomotor stimulation. Helium and pressure did not alter the locomotor activity of saline-injected controls. These results indicate that 12 ATA heliox does not antagonize morphine-induced locomotor activation. This finding supports the hypothesis that low level hyperbaric exposure antagonizes ethanol's behavioral effects in a manner analogous to a specific competitive pharmacodynamic antagonist. Taken with recent findings that exposure to 12 ATA heliox antagonizes the behavioral effects of diazepam, the present results strengthens the evidence linking the allosteric mechanisms mediating diazepam's and ethanol's effects on $GABA_A$ receptor function (Supported by NIAAA, NIH Grants RO1AA03972 and R01AA05234)

658.3

LOW-LEVEL HYPERBARIC ANTAGONISM OF DIAZEPAM'S ANTICONVULSANT PROPERTY IN C57BL/6J MICE. D.L. Davies*, B.L. Jones, M.L. Palomares and R.L. Alkana. Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Low-level hyperbaric exposure antagonizes several ethanol-induced behaviors including its locomotor depressing and anticonvulsant effects. Recent work found that pressure also antagonizes diazepam-induced locomotor depression suggesting that ethanol and diazepam may have a common pressure antagonizable site of action. The present study further explored this hypothesis by testing whether this pressure-related link between ethanol and diazepam extends to diazepam's anticonvulsant property. Drug-naive, male C57BL/6J mice were injected intraperitoneally with vehicle, 4.0, 8.0, or 24.0 mg/kg diazepam followed immediately by an intramuscular injection of 300 mg/kg of INH. The mice were then exposed to either 1 atmosphere absolute (1 ATA) air, 1 ATA helium-oxygen gas mixture (heliox) or 12 ATA heliox at temperatures that offset the hypothermic effects of helium. Diazepam increased the latency to onset of myoclonus in a dose dependent manner. Exposure to 12 ATA heliox antagonized diazepam's anticonvulsant effect at 8.0 and 24.0 mg/kg, but not 4.0 mg/kg. Diazepam also increased the latency to onset of clonus in a dose dependent manner beginning at 8.0 mg/kg. Exposure to 12 ATA heliox antagonized this anticonvulsant effect. These findings extend the acute behavioral effects of diazepam known to be antagonized by hyperbaric exposure and support the hypothesis that ethanol and diazepam share a common pressure antagonizable site of action in the $GABA_A$ receptor complex.(Supported by NIAAA grant AA03972).

658.4

ALCOHOL PREFERENCE IN RATS SELECTIVELY BRED FOR SACCHARIN CONSUMPTION. N.E. Badia-Elder*, S.W. Kiefer[1], and N.K. Dess[2]. Psychology Dept., Kansas State Univ.[1], Manhattan, KS 66506, Psychology Dept., Occidental College[2], Los Angeles, CA 90041.

Selectively bred high saccharin consuming (HIS) and low saccharin consuming (LOS) rats, obtained from The Occidental College in Los Angeles, were tested for taste reactivity to 10% alcohol, sucrose, quinine, and a sucrose/quinine mixture. After taste reactivity testing, a two-bottle consumption test with 10% alcohol and distilled water was given for 14 days. At the end of two-bottle testing, rats were tested a second time for taste reactivity to 10% alcohol. In the initial taste reactivity exposure, HIS and LOS rats did not differ in response to 10% alcohol, sucrose, quinine, or the sucrose/quinine mixture. Likewise, there were no significant differences between HIS and LOS rats in their second taste reactivity to 10% alcohol. However, there was an exposure effect in that all rats significantly increased ingestive responding and decreased aversive responding to 10% alcohol on the second test. HIS and LOS rats did not differ in alcohol consumption as measured with two-bottle testing. According to these results, HIS and LOS rats display similar patterns of alcohol preference as measured by taste reactivity and consumption.
(Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).

658.5

ALCOHOL AVERSION LEARNING IN RATS SELECTIVELY BRED FOR ALCOHOL SENSITIVITY. R.L. Elder*, N. Badia-Elder, S.W. Kiefer, and F.A. Martinson. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-5302.

Previous work reported that Low Alcohol Sensitive (LAS) rats failed to develop normal saccharin aversions, relative to High Alcohol Sensitive (HAS) rats, when alcohol was used as the illness agent. In the present experiment, HAS and LAS rats were compared with control rats in the acquisition of an alcohol aversion using lithium chloride as the illness agent. Rats were trained to avoid a 6% alcohol solution by pairing it with LiCl intubations (3% body weight of a 0.15 M solution). Rats were tested for taste reactivity to 6% alcohol on the day after training. This test was then followed with extinction trials with 6% alcohol in the drinking situation. No significant differences between groups were found in ingestive or aversive taste reactivity. There were also no significant consumption differences found between groups during aversion extinction. These data indicate that HAS and LAS rat lines are capable of developing normal alcohol aversions when the illness agent is LiCl.
(Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).

658.6

NALTREXONE AND TASTE REACTIVITY TO ALCOHOL IN RATS. K.G. Hill*, N.E. Badia-Elder, and S.W. Kiefer. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-5302.

Naltrexone, an opiate antagonist, has been shown to reduce alcohol consumption, particularly in high alcohol consuming rats. In the present study, it was determined whether naltrexone altered the taste reactivity responding of rats when presented with an intraoral infusion of alcohol. Two groups of rats were tested for their taste reactivity response to 10% alcohol 30 min after naltrexone treatment (1 mg/kg ip) and 30 min after saline treatment: High Ingestive Responding (HIR; n=7) rats, which were selectively bred for their taste reactivity to alcohol and which consumed high amounts of 10% alcohol; Sprague-Dawley (n=8) rats that had previous experience with a range of alcohol concentrations. Although naltrexone did not alter alcohol reactivity in the Sprague-Dawley rats, HIR rats did exhibit increased aversive responding and decreased ingestive responding following naltrexone treatment. The results provide preliminary evidence that naltrexone may alter alcohol consumption by altering its palatability.

(Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).

658.7

NALTREXONE ATTENUATES ETHANOL STIMULATED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS. R.R. KOHL and W.J. McBRIDE* Depts. of Psychiatry and Medicine, Medical Neurobiology Graduate Program, Indiana University School of Medicine, Indianapolis IN, 46202-4887.

The effect of naltrexone (NAL) on ethanol stimulated-dopamine (DA) release in the nucleus accumbens was studied in adult, female, Wistar rats using in vivo microdialysis in conjunction with HPLC with electrochemical detection. Rats were implanted with microdialysis probes 36-48 hours prior to the administration of ethanol alone (2.0 g/kg, i.p.) or ethanol following pretreatment with 20 mg/kg i.p. NAL. Experiments were conducted on awake, freely moving animals. The i.p. administration of 2.0 g/kg ethanol increased extracellular DA concentrations to approximately 160% of baseline (N=6) within 60 minutes and levels remained elevated for at least two hours. The i.p. administration of NAL (N=5) 30 minutes before injection of ethanol reduced ($p<0.001$) the ethanol induced increase in extracellular DA concentrations. There were no effects on extracellular levels of dopamine with i.p. injections of saline. In general, the extracellular levels of DA and serotonin metabolites did not change during any of the treatments. The results suggest a role for endogenous opioids in mediating the actions of alcohol on the mesolimbic DA system. (AA 09090, AA 08553)

658.8

NALOXONE FAILS TO BLOCK INHIBITORY EFFECTS OF ETHANOL ON SPONTANEOUS AND AMYGDALA-EVOKED SINGLE UNIT ACTIVITY OF NUCLEUS ACCUMBENS NEURONS. R.-S. Lee*, J.R. Criado, G.I. Berg, and S.J. Henriksen, Alcohol Research Center, Dept. of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037.

Previous studies from our group have shown diverse effects of ethanol on nucleus accumbens (NAcc) physiology. For example, systemic administration of ethanol reduces the firing rate of spontaneous and glutamate-activated NAcc neurons in both acute anesthetized and unrestrained rats. In contrast, ethanol did not affect the recruitment of fimbria-activated NAcc neurons. Others have demonstrated that naloxone, an opioid antagonist, reduces ethanol preference in rats. The functional significance of these inhibitory effects of ethanol on spontaneous and glutamate-activated NAcc neurons is still unknown. In view of the possible role of the NAcc in mediating the reinforcing properties of ethanol, we studied the effects of naloxone (5 mg/kg, i.p.) on ethanol-dependent inhibition of spontaneous NAcc neurons in acute anesthetized and unrestrained rats. Also, to further characterize the effects of ethanol on limbic-NAcc inputs, we studied the sensitivity of amygdala-activated NAcc neurons to intoxicating doses of ethanol. Systemic administration of ethanol (1.2-1.4 g/kg, i.p.) significantly reduced the firing rate of spontaneous NAcc neurons in both electrophysiological preparations. Similarly, ethanol inhibited the occurrence of amygdala-activated single units in NAcc neurons. Naloxone did not reverse these inhibitory effects. These findings suggest that some of the inhibitory effects of ethanol on NAcc physiology are independent of opioid mechanisms (Supported by ARC AA06420 to SJH).

658.9

THE EFFECT OF NALTRINDOLE ON EXPRESSION OF ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE. S. D. Dickinson and C. L. Cunningham*. Dept. of Medical Psychology, Oregon Health Sciences Univ., Portland, OR 97201.

Recent research has suggested a role of the endogenous opioid system in the reinforcing properties of ethanol. Research in our laboratory has shown that the expression of ethanol-induced conditioned place preference in mice can be attenuated by naloxone (1.5 - 10 mg/kg). However, at these doses, naloxone may act as a nonspecific antagonist, occupying both mu and delta opioid receptors. The current study was designed to determine whether naloxone's actions were delta-receptor mediated. Three groups of male DBA/2J mice underwent a Pavlovian conditioning procedure that paired injection of ethanol (2 g/kg, IP) with a distinctive tactile stimulus and injection of saline with a different stimulus. After four pairings of each type, a preference test was conducted in which mice were exposed to both tactile stimuli. Prior to the preference test, mice were injected with either 0, 3, or 10 mg/kg of naltrindole (NTI), a selective delta opioid receptor antagonist. All groups demonstrated a conditioned preference for the tactile stimulus that had been paired with ethanol. Unlike naloxone, NTI had no effect on the expression of the conditioned preference. This indicates that naloxone's ability to interfere with expression of ethanol place preference is not mediated via delta opioid receptors. Supported by an N. L. Tartar Fellowship.

658.10

IBOGAINE ATTENUATES ALCOHOL INTAKE IN ALCOHOL DRINKING RATS. Y.W. Lee, Amir H. Rezvani* and D.H. Overstreet. Skipper Bowles Center for Alcohol Studies, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7175.

Ibogaine, the principal alkaloid of the root bark of *Iboga tabernanthe* grown in West Central Africa, has been claimed to possess anti-addictive properties for cocaine and morphine. This study was designed to determine the effect of Ibogaine on alcohol intake in two strains of alcohol drinking rats. Alcohol preferring (P) and Fawn Hooded (FH) rats were injected IP with either vehicle or one of the doses (10, 30 and 60 mg/kg) of Ibogaine and their alcohol intake was measured 24 hr later. In other experiments the effects of acute and subchronic intragastric as well as acute subcutaneous administrations of Ibogaine in FH rats were determined. Intraperitoneal injections of Ibogaine significantly and dose-dependently attenuated alcohol intake in both strains. However, FH rats were less affected by the drug. Subcutaneous administration of the drug did not exert any effect. Both acute and subchronic intragastric administration of 60 mg/kg Ibogaine significantly reduced alcohol intake without development of tolerance. The fact that Ibogaine reduced alcohol intake when is injected intraperitoneally and intragastrically, but not subcutaneously, suggests the involvement of its metabolite(s) in reducing alcohol intake. Although the true mechanism(s) of action of Ibogaine in reducing voluntarily alcohol intake is not yet clear, the fact that FH rats with genetic serotonin dysfunction were less affected by this drug suggests that, in addition to other neurotransmitters, Ibogaine may interact with serotonergic systems in the brain to reduce alcohol intake.

658.11

EFFECT OF CHRONIC ALCOHOL ADMINISTRATION ON DIURNAL ACTH AND CORTICOSTERONE SECRETION IN INTACT AND ADRENALECTOMIZED RATS WITH CORTICOSTERONE REPLACEMENT. T. Chautard, T. Torda, R. Daoud, J.G.Ondo*, and R. Eskay. NIAAA, NIH, Bethesda, MD 20892.

The hypothalamo-pituitary-adrenal (HPA) axis is characterized by a circadian activity with lowest plasma corticosterone (CS) and ACTH levels near lights on and highest hormone levels near lights off. Intact or adrenalectomized (ADX) male rats implanted with CS pellets (plasma CS levels of 3 to 5 μg/dl) were fed every four hours for up to 10 days via an intragastric cannula with the Leiber-DeCarli liquid control diet (LCD) or ethanol diet (LED). Blood samples were taken via an implanted atrial cannula at the trough and zenith of the diurnal rhythm. In intact alcohol-treated rats, daily plasma CS levels were 2 to 10 fold hgher at the nadir, when compared to control rats, whereas daily plasma CS levels at the zenith were elevated less than 50 percent of the time. In ADX rats with CS replacement, plasma ACTH levels were significantly higher only during the trough in LED-treated rats compared with LCD-treated rats. Furthermore, chronic ethanol treatment decreased thymus and spleen weight in both intact and ADX rats with CS replacement. These results indicate that continuous alcohol exposure (150-300 mg/dl) leads to a persistent activation of the HPA axis, particularly during the nadir of the diurnal rhythm.

658.12

CENTRAL OXYTOCIN MODULATION OF SPONTANEOUS ETHANOL INGESTION IN RATS. R.E. Blackburn*, W.K. Samson, R.J. Fulton, E.M Stricker, and J.G. Verbalis. University of Pittsburgh, Pittsburgh, PA 15261 and University of North Dakota, Grand Forks, ND 58202.

Recent studies from our laboratories have implicated central oxytocin (OT) pathways in the inhibitory control of solute ingestion, whether the solute is taken as food or as a concentrated NaCl solution. These data led us to investigate whether central OT may be similarly involved in the control of ethanol (EtOH) ingestion, since EtOH also is a solute. In initial studies rats were pretreated with central injections of ricin A toxin conjugated to OT (rAOT, 5 μg icv; n=22) to irreversibly destroy brain cells bearing OT receptors, or with control injections of non-conjugated toxin (rA, 5 μg icv; n=8). When the pretreated rats were given access to standard chow, water, and a range of concentrated EtOH solutions (3%-15%), the rAOT-pretreated rats demonstrated 3-5 fold greater daily EtOH intakes compared to the rA controls at all EtOH concentrations tested ($p<0.01$). This marked EtOH preference in the rAOT-pretreated rats produced significantly greater degrees of tolerance, as measured by latencies to righting reflex after 4 g/kg EtOH ip (90±15 vs. 2±1 sec; $p<0.01$). In additional studies, a similarly potentiated EtOH ingestion was achieved when central OT receptors were transiently blocked with a non-peptide OT receptor antagonist (L366,948), which resulted in 5-10 fold greater EtOH intakes than controls at all concentrations tested (3-15%). These data indicate that central OT pathways likely participate in the inhibition of EtOH consumption in rats, and suggest that blockade of central OT receptors may provide a behaviorally relevant model for rapid initiation of spontaneous EtOH consumption in this species.

659.1

DISCRIMINATIVE AND AFFECTIVE STIMULUS PROPERTIES OF THE CALCIUM CHANNEL BLOCKER NIMODIPINE IN RATS.

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The L-type calcium channel blocker nimodipine, a 1,4-dihydropyridine (DHP) derivative, is effective in reducing alcohol intake and preference in animal models of alcoholism. In order to gain more insight in possible mechanisms underlying the alcohol intake-suppressing effects of this drug, the discriminative and affective (rewarding/aversive) stimulus properties of nimodipine were evaluated in Wistar rats. It was found that racemic nimodipine can function as a cue in a standard two-lever food-reinforced drug discrimination (DD) procedure and, in subsequent generalization tests, inverted U-shaped dose-response curves were obtained with nimodipine and nifedipine, another DHP. The nimodipine cue generalized completely to (+)-nimodipine (again, an inverted U-shaped curve was obtained), but only partially to (-)-nimodipine. Although effective as a discriminative stimulus, the quality of this stimulus appears to be dissimilar to that of ethanol as it was found that rats trained to discriminate ethanol from saline in a similar procedure did not generalize to nimodipine. The latter finding was confirmed in a cross-familiarization conditioned taste aversion (CF-CTA) procedure, an alternative method to reveal stimulus similarities between drugs. In addition to discriminative stimulus effects, nimodipine has also affective stimulus properties, as the compound was found to induce conditioned taste aversion (CTA), as well as, conditioned place preference (CPP). Racemic and (-)-nimodipine were active in CTA and CPP, whereas (+)-nimodipine failed to produce either CTA or CPP; suggesting that, in contrast to the DD effects, the affective stimulus effects are mainly mediated by the activity of (-)-nimodipine. Because the alcohol intake- and preference-reducing effects of nimodipine reside mainly in the (-)-enantiomer, the mechanism involved in these effects may be related to the affective stimulus properties of nimodipine.

659.2

THE USE OF A SUCROSE-SUBSTITUTION PROCEDURE TO ESTABLISH ETHANOL AS A POSITIVE REINFORCER IN AN OPERANT RUNWAY.

C.L. Czachowski* and A. Ettenberg. Behavioral Pharmacology Lab., Dept. of Psychology, Univ. of California, Santa Barbara, CA 93106.

The reinforcing properties of self-administered ethanol were examined in nondeprived rats trained to traverse a straight-arm runway for the opportunity to drink an ethanol solution in the goalbox. Animals were tested once each day in the runway where each trial culminated in 6 min of access to a drinking bottle containing 5-10% ethanol. In order to ensure reliable and significant levels of ethanol consumption, we employed a modified version of a sucrose-substitution procedure originally described by Samson (1986) in an operant lever-pressing paradigm. In the present study, subjects initially ran the alley for access to high concentrations of sucrose (20% w/v) without ethanol. Over the course of days/trials the concentration of sucrose was gradually reduced while the concentration of ethanol was increased from 0 to 10%. This procedure successfully resulted in a mean ethanol intake of 0.65 g/kg (S.E.M. $\pm$ 0.14) - a dose that rendered several animals ataxic and had clear behavioral/intoxicant effects. These data suggest that the runway/sucrose-substitution procedure may provide a unique model for investigating the reinforcing properties of high doses of ethanol in nondeprived animals.

659.3

EARLY TASTE AVERSION TRAINING IS EFFECTIVE IN PREVENTING THE ONSET OF ALCOHOL DRINKING IN P AND HAD RAT LINES.

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The objective of this study was to determine the effects of taste aversion training during adolescence on subsequent alcohol intake of the selectively bred alcohol-preferring P and high-alcohol drinking HAD lines of rats. Beginning at 30 days of age, male and female rat pups were fluid deprived 24 hrs before a 30 min access period to a 10% (v/v) ethanol solution. Following ethanol exposure, rats were given an i.p. injection of either saline or 0.15 M LiCl (10 ml/kg). A total of five training sessions were administered every other day with ad lib access to water on intervening training days. Twenty-four hrs after the last training trial, rats were given continuous free-choice between water and 10% ethanol for four weeks with food available ad lib. There were no obvious gender differences or line differences to the effects of taste aversion training. All LiCl-treated subjects avoided the usually preferred ethanol solution for the entire four week test period (intake in week 4 was 0.6 ± 0.1 g/kg/day; N=6), while saline-treated rats steadily increased their alcohol intake to 8.4 ± 0.7 g/kg/day during week 4 ($p<0.01$ vs LiCl-treated). Total volume of fluid intake did not differ between groups (39 ± 4 ml/day for saline-treated and 35 ± 5 ml/day for LiCl-treated group). Approximately 60% of the total fluid intake was 10% ethanol for the saline treated group; for the LiCl-treated rats this value was less than 3%. Rats in the saline and LiCl-treated groups gained weight at equivalent rates. These data suggest that early environmental intervention can prevent the onset of alcohol drinking in the selectively bred alcohol-preferring P and high alcohol-drinking HAD lines of rats. (AA07462, AA08553, AA07611)

659.4

USE OF ETHANOL INHALATION TO STUDY THE EFFECTS OF ETHANOL AND ETHANOL DEPENDENCE ON NEONATAL DEVELOPMENT IN MICE.

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The present study explores the use of ethanol inhalation as a model to study the effects of ethanol, ethanol dependence and withdrawal on development in neonatal mice.In these experiments 1-2 day old swiss webster mice along with their mother were put in an alcohol inhalation chamber and continously exposed to alcohol vapors for 6 or 12 days.Pyrazole was not used. At the end of exposure, when the animals were removed from the inhalation chamber they were observed for ethanol dependence, as manifested by withdrawal symptoms. It was found that:(a)the neonates developed substantial blood alcohol levels (180-300mg/dl); whereas the mothers had little (<5mg/dl) or no detectable blood alcohol concentrations; (b) physical dependence to ethanol was produced in the neonates, as evidenced by typical withdrawal symptoms of tremors and convulsions. The severity of withdrawal was related to the length of exposure; (c) exposure to ethanol vapors did not affect the weight gain or physical appearance of the neonates; (d) there was an ethanol exposure dependent effect on the brain weights; (e) exposure to vapors produced changes in behavior and ethanol sensitivity.These results indicate that the ethanol inhalation technque can be used to investigate the effects of ethanol, ethanol dependence and withdrawal on neonatal development in mice as a model for human fetal exposure during third trimester of pregnancy. This technique offers several advantages over other methods commonly used for neonatal alcohol exposure: (1)The pups do not suffer the stress of maternal separation,and can be exposed right after birth; (2) No surgical procedure is necessary; (3) Blood alcohol levels can be easily manipulated by altering the vapor concentration of alcohol; and (4) exposure to vapors does not effect the weight gain of the experimental animals. (Supported in part by NIAAA,NIH Research grants AA03972 and AA05234).

659.5

CO-MORBID DEPRESSION, DRUG DEPENDENCE, AND ALCOHOLISM

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We examined the relationship between a lifetime diagnosis of major depression in 6355 patients with drug dependency and/or alcoholism. Evaluations were performed in a personal interview on admission (383 questions) and follow-up data was gathered by a structured telephone interview (110 questions) at 6 and 12 months. The most common diagnosis of a substance use disorder was alcohol (51.3%), followed by cocaine(19.7%) and then marijuana dependence (12.3%). The rate of lifetime diagnosis of major depression was 43.7% and subclinical depression was 9.6%. Over half of the patients had two or more symptoms of depression and 35.9% had five or more symptoms of major depression. The rates of depression were significantly greater for females than males, for alcoholism than other drug dependencies. A lifetime diagnosis of major depression was significantly associated with poly-drug dependence.

NUMBER & FREQUENCY OF DRUG USE BY DEPRESSION DIAGNOSIS

	DAILY		WEEKLY		RECENT(WEEK)	
Number of Drugs	MALE	FEM.	MALE	FEM.	MALE	FEM.
0	21.7	31.6	20.7	30.8	22.3	36.8
1	27.8	38.7	29.3	39.8	23.1	33.9
2	39.0	58.3	38.2	47.7	27.9	36.9
3	55.9	52.6	56.2	58.0	37.0	47.1

$p > .00001$

659.6

NEURAL EXPRESSION OF C-FOS PROTEIN FOLLOWING AGGRESSIVE BEHAVIOR OR ETHANOL ADMINISTRATION IN MICE.

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We employed immunocytochemistry to detect the presence of c-fos protein in the brains of male wild mice one hour following i.p. treatment (20ml/kg) with 1)0.33 g/kg ethanol (EtOH) 2)1.67 g/kg EtOH 3).9% saline or 4).9% saline and an agonistic encounter with an unfamiliar conspecific male in a neutral arena 10 min. following injection. Mice subjected to an aggressive interaction or treated with 1.67 g/kg EtOH exhibited elevated c-fos expression in the accessory olfactory areas, corticomedial amygdala, medial preoptic area and entorhinal cortex relative to mice treated with 0.33 g/kg EtOH or saline. C-fos was also expressed in the piriform cortex of mice that fought and in the hippocampus of mice given the higher dose of EtOH. These results demonstrate c-fos activation in olfactory and limbic structures that have been implicated in the mediation of aggressive and sexual behaviors in other mammals. They also suggest that EtOH may affect mouse social behavior via activation of gene expression in the olfactory-limbic pathway.

659.7

TIME-DEPENDENT, QUANTIFIABLE WITHDRAWAL FROM ETHANOL IN THE RAT: EFFECT OF METHOD OF DEPENDENCE INDUCTION. D.Macey, G.Schulteis, S.C Heinrichs, and G. F. Koob*, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The importance of temporal factors in the establishment and maintenance of ethanol withdrawal measures in the rat was quantified using a tremor meter, observational rating scale, and acoustic startle test. Ethanol dependence was invoked in naive male Wistar rats either by ethanol liquid diet administration (n=24) or vapor chamber exposure (n=16) for 14-17 days prior to withdrawal in both cases. The rats maintained in the ethanol vapor chambers were divided into 3 groups with target BALs of 120-140, 170-190, and 220-240mg% to determine the effects of BAL concentration on withdrawal measures. Both test groups had appropriate controls (n=8). Repeated-measures trials were conducted at 2, 4, 8, 12, 24, 48, and 72hr postwithdrawal. The tremorogenic activity induced by ethanol withdrawal was quantified using a Plexiglas chamber possessing a piezo-electric film installed in the cage floor. Distortions of the cage floor produced a piezoelectric response which was amplified, filtered, and stored in an IBM computer. Following each tremor trial, subjects were rated for withdrawal signs: ventromedial distal flexion reflex, irritability, tail stiffness, and abnormal body posture/ splayed gait. For acoustic startle, movements were detected by a piezoelectric accelerometer beneath the Plexiglas chamber, digitized and recorded on an IBM computer. Mixed trials with sound pulses of 105 and 120dB were included in each test session. Data analysis revealed that most withdrawal signs reached peak intensities between 8 and 12hr postwithdrawal. Most quantified signs of withdrawal were greater in the vapor chamber subjects than in the liquid diet subjects, probably reflecting higher chronic BAL's in the former case. Results suggest that both liquid diet administration and vapor chamber exposure produce quantifiable, time-dependent measures of ethanol withdrawal.

659.8

REGIONAL CEREBRAL METABOLISM AFTER ETHANOL AS A FUNCTION OF PREFERENCE FOR THE DRUG. J. T. Metz*, H. de Wit, J. Singh, D. Dooley, J. Roemer, M. Jiang, T. Brown, J.-S. Chou, C.-T. Chen, M. Cooper. The University of Chicago, Chicago, IL 60637.

We have continued our studies of the relationship between regional cerebral metabolism of glucose (rCMglu) and subjective responses to drugs of abuse. In order to focus on individual differences in behavioral effects, we have applied PET volumetric and parametric comparison procedures which enable us better to characterize metabolic responses to the drug in different groups of subjects.

Subjects were 22 normal, healthy males aged 21 to 31. Subjects took part in two PET sessions in which they received 0.5 g/kg ethanol and placebo in a double-blind counter-balanced procedure. PET data were collected on a PETT VI using FDG as the metabolic tracer. In each session subjects also completed mood and drug liking questionnaires. On the basis of their self-reported preference for ethanol over placebo, subjects were categorized into two equal groups: high liking (HL, mean difference of liking for ethanol compared to placebo + 44.9, S.D. = 14.8) and low liking (LL, mean difference + 5.4, S.D. = 12.9). PET data sets for each subject were correlated with anatomical MRIs, transformed to standard volumes based on the Talairach atlas, interpolated, and averaged across subjects for HL and LL. For each group the ethanol condition was compared to the placebo condition by repeated measures voxel to voxel t-tests.

Both groups of subjects showed reductions in rCMglu in cerebellar regions. For the HL group these reductions were on the left side only, for the LL group, they were bilateral. Only the LL group, however, showed reduced rCMglu in occipital cortex and only the HL group showed increased rCMglu in left temporal areas. Compared to the LL group, the HL group was significantly lower on behavioral measures of anxiety and depression and significantly higher on friendliness and elation. Thus, we have demonstrated that subjective effects of ethanol are related to differences in rCMglu.

659.9

EXTRACELLULAR CONCENTRATIONS OF ETHANOL IN THE BRAIN FOLLOWING IP, IG OR CONSUMED ETHANOL IN RATS. K. Kiianmaa*, M. Nurmi[1] and J.D. Sinclair. Biomedical Research Center, Alko Ltd., and [1]Department of Zoology, University of Helsinki, Helsinki, Finland.

The distribution of ethanol in the brain was examined by studying the extracellular levels of ethanol in the nucleus accumbens of the alcohol preferring AA (Alko Alcohol) and alcohol avoiding ANA (Alko Nonalcohol) rats with *in vivo* microdialysis. Samples for the assay of ethanol with head-space GC were collected from freely moving animals at 1 min intervals after administration of ethanol (1 g/kg) IP or IG, as well as during voluntary drinking of 10 % (v/v) ethanol solution promoted by limiting the access to ethanol solution to 1 h daily. Tail blood was drawn every 5 min. The results show that there is a steep rise in brain ethanol concentration within minutes after IP injection, but there is no obvious difference between the two rat lines in the distribution of ethanol into the brain. Tail blood ethanol curves from the same animals peaked much later. With IG administration, however, the time courses were similar for brain and tail-blood ethanol. In the drinking study ethanol was found in the brain only a few minutes after the rats started to consume ethanol. The results so far give no indication that the differential ethanol consumption by AA and ANA rats could be explained in terms of differences in the distribution of ethanol to the nucleus accumbens. Nevertheless, the observed level of brain ethanol after voluntary drinking is sufficiently large and rises rapidly enough to provide positive reinforcement. Furthermore, the study shows that microdialysis coupled with head space GC gives detailed information of the brain ethanol curve in individual, freely moving animals after relatively small doses of ethanol.

659.10

CHRONIC INTRACEREBROVENTRICULAR ETHANOL ADMINISTRATION IMPAIRS NONSPATIAL MEMORY IN RATS. **C.L. Weaver, L.M. Copley, R.Z. Couturier, and G.L. Dunbar*. Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI 48858.**

Chronic IP injections of ethanol (EtOH) reduces behavioral flexibility in spatial learning tasks (Devenport et al., Behav. Neurosci., 103:1259-1266). We tested the effects of chronic ICV EtOH administration on spatial and nonspatial, working and reference memories on an eight-arm radial water maze (RAWM) task. Male rats were given chronic (28 days) ICV EtOH (20% in saline) or saline via mini-osmotic pumps. Both EtOH- or saline-treated rats could solve the spatial version of the task at unoperated control levels. Rats receiving the EtOH made significantly more reference memory (entries into channels that never had escape platforms) and working memory 'incorrect'(re-entries into channels previously visited within a trial that never had escape platforms) errors than both saline and unoperated controls during nonspatial testing. Our results indicate that chronic ICV EtOH can disrupt some aspects of both reference and working nonspatial memory. Supported by NSF Grant DUE 9352355 and Research Excellence Funds.

659.11

LATENCY OF CONDITIONING TRIAL AND EXTENT OF CHRONIC CONDITIONING EFFECT EtOH-INDUCED CONDITIONED PLACE PREFERENCE AND AVERSION. G.G. Blakley*, M.J. Lewis. Neurobehavioral Laboratory, Dept. of Psych., Temple U., Phila. PA 19122

The present experiment was designed to examine the effect of latency of conditioning trials and the extent of chronic conditioning upon a EtOH-induced place preference and aversion. Previous place preference studies suggest that ethanol may gain its reinforcing properties only after prior exposure to the drug. Moreover, research from our lab has suggests that the latency of the conditioning trial to the time of administration is an important factor in EtOH's rewarding properties. Male albino rats were subjected to pairings of a distinctive floor stimulus (CS+) and ip injections of varying doses of ethanol (0.25g/kg-1.0g/kg). Subjects received one trial per day for four consecutive days and were given a free choice test on the fifth day. Each subject received three consecutive recurrences of this venue. Conditioning sessions lasted 15 minutes and began at latencies of 0, 15 or 30 minutes post-injection. Significant interactions between dose, extent of conditioning, and latency with preference and aversion were observed. These data suggest that EtOH has both rewarding and aversive properties depending upon these experimental variables.

659.12

ACQUISITION OF A SPATIAL NAVIGATION WORKING MEMORY TASK IN THE MORRIS WATER MAZE: EVALUATION OF SPATIAL WORKING MEMORY IN ADULT RATS EXPOSED NEONATALLY TO ALCOHOL. [1]C.R. Goodlett*, [1]Stephanie D. Peterson, and [2]Kate O'Brien, [1]Dept. of Psychology, IUPUI, Indianapolis, IN 46202 & [2]Kalamazoo College.

Binge-like exposure to alcohol during the early postnatal brain growth spurt in rats reduces hippocampal CA1 pyramidal neuron density. Behaviorally, severe deficits in Morris water maze place learning are apparent in neonatally exposed rats tested as juveniles, but by adulthood place learning deficits are greatly attenuated. Because hippocampal damage is also linked to impaired spatial working memory processes, a spatial navigation task placing heavy demands on working memory was developed to assess whether neonatal alcohol induced long-lasting spatial working memory deficits. Sprague-Dawley rat pups were randomly assigned within litter to three treatment groups: an ethanol group was exposed to 4.5 g/kg/day of alcohol on postnatal days (PD) 4 through 9 via artificial rearing methods; a calorically matched gastrostomy control group was artificially reared from PD 4 through 9; a suckle control group reared normally throughout the period by lactating dams. This alcohol treatment (10.2% v/v in two consecutive feedings each day) produces mean peak blood alcohol concentrations of approximately 300 mg/dl. At 60 days of age, the rats were assigned either to the working memory trained group (WM) or to a random placement group. The WM groups were given 12 trials each day to find the hidden platform, with the trials divided into four sets of trial triplets in which the platform remained in the same location for each trial (separated by a 10 sec ITI) of the triplet. After completion of a triplet, the platform was moved to a new, random location, and sets were separated by 5-10 min; training continued for 10 days. Thus, the first trial of a set constituted the "information" trial, and the subsequent two trials constituted the "memory" trials. The random groups were given similar training procedures except the platform location was randomly determined for every trial. Neonatal alcohol exposure had no significant effect on performance of female rats; alcohol-treated male rats also learned the task but were less efficient at finding the platform on the "memory" trials at the terminal stages of training. Supported by Grant #AA09596.

659.13

MITIGATING EFFECTS OF COMBINED PRENATAL AND POSTNATAL EXPOSURE TO ETHANOL ON HIPPOCAMPALLY-MEDIATED MEMORY-BASED LEARNING IN THE INFANT RAT.

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We have previously shown that combined prenatal and postnatal exposure to ethanol (EtOH), compared with either pre- or postnatal exposure, has mitigating effects on deficits found in the partial reinforcement extinction effect, a hippocampally-mediated indicant of learned persistence (Diaz-Granados et al., *Behav. Neurosci.*, 107:1059, 1993). Our laboratory has also reported that infant rats, postnatally exposed to EtOH, show a significant deficit in patterned alternation (PA), a hippocampally-mediated memory-based discrimination task (Greene et al., Behav. Neurosci., 106:51, 1992). In the present experiment, we examined the effect of prenatal, postnatal, and combined exposure to EtOH on PA at a 60-s intertrial interval. Pregnant dams were intubated once per day from G12 to G18 with either a 35% EtOH solution (5g/kg) or an isocaloric dextrin solution. In the postnatal condition, pups were artificially reared from postnatal days 4 to 9. Pups in the EtOH condition received 4 consecutive feedings of an EtOH adulterated diet while controls received an isocaloric maltose-adulterated diet. All pups received unadulterated diet for the remaining 20 daily feedings. On days 17-18, pups were tested on PA. The results were that prenatal exposure to EtOH had no effect on memory-based learning, whereas postnatally exposed pups show a deficit. Subjects exposed to ethanol prenatally and postnatally showed better PA learning than subjects in the postnatal EtOH-only condition. This result, along with our previous work, suggests that prenatal exposure to EtOH has an alleviative effect on deficits in hippocampally-mediated learning resulting from postnatal exposure to EtOH. Supported by NIAAA grant AA07052.

DRUGS OF ABUSE: ALCOHOL III

660.1

INTERACTIONS OF ETHANOL WITH NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES.

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While the correlation between smoking and drinking is very large, little is known about the biological factors which regulate the co-use of nicotine (NIC) and ethanol (EtOH). Previous evidence has suggested that sensitivity to these agents is genetically correlated because EtOH may modulate nicotinic acetylcholine receptor (nAChR) function by stabilizing nAChRs in the nonfunctional, desensitized form. Here we report that ethanol affects neuronal nAChRs in a subtype-selective fashion. Neuronal nAChRs subtypes ($\alpha2\beta2$, $\alpha3\beta2$ and $\alpha4\beta2$) were expressed in *Xenopus* oocytes from cRNA and studied under two-electrode voltage clamp for responsiveness to bath-applied ACh, NIC and EtOH. While EtOH displayed no direct agonist or antagonist effects, it modulated the function of each of these receptor subtypes. At all receptor subtypes, there was a trend for co-application of EtOH to enhance responses to NIC. In oocytes expressing $\alpha4\beta2$ and $\alpha2\beta2$ receptors, application of NIC 2.5 min after the start of a 5 min application of EtOH attenuated responses to ACh applied 5 min following NIC (2.5 min after the end of the EtOH application). This effect was not seen at $\alpha3\beta2$ receptors. Dose-response and time-course analyses are currently being conducted at these as well as at other nAChR subtypes. These results suggests that EtOH may display complex pharmacological properties at specific neuronal nAChR subtypes. *Supported in part by training grant AA-07561. The cDNA clones were kindly provided by Dr. Jim Boulter of the Salk Institute.*

660.2

ETHANOL MODULATION OF RECOMBINANT ACETYLCHOLINE RECEPTOR CHANNELS EXPRESSED IN HEK-293 CELLS.

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The nicotinic acetylcholine receptor (AChR) is a ligand-gated ion channel located in the postsynaptic membranes of nerve, muscle and electric organ. The AChR from electric organ and muscle consist of four distinct subunits assembled into a $\alpha_2\beta\gamma$(or ϵ)δ pentamer. The modulation of acetylcholine (ACh) induced current by ethanol was studied in HEK-293 cells transfected with mouse $\alpha\beta\varepsilon\delta$ AChR subunit cDNAs. 40-48 hr after transfection whole-cell and out-side out patch clamp techniques were used to record ACh induced inward current from cells expressing AChR. ACh alone and in combination with ethanol (EtOH) were applied to the recorded cell and out-side out patch by a theta-tube rapid perfusion system that enabled complete exchange of solutions in 1-5 ms. ACh activated rapidly desensitizing inward currents with an EC_{50} of 20 μM. Co-application of EtOH (10-150 mM) potentiated, in a concentration-dependent manner, currents activated by 5 and 10 μM ACh. The potentiation was between 20-50% of the control response. With higher concentrations of ACh > 25 μM, EtOH seem to induce significant reduction in peak current amplitude. Further studies are being undertaken to elucidate the mechanism of ethanol modulation of AChR channels.

660.3

ACTIONS OF ETHANOL ON SYNAPTIC TRANSMISSION AND MEMBRANE PROPERTIES OF LAYER V CORTICAL NEURONS.

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Ethanol (EtOH) has an extremely complex profile of actions on the CNS, and attention has increasingly focused on factors that create selective vulnerability of specific cell types. The hypothesis tested here was that EtOH effects in the somatosensory cortex may be related to specific neural attributes of membrane function, including electrophysiological signature and morphology. Intracellular recording techniques combined with dye injections were used *in vitro* to investigate the actions of EtOH on synaptic transmission within layer V of the somatosensory cortex. Bath application of EtOH (1-100mM) revealed a large spectrum of effects on membrane parameters and postsynaptic potentials of cortical neurons. In some regular spiking neurons with "bursting" type characteristics, EtOH (10mM) did not change input resistance and rheobase but produced a blockade of accomodation. In this cell type, excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation of white matter or adjacent layers were monophasic and very sensitive to the depressant action of EtOH. On the other hand, in a population of "doublet" cells, the rheobase and input resistance were altered by EtOH (10mM). In response to stimulation of the white matter, these neurons displayed a characteristic biphasic EPSP, with early and late components. The late component was more strongly affected by EtOH. Neurons of the "intrinsic bursting" type also had biphasic EPSPs whose late components where preferentially depressed by EtOH. Finally, in certain "regular spiking" neurons, EtOH altered input resistance, blocked accomodation, decreased duration and amplitude of the slow afterhyperpolarization potential and had robust inhibitory effects on the late component of the EPSP. These results confirm that EtOH can exert variable effects on different neural elements of layer V neocortical circuits. The influence of EtOH on specific, vulnerable neuronal subtypes within the cerebrocortical circuitry may underlie its effect on specific tasks. (Supported by NIDA DA 08405 to FMS and DA 08349 to AEKK).

660.4

ALCOHOL ABUSE AND EXPRESSION OF $GABA_A$ NEUROTRANSMITTER RECEPTOR GENES.

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Neuropathological studies in chronic alcoholism and its related diseases (liver cirrhosis) suggest specific brain regions (eg. the superior frontal cortex) are selectively prone to damage(1). However, the mechanism of this damage and the development of dependence and withdrawal are not well understood. Evidence suggests the GABA-mediated neurotransmitter system is involved in the chronic effects of alcohol. Our studies have shown $GABA_A$ receptor properties (affinity and density) are altered in the SFC of human chronic alcoholics *cf* non-alcoholics(2). Also, the receptor binding pattern in alcoholics with cirrhosis is differ *cf* nom-cirrhotic alcoholics(2). The $GABA_A$ receptor is a heterooligomeric complex composed of 4 or 5 subunits with atleast 16 candidate genes able to code for the subunits; α_{1-6}, β_{1-4}, γ_{1-3}, δ and ρ_{1-2}. Recombinant studies have shown that the pharmacological properties of the receptor is determined by its subunit makeup(3). Localized brain damage and alterations in $GABA_A$ receptor properties in alcoholism are possibly due to variation in the receptor's subunit composition. $GABA_A$ mRNA subunit expression was assessed by S1 nuclease protection analysis in post mortem brain tissue (superior frontal and motor cortices) of non-alcoholics (ethanol consumption < 20 g/day), alcoholics (> 80 g/day), alcoholics with cirrhosis and non-alcoholic cirrhotics with human specific cDNA oligonucleotide probes. Regional-specific subunit expression was found to be altered in the alcoholic subgroups *cf* non-alcoholics.

1 Kril J. J. *et al* (1989) Acta Neuropathol. 79, 200-204.
2 Dodd P. R. *et al* (1992) J. Neurochem. 59, 1506-1515.
3 Pritchett *et al* (1989) Sci. 245, 1389-1391.

660.5

EFFECTS OF CHRONIC ETHANOL EXPOSURE ON $GABA_A$ RECEPTOR β2 and γ2 SUBUNIT mRNA LEVELS IN RAT BRAIN. L. L. Devaud*[1], D.R. Grayson[2] and A. L. Morrow[1], [1]UNC Sch. of Med., Chapel Hill, NC 27599 and [2]Allegheny-Singer Res. Inst., Pittsburgh, PA 15212.

Chronic ethanol ingestion results in alterations in $GABA_A$ receptor-mediated functions. These effects are associated with decreased $GABA_A$ receptor α1, α2, but not α3, subunit mRNA levels in cortex, while α1 mRNAs are reduced and α6 mRNAs are increased in cerebellum. However, maximal [^{3}H]zolpidem binding, which labels α1 subunit-containing type I BZD $GABA_A$ receptors, is increased in cortex and cerebellum following chronic ethanol exposure. Since type I BZD receptors appear to be comprised of α1, β2 and γ2 subunits in rat brain, we investigated whether chronic ethanol exposure alters these subunit mRNA levels. Poly A+ RNA was prepared from pooled tissue (8 rats/grp) in 3 separate experiments. Northern blot analysis showed no alterations in β2 or γ2 subunit mRNA levels in cortex or cerebella of ethanol-dependent vs. pair fed control rats. Competitive, quantitative PCR analysis was conducted using subunit specific primers and internal standards to measure γ2S and γ2L mRNA levels. In cortex, γ2S levels were decreased by 23 ± 4% and γ2L levels increased 14 ± 4% (n=3 exp). These results suggest that chronic ethanol exposure may elicit opposite effects on γ2 splice variant mRNA levels. Further studies with total RNA on individual animals are underway to extend the validity of these results. These data are consistent with our hypothesis that chronic ethanol exposure differentially alters $GABA_A$ receptor subunit mRNA levels which results in alterations in the subunit composition of $GABA_A$ receptors. (Supported by ES07126, AA09013 and Pharm. Man. Assoc. Found.)

660.6

AN *IN SITU* HYBRIDIZATION STUDY ON α_6-SUBUNIT mRNA OF $GABA_A$ RECEPTORS OF PENTOBARBITAL (PB) TOLERANT / DEPENDENT RAT BRAINS T. Ito, T. Suzuki, D.K. Lim and I. K. Ho.* Department of Pharmacol. and Toxicol., Univ. of MS Med. Ctr., Jackson, MS 39216.

Our previous *in situ* hybridization study revealed that contents of α_1-, β_3- or γ_2- receptor subunit mRNA were altered in discrete regions of the brain of PB tolerant and dependent rats. As α_6-subunit mRNA is localized in granular layer of the cerebellum, *in situ* hybridization study using α_6-subunit mRNA as a target was performed to elicit cerebellum specific alterations in $GABA_A$ receptor in mRNA level. In the PB-tolerant group, rats (male SD rats, body weight 220-250g) were made tolerant to PB by continuous i.c.v. infusion of PB (300 mg/10ml/hour) for 6 days using osmotic minipump (Alzet model 2ML1). Control group received vehicle infusion for the same duration. In the PB-dependent group, the PB infusion was discontinued on the seventh day and assessed dependence 24 hours after discontinuance. Acute study was also done (dose: 60 mg of PB/kg). Frozen brain sections mounted on gelatine coated slide was used as samples. The content of α_6-subunit mRNA was increased in the PB tolerant rats (149±7 [$p<0.05$], % of control), whereas no change was observed in PB dependent (120±12) and acutely treated animals (105±1). These results suggest tolerance to PB induced alteration in α_6-subunit mRNA content and this finding might coincide with changes in characteristics of $GABA_A$ receptor bindings in the cerebellum. (Supported by NIDA 4480)

660.7

GABAERGIC NEURONS IN THE EXTENDED AMYGDALA MODULATE ALCOHOL REINFORCEMENT. P. Hyytiä* and G. F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The GABA/benzodiazepine receptor complex has been implicated in various behavioral actions of alcohol, including the reinforcing effects, but the specific brain sites where GABA mediates alcohol reward remain to be characterized. The principal components of the extended amygdala (Heimer and Alheid 1991) contain large numbers of GABAergic neurons and may provide potential sites for the modulation of alcohol reinforcement by GABA. The present experiments assessed the role of two of these components, the central nucleus of the amygdala (CeA) and the shell of the nucleus accumbens (AccSh), in the regulation of alcohol-reinforced responding. Wistar rats were trained to orally self-administer alcohol (10% w/v) during daily 30-min sessions using a saccharin fading technique. Once stable alcohol responding was established, the animals were implanted stereotaxically with bilateral guide cannulae either above the CeA or the AccSh. After recovery, the rats were tested after intracerebral injections of a competitive $GABA_A$ antagonist SR 95531 (0-16 ng). SR 95531 injections into both sites dose-dependently decreased alcohol-reinforced responding without affecting responses for water. Injections into the CeA significantly reduced responding at doses of 2 and 4 ng, whereas larger doses were required in the AccSh. These findings provide evidence that GABAergic neurons in the extended amygdala, particularly in the CeA, may be involved in the mediation of alcohol reinforcement.

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660.8

CHANGES IN GABAERGIC SYNAPSES OF DENTATE GRANULE CELLS DURING CHRONIC ETHANOL TREATMENT. E. Fifková*, H. Eason, K. Bueltmann, P. Schaner. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Ethanol-sensitive LS_{IBG} and ethanol-insensitive SS_{IBG} mice were exposed to ethanol (23.5% ethanol-derived calories) for 4 months. Half of the animals were sacrificed at this time and the other half was withdrawn from the ethanol diet for 1 month. GABA immunoelectron microscopy was applied to study the impact of the treatments on the density and length of synaptic contacts of the dentate granule cell bodies. The majority of synapses on dentate granule cell bodies is GABAergic (87%). Out of these, 57% are symmetrical synapses with polymorph vesicles and 30% are asymmetrical synapses with round vesicles. These proportions are similar in the LS and SS line. The synaptic density was not affected by the ethanol treatment. However the synaptic length of symmetrical synapses in the LS line was reduced during withdrawal by 20%. No changes either in density or length were seen in the synaptic contacts of the SS mice. This result adds to the numerous other reports in the literature showing differences between the LS and SS line in response of their respective GABAergic systems to ethanol. The observed decreased length of GABAergic synapses on the dentate granule cells complements the loss of GABAergic synapses on dentrites. Both changes indicate reduced inhibition during withdrawal. Since a loss of excitatory synapses in the dentate molecular layer preceded the changes in the GABAergic system, the reduced inhibition during withdrawal could indicate adaptation to the ethanol-induced decreased excitability of the region. Such a reduction of GABAergic parameters is in agreement with the impairment of inhibitory synaptic transmission seen in the hippocampus following chronic ethanol exposure (Durand and Carlen, Science, 224:1359, 1984). Supported by grants AA06196 and AA00130.

660.9

INTRA-STRATIAL ADMINISTRATION OF FLUMAZENIL (FL) TO DIAZEPAM (DZ) DEPENDENT RATS. E.P. Wala*, J.W. Sloan, X. Jing, Dept. of Anesthesiology, College of Medicine, Univ. of Ky., Lexington, KY 40536.

Precipitated abstinence (PA) was characterized in DZ dependent rats following microinjections (MI) of FL into the corpus striatum. Rats (n=6) were implanted with a guide cannula, which also served as a recording electrode, in the parietal cortex (AP=8.08, RL=3, V=8). EEGs were recorded from the hippocampus (AP=3.7, LL=2,V=6 and AP=4.7, LL=2, V=6.2), cerebellum (AP=-0.16, LL=3, V=8.4 or AP=-2.6, LL=2, V=5) and frontal cortex (AP=10.7, LL=1,V=10). Stable plasma levels of DZ and its metabolites were maintained by weekly subcutaneous implantations of silastic capsules containing DZ (3 x 180 mg DZ/cap/week). After the third implantation rats were MI at weekly intervals with 1 μl of FL (25 μg in DMSO) or DMSO vehicle into caudate putamen (C-P)(V=6), globus pallidus (G-P)(V=3.8) or either FL (40 mg/kg) or DMSO vehicle was administered systemically as an IV bolus. EEG was recorded and rats were simultaneously observed for signs of PA and behavioral states for 10 min before and 40 min after treatments. EEGs indicated convulsive activity. PA scores (PAS) were significantly different from DMSO when FL was MI into G-P (PAS 5 min) and C-P (PAS avg). Behavioral scores after FL were not different from DMSO. Head and body tremors and clonic convulsions (1 rat) were observed following MI of FL to G-P and C-P. FL tends to evoke more turning, writhing, digging, wet dog shakes and ataxia in G-P and more twitches and jerks, stretching, blinking, chewing and poker tail in C-P. IV injections of FL induced seizures, tachypnea and a variety of signs and symptoms of PA. The data indicate that C-P and G-P make different contributions to the abstinence syndrome which may be related to localization of BZ receptors in corpus striatum. Supported by NIDA Grant DA02195.

660.10

ETHANOL INCREASES TAURINE BUT NOT GLUTAMATE NOR GABA IN THE NUCLEUS ACCUMBENS USING MICRODIALYSIS. A. Dahchour,(1) E. Quertermont, (1) Ph. Durbin, (2) and Ph. De Witte* (1). (1) Lab. Psychobiology. Univ. of Louvain - 1 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium. (2) LIPHA - 115 Av. Lacassagne, 69003 France.

The extracellular concentration of glutamate, taurine and Gaba was simultaneously estimated into the nucleus accumbens following an IP injection of 3g/kg ethanol (15% v/v) using the microdialysis technique in vivo. The level of taurine significantly increased for 40 min. after the acute ethanol injection while the other amino acids remained unchanged. Owing to its action on alcohol intake in rats and humans, Acamprosate was chosen to analyse the alterations affecting amino acids within the nucleus accumbens. The IP injection of 1g/kg Acamprosate produced a dramatic increase of taurine reaching 210% of the initial baseline after 300 min, no change was observed in the level of the other amino acids estimated. In rats pretreated with 400 mg/kg PO Acamprosate for 3 weeks, the acute injection of 3g/kg ethanol induced a higher increase in the extracellular taurine when compared to the effect of ethanol alone.

Our data thus supported the hypothesis of a cross-tolerance between Acamprosate and ethanol on the extracellular taurine as estimated by the microdialysis of the nucleus accumbens.

660.11

CHRONIC SHORT-TERM ETHANOL TREATMENT IMPAIRS LTP AND GABAERGIC INHIBITION IN HIPPOCAMPUS. N. Hori[1], K. Yamamoto[1], T. Kosaka[1] and D.O. Carpenter[2]. [1]Faculty of Dentistry & Medicine, Kyushu Univ, Fukuoka 812, Japan, [2]Wadsworth Labs, NYS Dept. Health, Albany, NY 12201.

Chronic alcoholism results in functional and morphological changes in the mammalian brain. Many animal models of alcoholism have used rats which were given long-term (greater than 7 months) ethanol treatment. However, the time dependence of physiological and morphological changes occurring at the onset of chronic alcohol ingestion dependency have been studied less. We have focused on physiological and morphological changes in rat brains exposed to alcohol for 2 to 4 months. Rats (200 g starting weight) were given alcohol in drinking water (10% ethanol and 5% sucrose) for 2 to 4 months. (Each rat consumed 25 to 30 mL/day and grew to 300 g during this period). Standard methods of making slices and recording field potentials and intracellular activity were used. A monopolar electrode was placed on both the mossy fibers and the Schaffer collaterals for synaptic activation of CA3 and CA1, respectively. Lucifer yellow was injected into neurons during intracellular recordings to study morphology.

The field potentials in CA3 from animals treated with alcohol for 90 days were of greater amplitudes than controls, and on intracellular recording the discharge rate was not as sensitive to bicuculline (10 μM). Iontophoretic application of GABA resulted in no response in CA3 and very little response in CA1 as compared to control rats. LTP induced in CA1 of chronic treated rats was significantly less than LTP in control slices. (Ethanol treated rats 114.42 $\pm$ 8.2% n=4; Normal rats 142.41 $\pm$ 10.7 n=4). These observations were in contrast to the effects of acute alcohol perfusion to the slice, in that 5 mM ethanol enhanced LTP in control rats (163.9 $\pm$ 13.0%) while 25% ethanol depressed LTP. Lucifer yellow injections show that some fine processes of the dendrites disappeared when compared to control rats.

660.12

DECREASE OF PAIRED-PULSE INHIBITION IN CA1 AREA OF HIPPOCAMPAL SLICES OF CHRONIC INTERMITTENT ETHANOL TREATED RATS. M.H. Kang[1], I. Spigelman[2], D.W. Sapp[1], and R.W. Olsen*[1]. [1]Dept. of Pharmacology, UCLA School of Medicine, [2]School of Dentistry, Los Angeles, CA 90024.

Chronic intermittent ethanol(CIE)-treated rats showed severe withdrawal signs including a persistent kindling-like decrease in pentylenetetrazol(PTZ) seizure threshold (Kokka et al., Alcohol Clin. Exp. Res., 525-531, 1993). To understand the cellular mechanism underlying this plasticity, the involvement of $GABA_A$ receptors, the target of ethanol and PTZ pharmacological effects, was studied using biochemical and electrophysiological functional assays. We observed a significant decrease in muscimol(100 μM)-induced $^{36}Cl^-$ efflux in hippocampal slices from CIE rats. Paired-pulse inhibition, which is considered to be predominantly due to $GABA_A$-mediated recurrent inhibition, was significantly(p<.005) decreased in the CA1 area of hippocampal slices from CIE rats measured 2 days post-ethanol. This study suggests that a hypofunction of $GABA_A$ receptors in the hippocampus may play a key role in kindling-like decrease in the seizure threshold in the CIE rat model of alcohol dependence. Supported by AA07680.

660.13

THE EFFECT OF PRENATAL ALCOHOL EXPOSURE ON POSTNATAL NMDAR1 SUBUNIT EXPRESSION *in vivo* AND *in vitro*. M. Maguire, V. Rema, A. Banerjee, V. Anita, L.M. Smith* & F.F. Ebner Inst. for Dev. Neuroscience, Kennedy Center, Vanderbilt University, Nashville, TN 37203

NMDA receptors are important during development and later for adjusting synaptic efficacy. Following the ingestion of 6.5% ethyl alcohol in the diet of pregnant rats throughout gestation (Exp), we analyzed the density and location of NMDA receptors in Exp and Con (no alcohol) cultured cortical neurons and in the brains of rat pups after 1 day to 1 month of alcohol-free conditions. Antibodies to NMDAR1 subunit were used to assay the effect of alcohol on development of this glutamate receptor subtype subunit. Both Exp cultured neurons and brain sections showed reduction in the NMDAR1 immunoreactivity in cortex at postnatal ages up to 3 weeks compared to controls, with a remarkable variation in the intensity of staining from cell to cell. The variability diminishes in cultures after roughly 3 weeks. P-14 is the time of greatest alcohol effect. Exp cultures show intense cell clustering, greater cell survival and some very large neurons. By P-30 the clustered cells show high R1 levels and very short processes. Con cultures at P-30 contain fewer cells with much greater neuropil development. A striking flip-flop effect of alcohol on thalamic cells *in vivo* is present at P-14 when expression of NMDAR1 by sensory relay cells is reduced to near background levels and over-expression by cells in the midline/ intralaminar nuclei is intense. These observations suggest that prenatal alcohol exposure produces subtle effects on postnatal NMDAR1 expression that change as a function of age during a very dynamic phase of cortical development. The effect of these alterations in NMDA receptor expression is now being assayed using functional/behavioral techniques. (supported by HD-1052-14 and NS-13031)

660.14

FACILITATORY EFFECTS OF ALCOHOL UPON DEFENSIVE RAGE BEHAVIOR IN THE CAT ARE BLOCKED BY AN NMDA RECEPTOR ANTAGONIST. K. Schubert*, J. Perez, M.B. Shaikh, L. Pohorecky, D. Benjamin, and A. Siegel. Laboratory of Limbic System and Behavior, Department of Neurosciences, New Jersey Medical School, Graduate School of Biomedical Sciences, UMDNJ, Newark, New Jersey 07103 and Center for Alcohol Studies, Rutgers University, Piscataway, New Jersey 08855.

This study was designed to determine the effects of alcohol upon: (1) defensive rage behavior (DR) elicited from the medial hypothalamus (MH), and (2) MH facilitation of DR elicited from the dorsal midbrain periaqueductal gray (PAG). *Experiment I:* peripheral administration of alcohol (0.02, 0.5 and 1.0 g/kg, I.P.) reduced latency responses in a dose and time dependent manner, the maximal effect occurring at 60 min, postinjection, at the highest dose level. *Experiment II:* peripheral administration of alcohol at the same dose levels markedly enhanced MH-facilitation of DR elicited from the PAG in a dose and time dependent manner. *Experiment III:* microinjections of the NMDA receptor antagonist, AP-7, into PAG-DR sites blocked MH facilitation of DR elicited from the PAG. *Experiment IV:* microinjections of AP-7 into PAG-DR sites blocked the enhancement of alcohol induced MH facilitation of DR elicited from the PAG. These results indicate that: (1) alcohol administration facilitates the occurrence of DR; and (2) the excitatory effects of alcohol upon rage may be manifest through the MH-PAG pathway which involves NMDA receptors.
[Supported by NIH Grant NS 07941-24].

660.15

SENSITIVITY OF MUTANT NMDA RECEPTORS TO INHIBITION BY ETHANOL. Tooraj Mirshahi and John J. Woodward*, Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Ethanol has been shown to inhibit the NMDA subtype of glutamate receptors. Recent cloning of different subunits of the NMDA receptor has made it possible to study their function in expression systems. Three mutant NMDAR1 subunits showing decreased sensitivity to Mg^{++} blockade (N616R and N616Q) or decreased Ca^{++} permeability (F609L) were tested for their sensitivity to ethanol inhibition. The mutant receptors were expressed along with the NMDAR2A subunit in *Xenopus* oocytes. Membrane currents were measured using conventional two-electrode voltage-clamp methods. Cells were clamped at -80mV and currents were recorded in presence of 100μM NMDA and 10μM glycine with varying concentrations of ethanol. Ethanol (25-100 mM) inhibited the NMDA-induced current in all receptor combinations tested. The wild-type receptor (NR1/NR2A) was inhibited by 22, 35 and 47% at 25, 50 and 100 mM ethanol respectively. The ethanol sensitivity of the mutant receptor combinations (N616Q, N616R and F609L) was not significantly different from that of the wild-type receptor. These results suggest that mutations which reduce the Mg^{++} sensitivity of the NMDA receptor do not alter the inhibitory effects of ethanol. Supported by NIAAA AA08089 and NIDA07027.

660.16

EFFECTS OF AN ALCOHOL INJECTION ON AMINO ACIDS AND RELATED COMPOUNDS IN RAT PLASMA, AORTA, HEART, BRONCHI AND PANCREAS. R. Abdel-Nabi, L. Milakofsky, J. M. Hofford, W. H. Vogel, and T. A. Hare*. Thomas Jefferson Univ., Dept. of Pharmacology, Phila., PA 19107 and Penn State Univ., Dept. of Chemistry, Berks Campus, Reading, PA 19610.

An acute injection of ethanol (EtOH) has been shown to affect the levels of plasma amino acids and related compounds in rats (Milakofsky, et. al., *Biochem. Pharmacol.* **35**:3885, 1986). In order to explore if EtOH would also affect these compounds in certain tissues, rats were injected with 2 g/kg of EtOH, sacrificed 30 min later and plasma, aorta, heart, bronchi and pancreas were obtained. Analysis involved HPLC with fluorometric detection. In plasma, ARG (32%), ASP (29%), SER (18%), GLY (20%), ALA (25%) and ß-ALA (26%) were significantly decreased. Ammonia (36%) and TAU (26%) were decreased in the aorta; ASP (50%) and LEU (25%) in the heart; CIT (28%), ASP(49%), TAU (25%), ASN (14%) and ALA (34%) in the pancreas and TAU (31%), ILE (34%), ethanolamine (39%) and HIS (30%) in the bronchi. Increases were found with phosphoserine (44%) in the aorta; ORN (37%), GLU (28%), GABA (41%) and CAR (37%) in the heart; VAL (56%), ILE (73%), LEU (33%) and HIS (30%) in the pancreas. All other compound were unaffected. These results indicate that an acute injection of EtOH can affect certain amino compounds in various tissues of rats. (Supported in part by the Anesthesia Research Foundation, Wilmington, DE).

660.17

ROLE OF PROTEIN KINASE C IN ETHANOL INDUCED INHIBITION OF METABOTROPIC - GLUTAMATE RECEPTOR FUNCTION IN PRIMARY CULTURES OF ASTROCYTES. T.L. Smith* and M.S. Bitrick, Research Service (151), Dept. of Veterans Affairs Med. Ctr.., Tucson, AZ 85723

Chronic ethanol (E) exposure selectively inhibits metabotropic-glutamate receptor-stimulated polyphosphoinositide hydrolysis in astrocytes (Smith, Alcohol, in press 1994). Because this receptor system is highly sensitive to modulation by protein kinase C (PKC), the aim of the present study was to determine whether the inhibiting effect of E could be reversed in the presence of staurosporine or calphostin C. Astrocytes were cultured in DMEM with 5% FCS in 35 mm dishes in the absence or presence of 100 mM E for 4 days. In some studies, control and E treated cells were co-incubated with 10 nM staurosporine for 3 days or with 2 μM calphostin C 10 mins prior to addition of agonists. After a 24 hr. preincubation with [^{3}H]Inositol (1μ Ci/ml), astrocytes were stimulated with 1S, 3R - ACPD and subsequent [^{3}H]InP determined. Chronic E significantly inhibited the InP response to 1S, 3R - ACPD. Moreover, exposure for 10 min to 10nM phorbol ester (PMA) further inhibited the response in E treated cells, indicating that the effects of E and PKC activation were additive. The results suggest that the inhibitory effect of chronic E is not mediated primarily by PKC. (supported by a research grant from the Dept. of Veterans Affairs, Washington, DC).

660.18

MODULATORY EFFECTS OF ACUTE ETHANOL ON METABOTROPIC GLUTAMATE RESPONSES IN CULTURED PURKINJE NEURONS. J.G. Netzeband* and D. L. Gruol. Dept. Neuropharmacology and the Alcohol Research Center, The Scripps Research Institute, La Jolla, CA 92037.

Of the many actions attributed to ethanol in the brain, little attention has been given to possible interactions with metabotropic glutamate responses that may be involved in synaptic transmission and plasticity. To this end, modified organotypic cultures were prepared from embryonic day 20 rat cerebella and extracellular recordings were made from Purkinje neurons at 21-37 days in culture (37 °C). Metabotropic glutamate responses were induced by pressure ejection of (a) 300 μM (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD] or (b) 5 μM quisqualate (50 μM DNQX was used to block the ionotropic componants of quisqualate-mediated responses). Both (1S,3R)-ACPD and quisqualate produced biphasic responses consisting of an initial brief excitatory phase (5-20 s) followed by a prolonged inhibitory phase (10 s to 2.5 min). These agents also induced increases in the appearance of burst-like activity. In the presence of 66 mM ethanol, (1S,3R)-ACPD-mediated responses exhibited a decrease in the magnitude of the excitatory phase and an increase of the total response duration, with no change in the induction of burst activity compared to controls. In comparison, 66 mM ethanol had much different effects on quisqualate-mediated responses. For quisqualate, ethanol decreased the total response duration and the induction of burst activity, but had no effect on the magnitude of excitation. These results indicate that acute ethanol exposure has complex modulatory actions on metabotropic glutamate responses in cultured Purkinje neurons. *Supported by ARC 6420 and AA 0756.*

DRUGS OF ABUSE: ALCOHOL IV

661.1

EMBRYONIC ETHANOL EXPOSURE ALTERS THE DEVELOPMENT OF SEROTONINERGIC NEURONS AND FIBERS IN CHICK SPINAL CORD. B. Mendelson* and A. Driskill. Dept. of Anatomy, University of Arkansas for Med. Sci., Little Rock, AR 72205.

Embryonic exposure to ethanol (ETOH) often produces motor dysfunction consisting of poor balance, altered gait and decreased tendon (stretch) reflexes. To determine if alterations in the development of serotoninergic somata and fibers in the spinal cord contribute to these symptoms, chick embryos were exposed to ETOH. Subsequently, the development of serotoninergic neurons and fibers was studied with immunohistochemical techniques using a polyclonal antiserum to serotonin (5HT). Groups of chick embryos were treated with 10% ETOH in sterile Tyrode's to produce blood ETOH concentrations of either 0.15-0.2% w/v (medium dose) or 0.275-0.325% w/v (high dose). Control embryos were treated with equivalent volumes of sterile Tyrode's. The ETOH administration began on embryonic day 1 (E1) and continued until the animals were sacrificed. Serotonin immunoreactivity (5HT-IR) was examined in the first 3 lumbosacral spinal cord segments in animals sacrificed on E10, E12 and E14. At all stages analyzed, the density of 5HT-IR fibers was less in both ETOH-treated groups than in control embryos. Camera lucida drawings of 5HT-IR neurons were analyzed for number of primary dendrites (those originating directly from the soma) and secondary dendrites (any dendrite originating from a dendrite). At all stages examined, the number of secondary dendrites was significantly less in both ETOH-treated groups as compared to controls. There were significantly fewer primary dendrites in both ETOH-treated groups at E12 and E14 and in the embryos treated with the high ETOH-dose at E10. These ETOH-induced alterations in the serotoninergic system may be involved in the motor dysfunction observed after embryonic ETOH exposure.

(Supported by AA09205).

661.2

IMPACT OF ETHANOL ON DEVELOPMENT OF ORGANOTYPIC CULTURES FROM NEOCORTEX AND SPINAL CORD.

D.L. Davies* and B. Mendelson, Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

Development of the CNS is adversely influenced by exposure to ethanol. As part of an endeavor to assess the effects of ethanol on neural development in the absence of confounding systemic variables, organotypic cultures were histologically evaluated following ethanol exposure. Organotypic cultures were prepared from the neocortex of 4-day-old postnatal rats and the spinal cords of embryonic day 10 chicks. Groups of cultures from both sources were incubated in medium containing 0%, 0.2% or 0.5% (w/v) ethanol; cultures were serially harvested for evaluation after 1, 3, 6 and 9 days of *in vitro* ethanol exposure. At these timepoints, both control and ethanol exposed cultures from neocortex and spinal cord retained features of normative cytoarchitecture. Also, these cultures exhibited a dense feltwork of astrocytes identified by immunolocalization of glial fibrillary acidic protein. Throughout the culture interval, serotoninergic neurons in the spinal cord were immunocytochemically detected in control and ethanol exposed cultures. The somata and dendritic arbors of motoneurons and particular classes of interneurons were labeled by placement of small crystals of DiI at specific locations on the fixed spinal cord. In the neocortex, ethanol exposed cultures exhibited smaller dimensions than control cultures; this was more evident at the high (0.5%) ethanol concentration and may reflect lower cell viability. These studies suggest the utility of organotypic cultures as alternative models for delineation of the direct effects of ethanol on development and cellular interactions in the CNS.

Supported by NIH grants AA07145 (DLD) and AA09205 (BM).

661.3

ALCOHOL SELF-ADMINISTRATION, DOPAMINE AND AGGRESSION IN RATS. A.M.M. van Erp, H.H. Samson* and K.A. Miczek. Department of Psychology, Tufts University, Medford, MA 02155

The objective was to investigate whether ethanol, when self-administered orally, would enhance aggression in residents confronting an intruder. Male Long-Evans rats, housed with a female, attacked a smaller male intruder in their home cage during 5 min tests. Rats which consistently attacked were trained to drink a 10% ethanol solution during 15 min access in their home cage, using a sucrose-fading technique. After ethanol intake stabilized, intruder tests were conducted 2/wk, starting 5 min after the ethanol session. "Control" fights were scheduled before or more than 3 h after the ethanol session (BAC levels 0 mg/kg). Blood samples were taken directly after the fight from the orbital sinus, under isoflurane anesthesia. Under these conditions, rats drank up to 1.0 g/kg, resulting in BAC levels in a range of 10-80 mg/dl, but mostly 20-40 mg/dl. Neither intoxication nor sedation were observed. Aggressive behavior after ethanol self-administration was enhanced in some animals and unchanged in others, similar to previously reported results from tests with experimenter-administered ethanol. The magnitude of the increase in attack and threat responses ranged from 20-50% above control. Individual differences are currently being investigated using microdialysis, to monitor changes in catecholamines in the nucleus accumbens during ethanol drinking and subsequent intruder tests. Preliminary results show that dopamine release is increased during an intruder confrontation; this effect is enhanced and prolonged when the confrontation is preceded by ethanol self-administration. These results suggest that the ethanol-induced increases in aggressive behavior may be modulated by mesocorticolimbic dopamine.

661.4

ETHANOL MODIFIES EXTRACELLULAR LEVELS OF DOPAMINE, SEROTONIN, AND CORTICOTROPIN RELEASING FACTOR IN THE LIMBIC FOREBRAIN: STUDIES IN RATS WITH DIFFERENT HISTORIES OF ETHANOL EXPOSURE. A. Smith*, L.H. Parsons, E. Merlo Pich, P. Hyytiä, G. Schulteis, M.T. Lorang, M.F. Yackey, G.F. Koob, and F. Weiss. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

A series of studies was conducted to further examine the neurobiological basis of alcohol preference and reinforcement. The objective of the first experiment was to determine whether Alcohol Preferring (P), Nonpreferring (NP), and Wistar (W) rats can be distinguished on the basis of differences in basal accumbal dopamine (DA) and serotonin (5-HT) function as measured by the "no-net-flux" quantitative microdialysis method. The results suggest a more similar profile of DA and 5-HT release in NP and W as opposed to P rats. Specifically, P rats showed reduced basal extracellular DA and a tendency toward lowered 5-HT levels compared to NP and W rats. A second experiment, designed to identify neurochemical substrates of ethanol dependence, examined the effects of chronic ethanol and subsequent withdrawal in W rats on the efflux of DA and 5-HT in the nucleus accumbens (NAC), and of corticotropin releasing factor (CRF) in the central nucleus of the amygdala (CNA). Ethanol withdrawal produced significant decreases in accumbal DA and 5-HT efflux below pre-withdrawal baselines and a substantial elevation in CNA dialysate CRF concentrations. These data may implicate extracellular DA and 5-HT deficiencies and enhanced CRF release in withdrawal distress and in the maintenance of ethanol dependence. Finally, the neurochemical basis of ethanol-seeking behavior in dependent subjects was explored by monitoring dialysate DA and 5-HT concentrations in the NAC of W rats operantly self-administering ethanol during withdrawal. Ethanol reinstated DA efflux to pre-withdrawal values within 10 minutes. In contrast, 5-HT efflux remained below pre-withdrawal levels, suggesting that DA may play a more prominent role than 5-HT in the motivational effects of ethanol in dependent rats. Supported by NIAAA AA 08164, AA 06420 and NIDA DA 08426 (F.W. and G.F.K.).

661.5

NO REDUCTION OF SPONTANEOUSLY ACTIVE MESOLIMBIC DOPAMINERGIC NEURONS IN ETHANOL-WITHDRAWN RATS.

Marco Diana, Marco Pistis, Annalisa Muntoni & Gianluigi Gessa. "B.B. Brodie" Dept. of Neuroscience, Univ. of Cagliari, Italy.*

It has recently been shown that mesolimbic dopaminergic (DA) neurons are drastically reduced in their spontaneous electrophysiological activity during ethanol withdrawal syndrome. However, the reduction in firing rate, burst firing and spikes/burst is not accompanied by a reduction in the number of spontaneously active DA neurons as evidenced by a cells/track index unchanged between ethanol-withdrawn and saline-treated control rats (Diana et al 1993, PNAS 90, 7966-7969). In spite of this result, a recent report (Shen & Chiodo 1993, Brain Res. 622, 289-293) contends that there is a reduction in the number of spontaneously active dopaminergic neurons recorded from ethanol-withdrawn in chloral hydrate anesthetized rats. Since there is a possibility that the use of anesthetics may be a possible confounding factor, we repeated the experiments in unanesthetized and chloral hydrate anesthetized rats during ethanol withdrawal syndrome. While in unanesthetized rats the cells/track index was found again unchanged (as compared to saline-treated control rats), in chloral hydrate anestetized rats we found a profound reduction (as compared to saline-treated control rats) of the same index (<62.5%), thereby confirming Shen & Chiodo's report. However, apomorphine administration did not reverse the reduced cells/track index and did not increase firing rate of "silent" DA neurons identified antidromically from the nucleus accumbens. In conclusion, the present results suggest that there is indeed a reduction in the number of spontaneously active mesolimbic DA neurons recorded from chloral hydrate anesthetized rats but 1) this is confined to the anesthetized preparation and 2) this finding does not support the presence of depolarization inactivation of mesolimbic DA neurons in ethanol withdrawn rats.

661.6

DEFICIENCIES IN DOPAMINE AND SEROTONIN CNS SYSTEMS ASSOCIATED WITH HIGH ALCOHOL PREFERENCE. W.J. McBride, J.K. Chamberlain*, E. Chernet, B. Bodart, L. Lumeng and T.-K. Li. Depts. Psych. & Med., Indiana Univ. Sch. Med. and VA Med. Ctr., Indianapolis, IN 46202-4887.

The hypothesis that high alcohol preference is associated with abnormalities in certain dopamine (DA) and serotonin (5-HT) CNS systems was investigated. In one study, the contents of DA and 5-HT were determined in 4 CNS regions of adult male rats in the F_2 generation obtained by intercrossing the selectively bred alcohol-preferring P and alcohol non-preferring NP lines. Rats with the highest (N=11) and lowest (N=15) alcohol intakes (6.3±0.3 vs 0.4±0.6 g/kg/day; $p<0.001$) were used for HPLC analysis. Lower contents of DA (46±2 vs 61±3 pmol/mg tissue; $p<0.001$) and 5-HT (6.3±0.3 vs 7.0±0.2 pmol/mg tissue; $p<0.08$) were observed in the nucleus accumbens (ACB) of the high compared with the low drinkers. These findings agree with differences previously obtained between the parent P and NP rats. In a second study, the densities of 5-HT_{1A} and 5-HT_2 receptors were determined in alcohol-naive selectively bred high-alcohol (HAD) and low-alcohol (LAD) drinking lines of rats (N=6/line). In contrast to findings with the P and NP lines, no differences were observed in the densities of 5-HT_{1A} sites in several cerebral cortical areas between HAD and LAD rats. In agreement with differences found between the P and NP rats, the densities of 5-HT_2 sites were lower in layer 4 of the medial prefrontal and frontal cortices and in the lateral (core) ACB of the HAD than LAD group. Overall, the results support the hypothesis that deficiencies in certain DA and 5-HT CNS systems are associated with high alcohol drinking behavior. (AA08553, AA07462, AA07611).

661.7

INVOLVEMENT OF THE DOPAMINE AXIS IN A VERVET MONKEY MODEL OF ALCOHOL ABUSE. **R.M.Palmour, D. Mash#, F.R. Ervin and S.N. Young*, Dept Psychiatry, McGill Univ. Sch. Med., Montreal Qué CANADA H3A 1A1 & #Dept Neurology, U Miami Sch. Med., Miami FLA**

Approximately 15% of vervet monkeys voluntarily consume large quantities (>5 g/kg/day) of unsweetened beverage alcohol (Ervin et al., 1990). Several convergent lines of evidence suggest that dopaminergic neurotransmission may differ between Alc preferring (AP) and avoiding (AA) animals. As noted previously (Mash et al., 1993), the density of DA transporters is high in abstinent AP monkeys and reduced in AP animals exposed to chronic Alc. Second, baseline CSF HVA concentrations, determined prior to initial alcohol screening, were lower in AP monkeys (251 ng/ml vs. 202 ng/ml), while other CSF amines and metabolites did not differ between groups. Paradoxically, alcohol consumption was reduced 32% by administration of an amino acid drink devoid of phenylalanine and tyrosine (n = 18). As compared to placebo, this manipulation lowered CSF HVA levels by 40% and 27% at 5 hr and 16 hr after amino acid administration, while CSF MHPG levels fell to 74% and 81% of control values. Reduced consumption of Alc persisted for up to 72 hr.

We also found that mazindol (a non-selective antagonist of catecholamine transport) and GBR12909 (a selective DAT inhibitor) reduced alcohol consumption by 20 to 42% when given repeatedly for10 days to monkeys drinking in a scheduled access paradigm. These compounds had similar and dose-dependent effects upon alcohol consumption, but Maz significantly reduced water consumption as well. The aggregate data are consistent with an extensive literature implicating the dopamine reward system in ingestive and addictive behaviors, and suggest that further evaluation of DA receptors, as well as molecular and biochemical markers of DA neurotransmission is warranted in AP vervet monkeys.

661.8

PHARMACOLOGICAL MANIPULATION OF ALCOHOL CONSUMPTION IN VERVET MONKEYS. **F.R. Ervin*, R.M. Palmour, M.H. Dongier, Dept Psychiatry, McGill Univ. Sch. Med., Montreal Qué CANADA H3A 1A1**

Approximately 15% of vervet monkeys spontaneously consume large quantities (>5 g/kg/day) of unsweetened beverage alcohol (Alc) in the absence of any biological or behavioral coercion (Ervin et al., 1990). Alc consumption during 2 or 4 hour scheduled access is highly correlated with 24hr Alc consumption (r = 0.712 and 0.633, p<.001), and mean Alc: water preference ratios increase as the duration of availability decreases (24 hr = 1.14, 4 hr = 3.08, 2 hr = 6.70). The data suggest that these animals are drinking to maintain a pharmacological effect, rather than for taste preference.

We have begun to survey the efficacy of pharmacological interventions acting, respectively, on endogenous opiate, serotonin and dopamine systems as possible modulators of Alc selection. Opioids: Naltrexone (5 or 10 mg/day), administered daily for 10 days to monkeys in a chronic scheduled access paradigm, moderately reduced Alc consumption (20-38%) and the Alc:water preference ratio, as compared to placebo. SCH 23354 (an inhibitor of neutral endopeptidases) at 30 mg/day had a similar effect. Serotonin: Buspirone (2 mg), given daily for 5 days to 10 animals under scheduled access, produced a moderate increase in mean ethanol consumption (31%), a signifi cant reduction in the volume of water consumed during the Alc access period (-42%) and a highly significant increase in Alc:Water ratio (2.56-fold over placebo). Dopamine: Long-acting bromocriptine (25 mg depot) transiently decreased Alc consumption (12-20%) in monkeys drinking <5 g/kg/day, but increased consumption (32-36%) in monkeys drinking >5 g/kg/day. D1 antagonists, such as SCH23390, abruptly (within 1 hr) and sharply (32-76%) decreased Alc intake, but increasing doses were required to maintain the effect. These effects, though pharmacologically quite modest, are within the range expected to be useful for treating alcohol abuse and dependence.

661.9

ALTERED DENSITIES OF DOPAMINE SYNAPTIC MARKERS IN ALCOHOL-PREFERRING VERVET MONKEYS. D. C. Mash, J. Staley, M. Basile*, F. M. Doepel, J. Wagner, F. R. Ervin, and R. P. Palmour, Depts. of Neurology, Pharmacology & Pathology, Univ. of Miami School of Medicine, Miami, FL., 33101 and Dept. Psychiatry and Centre for Human Genetics, McGill University, Montreal H3A1A1, Canada.

The binding characteristics of dopamine synaptic markers were studied in four different treatment groups: alcohol-avoiding, "predrinking" alcohol-preferring, short-term alcohol induction, and long-term alcohol exposure. We have used radioligand binding and autoradiography to quantify and map the status of D1 dopaminergic receptors in alcohol-preferring vervet monkeys. Our analysis demonstrates that the number of D1 receptors labeled with $[^3H]$SCH 23390 are elevated over limbic sectors of the basal forebrain and striatum. Saturation binding analysis demonstrates that elevated number of binding sites seen in autoradiographic studies reflects an increase in the density (Bmax) with no change in the affinity (KD values) of the radioligand. Analysis of the autoradiograms taken from alcohol-preferring monkeys allowed unlimited access to ethanol for 5 or 35 days revealed comparable densities to the "pre-drinking" region-of-interest measurements. These data suggest that D1 receptors do not show tolerance to the effects of ethanol in alcohol-preferring monkeys given voluntary access. In alcohol-preferring vervet monkeys, the D1 receptor antagonist SCH 23390 sharply reduced alcohol consumption. It has been suggested that expression of D1 receptor-mediated behaviors are important for the rewarding effects of drugs of abuse. We have demonstrated a marked elevation in dopamine transporter (DAT) densities with no change in the number of vesicular transporters in alcohol-preferring vervet monkeys. Voluntary consumption of ethanol resulted in a return to "normalized" densities of DAT. The elevated expression of D1 receptor number may reflect a compensatory response to lower dopaminergic tone in alcohol-preferring vervet monkeys. (Alcoholic Beverage Medical Research Foundation & AA09562).

661.10

MEDIAL FRONTAL SEROTONERGIC SYSTEMS CORRELATE WITH VOLUNTARY ALCOHOL CONSUMPTION IN RATS A.W.Deckel*, E. Vavrousek-Jakuba, W.J. Shoemaker, Dept. of Psychiatry, Alcohol Research Center, Univ. of Conn. Sch. of Med., Farmington, CT 06030.

This experiment examines the relationship between dopamine (DA), norepinephrine (NE) and serotonin (5-HT) and their metabolites to voluntary alcohol consumption. Seven Wistar male rats were anesthetized, pretreated with desimipramine and pargylline and then infused bilaterally with 6-OHDA into the frontal region. Thirteen rats had vehicle infusions (sham lesions) and 3 were untreated. All rats were trained to drink ethanol-containing solutions in a modified sucrose fading paradigm. Four weeks after initiation of the sucrose fading protocol, rats drank a solution containing 10% ETOH/5% sucrose for 2 weeks (wk 1 and wk 2), followed by a 10% ETOH/ 3% sucrose solution for two additional days. For each time period, the g ETOH/kg body weight/day was computed. Following sacrifice, monoamine levels in 5 brain regions were analyzed by HPLC/ED. Lesioned animals had significant depletions in DA, HVA and NE, but showed no changes in their 5-HT or HIAA levels, in the FCx. Lesioned animals did not consume significantly different levels of ETOH compared to controls. Regardless of lesion condition, however, animals with low levels of FCx HIAA, as determined by median split, consumed more ETOH than those with high levels of HIAA.

	10% ETOH 5% sucrose (wk 1)	10% ETOH 5% sucrose (wk 2)	10% ETOH 3% sucrose
Low HIAA	*1.53/.35***	*1.51/.37***	*1.01/.34 *
High HIAA	*1.02/.34*	*1.00/.25*	*.62/.19*

(mean intake in g ETOH/kg b.wt. per day / s.d.; *= p<.05; **=p<.01)

These results suggest that medial frontal serotonergic systems are important in regulating alcohol consumption in the rat, and further extend the experimental literature implicating serotonin in the regulation of alcohol-related behaviors.

661.11

CLOMIPRAMINE ALTERS THE RESPONSE OF VENTAL TEGMENTAL AREA (VTA) NEURONS TO ETHANOL-INDUCED EXCITATION, R.D. Trifunović and M.S. Brodie*, Dept. Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL 60612.

Excitation of dopamine neurons of the ventral tegmental area (VTA) may be a critical factor for the rewarding effect of ethanol (EtOH). EtOH increases the firing rate of VTA neurons *in vivo* and *in vitro*, and the potency of EtOH to excite VTA neurons is increased by serotonin (5-HT). Drugs which block the reuptake of 5-HT, like zimelidine and clomipramine (CLOM), have been used clinically to decrease drinking in alcoholics. Our objective was to test whether clompiramine, at concentrations which block 5-HT reuptake, alters the response of VTA neurons to EtOH, as we have shown for 5-HT.

Coronal brain slices containing the VTA were prepared from young adult F344 rats. Concentrations of EtOH (40 - 120 mM) were tested in the absence and presence of CLOM (125 nM to 2 μM). All neurons (n = 21) studied had electrophysiological characteristics typical of dopamine-containing neurons and were excited by EtOH in a concentration-dependent manner. Clomipramine itself had little or no effect on the firing rate of VTA neurons. In the presence of 500 nM CLOM, EtOH-induced excitations were approximately doubled; with CLOM, the mean excitatory effect of EtOH increased from 10% to 20% for 40 mM EtOH, and increased from 34% to 58% for 120 mM EtOH. This potentiation is consistent the action of CLOM to block 5-HT reuptake, and with our earlier findings that 5-HT potentiates the excitatory effects of EtOH on VTA neurons. At higher clomipramine concentrations (1 - 2 μM), there was no significant enhancement of EtOH-induced excitation. These higher concentrations are associated with reuptake blockade of norepinephrine and dopamine, which may obscure the effect of enhanced 5-HT availability. Grant Support: PHS AA-09125; Alcoholic Beverage Medical Research Foundation.

661.12

CHRONIC ETHANOL EXPOSURE ALTERS DOPAMINE D_2 RECEPTOR mRNA EXPRESSION IN NUCLEUS ACCUMBENS IN RATS. B.A. Blanchard* & P.K. Rudeen, Dept. of Pathology & Anatomical Sciences., Univ. of Missouri School of Medicine, Columbia, MO 65212.

Brain dopamine (DA) systems are believed to mediate the reinforcing properties of drugs of abuse, including ethanol (EtOH). Chronic EtOH exposure in rats has been shown to alter DA D_2 receptor populations, with the direction of change possibly dependent on the dose of EtOH and the period of exposure. The present study examined effects of chronic EtOH exposure on expression of the DA D_2 receptor gene in the terminal regions of the mesolimbic and nigrostriatal DA pathways: the nucleus accumbens and striatum. Adult female Long-Evans rats were exposed to EtOH via liquid diet (35% EtOH-derived calories) for periods of either two or six weeks. Following EtOH exposure, DA D_2 receptor mRNA content in the core and shell regions of the nucleus accumbens, and medial and lateral regions of the striatum was examined using *in situ* hybridization histochemistry. After two weeks of EtOH exposure, there were no significant changes in DA D_2 receptor gene expression in any region of either structure. However, six weeks of EtOH exposure produced a significant decrease (approximately 40% below control levels) in DA D_2 receptor mRNA content in the core region of the nucleus accumbens. There were no significant changes in the nucleus accumbens shell, nor in either region of the striatum. The findings suggest that chronic EtOH exposure may alter DA receptor populations by altering gene expression in regions believed to be important in drug reward.

661.13

ETHANOL INTERACTIONS WITH GABA AND β-ADRENERGIC MECHANISMS ON CEREBELLAR NEURONS IN LAS AND HAS RATS. **Ronald K. Freund, Donatella P. Donatelli, and Michael R. Palmer*. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.**

We previously found that ethanol-induced depressions of cerebellar Purkinje neurons in Sprague-Dawley rats are mediated by a $GABA_A$ mechanism, but that ethanol alone only potentiated GABA responses on 20% of these cells unless concomitantly applied with a β-adrenergic agonist. Timolol, a β-adrenergic antagonist, not only blocks these ethanol-induced potentiations of GABA, but also antagonizes ethanol-induced depressions on 20% of these neurons as well. These data may suggest that synaptically-released norepinephrine alters the responsiveness of this subpopulation of Purkinje neurons to ethanol actions under the conditions of our experiments. In the present study, we investigated these ethanol actions in two lines of rats which have been selectively bred for high (HAS) and low (LAS) alcohol sensitivity. We recorded single cerebellar Purkinje neurons from one barrel of a multibarrel micropipette while locally applying drugs from other barrels of the same pipette in urethane-anesthetized rats. Similar to Sprague-Dawley rats, we found that timolol reduced ethanol-induced depressions in 16% of the neurons tested in HAS rats. On 62% of the LAS neurons, however, the ethanol responses were antagonized by timolol application. Based on this observation, we hypothesised that ethanol would more frequently potentiate GABA-induced depressions in LAS than in HAS rats. Indeed, we observed this phenomenon in 69% of LAS neurons studied, but only in 20% of HAS neurons. These data suggest that there is a difference in the interaction of ethanol with GABA mechanisms between HAS and LAS rats which may be regulated by the activity of endogenous β-adrenergic mechanisms.

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661.14

ENDOGENOUS NORADRENERGIC MECHANISMS REGULATE ETHANOL ACTIONS IN RAT CEREBELLUM. **Michael R. Palmer, Ronald K. Freund*, Anya M-Y. Lin, and Yun Wang. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262 and National Defense Medical School, Taipei, Taiwan, R.O.C.**

We previously found that ethanol-induced depressions of cerebellar Purkinje neurons in Sprague-Dawley rats are mediated by a $GABA_A$ mechanism, but that ethanol alone only potentiated GABA responses on 20% of these cells unless concomitantly applied with a β-adrenergic agonist. In the present study we found that timolol, a β-adrenergic antagonist, not only blocks these ethanol-induced potentiations of GABA, but also antagonizes ethanol-induced depressions on 20% of these neurons as well. These data may suggest that synaptically-released norepinephrine alters the responsiveness of this subpopulation of Purkinje neurons to ethanol actions under the conditions of our experiments. Supporting this conclusion, we find that phencyclidine, a agent that facilitates noradrenergic synaptic function in the cerebellum, reversibly potentiates the depressant effects of locally applied ethanol. Furthermore, we investigated the influence of acute morphine withdrawal on the sensitivity of Purkinje neurons to ethanol-induced depressions, a condition in which there is increased noradrenergic synaptic input from the locus coeruleus. We found that after 7 days of chronic morphine treatment, the systemic injection of naloxone, a morphine antagonist that precipitates withdrawal in chronically morphine-treated animals, enhanced the depressant responses of cerebellar Purkinje neurons to local applications of both ethanol and PCP. These data suggest that endogenous β-adrenergic mechanisms regulate ethanol actions in the cerebellum.

(Supported by USPHS grant AA05915. MP is supported by ADAMHA Research Scientist Development Award AA00102.)

661.15

SUPPRESSIVE EFFECTS OF ALCOHOL UPON PREDATORY ATTACK BEHAVIOR IN THE CAT ARE BLOCKED BY A SUBSTANCE P, NK_1 RECEPTOR ANTAGONIST. M.B. Shaikh*, Y.C. Han, L. Pohorecky, D. Benjamin, and A. Siegel, Laboratory of Limbic System & Behavior, Department of Neurosciences, New Jersey Medical School, Graduate School of Biomedical Sciences, UMDNJ, Newark, New Jersey 07103, and Center for Alcohol Studies, Rutgers University, Piscataway, New Jersey, 08855.

This study was designed to determine the effects of alcohol upon quiet biting "predatory" attack behavior (QBA) elicited from the lateral hypothalamus (LH) and the mechanism within the medial hypothalamus (MH) involved in suppression of this response. *Experiment I:* peripheral administration of alcohol (0.02, 0.5 and 1.0 g/kg, I.P.) resulted in a dose and time dependent suppression of QBA in which the maximal effect (42% suppression) was observed 60 min, postinjection, when tested with 5% alcohol. *Experiment II:* peripheral administration of alcohol at the same dose levels also increased medial amygdaloid (ME)-induced suppression of QBA in a dose and time dependent manner. *Experiment III:* microinfusion of the substance P (SP)-NK_1 antagonist, CP 96,345, into MH blocked alcohol-induced enhancement of the suppressive effects of ME stimulation upon the LH. These results suggest the following conclusions: (1) that alcohol suppresses QBA; and (2) that the underlying mechanism for suppression of this response may involve agonistic-like effects of alcohol upon SP receptors within the MH, which receive direct inputs from an SP pathway originating in the ME.

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661.16

ETHANOL MODULATES MEMBRANE CURRENTS OF NEONATAL RAT PURKINJE NEURONS *IN VITRO*. H.K. Strahlendorf*, Y. Wang and J.C. Strahlendorf. Physiol. & Pharmacol., Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX. 79430

Brief exposure to ethanol (ETOH) during critical growth periods causes abnormal development and death of Purkinje neurons (PNs). Mechanisms may relate to ETOH disruption of membrane ionic currents seminal for normal development. Whole-cell patch clamp recordings of PNs in slices of neonatal rat cerebellum (4 to 15 days) revealed multiple actions of low (5 mM) to moderate (50 mM) concentrations of ETOH. Under voltage-clamp at -60 mV, 4 min exposures to 5 mM ETOH most often enhanced the cationic inward rectifier I_h (12-52%,n=6) and consistently elicited periods of brief spontaneous inward currents. During recovery, a transient inward current shift occurred that returned to control levels within 90 sec. ETOH at 50 mM suppressed I_h markedly (15-44%,n=8) and also elicited an inward current shift during recovery that did not return to pre-ETOH levels. At that time all electroresponsiveness was lost. These data reveal effects of ETOH on developing PNs potentially important to its toxicity.

661.17

EFFECTS OF ETHANOL ON MIDDLE LATENCY AUDITORY EVOKED POTENTIALS IN THE RAT. H. Miyazato, R.D. Skinner* and E. Garcia-Rill, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

The P1 middle latency auditory evoked potential in the human peaks at a 50-60 msec latency, is present during waking and REM sleep, but absent during slow wave sleep; i.e., it is present during periods of cortical desynchronization. Recently, we described the presence of the P13 potential (peak 11-15 msec) in the rat which correlates with the human P1 potential and wave A in the cat, the feline equivalent of the P1 (Skinner, et al., 1993). The present study was undertaken to determine the effects of ethanol on the behavior of the rat P13 potential.

Under barbiturate anesthesia, cortical leads were implanted at the vertex and over the auditory cortex. An intragastric tube was inserted and routed to exit in the dorsal neck. Auditory stimuli (intensity 103 dB, rate 0.2 Hz) evoked P13 potentials referenced to a frontal sinus screw were recorded and averaged from male rats following intragastric ethanol administration (0.5, 1, 3, or 5 gm/Kg, 40% in water). Following a 0.5 gm/Kg dose of ethanol there was no effect on the P13 potential. However, following a dose of 1 gm/Kg, the P13 potential was reduced to 60% at 5 min and then recovered by 15 min. Larger doses of ethanol (3 and 5 gm/Kg) reduced the P13 potential to 30% and 20%, respectively. The P13 potential recovered after 30 min following the 3 gm/Kg dose, and after 75 min following the 5 gm/Kg dose. Simultaneous recording of the averaged auditory evoked responses (Pa) over the auditory cortex showed only a slight effect on this potential at all ethanol doses tested.

Our results suggest that ethanol reduces the amplitude of the P13 potential and delays its recovery in a dose-dependent manner. Thus, the behavior of this potential indicates that ethanol decreases the ability of the reticular activating system to generate a normal arousal response.

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661.18

INTRAGASTRIC ETHANOL LOWERS THE PEAK FREQUENCY OF TYPE I THETA. S. Morzorati*, T.E. Breen. Indiana Univ. Sch. Med., Inst. Psych. Res., Indianapolis, IN 46202.

Theta is a rhythmic slow wave recorded from the hippocampus during wakefulness and sleep. Type I theta has a frequency between 6-10Hz and occurs during ambulation; type II theta has a frequency between 4-9Hz and occurs during alert immobility. Neurons in the medial septum/diagonal band of Broca and entorhinal cortex pace theta activity, sending rhythmic discharges to hippocampal interneurons which, in turn, communicate with hippocampal pyramidal cells, the source of theta activity.

Intravenous administration of ethanol has been shown to disrupt the rhythmic discharges of medial septal cells in freely moving rats (Givens and Breese, JPET 253:95, 1990). Given this fact, experiments were designed to investigate the effect of ethanol on type I theta.

Male Wistar rats (N=5) were trained to ambulate steadily on a treadmill and implanted with chronic intragastric cannulae and hippocampal EEG recording electrodes. Water or ethanol (0.5, 1.0, 2.0 g/kg body wt) was infused following an overnight fast. EEG was then recorded while ambulating at 5 min intervals for 120 min. The data were subjected to power spectral analysis and the peak frequency of the power spectra determined.

A preliminary analysis of variance indicated that all doses of ethanol significantly ($p<0.0001$) decreased the peak frequency of type I theta. The decrease was dose-dependent. Taken together, these and previous data indicate that ethanol decreases theta frequency, perhaps by disrupting the rhythmic activity of medial septal cells.

(Supported by AA07611, AA07462)

661.19

INHIBITION OF CALCIUM ENTRY BY CADMIUM CHANGES ETHANOL INHIBITION INTO EXCITATION IN RAT LOCUS COERULEUS NEURONS. S.S. Osmanovic* and S.A. Shefner, Department of Physiology and Biophysics, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612-7342.

We have previously shown that ethanol can inhibit the spontaneous firing of rat locus coeruleus (LC) neurons. In the present study, intracellular recording in completely submerged brain slices from Fisher 344 rats was used to study the involvement of Ca^{2+} entry in the ethanol-induced inhibition of LC neurons. Ethanol was tested before and after application of the calcium channel blocker Cd^{2+} or low Ca^{2+}/high Mg^{2+} media. In 7 LC neurons which showed ethanol-induced inhibition, the mean spontaneous firing rate in control media was 0.72 $\pm$ 0.18 Hz (S.E.M.) and 0.28 $\pm$ 0.09 Hz after bath application of 100 mM ethanol. The mean ethanol-induced inhibition of firing, calculated with each cell as its own control, was 49.4 $\pm$ 9.1%. The ethanol-induced inhibition of spontaneous firing was associated with no change in resting membrane potential (n=5) or membrane hyperpolarizations of 2-4 mV (n=2). Effects of ethanol were retested after superfusion with media containing Cd^{2+} (100 - 500 μM) for 15 - 25 min. In the presence of Cd^{2+}, ethanol caused membrane *depolarization* and *increased* the firing rate in all 7 neurons tested; the mean depolarization and increase in firing rate were 2.7 $\pm$ 0.36 mV and 91 $\pm$ 36.8%, respectively. The effect of ethanol in Cd^{2+} was significantly different than in control as determined by a paired t-test ($p< 0.05$). Ethanol-induced inhibition was also changed to excitation when the Ca^{2+} concentration in the media was reduced from 2.4 to 0.25 mM and the Mg^{2+} concentration increased from 1.3 to 10 mM (n=5). These data indicate that ethanol-induced inhibition of spontaneous firing in LC neurons is dependent on Ca^{2+} entry. Another previously reported action of ethanol on LC neurons is reduction of action potential duration. This action of ethanol was not affected by Cd^{2+} indicating that ethanol effects on spontaneous firing and action potential duration are mediated by separate mechanisms.

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DRUGS OF ABUSE: ALCOHOL V

662.1

EFFECT OF ETHANOL ON FORSKOLIN-STIMULATED CYCLIC AMP ACCUMULATION IN CULTURED HUMAN NEUROBLASTOMA CELLS S. M. Rezazadeh,* H. Lal, and M.W. Martin. Department of Pharmacology, University of North Texas Health Science Center, Ft. Worth, Texas, 76107.

Previous reports indicate that ethanol (ETOH), in some membrane preparations, stimulates adenylate cyclase (AC) activity. Also, acute exposure of some cell types to ETOH increases extracellular adenosine by blocking its reuptake. Thus, adenosine, by stimulating A2 adenosine receptors, may contribute to the enhanced cyclic AMP (cAMP) responses observed in the presence of ETOH. The purpose of this study was to determine whether ETOH directly modifies AC activity independent of endogenous adenosine. Accumulation of cAMP in intact SK-N-SH neuroblastoma cells was measured in the presence of the phosphodiesterase inhibitor Ro 20-1724 and adenosine deaminase. Synthesis of cAMP was stimulated by forskolin (FSK), CGS21680 (CGS), an A2 adenosine agonist, or isoproterenol (ISO). FSK which produces a receptor-independent activation of cAMP synthesis, increased the cAMP levels by 30-40 fold with an EC_{50} of 6 μM. In the presence of 1 mM FSK, stimulation of cAMP synthesis by either CGS or ISO was markedly potentiated. Ethanol (>40 mM) inhibited FSK-stimulated cAMP synthesis in a concentration-dependent manner. Although ETOH (160 mM) produced little or no change in the response of ISO or CGS alone, the response to these agonists in the presence of FSK (1 mM) was significantly inhibited by ETOH. To investigate the effect of long-term ETOH exposure, cells were pre-incubated with 80 or 160 mM ETOH for 2-48 hours. The inhibitory effect of ETOH on FSK response was not attenuated after a previous ETOH exposure. This may indicate that tolerance does not develop to this effect of ETOH. It is concluded that ETOH inhibits FSK-dependent, but not receptor-dependent, AC activity and tolerance does not develop to this pharmacologic effect. These results suggest that ETOH may alter the interaction between Gs, FSK, and the AC catalytic subunit. (Supported by NIAAA-A06890 and UNTHSC Grants).

662.2

POSSIBLE MECHANISM OF ACTION FOR ETHANOL INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN C6 GLIOMA CELLS. P. J. Syapin* and J. D. Militante. Dept. of Pharmacology, Texas Tech Univ. Health Sci. Center, Lubbock, TX 79430.

Growth of C6 glioma cells in ethanol for 10 days potently inhibits inducible nitric oxide synthase (iNOS) activity (IC50 ≈ 30 mM), measured as nitrite accumulation during 24 h exposure to 400 ng/ml phorbol 12-myristate 13-acetate (PMA) plus 500 ng/ml lipopolysaccharide (LPS). Ethanol for 24 h during stimulation with PMA+LPS was less effective (IC50 ≈ 140 mM), indicating a change in cell sensitivity after chronic ethanol (Syapin, submitted for publication). Induction of iNOS activity requires both gene expression and interaction of the apoprotein with essential co-factors. Thus, ethanol could inhibit by decreasing gene induction or enzyme activation. The time-course for reversal of inhibition upon withdrawal from chronic ethanol was long (>72 h), suggesting down-regulation of important components of the gene induction pathway. *In vitro* experiments suggest decreased iNOS activity in cytosol from chronic ethanol-exposed cells, consistent with a decrease in gene expression. Using nitrite accumulation to measure iNOS activity, concentration-response curves on cells exposed to 50 mM ethanol showed reduced maximum responses to both PMA (25-400 ng/ml) plus 500 ng LPS/ml and LPS (25-500 ng/ml) plus 400 ng PMA/ml ($p < 0.001$), but potency changed only for LPS (3-4 fold less; $p < 0.05$). In other studies, acute inhibition occurred only when ethanol and LPS were both present. Because the induction of glial iNOS by LPS requires early activation of protein tyrosine kinase, ethanol may act to decrease signal transduction by this pathway. Experiments are investigating this possibility. These data suggest a new mode of action for ethanol effects in the brain.

662.3

INFLUENCE OF NITRIC OXIDE SYNTHASE INHIBITOR ON THE DEVELOPMENT OF RAPID TOLERANCE TO ETHANOL. J.M. Khanna*, G.S. Morato, A. Chau and H. Kalant. Department of Pharmacology, University of Toronto, University of Santa Catarina, Brazil and Addiction Research Foundation, Toronto, Canada.

We recently reported that the nitric oxide (NO) synthase inhibitor L-nitro-arginine (L-NA) blocks the development of rapid tolerance to the motor incoordinating effect of ethanol (E) in the tilt-plane test. In the present study, three experiments were carried out using the same test. The first assessed the acquisition of rapid tolerance to E in rats pretreated with L-NA prior to E on both days 1 and 2. In the second, the effect of L-NA given prior to behavioral testing with E on day 1 was compared to the effect of L-NA given after the behavioral testing on day 1. The third investigated the effects of L-NA on the enhancement of rapid tolerance to E by D-cycloserine (CS), an agonist at the N-methyl-D-aspartate (NMDA) receptor. The results demonstrated that L-NA prevents the development of rapid tolerance to E when injected prior to E either on both days or only on day 1. Furthermore, tolerance was blocked only when L-NA was injected before but not after behavioral testing. L-NA also blocked the enhancement of rapid tolerance to ethanol induced by CS. L-NA did not influence brain or arterial blood E concentrations on day 2. These data argue against state-dependent learning and confirm and extend our previous results that NO plays a role in the development of rapid tolerance to E. Moreover, considering that the NMDA receptor and NO participate in the mechanisms underlying learning and memory, our data also support the view that NO is involved in the enhancement of E tolerance by CS.

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662.4

THE EFFECT OF REPEATED ETHANOL WITHDRAWAL ON ULTRASONIC VOCALIZATION AND THE INDUCTION OF C-FOS PROTEIN. S.S. Moy*, D.J. Knapp, G.E. Duncan, and G.R. Breese. Brain and Development Research Center, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27599-7250.

Repeated withdrawals from chronic ethanol consumption have been linked to an exacerbation of withdrawal symptoms, suggesting that multiple exposures and withdrawals from ethanol lead to a progressive sensitization not apparent with continuous ethanol administration. Previous research has shown that both ultrasonic vocalizations and the expression of fos-like proteins are increased during withdrawal from chronic ethanol administration. In the present experiment, rats given repeated withdrawals from ethanol, rats given the same amount of ethanol continuously, and control animals were compared on the basis of ultrasonic vocalizations in response to air puff and fos-like immunoreactivity (Fos-LI). Behavioral testing occurred seven to eight hours following ethanol withdrawal on the final day of the experiment. The results showed that both withdrawal groups spent more time vocalizing in comparison to the control animals, while the repeated withdrawal group demonstrated increased vocalization in comparison to the continuous ethanol group. Immunohistological analysis of c-fos activity showed a similar trend for specific brain regions. In particular, Fos-LI in the medial prefrontal cortex, ventrolateral orbital cortex, piriform cortex, retrosplenial cortex, locus coeruleus, and taenia tecta was enhanced more by repeated withdrawals than by a single withdrawal. These data support the hypothesis that multiple ethanol exposure/withdrawal cycles result in a more severe withdrawal syndrome than a single ethanol withdrawal. AA-08024, NS26595, & HD07201.

662.5

EXPRESSION OF HEAT SHOCK PROTEINS FOLLOWING EXPOSURE TO CHRONIC ETHANOL. Ellen T. Ott-Reeves, J. Mark Sherman, Clayton M. Pickering and John D. Lane*. Department of Pharmacology, UNTHSC-FW, 3500 Camp Bowie Blvd., Fort Worth, TX 76107 USA.

Heat shock proteins are induced in cultured cells when the culture medium contains 5% ethanol. Patients with a history of chronic ethanol exposure show high levels of heat shock proteins. In this experiment, male Fisher 344 rats were taught to orally self-administer ethanol on an FR4 schedule, using sucrose fading. These rats showed only low response rates for ethanol alone. Rats were fed a liquid diet containing ethanol, then retested over a range of ethanol concentrations. Ethanol intake increased over levels prior to administration of the liquid diet. Rats were again fed the liquid diet for 2 weeks. Tissues from heart, liver, kidney, gastrointestinal tract, skeletal muscle and multiple brain regions were harvested and *in situ* hybridization was performed using oligonucleotide probes specific for either inducible or constitutive 70kd heat shock proteins. Both inducible and constitutive mRNAs were found in control and ethanol rats. In brain, the highest levels of both inducible and constitutive mRNAs were found in the cerebellar granular layer, hippocampus, dentate gyrus and hypothalamic nuclei. In kidney, the inducible probe showed uniform distribution; however, the constitutive form showed a specific pattern with highest levels of mRNA in the medulla. All other organs showed more uniform distribution of both the inducible and constitutive mRNAs. In regions showing a statistically significant difference, levels of inducible mRNAs were greater than constitutive in both ethanol and control rats.

662.6

ENHANCED (Na+K)-ATPase EXPRESSION IN MOUSE BRAIN AFTER CHRONIC ETHANOL ADMINISTRATION. Y.M. Chen, P.M. Wixom* and A.Y. Sun, Dept. of Pharmacology, University of Missouri, Columbia MO 65212.

It is the general hypothesis that the primary mode of action of ethanol is the alteration of membrane structure and function including the conformation of receptors and ion channels essential for neurotransmission and signal transduction. (Na+K)-ATPase is of interest because of its major role in Na+ homeostasis and its membrane dependence. In the past we have demonstrated the inhibitory effect of ethanol on (Na+K)-ATPase in vitro, and chronic administration of ethanol has led to an increase in the specific activity of this enzyme. However, the issue of whether ethanol affects (Na+K)-ATPase under physiological conditions remains unsettled. In this study, adult mice were treated with a daily dose of 5 g/kg of ethanol for 28 days. The RNA was isolated from brain samples and probed for the (Na+K)-ATPase mRNA using Northern blot analysis. We have found an increased expression of (Na+K)-ATPase in the chronically treated alcohol group as compared with controls. This result was further substantiated by the increased protein phosphorylation of this enzyme after chronic ethanol administration. Thus we have demonstrated that ethanol may directly or indirectly affect (Na+K)-ATPase in situ, leading to the increased synthesis of this enzyme through adaptive mechanisms. (Supported by NIH Grant #AA02054).

662.7

IMMOBILITY IN A SWIM TEST IS NOT ASSOCIATED WITH A GENETIC PREDISPOSITION TO ALCOHOL PREFERENCE. C.D. Godfrey, J.C. Froehlich*, L. Lumeng, T.-K. Li and J.M. Murphy. Dept. Psych., Purdue Sch. Sci.; Inst. Psychiat. Res. and Dept. Medicine, Indiana Univ. Sch. Medicine, IUPUI, Indianapolis, IN 46202.

Alcohol-preferring P rats exhibit less immobility ("behavioral despair") in a forced swim test than -nonpreferring NP rats (Godfrey et al., Soc. Neurosci. Abs. 18:541, 1992; Overstreet et al., Alcohol: Clin. Exp. Res. 16:407, 1992). However, the AA alcohol drinking rats exhibit more immobility than nondrinking ANA rats in a similar test (Korpi et al., 1988). The present study compared high alcohol drinking HAD rats with low drinking LAD rats on a swim test and examined the effects of desipramine (10 or 20 mg/kg). Adult male HAD and LAD rats (n=9/line/dose) were placed in a round tub filled with water (21°C). The rats were unable to touch the bottom or escape from the tub. Behavior was videotaped for 10 min on each of two consecutive days and scored for time spent immobile. HAD rats were more immobile ($p<0.05$) than LAD rats; 124±10 vs. 91±10 sec (mean±SEM), respectively. Desipramine attenuated ($p<0.05$) time spent immobile in LAD but not in HAD rats which contrasts with the attenuation of immobility seen previously in both P and NP rats, with greater attenuation in NPs. Immobility in the swim test is not associated with a genetic predisposition to alcohol preference. Attenuation of immobility by desipramine is seen in both alcohol-nonpreferring (NP and LAD) lines but not in both -preferring lines. (AA07611, AA08553)

662.8

REPEATED ETHANOL WITHDRAWAL EXPERIENCE DIFFERENTIALLY INFLUENCES WITHDRAWAL-RELATED SEIZURES AND 'ANXIETY' RESPONSES IN MICE. H.C. Becker*, K.G. Fernandes, and R.T. Weathersby. VA Medical Center and Medical University of South Carolina, Charleston, SC 29401

We previously demonstrated an exacerbation of withdrawal seizures in adult C3H mice that experienced multiple cycles of ethanol (EtOH) withdrawal in comparison to mice withdrawn from EtOH a single time, even when total EtOH exposure is equated across groups. This study was designed to examine whether similar results may be obtained in another mouse strain, and with another symptom of withdrawal (anxiety). Adult male NIH Swiss mice were exposed to EtOH vapor prior to withdrawal. A multiple withdrawal (MW) group received 3 cycles of 16 hr EtOH vapor separated by 8 hr periods of abstinence; a continuously exposed (CE) group received the same total amount of EtOH (48 hr) uninterrupted; a single withdrawal (SW) group received a single 16 hr bout of EtOH exposure; and controls received no EtOH exposure. Following the final withdrawal cycle, mice were tested for handling-induced convulsions (HIC) or performance on an elevated plus-maze. Blood EtOH levels upon final withdrawal were similar among EtOH-exposed groups (180-200 mg%). The groups did not differ in % time on or % entries into open arms in the plus-maze at 8 and 24 hrs post-withdrawal. Total arm entries were depressed in the MW and CE groups at 8 hrs, but not at 24 hrs. In contrast, severity of withdrawal seizures was significantly greater in the MW group in comparison to other groups. Mean area under the 24 hr HIC curve was 21.3 ± 2.0, 10.6 ± 4.7, 13.3 ± 3.0, and 1.0 ± 0.3 for MW, SW, CE, and control groups, respectively. These results suggest that in this mouse strain, repeated EtOH withdrawal experience exacerbates the severity of subsequent withdrawal seizures, but does not influence anxiety, as measured in the plus-maze test. Other mouse strains are being used to continue to explore this research question. Supported by VA Medical Research Service and NIAAA.

662.9

ULTRAESTRUCTURE CHANGES IN THE RAT CEREBRAL CORTEX DUE TO CHRONIC INTAKE OF ETHANOL J. Trujillo Santa-Ana* and G. Espinosa Villanueva. Departamento de Anatomia. Facultad de Medicina, U.N.A.M. México, 04510.

Alcohol has always been the major drug used. Alcohol's abuse is related with a deleterious effect on the organism and the behavior. This, is especifically true for the nervous system.
In this study, young Wistar rats of 8 weeks were divided in two groups: control (C, n=9) and experimental (E, n=9). The E group drank ethanol at 15% (v/v) for the last two months up to 5 months of age, and then they were sacrificed and processed for electronic microscopy (EM).
Brain of C and E groups were compared. The E group showed mild microencephaly, in most of the individuals. The light microscopy showed a decrease in the thickness of each layer examined. Some alterations, in patches, in subcellular structures: increase in the interlaminar space, altered distribution of both microtubules and neurofilaments, and the myelin sheats, were show trough EM.
These results with 2 months of ethanol intake, show a mild structural damage effects on the cerebral cortex, due to chronic ethanol intake. More studies are needed in order to relate this damage with the behavior in the rat.

662.10

ETHANOL PARTIALLY NORMALIZES VESTIBULAR PRONATION OF PONTINE-DAMAGED RATS BUT DISRUPTS THAT OF UNDAMAGED RATS. Rebecca M. Chesire* & Barbara E. Digman. Coll. Health Sciences & Psychol. Dept., Univ. Hawaii, Honolulu, HI 96822.

Acute or chronic ethanol (ETOH; 5-40%) does not abolish excessive forward locomotion in rats with damage of the nucleus reticularis tegmenti pontis (NRTP). Although ETOH interferes with motor activity in intact rats, it can improve some motor functions of NRTP-damaged rats. Pronation of the head and torso from a supine position in the air using an integrated sequence of movements is an index of neural integrity that is compromised by basal ganglia dysfunction. In NRTP-damaged rats, disruption of pronation is characterized by a change in form from lateral to ventroflexed righting. 11 female and 6 male Long-Evans hooded rats were lesioned electrolytically in the NRTP (1mA anodal current/15s bilaterally; n=8) or were used as controls (n=9). Testing was done over ≈ 50 days during availability of tap water (TW), TW+25% sucrose (SU), or 5-30% ETOH in TW+SU in water bottles. A challenge dose (CD) of 30%ETOH was administered i.p. after 8hr withdrawal. Righting was tested by holding the animals in a supine position ≈ 35cm above a 7cm thick pillow and releasing them (lateral form=1, ventoflexion=zero; 4 trials/animal/substance&dose). On TW, NRTP-damaged rats showed more ventroflexed than lateral righting (x=.5) and undamaged rats showed lateral righting exclusively (x=1.0). NRTP-damaged rats normalized slightly on SU (x=.6), but unoperated rats began to ventroflex (x=.87). At each level of ETOH, NRTP-damaged rats showed more lateral righting than on TW (x=.68 for 5-30%ETOH & .75 at 30%CD;range=.58-.75 vs. TW=.5), and unoperated rats showed less (x=.76 for 5-30%ETOH and .88 at 30%CD). The results indicate that ETOH can improve vestibular function in NRTP-damaged rats, and further support an appetitive role for ETOH in the presence of pontine damage.

662.11

***IN UTERO* ETHANOL EXPOSURE RETARDS GROWTH & ALTERS MORPHOLOGY OF CORTICAL CULTURES: GM1 REVERSES EFFECTS.** H. Laev*, B.L. Hungund, V. Gokhale, & S.E. Karpiak. Divs. of Neuroscience, & Analyt. Psychopharm., NYSPI; Dept. Psychiatry, Columbia U. (P&S) NY.

Ethanol, a developmental neurotoxin, affects plasma membrane physico-chemical properties. Its multiple actions in the developing CNS are complex, affecting embryogenesis, cell migration, differentiation & synaptogenesis. In a prior study using a model for the *fetal alcohol effects*, the neuroprotective lipid GM1 reduced fatty acid ethyl ester accumulations in rat fetuses exposed to ethanol *in utero* (GD7&8 and GD 13&14) *(Hungund et al., 1993)*. This study was initiated to further describe the *in utero* effects of ethanol, and, the capacity of GM1 treatment to ameliorate such changes. Sprague-Dawley CVF dams were exposed to ethanol (23.4 gr/kg i.g. *supra*). GM1 ganglioside (10mg/kg/i.m.) was given 24hrs & 1hr prior to each ethanol exposure. Cortical cultures were derived from GD15&20 fetuses. Since GM1 is highly localized on the cellular plasma membrane outer surface of CNS cells, we have used it as a marker molecule to assess cell integrity. We used cholera toxin/antitoxin/fluorescence immunohistochemistry to localize GM1. Cultures were stained at 24, 48, 96hrs and 7 & 14 days after plating. Results indicate that the brief *in utero* exposures to ethanol affected cell growth and morphology. We observed a marked retardation in cell development and arborization as early as 24hrs after plating. Also, there was a reduced number of viable cells (24hrs thru 14days). Control cells showed continuous staining for GM1 along outer plasma membranes. Ethanol exposed cells exhibited decreased and discontinuous membrane staining for GM1. This reduced staining is associated with the loss of membrane integrity *(Laev et al., 1993)*. These *in utero* ethanol induced pathologies are remarkably diminished in those cultures derived from fetuses of dams treated with GM1.

662.12

EFFECT OF ALCOHOL EXPOSURE DURING DEVELOPMENT ON THE AMYGDALA REGION. C. R. Miyazaki and S. J. Kelly*. Department of Psychology, University of South Carolina, Columbia SC 29208.

The early postnatal period in the rat is roughly equivalent to the third trimester in humans with respect to birth. Exposure to alcohol during this period has been shown to decrease the DNA concentration in the amygdala region of male rats at both 45 and 90 days of age. Female rats showed no effects. The decrease in DNA concentration suggests that there is an alcohol-induced decrease in cell number in the amygdala region, although the DNA concentration does not differentiate among cell types. In order to continue our investigation of the amygdala region, rats of both sexes were artifically reared and exposed to 5 g/kg/day of ethanol, artificially reared and not exposed to ethanol, or reared normally with dams. Rats were weaned at 21 days of age and housed with a same sex conspecific. At adulthood (130 days), the rats were deeply anesthetized with sodium pentobarbital and perfused intracardially with saline followed by 10% formaldehyde in phosphate buffer. The brains were removed and post-fixed in 10% formaldehyde for at least a week. The brains were cut into 40 um sections, mounted on slides, stained Luxol Fast Blue, and then counterstained with cresyl violet. A section at the level of the stria terminalis was identified and the area of the amygdala region was measured. Among female rats, no differences were found in the areal measurements of the amygdala region. (Supported by NIAAA Grant AA08080 to S.J.K.)

662.13

SEIZURE SUSCEPTIBILITY DURING WITHDRAWAL FROM ACUTE AND CHRONIC ETHANOL. D.A. Finn*, A.J. Roberts and J.C. Crabbe. Dept. Veterans Affairs & Dept. Med. Psychology, Oregon Health Sci. Univ., Portland, OR 97201.

The present studies were conducted to determine whether Withdrawal Seizure Prone (WSP) and Resistant (WSR) mice, which were selectively bred for differences in handling-induced convulsions following chronic ethanol (EtOH) inhalation, differed in seizure susceptibility to pentylenetetrazol (PTZ) and N-methyl-d-aspartate (NMDA) following chronic EtOH. Male WSP and WSR mice were exposed to EtOH vapor or air for 24 hrs. Once peak withdrawal had been attained, the animals were administered PTZ or NMDA via tail vein infusion. The threshold dose for onset to PTZ-induced tonic hindlimb extension (THE) was decreased in the EtOH vs. air exposed WSP and WSR mice. In contrast, the threshold dose for onset to NMDA-induced THE was unchanged in the EtOH exposed WSPs and increased in the EtOH exposed WSRs vs. their respective air exposed counterparts, suggesting a different time course for adaptation of GABA vs. NMDA receptor systems to chronic EtOH. Additional studies evaluated whether the decreased susceptibility to NMDA-induced seizures during withdrawal was due to an interaction with corticosterone (CORT), since circadian fluctuations in CORT alter seizure susceptibility to kainic acid (KA). WSP and WSR mice were administered EtOH (4 g/kg) or saline and were tail infused with KA during peak acute withdrawal. Separate groups were injected at 4:30 a.m. or 12:30 p.m., so that peak withdrawal would occur at the previously demonstrated minimum (a.m.) or maximum (p.m.) in seizure susceptibility to KA. The threshold dose for onset to KA-induced THE was increased in EtOH vs. saline injected WSP mice in both the a.m. and p.m. The threshold dose in EtOH vs. saline injected WSR mice was unchanged in the a.m. and increased in the p.m. These results suggest that the decreased susceptibility to NMDA receptor specific convulsants during peak withdrawal does not appear to be due to an interaction with CORT. Supported by grants from the VA and AA 08261 (JCC).

663.1

INHIBITORY EFFECT OF THE CALCIUM ANTAGONIST ISRADIPINE ON NICOTINE INTREVENOUS SELF-ADMINISTRATION IN DRUG-NAIVE MICE. W.Fratta*, M.C.Martellotta [1], A.Kuzmin [1], E.Zvartau [1]. Department of Neuroscience, University of Cagliari, Italy. [1]Department of Pharmacology, Pavlov Medical Institute, St. Petersburg, Russia.

Several reports indicate that nicotine positive reinforcing effect is associated with a selective activation of the mesolimbic dopamine system. Thus, in common with other drugs of abuse, such as morphine and cocaine, nicotine increases dopamine release in rat striatum and nucleus accumbens. We have recently shown that isradipine, an L-type dihydropyridine Ca^{2+} channel antagonist, inhibits the reinforcing properties of cocaine and morphine both in rats and mice. This effect is likely due to an inhibition of cocaine- and morphine-induced dopamine release. Here we show that isradipine dose-dependently and stereospecifically inhibits nicotine intravenous (i.v.) self-administration in drug-naive mice. In fact, nicotine induces a dose dependent i.v. self-administration response in drug-naive mice. Pretreatment with (±) isradipine dose-dependently inhibits nicotine i.v. self-administration in drug-naive mice. Furthermore, testing of both the isomers of isradipine reveals that only pretreatment with (+)isradipine is active in inhibiting nicotine i.v. self-administration in drug naive mice, while pretreatment with the (-)isomer is completely inactive. The possibility that the isradipine inhibitory effect might be related to an inhibition of nicotine-induced dopamine release seems to be very likely. Furthermore, these results might provide an indication for new therapeutic strategies in tobacco addiction.

663.2

NICOTINE ALLEVIATION OF NICOTINE ABSTINENCE IS NALOXONE-REVERSIBLE M.C. Payne, O.B. Wilson*, J.R. Lake, V.A. Carter, J.S. Cunningham and D.H. Malin. *Baylor College of Medicine, Houston, TX 77030 and University of Houston-Clear Lake, Houston, TX 77058.

Recently, a rodent model of nicotine abstinence syndrome has been developed based on the frequency of spontaneous behavioral signs following termination of continuous nicotine tartrate infusion in rats. In this model, nicotine abstinence syndrome can be precipitated by naloxone and reversed by morphine as well as nicotine itself. The observed signs closely resemble those typical of rat opiate abstinence syndrome. To test the hypothesis that nicotine alleviation of nicotine abstinence is partly mediated by release of endogenous opioids, 28 rats were infused for 7 days with 9 mg/kg/day nicotine tartrate in saline via Alzet osmotic minipumps. Twenty-one hours following termination of nicotine infusion, 14 rats received 9 mg/kg naloxone s.c. and 14 received saline; 5 minutes later half of each group received 0.35 mg/kg nicotine s.c. and half received saline. Rats were then observed for 15 minutes under "blind" conditions.

Anova revealed a significant interaction effect of naloxone and nicotine injections. Post-hoc comparisons revealed that nicotine significantly reduced nicotine abstinence signs in the absence of naloxone but not in its presence. Conversely, naloxone had a significant effect in the presence of nicotine but not in its absence. The results are consistent with the hypothesis that there is an endogenous opioid peptide component in nicotine dependence and abstinence.

663.3

AMPHETAMINE WITHDRAWAL-RELATED BEHAVIORS INDUCED BY MEDIAL PREFRONTAL CORTICAL INJECTIONS OF C-FOS ANTISENSE OLIGONUCLEOTIDES IN THE RAT. A.M. Persico*, C.W. Schindler, S. Davis, E. Ambrosio and G.R. Uhl. Mol. Neurobiol. Branch, ARC/NIDA/NIH, Baltimore, MD 21224; Depts. Neurol. & Neurosci. JHUSM; Dept. NeuroPsich.Sci., S. Raffaele Hosp., Milan, Italy; Dept. Psicobiol., UNED, Madrid, Spain.

Prefrontal areas display most prominent decreases in immediate-early-gene expression during amphetamine withdrawal. We employed antisense technology *in vivo*, to assess whether prefrontal c-*fos* may play a role in behavioral correlates of rat amphetamine withdrawal.

Phosphorothioated antisense 18-mers aimed at the translation start site of the c-*fos* mRNA display a half-life of about 3 hrs, in contrast to 15-30 min for phosphodiesters. Intracerebral medial prefrontal cortical injections of 2nM antisense, but not missense, phosphorothioated oligos, block c-*fos* mRNA translation, while exerting variable and inconsistent effects on c-*fos* mRNA levels. During c-*fos* translational blockade, animals display marked reductions in linear and repetitive locomotor behavior when exposed to a novel environment. No change in locomotor activity is evident in animals accustomed to the activity monitor. These changes closely mimic those recorded during amphetamine withdrawal and suggest that prefrontal c-*fos* may play a role in the chain of neurobiological events responsible for amphetamine withdrawal-induced behavioral alterations.

663.4

AMPHETAMINE REGULATION OF GENES ENCODING CELLULAR REGULATORS. *X.B. Wang*@, D. Vandenbergh@, and G.R. Uhl#@.* @Mol. Neurobiology Br., NIH/NIDA, IRP; #Depts. Neurol. & Neurosci., JHUSM, Balto., MD 21224.

Psychostimulant drugs such as amphetamine can induce dependence syndromes and presumed corresponding long-term changes in the CNS. Altered expression of genes may provide possible biochemical contributions to these drug induced long-term CNS changes. We have analyzed alterations in gene expression in brains of rats sacrificed 4 hours after injection of d-amphetamine (7.5 mg/kg, i.p.), using differential display PCR followed by differential hybridization. More than 20 bands which were changed after drug administration could be identified after amplification with two primer sets. Some were represented in all brain regions analyzed, and some showed region-specific patterns. Sequence analysis of two differentially-displayed products revealed high homologies with known genes. One product revealed 97% homology with the reported sequence of a calcium activable calmodulin-dependent phosphatase, calcineurin. Northern analysis showed that striatal calcineurin mRNA increased by 40% after d-amphetamine treatment. The second cDNA encoded a transducin-like factor. Hybridizing mRNA showed marked, approximately 10-fold, increases in striatum with changes in cortex and thalamus also notable. Products of genes acutely regulated by d-amphetamine are candidates for participation in drug-induced long-term changes in the CNS.

663.5

TRANSCRIPTION FACTORS INVOLVED IN THE METHAMPHETAMINE-INDUCED INCREASE IN STRIATAL DYNORPHIN EXPRESSION: EFFECTS OF CYCLOHEXIMIDE. D. Bronstein, I. Merchenthaler, K. Pennypacker, I.Perez Otano, P. Hudson, D. Chalmers* & J.-S. Hong. U. Michigan, Ann Arbor, MI & LMIN, NIEHS, Research Triangle Park, NC 27709.

The mechanism by which activation of dopamine receptors in the striatum leads to increased expression of the opioid peptide dynorphin (DYN) has been the subject of recent debate. In this study, the role of newly synthesized immediate early genes (e.g., c-fos, c-jun) in mediating the increased expression of DYN was addressed by using the protein synthesis inhibitor, cycloheximide (CHX). Adult male rats were pretreated bilaterally with vehicle or CHX (250 µg in 10 µl, I.C.V.), then injected with METH (10 mg/kg, s.c.) and sacrificed 6 hr later. Whereas METH caused only a slight reduction in DYN-ir concentrations in the striata of vehicle-pretreated animals, it caused a significant (35%) reduction in DYN levels in CHX-pretreated animals. This decrease was interpreted to indicate that CHX blocked the biosynthesis of new DYN-ir protein following the METH-induced release/depletion of intraneuronal DYN-ir. However, Northern analyses indicated that METH induced DYN mRNA levels to a comparable degree in CHX and control rats, suggesting that *de novo* biosynthesis of transcription factors (e.g., c-fos and c-jun) was not necessary to induce DYN mRNA expression. Rather, it supports recent data implicating phosphorylation of pre-existing CREB proteins (and subsequent binding to the CRE site) as a critical step in the regulation of DYN expression. We are presently assessing directly whether CHX affects the expression of transcription factor proteins or the binding activity to AP-1 or CRE DNA elements following METH adminstration.

663.6

BEHAVIORAL SENSITIZATION TO AMPHETAMINE IS CORRELATED WITH A GREATER INCREASE IN C-*FOS* mRNA LEVELS IN THE STRIATUM OF RATS. R. Rivest*[1], R.A. Wise[2], T. Di Paolo[1] and S. Rivest[3] [1]School of Pharmacy, [3]Mol. Endo. Lab., CHUL, Laval university, Quebec and [2]CSBN, Dept. of Psychol., Concordia Univ., Montreal.

Most studies exploring the neural basis of behavioral sensitization converge on the mesoaccumbens dopaminergic pathway. The mechanisms underlying this phenomenon may, however, be more complex and recent data suggest the participation of additional neurotransmitters. To better understand the mechanism of behavioral sensitization, we have performed *in situ* hybridization analysis of c-*fos* mRNA in the brain of animals sensitized to amphetamine. Male Long-Evans rats received daily injections of saline or amphetamine (1 mg/kg/ml) for 10 consecutive days. One week later they were given an acute challenge injection of saline or amphetamine (0.5 mg/kg). The animals were sacrificed 45 min or 3 h after the last injection and their brains were collected for further *in situ* hybridization analysis for c-*fos* mRNA. Amphetamine (1mg/kg) increased locomotor activity and the increase was progressively greater as animals received repeated injections. The challenge dose of 0.5 mg/kg in animals previously tested repeatedly with saline produced a slight increase in locomotor activity or c-*fos* mRNA levels in the striatum. The same treatment in animals previously tested with amphetamine resulted in a significantly greater increase in locomotor activity and c-*fos* mRNA levels. Characterization of the neurons in which c-*fos* responses to amphetamine are increased in sensitized animals is presently under investigations. Supported by the MRC.

663.7

PSYCHOSTIMULANT-INDUCED ENHANCEMENT OF FOS-LIKE IMMUNOREACTIVITY IN THE RAT CEREBELLUM. M.A Klitenick*, C.-S. Tham and H.C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z3

Previous studies have demonstrated increased locomotor activity and immediate-early gene expression in the cerebellum following the administration of various psychostimulants. In the present study, immunohistochemical techniques were used to assess the pattern of Fos-like immunoreactivity (FLI) in the cerebellum following acute, systemic administration of amphetamine and cocaine. Amphetamine (1.5, 6mg/kg) increased locomotor which could be blocked by pretreatment with the D1 dopamine receptor antagonist SCH 23390 (1 mg/kg). Within the cerebellum, amphetamine elicited a dose-dependent increase in FLI that was markedly attenuated by SCH 23390 pretreatment. In contrast, SCH 23390 pretreatment abolished the dose-dependent increase in locomotor activity and FLI following cocaine (10, 20 mg/kg). Psychostimulant-induced FLI was restricted to the granule cell layer within each of the midvermal cerebellar lobules (I-X) and distributed in dense clusters extending from the molecular layer to the Purkinje cell layer. Studies assessing the effects of acute, systemic caffeine administration on FLI in the cerebellum are in progress. Preliminary results indicate that caffeine (15 mg/kg) produces a somewhat different pattern of cerebellar FLI, with greater involvement of the molecular layer then seen following amphetamine and cocaine.

663.8

NBQX INHIBITS AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY BUT NOT C-FOS INDUCTION. A. Dalia, N.J. Uretsky, and L.J. Wallace*. College of Pharmacy, Ohio State University, Columbus, OH 43210.

Administration of amphetamine activates the dopaminergic and the AMPA subtype of glutamate receptors as well as induces c-fos protein in the nucleus accumbens. A role for basal levels of c-fos in expression of locomotor activity is derived from the observation that administration of c-fos antisense oligonucleotide into the nucleus accumbens 5 hours before amphetamine blocked the locomotor response. Therefore, a possible correlation between activation of glutamatergic receptors, increases in c-fos protein, and stimulation of locomotor activity was explored. A behaviorally relevant dose of amphetamine (1 mg/kg, ip) elicited a small increase in c-fos protein and stimulated locomotor activity. Pretreatment with NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxalinedione) (30 mg/kg, ip), an AMPA receptor antagonist, had no effect on amphetamine-induced increase in c-fos protein but inhibited locomotor activity. NBQX alone had no effects on c-fos levels or locomotor activity. MK-801 (0.1 mg/kg, sc), an NMDA receptor antagonist, did not increase c-fos but stimulated locomotor activity. These data suggest that induction of c-fos is not correlated with stimulation of locomotor activity. (Supported by DA07722 and DA06776).

663.9

NBQX INHIBITS LOCOMOTOR ACTIVITY INDUCED BY APOMORPHINE IN DRUG-NAIVE BUT NOT SENSITIZED RATS. P. Laudrup* and L.J. Wallace, Ohio State University, College of Pharmacy, Columbus, OH-43210.

The development of sensitization to apomorphine (apo), cocaine, and amphetamine is considered to be mediated by changes in dopamine neurotransmission. Since the acute effects of these agents involve activation of the AMPA receptor subtypes of glutamate receptors, the present study investigates whether the sensitized state is also characterized by a change in glutamate neurotransmission. On day 0, male rats were conditioned to the test cages. At each treatment day they were habituated for one hour, injected (NBQX i.p. and apo s.c.) and distance travelled measured for two hours by automated image analysis. In drug-naive rats, NBQX (10 mg/kg), an AMPA antagonist, attenuated locomotor activity induced by apo (1 mg/kg) to 45%. However, when pretreated with apo (5 mg/kg) every third day during 13 days, the locomotor activity induced by apo (1 mg/kg) was increased, while NBQX only attenuated to 93%. Preliminary results indicate that sensitization to cocaine and amphetamine also decreases the inhibitory effect of NBQX. These results indicate that sensitization and a change in response to NBQX occurs simultaneously. Additional studies will elucidate if these changes is a part of sensitization and further describe the nature of the alterations. (Supported by a gift of NBQX from Novo Nordisk A/S and grant number DA07722).

663.10

COMPETITIVE AND NONCOMPETITIVE NMDA-RECEPTOR ANTAGONISTS BLOCK NICOTINE-INDUCED DOPAMINE OVERFLOW. E. Museo* and A. Pert, Biological Psychiatry Branch, National Inst. of Mental Health, Bethesda, MD 20892.

The psychomotor stimulant effects of nicotine are believed to be due, at least in part, to the activation of a dopaminergic substrate; one dopamine (DA)-containing region that appears to be involved is the nucleus accumbens (nAcb). We previously reported that the systemic or intra-nAcb administration of nicotine stereoselectively facilitates DA overflow in this region. The possibility has been raised that nicotine's actions on DA overflow are dependent on the integrity of glutamatergic transmission in the nAcb. The present experiment tested this hypothesis using two NMDA-receptor antagosists: the noncompetitive antagonist MK-801 and the competitive antagonist AP-5. Male, Sprague-Dawley rats were anaesthetized with chloral hydrate and a microdialysis probe was surgically positioned in the nAcb. All pharmacological treatments were administered through the probe and dialysate samples were collected and analyzed for DA content. Nicotine (100 μM), on its own, increased nAcb DA overflow by approximately 50 percent; the intra-nAcb administration of either the noncompetitive antagonist MK-801 (100 μM) or the competitive antagonist AP-5 (100 μM) prior to and during the administration of nicotine (100 μM) significantly reduced the magnitude of this effect. It appears that the blockade of glutamatergic transmission is sufficient to disrupt the actions of nicotine on DA overflow in the nAcb.

663.11

EFFECTS OF ACUTE AND REPEATED AMPHETAMINE ADMINISTRATION ON EXTRACELLULAR GLUTAMATE AND ASPARTATE LEVELS IN RAT VENTRAL TEGMENTAL AREA AND NUCLEUS ACCUMBENS. Y. Li*, C.-J. Xue and M.E. Wolf. Dept. of Neuroscience, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Behavioral sensitization refers to the progressive enhancement of the locomotor stimulatory effects of stimulants such as amphetamine (AMP) during their repeated administration. Considerable evidence indicates an important role in sensitization for mesoaccumbens DA neurons, originating in the ventral tegmental area (VTA) and projecting to nucleus accumbens (NAc). Recent work also supports a role for excitatory amino acid (EAA) transmission, since: 1) coadministration of N-methyl-D-aspartate antagonists prevents the development of sensitization, 2) it also prevents functional changes in the mesoaccumbens DA system which accompany sensitization, and 3) EAA receptor sensitivity in VTA and NAc is altered in AMPH-sensitized rats. These findings suggest that AMP administration may be associated with changes in EAA release in the VTA or NAc. However, using *in vivo* microdialysis in behaving rats, we found that AMP (2.5 mg/kg) failed to alter extracellular levels of aspartate or glutamate in the NAc of naive or AMP-sensitized rats. Preliminary results in VTA are similar. Studies are underway to determine whether the efficiency of the glutamate transporter is masking AMP-induced changes in EAA release. In other studies, the quantitative "no net flux" method was used to determine basal extracellular levels of glutamate in NAc (estimated at 1.9 uM). This method will be used to examine the effects of repeated AMPH administration on basal EAA levels and *in vivo* turnover rate. Supported by USPHS Grant DA 07735.

663.12

LESIONS OF PREFRONTAL CORTEX OR AMYGDALA, BUT NOT FIMBRIA FORNIX, PREVENT SENSITIZATION OF AMPHETAMINE-STIMULATED HORIZONTAL LOCOMOTOR ACTIVITY. S.L. Dahlin, X.-T. Hu, C.-J. Xue and M.E. Wolf*. Dept. of Neuroscience, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

A role for mesolimbic DA neurons in behavioral sensitization to amphetamine (AMPH) is well established. However, the fact that coadministration of N-methyl-D-aspartate antagonists prevents the development of both AMPH sensitization and associated changes in the mesoaccumbens DA system suggests that excitatory amino acid (EAA)-dependent events occur very early in sensitization and are critical to its initiation. This study sought to identify EAA projections required for sensitization, focusing on projections to nucleus accumbens (NAc) or ventral tegmental area (VTA). The major EAA projections to NAc originate in prefrontal cortex (PFC), basolateral amygdala (AMY) and hippocampus (HPC). PFC and AMY also send EAA projections to the VTA. Ibotenic acid lesions of PFC or AMY and electrolytic lesions of fimbria fornix were performed in rats. After 1 week, rats were treated with water or 2.5 mg/kg amphetamine (AMP) for 6 days and challenged with AMP on day 8. Activity was tested in photobeam cages on days 1 and 8. On day 1, control and sham-lesioned rats exhibited an early phase of stereotypy followed by a phase of horizontal locomotor activity; both phases exhibited sensitization on day 8. Lesions of fimbria fornix produced a general disinhibition of locomotor activity, but did not interfere with sensitization. Lesions of AMY or PFC failed to prevent sensitization of stereotypy, but eliminated sensitization of horizontal locomotion. Since both PFC and AMY, but not HPC, send EAA projections to VTA, these data may suggest that EAA projections from PFC and AMY to VTA are critical to the initiation of sensitization. USPHS Grant DA 07735.

663.13

BOTH TYPICAL AND ATYPICAL NEUROLEPTICS BLOCK PHENCYCLIDINE (PCP)-INDUCED STRIATAL AND BEHAVIORAL EXCITATION IN FREELY MOVING RATS. I. M. White*, G. S. Flory, I. Speciale, and G. V. Rebec. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405

The striatum plays a critical role in the behavioral excitation induced by amphetamine and other indirect dopamine agonists. In freely moving rats, amphetamine typically increases the activity of striatal neurons, and this effect is blocked by dopamine antagonists, which also attenuate amphetamine-induced behavioral effects. Phencyclidine (PCP), a non-competitive NMDA antagonist with little or no affinity for dopamine receptors, induces a similar pattern of behavioral excitation, but little information is available on the striatal mechanisms underlying this effect. In the present series of experiments, we assessed the effects of PCP (1.0 - 5.0 mg/kg) on single-unit activity in the striatum of awake, behaving rats. Like amphetamine, PCP routinely increased striatal activity, and this effect was reversed by subsequent injection of either haloperidol (1.0 mg/kg) or clozapine (20.0 mg/kg), typical and atypical neuroleptics, respectively. To the extent that neuroleptics act primarily as dopamine antagonists, our results suggest a striatal dopamine-NMDA interaction in the behavioral effects of PCP.

Supported by USPHS Grant DA 02451.

663.14

THE ROLE OF DOPAMINE D3 RECEPTORS IN BEHAVIORAL SENSITIZATION, SENSITIVITY TO APOMORPHINE, AND BASAL DOPAMINE SYNTHESIS. P.M. Robinet*, B.A. Mattingly, J.K. Rowlett & M.T. Bardo. Departments of Psychology, Morehead State Univ., Morehead, KY 40351 and Univ. of Kentucky, Lexington, KY 40506.

Behavioral sensitization to repeated treatments of the mixed D1/D2 dopamine (DA) receptor agonist apomorphine (APO) can be blocked by the D1 antagonist SCH 23390 but not D2 antagonists. Further, APO increases basal DA synthesis as does the D2/D3 agonist quinpirole. This effect is blocked by the D2 antagonist eticlopride. While behavioral sensitization to APO appears to be D1 mediated D2 receptors may be involved in the increased DA synthesis. The current study assessed the role of DA D3 receptor activation in locomotor activity, sensitivity to APO and basal DA synthesis. In two experiments, the D3 selective agonist 7-OH-DPAT was administered at 24 hour intervals for 10 consecutive days (0.0, 0.01, 0.1, 1.0 mg/kg). Following each injection locomotor activity was recorded for 20 min. On day 11 in experiment 1, all rats received a challenge injection of APO (1.0 mg/kg) and their locomotor activity was recorded. On day 11 in experiment 2, all rats were injected with the DOPA decarboxylase inhibitor NSD1015 (100 mg/kg). After 30 min the brains were removed and DA synthesis was assessed by measuring DOPA accumulation in the extracted tissue. Locomotor activity was initially inhibited at the higher doses (0.1, 1.0 mg/kg) but increased to control levels by day 10. The low dose (0.01 mg/kg) also inhibited activity initially but in contrast to the higher doses, this inhibition increased with repeated treatment. No increase in sensitivity to APO was found at any dose. Also, chronic treatment did not significantly increase basal DA synthesis. These findings suggest that the development of behavioral sensitization to APO, and the accompanying increase in basal DA synthesis, is not mediated by D3 receptor activation. (Supported by grants from the Kentucky/NSF EPSCoR committee and Morehead State University).

663.15

AMPHETAMINE- AND COCAINE- INDUCED FOS IN THE RAT STRIATUM DEPENDS ON D_2 DOPAMINE RECEPTOR ACTIVATION. D.N. Ruskin* and J.F. Marshall. Department of Psychobiology, University of California, Irvine, CA 92717.

Amphetamine or cocaine injection causes expression of the immediate-early gene *c-fos* in the striatum. Previous studies have shown that dopamine D_1 receptor activation is necessary for this effect, but have not established a consistent role for D_2 receptors. We have investigated the involvement of D_2 receptors in indirect agonist-induced striatal Fos-like immunoreactivity using the selective D_2 antagonist eticlopride. Rats received eticlopride (0.5 mg/kg) or saline 30 min before receiving amphetamine (5 mg/kg), cocaine (40 mg/kg), or saline. All injections were I.P. Two hr after the second injection, the rats were perfused with 4% paraformaldehyde and their brains sectioned at 40 μm through the striatum for immunocytochemistry. Eticlopride treatment caused Fos expression by itself, but also decreased Fos expression in the central striatum due to amphetamine or cocaine by 90% and 85%, respectively. In striatonigral neurons, identified by labeling with the retrograde tracer Fluorogold iontophoresed into the substantia nigra pars reticulata, the blockade of stimulant-induced Fos-like immunofluorescence by eticlopride was nearly complete, with decreases of 98% for amphetamine and 94% for cocaine. In striatonigral neurons, the D_2 antagonist alone induced minimal Fos. We conclude that activation of both D_1 and D_2 receptor classes by dopamine agonists is necessary for induction of Fos in the striatonigral cells of normal rats. These results provide an important parallel to behavioral and electrophysiological work that also demonstrates D_1/D_2 interdependence in the control of normal basal ganglia functions.

663.16

EFFECT OF INTRAVENOUS SELF-ADMINISTRATION OF *d*- and *l*-AMPHETAMINE ON NUCLEUS ACCUMBENS (NAc) DOPAMINE LEVELS. K Leeb* and R.A. Wise. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Rats given limited access to a variety of stimulant drugs self-administer these drugs with very regular inter-response rates (Pickens and Thompson, 1968; Yokel and Pickens, 1974). To date, the variables responsible for maintaining such a regular pattern of responding for drug have not been fully established. Because amphetamine (AMPH) increases extracellular levels of dopamine (DA) in the NAc we investigated whether fluctuations in dopamine correlate with responding for drug. Male Long-Evans rats with chronic intravenous catheters and intra-accumbens guide cannulae were trained to lever-press for infusions of either 0.25 mg/kg *d*-AMPH or 0.75 mg/kg *l*-AMPH in 4h daily sessions. Once stable self-administration was established animals underwent microdialysis during drug self-administration. Dialysate samples were taken every 5 min and frozen for subsequent determination of DA concentration using HPLC with electrochemical detection. Self-administered AMPH caused rapid elevation of DA levels to 300-500% of baseline, and these elevations were sustained, more or less, for the remainder of the session. However, there were phasic fluctuations in DA, superimposed on the tonic elevations, that were time-locked to lever-presses and the consequent drug injections; DA levels usually increased after each injection and fell prior to the next response. Thus the initiation of a drug-seeking response (e.g., a lever-press) was triggered in the absence of dopamine depletion and, indeed, long before dopamine concentration fell significantly toward basal levels.

663.17

STIMULANT-INDUCED FACILITATION OF SELF-STIMULATION IS POSITIVELY CORRELATED WITH MEDIAL DISTANCE OF ELECTRODE FROM SUBSTANTIA NIGRA. S.A. Khan*, S.L. Fledderjohn, V.L.Tsibulsky, S.R. Grocki, R.A. Frank. University of Cincinnati, Cincinnati, OH 45221-0376.

We studied the effects of amphetamine and cocaine on self-stimulation train-duration thresholds in rats implanted with bipolar electrodes (0.2 mm) in A9 and A10 dopaminergic regions of the midbrain. Amphetamine (1.0mg/kg, n=29) or cocaine (15mg/kg, n=14) was injected IP 15 min prior to self-stimulation testing. Electrode placements for 43 male rats were independently verified by three researchers and compared with drug effects. The distance between the electrode placement and the brain midline was measured. The correlation of this distance with stimulant-induced facilitation of self-stimulation was $r = -.43$, $p < .004$. Inspection of the data suggested that stimulation sites closest to substantia nigra, pars compacta showed the least stimulant-induced facilitation of self-stimulation. The correlation between drug effect and distance of electrode from the substantia nigra was $r = .44$, $p < .003$. The findings indicate that amphetamine and cocaine may differentially influence self-stimulation obtained from the A9 and A10 regions.

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663.18

RESERPINE ABOLISHES AMPHETAMINE-INDUCED DOPAMINE RELEASE IN VENTRAL MIDBRAIN NEURONAL CULTURE. C. St. Remy, S. Rayport and D. Sulzer*. Depts Psychiatry, Neurology, Anatomy & Cell Biology; Ctr Neurobiology & Behavior, Columbia Univ; Dept Neuropathology, NYS Psychiatric Institute, NY 10032.

Amphetamine-induced (AMPH-induced) dopamine (DA) release has been suggested to occur via actions at plasma membrane uptake transporters or at synaptic vesicles. Since several studies have reported that reserpine, which depletes vesicular DA, does not reduce AMPH-induced DA release, action at the vesicular level has been controversial. However, in these studies reserpine was administered *in vivo* 18 to 24 h prior to AMPH, providing sufficient time for increased DA synthesis or other time-dependent effects. To circumvent these issues, we used postnatal ventral midbrain DA neuron cultures, in which stimulation-dependent, AMPH-induced DA release and total cellular DA content can be reliably and concurrently measured. We found that reserpine (100 nM) reduced stimulation-dependent (40 mM KCl for 3 min) DA release by 60% after 15 min and by 90% after 60 min. At a higher concentration, reserpine (1 μM) reduced stimulation-dependent DA release to undetectable levels and total cellular DA by 95% at 90 min. In control cultures, 10 μM AMPH induced a 250% increase in extracellular DA, while following reserpine (1 μM for 90 min) AMPH induced only a 3% increase. Strikingly, AMPH released over 3-fold more DA from untreated cultures than the total DA content of reserpinized cultures. Together with *in vivo* microdialysis studies (Sabol et al., *Soc Neurosci Abstr*, 1993), this indicates that AMPH releases DA primarily from the vesicular pool, consistent with the weak base model of AMPH action (Sulzer et al., *J Neurochem*, 1993).

663.19

AGE-DEPENDENT PERSISTENCE OF FENFLURAMINE EFFECTS ON MONOAMINERGIC NEURONS IN REAGGREGATE TISSUE CULTURE. L. Won*, P.C. Hoffmann and A. Heller, Dept. of Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL 60637.

The three-dimensional reaggregate tissue culture system provides a useful approach to examine the effects of drug exposure on the subsequent development of monoaminergic neurons. Utilizing this system, we have previously demonstrated that exposure of 15 day old reaggregates, containing developing monoaminergic cells (equivalent in development to postnatal day 7 neurons), to 10^{-5}M (±) fenfluramine (FEN) for 7 days resulted in significant reductions of reaggregate dopamine (DA) and serotonin (5-HT) levels and this deficit persisted for 3 weeks following cessation of drug exposure. In order to examine the possible age-dependency of such effects, 2 month old reaggregate cultures composed of murine mesencephalon and corpus striatum cells (equivalent in development to adult neurons) were exposed to 10^{-5}M (±) FEN for 7 days and then were allowed to recover in drug-free medium for 3 weeks. At the end of the 7 day exposure to FEN, DA and 5-HT levels in reaggregates were significantly depressed to 35% and 69% of control levels, respectively. By the end of the 3 week recovery period, DA levels in FEN-treated reaggregates had attained similar values to those in control cultures whereas 5-HT levels in reaggregates exposed to FEN remained significantly depressed (52% of control). Thus, in reaggregate culture, both DA and 5-HT neurons are susceptible to the persistent effects of FEN during early development. With mature neurons, such longlasting effects are only observed on 5-HT neurons as is seen in the intact adult brain. Supported by MH42134.

663.20

THE STIMULATION OF LOCOMOTION BY MK-801 IS MEDIATED BY THE ACTIVATION OF MESO-LIMBIC DOPAMINERGIC NEURONS. S. Narayanan, A. Dalia, L.J. Wallace, N.J. Uretsky*, Ohio State University, College of Pharmacy, Division of Pharmacology, Columbus, Ohio 43210

Low doses of MK-801 (0.05-0.25 mg/kg), a noncompetitive antagonist of NMDA receptors, stimulates coordinated locomotor activity by enhancing dopamine (DA) neurotransmission in the nucleus accumbens (N.Ac). This stimulation may be related to the ability of this drug to increase the firing rate of meso-limbic DA neurons. To test this hypothesis, we have studied the locomotor response to MK-801 in rats after administration of baclofen, a GABA-B agonist that inhibits the firing rate of meso-limbic DA neurons, into the ventral tegmental area (VTA). MK-801 (0.1 mg/kg, i.p.) stimulated locomotor activity, and this effect was inhibited by baclofen (0.15 nmoles per side) injected into the VTA. This inhibitory effect appears to be related to an action on meso-limbic DA neurons, since baclofen did not inhibit the locomotor response to apomorphine (5 mg/kg, s.c.), which is mediated by the activation of DA receptors postsynaptic to the DA terminals. To determine whether the locomotor response to systemic MK-801 is due to its action in the VTA or the N.Ac, MK-801 (1-20 μg) or vehicle was injected into each of these sites and produced a stimulation of locomotor activity; the potency and maximum response to MK-801 were greater after its injection into the VTA. The locomotor response to MK-801, 10 μg (30 nmoles) per side into the VTA, was inhibited by the coadministration of baclofen (0.15 nmoles per side). In contrast baclofen did not inhibit the locomotor response to MK-801 (10 μg per side) injected into the N.Ac. Thus, the locomotor stimulant effect of systemic MK-801 may be mediated by an action of this drug in the VTA, producing an increase in the activity of DA neurons, resulting in an enhancement in DA neurotransmission in the N.Ac. Supported by grant DA07722

663.21

ADMINISTRATION OF PERTUSSIS TOXIN IN THE VENTRAL TEGMENTAL AREA DOES NOT ENHANCE THE LOCOMOTOR STIMULATION PRODUCED BY APOMORPHINE. J.J. Byrnes*, D.M. Weinstein, S. Narayanan, N.J. Uretsky, L.J. Wallace, Ohio State University, College of Pharmacy, Division of Pharmacology, Columbus, Ohio 43210

Repeated administration of amphetamine, cocaine, or apomorphine produces a progressively enhanced locomotor response. Recently it has been found that the bilateral administration of pertussis toxin (PTX) into the ventral tegmental area (VTA), the location of the cell bodies of mesolimbic dopaminergic neurons, produces an enhanced locomotor response to amphetamine and cocaine similar to the changes seen in animals sensitized to these agents. This suggests that changes produced by PTX mediate the development of sensitization to drugs that act presynaptically to increase dopaminergic transmission. In the present study we investigated whether administration of PTX into the VTA produces a sensitized locomotor response to apomorphine, which directly activates dopamine receptors. Rats were injected into the VTA with PTX (0.5 μg/ 0.5 μl per side) or vehicle, and 14 to 21 days later, the locomotor responses to amphetamine (0.5 mg/kg, i.p.) or apomorphine (1 and 5 mg/kg, s.c.) were determined. Animals treated previously with PTX showed a markedly enhanced locomotor response to amphetamine compared to vehicle-treated animals. However, the response of PTX-treated animals to either dose of apomorphine was not significantly greater than that of vehicle pretreated controls. Thus, while pretreatment with PTX in the VTA mimics some of the components of sensitization produced by drugs that indirectly stimulate central dopamine systems, it is not sufficient to produce a sensitized response to a directly acting dopamine agonist. (Supported by DA07722 and DA06776)

663.22

REDUCED RESPONSIVENESS OF D1 RECEPTOR DEFICIENT MICE TO THE EFFECTS OF D1 SELECTIVE AGONISTS, ANTAGONISTS AND D-AMPHETAMINE IN TESTS OF LOCOMOTOR ACTIVITY AND CATALEPSY. L.H. Gold*, M. Xu, I. Polis, C. Heyser, S. Tonegawa and G.F. Koob, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, 92037 and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

Dopamine one (D1)- receptor deficient mice were created through introduction of a deletion in the D1 gene of mouse embryonic stem (ES) cells by homologous recombination. ES cells carrying the D1 deletion were injected into mouse blastocysts to generate chimeric mice which were then bred to obtain homozygous mutant mice. The complete absence of D1 receptors was verified by genomic Southern analysis. In wild-type mice, the D1 agonist SKF81297 (1.2, 4.8, and 7.4 mg/kg, s.c.) produced a dose-dependent increase in locomotor activity compared to saline levels, while SKF81297 had no effect in D1-deficient mice. The D1 antagonist SCH23390 (10, 30 μg/kg, s.c.) produced a dose dependent reduction in locomotor activity in the wild-type mice, while not altering locomotion in the D1-deficient mice. SCH23390 (0.05, 0.1, 0.2 mg/kg, s.c.) also produced dose-dependent increases in catalepsy in the wild-type mice, measured as percent of time immobile in an elevated ring-procedure, but was ineffective in the D1-deficient mice. Interestingly, the D1-deficient mice exhibited a marked attenuation of the locomotor stimulation produced by d-amphetamine (1, 3 mg/kg, s.c.), an indirect dopamine agonist, compared to wild-type mice. These results demonstrate that D1 receptor ligands are ineffective in D1 receptor deficient mice in behavioral assays of motor activity, and support the further use of these mice to investigate the relationship between D1 and D2 receptors in the behavioral actions of psychostimulant drugs and drug reinforcement.

663.23

CORRELATIONS OF NOVELTY AND AMPHETAMINE INDUCED LOCOMOTION IN RATS WITH MICRODIALYSIS MEASUREMENTS OF DA NEURONAL FUNCTION A. Pap* and C.W. Bradberry, Yale Univ. Sch. Med., Dept. of Psychiatry and the West Haven VA Hospital, West Haven, CT 06516

Using a circular runway locomotor chamber, we have been investigating biochemical correlates of novelty induced locomotion. Previous work by Piazza, et al. indicates this is predictive of amphetamine induced locomotion and of amphetamine self-administration. Novelty and amphetamine induced locomotion were assessed on two separate days, separated by at least two days. Individual locomotor scores from the two sessions were highly correlated. Following amphetamine challenge, animals were implanted with microdialysis guide cannulae overlying the nucleus accumbens, and after at least 48 hours, were implanted with microdialysis probes which were left in place overnight for experiments the following day. The first study examined the correlation of locomotor scores (both novelty and amphetamine) with basal DA (as assessed by in vitro probe recovery), and peak DA levels following local infusion (via the microdialysis probe) of 3 μM amphetamine for one twenty minute collection period. Significant correlations were seen between locomotor scores and basal DA, but not between locomotor scores and peak DA following amphetamine infusion expressed either as an absolute level, or as a percent of basal. Basal levels also did not correlate with peak levels. In a separate study, 3 μM DA was infused, and the in vivo recovery was determined. Justice's group has demonstrated that in vivo recovery is primarily a function of DA uptake. In this group, a non-significant trend ($p = 0.10$) toward a correlation was seen between in vivo recovery and locomotor-induced novelty, but not with amphetamine induced novelty. Supported by DA 08073, DA 08227, The Yale VA Alcoholism Research Center, and a NARSAD Young Investigator Award to CWB.

663.24

SELECTIVE INVOLVEMENT OF DOPAMINE D1 RECEPTORS OF THE VTA IN THE BEHAVIORAL SENSITIZATION INDUCED BY INTRA-VTA AMPHETAMINE INJECTIONS. Y. Bijjou, L. Stinus, M. Le Moal and M. Cador*, Unité INSERM U259, rue Camille Saint Saëns 33077 Bordeaux Cedex, France.

In agreement with previous studies, we demonstrated the complete independence of the neurobiological substrates responsible for the induction and the expression of the behavioral sensitization to amphetamine (AMPH). Using a local approach, we showed that AMPH injected at the level of the dopamine (DA) cell bodies (0, 1, 2.5, 5 μg/0.5μl/side) dose-dependently induced a behavioral sensitization to a later challenge with AMPH administered into the nucleus accumbens (1 μg/μl μl/side) whereas the repeated administration of AMPH into the nucleus accumbens (0, 1, 3, 10 μg/μl/side) did not produce such effect. In order to specify the mechanisms through which AMPH acts in the VTA, we studied the respective involvement of VTA DA-D1 and -D2 receptors in the behavioral sensitization produced by intra-VTA administration of AMPH. In Experiment I, different groups of rats received 4 injections (1 every other day) of different doses of the D1 antagonist SCH-23390 (0, 0.01, 0.1, 1 μg/0.5μl/side) in the VTA followed immediately by intra-VTA administration of AMPH (5 μg/0.5μl/side) or its solvent. Two, 4 and 12 days following the last intra-VTA injection, peripheral challenges were performed with saline (day2) and AMPH (0.5 mg/kg, s.c., day 4 and 12) respectively. Locomotor activity was measured following each injection. In Experiment II, the same protocol as Experiment I was performed but the DA-D2 antagonist sulpiride (10 μg/0.5μl/side) or the serotonin 5-HT2 antagonist ketanserin (1 μg/0.5μl/side) was used for the VTA pretreatment. Results demonstrated that: 1) VTA AMPH pretreatment induced behavioral sensitization as revealed by the potentiation of the response to later peripheral challenges with AMPH. 2) VTA DA-D1 receptor blockade dose-dependently prevented this behavioral sensitization. 3) neither DA-D2 nor 5-HT2 receptor blockade at the doses used had any effect. In conclusion, these results demonstrate clearly the selective involvement of the dopamine D1 receptors of the VTA in the induction of behavioral sensitization to AMPH.

663.25

PRE- AND PERINATAL METHAMPHETAMINE EXPOSURE PRODUCES NEUROCHEMICAL CHANGES IN MATERNAL BUT NOT IN DEVELOPING RAT BRAIN. X.M. Meng, G.D. Newport, M.G. Paule* and S.F. ALI. Neurochemistry Laboratory, Division of Neurotoxicolology, NCTR/FDA, Jefferson, AR 72079-9502.

Methamphetamine (METH) is one of the drugs of abuse known to cause neurotoxicity in the adult rodent and nonhuman primate. Little attention has been focused on the evaluation of pre- or perinatal exposure to METH on the developing brain. The present study was designed to evaluate whether pre- or perinatal exposure to METH produces changes in monoamine levels in maternal or developing pup brain. Pregnant CD rats were injected sc with either 0, 2.5 or 5 mg/kg METH daily on gestational days 6 through postnatal day (PND) 21. Pups were sacrificed on PNDs 1, 7, 14 or 21 and dams were sacrificed 24 hours after the last dose (PND 22). Monoamine levels were measured by HPLC/EC. Pre- and perinatal exposure to METH had little effect on monoamine levels in different brain regions at PNDs 1, 7, 14 and 21. However, in maternal brain, dopamine levels were significantly decreased in the caudate nucleus. It is concluded that pre- and perinatal exposure to METH at these doses produces significant alterations in the maternal but not in the developing rat pup brain monoamine levels.

663.26

THE EFFECT OF BODY TEMPERATURE ON THE RAPID DECREASE IN TRYPTOPHAN HYDROXYLASE ACTIVITY INDUCED BY 3,4-METHYLENEDIOXYMETHAMPHETAMINE. S. Che, J.W. Gibb, G.R. Hanson and M. Johnson*, Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Brain tryptophan hydroxylase (TPH) activity decreases within 2 hours after a single administration of 3,4-methylenedioxymethamphetamine (MDMA). In this study, the effect of body temperature on this rapid decrease of TPH activity was examined. Male Sprague Dawley rats (180-220 g) were administered a single dose of MDMA (20 mg/kg) or saline, and were maintained at 25°C or 7°C for 2 hours. After this period of time, rectal temperature was measured and the animals were killed. The body temperature of the MDMA-treated rats kept at 25°C was increased by 1.2°C when compared to their control group, whereas the body temperature of MDMA-treated rats kept at 7°C was decreased by 1.1°C. The rectal temperature of control animals maintained in the cold room was not altered. TPH activity measured in the hippocampus, striatum and frontal cortex of hyperthermic rats treated with MDMA was decreased to 60%, 64% and 70% of control, respectively. However, under hypothermic conditions, MDMA administration had no effect on TPH activity in the hippocampus, striatum or frontal cortex. These results suggest that body temperature may affect the rapid decrease of TPH activity induced by MDMA. (Supported by USPHS grants DA 00869 and DA 04222)

663.27

THE EFFECTS OF RISPERIDONE AND MDL 28,133 ON AMPHETAMINE-INDUCED SELF-STIMULATION THRESHOLDS. S.R. Grocki*, V.L. Tsibulsky, B.A. Daschevsky, R.A. Frank. University of Cincinnati, Cincinnati, OH 45221-0376.

This experiment assessed the ability of D_2 - $5HT_2$ antagonists to reverse amphetamine-induced facilitation of brain stimulation reward. It has been shown previously that specific $5HT_2$ antagonists alone do not reverse amphetamine-induced facilitation of self-stimulation in the VTA. We speculated that the mixed antagonists would have a greater effect than more specific antagonists. Risperidone (.1 & .2 mg/kg) and MDL 28,133 (1.5, 3, 5, 6, & 7.5 mg/kg) were injected 15 min. prior to amphetamine. Both doses of Risperidone and the two highest doses of MDL 28,133 significantly decreased the effects of amphetamine. However, the ability of both drugs to reverse amphetamine's effects could be predicted from the elevation in thresholds produced by the antagonists alone. Therefore, it seems likely that the ability of the mixed antagonists to reverse amphetamine's effects is due to their D_2 component.

This project was supported by NIDA grant # DA 04483 and a grant from Marion Merrell Dow. Acknowledgements to Marion Merrell Dow for supplying MDL 28,133 and Janssen Research Foundation for supplying Risperidone.

663.28

THE EFFECTS OF ETICLOPRIDE, MDL 100,907 AND THEIR COMBINATION ON AMPHETAMINE- AND COCAINE-INDUCED FACILITATION OF SELF-STIMULATION. V.L.Tsibulsky*, B.A.Dashevsky, R.A.Frank. Brain Stimulation Lab, University of Cincinnati, Cincinnati, OH 45221-0376.

It was speculated that the combination of D_2 and $5HT_2$ antagonists could be more effective against stimulant-induced facilitation of brain stimulation reward in VTA than either compound separately. We used the specific D_2 antagonist eticlopride and specific $5HT_2$ antagonist MDL 100,907 to test this hypothesis. ETIC (0.01-0.066 mg/kg) and MDL 100.907 (0.66-2.0 mg/kg) or their combinations were injected 15 min prior to amphetamine (0.33-1.0 mg/kg) or cocaine (7.5-30 mg/kg). ETIC decreased the effects of both stimulants, while MDL 100,907 had no effect either alone or in combination with the D_2 antagonist. These results indicate that $5HT_2$ receptors are not involved in mediation of VTA brain stimulation reward or stimulant-induced facilitation of self-stimulation.

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663.29

SEROTONERGIC RELEASE INDUCED BY MDMA, MDA AND FENFLURAMINE IN CULTURE: EFFECTS OF CHRONIC ADMINISTRATION. *C.E. Hyde*, C.E. Hollingsworth, B.A. Bennett* Dept Physiol/Pharmacol, Bowman Gray Sch. Med., Wake Forest Univ.,Winston-Salem, NC 27157

Substituted amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methlyenedioxyamphetamine (MDA) and fenfluramine (FEN) are agents that are selective for serotonergic neurons. These compounds are thought to exert their effects by releasing serotonin (5HT) from presynaptic nerve terminals. Here we report on the acute mechanism by which MDMA, MDA and FEN induce 5HT release in primary cultures of fetal rat neurons. Data indicate that release of 5HT by these agents is concentration dependent. Furthermore, these agents promote release in a manner that is not affected by calcium channel blockers (1 μM conotoxin GVIA, 10 μM nifedipine) or by the absence of calcium from the media (calcium free media with 100 μM EGTA). In addition, depletion of the vesicular pool by continuous potassium stimulation (50 mM which eliminates potassium induced 5HT release) had no effect on 5HT release by these agents. However, fluoxetine (50nM), an agent known to inhibit the transporter, attenuated the acute release of 5HT induced by these compounds. This data is consistent with the notion that release by these agents is via a non-vesicular, transporter-mediated event. Studies were initiated to examine adaptive changes in serotonergic function following a chronic (5 day) exposure to MDMA, MDA, and FEN. Data indicate that 5 day exposure to 1 μM MDMA, MDA, and FEN cause a marked decrease in release capabilities of these neurons following a challenge exposure. These studies agree with recent reports of MDMA- and FEN-induced reductions in 5-HT transporter mRNA. Further studies will determine if these changes in 5HT release are a reflection of decreased transporter number (Vmax). *Supported by NIDA grants 05073, 06634 (BAB), 07246 (CEH).*

663.30

AMPHETAMINE INFUSION INTO THE MEDIAL SEPTAL AREA INCREASES BEHAVIORAL AND EEG INDICES OF AROUSAL: POSSIBLE INVOLVEMENT OF NORADRENERGIC β-RECEPTORS C.W. Berridge*, S. Bolen, S.L. Foote. Dept. Psychiatry, Univ. Calif., San Diego, CA 92093.

Previous studies indicated that the medial septal/diagonal band region (MS) is a site at which the LC-noradrenergic system, via actions at noradrenergic β-receptors, acts to influence forebrain EEG. Given that stimulants, such as amphetamine, enhance noradrenergic transmission it is hypothesized that stimulants may enhance arousal, in part, through the enhanced release of norepinephrine within MS. As an initial step in the testing of this hypothesis, three series of studies were conducted. First, the EEG effects of small infusions (150 nl) of AMPH (3.75 ug) or vehicle into MS were examined in the halothane-anesthetized rat. Infusions were made into MS, nucleus accumbens, striatum, or the substantia innominata/ nucleus basalis region. Unilateral MS AMPH (but not vehicle) infusions elicited bilateral forebrain EEG activation. AMPH had no EEG effects in sites other than MS. Second, to assess the degree to which β-receptors contribute to EEG activation following systemic AMPH administration, vehicle or the β-antagonist, timolol (TIM; 75-150 ug), was administered ICV (2 ul) 15-30 min prior to an intravenous AMPH injection (0.1 mg/kg) in the halothane-anesthetized rat. Pretreatment with TIM (but not vehicle) blocked the AMPH-induced activation of forebrain EEG. Finally, the effect of AMPH infused into MS on behavior in unanesthetized rats was examined. Unilateral infusions (150 nl) of vehicle or AMPH (3.75 ug) were made into MS or adjacent brain regions in resting rats in which a 33 ga. needle loaded with drug or vehicle had been inserted via a 26 ga. guide cannulae 90 min prior to infusion. AMPH significantly increased waking behavior (approx. 40 min) in the 60 min following the infusion, compared to vehicle (approx. 5 min). AMPH had no significant effects on waking behavior when infused unilaterally into nucleus accumbens at this dose and volume of infusion. These results suggest that actions at β-receptors within MS may contribute to the arousal-enhancing properties of stimulants.

663.31

The Delta-Opioid Receptor Antagonist Naltrindole Attenuates Amphetamine-Induced Increases in Extracellular Dopamine In the Striatum of Rats. C.A.Schad*, J.B.Justice, Jr.[1], and S.G.Holtzman, Departments of Pharmacology and Chemistry[1], Emory University, Atlanta, Georgia, 30322.

The opioid receptor antagonist naloxone has been shown to attenuate both the behavioral and neurochemical effects of amphetamine in rats. Furthermore, the amphetamine-induced increase in locomotor activity is attenuated by intracisternally-administered naltrindole (NTI), a selective delta-opioid receptor antagonist. This suggests a role of central delta-opioid receptors in the mediation of the behavioral effects of amphetamine. Therefore, this research was designed to look at the effects of NTI on the amphetamine-induced increase in extracellular dopamine (DA) in the brain of rats.

Microdialysis was performed on adult male rats (300-350g) that were pretreated with an intracisternal (IC) injection of 3, 10, or 30μg NTI or vehicle. Pretreatment injections were followed 15 min later by cumulative doses of subcutaneous *d*-amphetamine (0.0, 0.1, 0.4, 1.6, 6.4 mg/kg) at 30 min intervals. The microdialysis probes were perfused at a flow rate of 0.6μl/min and dialysate samples were collected every 10 min from either the nucleus accumbens (NACC) or striatum (STR).

Amphetamine dose-dependently increased extracellular DA in both the NACC and STR, as reported previously. NTI, 3, 10, and 30μg IC, significantly reduced the DA response to amphetamine in the STR. In contrast, 30μg of NTI did not modify the DA response to amphetamine in the NACC. These findings suggest that delta-opioid receptors play an important role in mediating the amphetamine-induced increase in extracellular DA in the STR, but not in the NACC.

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663.32

GENETICALLY VARIABLE CYTOCHROME P4502D1 IN THE BRAIN

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The cytochrome P4502D1 is an hepatic enzyme which is responsible for the metabolism of more than 30 drugs, including many centrally active drugs such as the tricyclic antidepressants, codeine, amphetamines and neuroleptics. There is genetic variability in the P4502D1 gene resulting in some people (7-10% of Caucasians) having no P4502D1 function. Catalytic, pharmacological and molecular studies have identified the genetically variable cytochrome P4502D1 (debrisoquine hydroxylase) in mammalian brain. There is significant overlap between substrates/inhibitors for the dopamine transporter and for the P4502D1 (i.e., (-)cocaine, MPTP, methylphenidate and d-amphetamine) suggesting that there may be a coordinated function between these two proteins.

RT-PCR were used to detect the presence of P4502D1 mRNA in brain regions and in multiple immortalized cell lines. Variation between rat brain regions in the amount of P4502D1 RNA was observed, as initially suggested by canine CNS activity studies. However differences were found between human and rat brain regional levels of P4502D1 mRNA. Cell lines provide an homogeneous population of cells in which to study the CNS P4502D1. Many of the immortalized cell lines studied contained the P4502D1 mRNA. Treatment of cell lines with forskolin differentiated the cells and altered the levels of the transcript for the enzyme. These preliminary studies suggest 1) that there is regional variation in the levels of CNS P4502D1 mRNA, 2) human and rodent brains may have different regional expression of the P4502D1, and 3) that cell lines may allow us to study the regulation of CNS P4502D1 and further our understanding of the roles for this genetically variable enzyme in the brain. Genetic differences in human brain metabolism of P4502D1 substrates (drugs, toxins, endogenous opiates or neurotransmitters) may effect drug and toxin response, and/or drug addiction. Supported by NIH and MRC of Canada.

DRUGS OF ABUSE: COCAINE—DOPAMINE

664.1

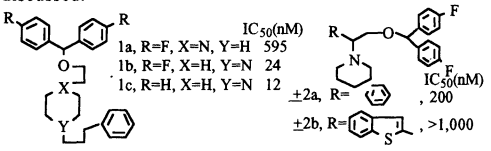
SPECT Imaging of Dopamine Transporters in Cocaine Dependent and Healthy Control Subjects with [123I]β-CIT. R.T. Malison*, E.A. Wallace, S. Best, M. Gandelman, S.S. Zoghbi, Y. Zea-Ponce, R.M. Baldwin, D.S. Charney, P.B. Hoffer, T.R. Kosten, R.B. Innis. Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06519.

Post-mortem studies of cocaine overdose victims have shown elevations in striatal dopamine (DA) transporters relative to healthy control brains. We examined whether acutely abstinent (< 72 hr) cocaine addicts have increased levels of striatal DA transporters compared to drug-naive controls, and whether posited increases would persist with sustained (2-4 weeks) drug abstinence. Cocaine dependent and healthy control subjects (n = 8 per group; ages 35 ± 10 vs. 33 ± 8 yr, p = NS) were injected with 10 mCi [123I]β-CIT and imaged from 24-30 hr post-injection under sustained equilibrium conditions. The ratio of specific to nonspecific brain uptake (i.e., (striatum - occipital)/occipital), a measure proportional the binding potential (B_{max}/K_D), was used for all comparisons. Preliminary results suggest a statistical trend (p=0.09; two-tailed) toward elevations in striatal DA transporters in acutely abstinent cocaine subjects in comparison to healthy controls (10.2 ± 2.7 vs. 8.2 ± 1.6; mean ± SD). Consistent with apparent increases (~25%) in [123I]β-CIT binding relative to controls, were within subject reductions (17 ± 18%; range, 7.8 - 33.3%) in cocaine addicts (n=5) after sustained (2-4 weeks) as compared to acute (< 72 hr) drug abstinence (12.2 ± 2.4 vs. 10.1 ± 1.7; p = 0.05). These results are consistent with preclinical studies which suggest an upregulation of DA transporters in response to chronic cocaine administration, changes which appear plastic and may "normalize" in the continued absence of the cocaine.

664.2

Potent and selective probes for the dopamine (DA) transporter. A. K. Dutta, B. K. Madras, P. C. Meltzer* and M. E. A. Reith. Organix, Inc., Woburn, MA 01801. Harvard Medical School, NERPC, Southborough, MA, 01772. University of Illinois, Peoria, IL 61656.

In order to asses the molecular determinants of the activity of the highly potent and selective GBR compounds for the DA transporter, several analogs were synthesized. Binding studies (IC_{50}) were performed on striatal tissue labelled with [3H]CFT. Changing the piperazine ring into piperidine in the GBR molecule did not affect the potency and selectivity for the DA transporter when the nitrogen atom was in the correct position (e.g. **1b-c**). Furthermore, the compound **1b** had very little (<3%) nonselective piperazine acceptor binding activity compared with the conventional GBR compounds. Compounds **2a-b** represent a new class of analogs. These compounds were synthesized in the racemic form and **2a** was found to have moderate affinity for the DA transporter. Detailed synthesis and binding studies of these compounds will be discussed.



664.3

IN CONTRAST TO INACTIVE S-COCAINE, A NOVEL SERIES OF S-COCAINE CONGENERS DISPLAY HIGH AFFINITY AND SELECTIVITY FOR THE DOPAMINE TRANSPORTER. B.K. Madras*, A.Y Liang and P.C. Meltzer. Harvard Medical School, NERPRC, Southborough, MA 01772-9102 and Organix, Inc. Woburn, MA 01801.

A novel series of benztropine analogs of cocaine were synthesized and their affinities for monoamine transporters were determined using [3H]WIN 35,428 to label the dopamine (DA) transporter and [3H]citalopram to label the serotonin (5HT) transporter. In sharp contrast to the active R-forms of cocaine and cocaine congeners (e.g. WIN 35,428), difluoropine* (4β-carbomethoxy-3α-methoxydifluorophenyltropane) and congeners displayed reverse stereoselective effects. The S-form was 200 times more potent (IC_{50}: 10.9 ± 1.2 nM) than the R-form, was highly selective for the DA/5HT transporter (324-fold) and was more potent than the 4β-dihydro analog. Unlike the phenyltropane series, other substituents on the aromatic ring tended to reduce the affinity for the DA transporter. This series of compounds challenges recent views on the binding domain of cocaine/cocaine congeners and offers unique opportunities for probing the binding terrain of the dopamine transporter. DA06303, RR00168, Boston Life Sci., Inc.; *reg.Tm.

664.4

INVOLVEMENT OF AUTOREGULATION IN THE EFFECT OF COCAINE (COC) ON MONOAMINES MEASURED BY DIALYSIS IN THE RAT VENTRAL TEGMENTAL AREA (VTA). M.E.A. Reith* and N.-H. Chen, Dept. of Basic Sciences, University of Illinois College of Medicine, Peoria, IL 61656.

Dopamine (DA) cell bodies in the VTA have been implicated in the rewarding and stimulatory activity of cocaine. The VTA is innervated by serotonin (5-HT)- and norepinephrine (NE)-containing afferents from the raphe nuclei and locus coeruleus, in addition to reciprocal efferent connections. In the present study, DA, 5-HT, and NE were measured simultaneously in dialysates from the VTA of freely moving rats, and the effect of COC was measured in the presence or absence of monoamine autoreceptor antagonists in the perfusate. Sulpiride (D_2/D_3, 10 μM) increased DA output induced by local (30 μM) or systemic (20 mg/kg i.p.) COC, methiothepin (5-HT_1/5-HT_2, 20 μM) promoted local COC-induced 5-HT output, and idazoxan (α_2, 100 μM) enhanced local COC-induced NE output. This is consonant with activation of autoreceptors in the VTA counteracting the effect of focal COC; possibly, modulation by somatodendritic 5-HT and NE autoreceptors is more important following systemic COC administration. Sulpiride promoted COC-induced NE output without modifying basal 5-HT/NE output, whereas methiothepin and idazoxan increased the basal output of all three amines without modifying COC-induced output of DA/NE or DA/5-HT, implying complex monoamine interactions. Sulpiride stimulated while methiothepin or idazoxan depressed COC (20 mg/kg)-induced motor activity. The analysis of behavioral/neurochemical relationships revealed a positive correlation between dialysate DA output and motor activity in the sulpiride/COC and methiothepin/COC groups, consonant with VTA DA reflecting DA cell firing, and a negative correlation was revealed between dialysate NE output and motor activity in the COC alone and idazoxan/COC groups, consonant with previous DA/NE balance theories. Supported by NIDA 03025.

664.5

FUNCTIONAL AND PHARMACOLOGICAL MODULATION OF SINGLE-UNIT ACTIVITY IN THE VENTRAL TEGMENTAL AREA AND RELATED REGIONS IN THE AWAKE, BEHAVING RAT. Kosobud, A.E., and Chapin, J.K. Hahnemann University, Philadelphia, PA, 19102, USA.

The ventral tegmental area (VTA) is a central element in a system that mediates the reinforcing properties of natural stimuli (such as food), brain stimulation and drugs of abuse. In the present studies, bundles of 4-8 microwire electrodes were chronically implanted in the VTA and target regions, including nucleus accumbens (NA), ventral pallidum (VP), mediodorsal thalamus (MDT) and prefrontal cortex (PFC) of male Wistar rats. Following recovery from surgery, simultaneous recordings from single neurons and unit clusters were obtained in unrestrained rats during pharmacological and behavioral studies. A substantial number of neurons in the VTA and target regions displayed no change in firing rate relative to behavior, or were unaffected by the peripheral administration of cocaine, diazepam, morphine, apomorphine or haloperidol in doses that have been shown to modulate firing rates in anesthetized rats. This suggests that in the awake rat, functional circuits may be more resistant to disruption by drugs. VTA neurons displaying the electrophysiological characteristics of dopaminergic neurons were relatively active in rats at rest, and reduced their firing rates during movement. These neurons were inhibited by apomorphine, and this inhibition coincided with the appearance of behavioral stereotypies (sniffing and head-weaving). This pattern was duplicated in neurons in MD thalamus, suggesting that functional patterns of activation are reflected in multiple sites throughout a neural circuit. A second class of VTA neurons, apparently non-dopaminergic, showed patterns of activity that were exactly the reverse of the VTA DA neurons: i.e. their firing rate was decreased in rats at rest, increased during movement, and excited by peripheral administration of apomorphine. Haloperidol reversed both the behavioral and electrophysiological effects of apomorphine. Supported by NIDA DA 08349 and Hahnemann University RIG 990414.

664.6

ELEVATIONS AND PHASIC FLUCTUATIONS IN NUCLEUS ACCUMBENS DOPAMINE DURING IV COCAINE SELF-ADMINISTRATION. R. A. Wise,*[1] P. Newton,[2] K. Leeb,[1] B. Burnette,[2] D. Pocock,[1] and J. Justice.[2] Ctr Stud Behav Neurobiol Concordia Univ., Montréal, PQ, CANADA H3G 1M8[1] and Dept Chem, Emory University, Atlanta GA 30322[2].

Fluctuations in extracellular nucleus accumbens dopamine and DOPAC levels were monitored in 1-min microdialysis samples from rats engaged in intravenous cocaine self-administration. Dopamine levels were elevated to 200-600% of baseline during cocaine self-administration, fluctuating phasically between responses. Each injection caused a phasic, short-latency increase in dopamine levels, with a decrease prior to the next lever press. This pattern was seen regardless of whether the dose per injection was fixed or varied unpredictably within the sessions. The magnitude of the phasic fluctuations was on the order of 20% of the magnitude of the sustained within-session elevations; dopamine levels never fell close to baseline levels before the animal responded and received another injection. Fluctuations of the same magnitude and with the same time-course were seen when cocaine was injected independent of the behavior of the animals. DOPAC levels increased in the first minutes and stayed elevated during the period of drug availability; there was no significant recovery between injections. These data are consistent with the possibility that falling dopamine levels trigger successive responses in the intravenous cocaine self-administration paradigm, but they offer no support for the hypothesis that extracellular dopamine is depleted at the time of response initiation within a self-administration session.

664.7

ACUTE COCAINE ALTERS DOPAMINE SYNTHESIS IN THE NUCLEUS ACCUMBENS OF RATS PRETREATED WITH SALINE, BUT NOT COCAINE OR CPP. D. Johnson,* P. Buckley, M. Kozak, and D. Mokler. University of New England, College of Osteopathic Medicine, Biddeford, Me. 04005

A paradigm to develop rapid sensitization to the locomotor-activating effects of cocaine and the role of competitive NMDA receptor antagonists in rapid sensitization was tested. All rats receiving acute cocaine injections (15 mg/kg) showed higher activity counts ($P < .01$) compared to saline-injected controls. No differences were seen among rats receiving acute cocaine which were pretreated once daily for two days with either cocaine or the competitive NMDA receptor antagonist CPP (2 mg/kg). Neurochemical analysis of norepinephrine (NE), dopamine (DA), DOPAC, HVA, 5-HT, and 5-HIAA in brain homogenates showed decreased ($P < .05$) levels of DA and HVA in the nucleus accumbens (NAC) of cocaine injected rats pretreated with saline, compared to saline injected rats pretreated with saline. Neurochemistry in rats injected with cocaine and pretreated with cocaine, CPP, or both was not different from controls in any brain region examined. These results suggest that this paradigm is inadequate to produce locomotor sensitization to cocaine. However, acute cocaine in this model does produce changes in DA synthesis in the NAC of saline-pretreated rats, but prior exposure to cocaine, CPP, or both negates this effect. Further behavioral paradigms are being tested, using microdialysis to measure neurocorrelates.

664.8

PHASIC FIRING PATTERNS OF ACCUMBENS NEURONS ARE RELATED TO CONDITIONED STIMULI ASSOCIATED WITH DRUG REINFORCEMENT DURING COCAINE SELF-ADMINISTRATION IN RATS.

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We have previously reported that a subset of nucleus accumbens (NA) neurons exhibit phasic changes in firing rate during cocaine self-administration and water reinforcement in rats (Brain Res 626:14-22,1993; Neurosci Abst 19:1857,1993). Of 244 NA neurons recorded in 14 self-administering rats, 24% (58 cells) exhibited 4 distinctly different neuronal firing patterns time-locked to the drug reinforced response (0.33 mg cocaine/inf, 5.8 sec) in which drug infusion was paired with a tone-houselight CS complex (20 sec). The purpose of the present study was to investigate the role of the CS in the firing patterns of NA cells. NA phasic activity was not related solely to a pharmacological action of cocaine since it was absent during randomly initiated drug infusions (no CS). Results indicate that NA phasic activity could be triggered by the CS alone since similar patterned discharges occurred independent of drug delivery. However, although the CS was present on all trials, phasic activity time-locked to its occurrence did not emerge until after stable self-administration responding. This onset could be prolonged further within the session by: 1) decreasing the dose of cocaine (0.16, 0.08 mg/inf) and, 2) pretreatment with the dopamine D_1 antagonist SCH23390 (10 μg/kg). This indicates that a crucial level of dopamine in the NA must be achieved before the CS can elicit NA phasic activity. In addition, the timecourse of CS related NA firing to cyclic dopamine levels in the NA during the session suggests that phasic activity triggered by the CS does not reflect changes in dopamine levels.

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664.9

TOLERANCE TO COCAINE INDUCED ELEVATION OF EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS FOLLOWING SEVEN DAYS OF WITHDRAWAL. W.M. Meil*, J.M. Roll and R.E. See. Department of Psychology, Washington State University, Pullman, WA, 99164-4820.

Time-dependent changes in mesolimbic dopamine (DA) function are believed to play a role in behavioral sensitization and drug craving experienced during withdrawal from chronic cocaine administration. The present study utilized intravenous (i.v.) cocaine self-administration coupled with intracranial microdialysis in rats to investigate time dependent changes during withdrawal from chronic cocaine exposure. Following 2 weeks of i.v. cocaine self-administration, rats were allowed contingent access to cocaine at 1 and 7 days of withdrawal while extracellular levels of DA were measured from the nucleus accumbens (NAcc). A second group of animals received yoked, noncontingent cocaine for 2 weeks and were then administered noncontingent cocaine on days 1 and 7 of withdrawal. In addition, a third group of animals received 2 weeks of yoked saline followed by noncontingent cocaine 1 day after withdrawal. There were no significant differences between groups for the overall cocaine dosage or temporal pattern of infusions on days 1 and 7 of withdrawal. Basal extracellular DA concentrations did not differ between any treatment groups at either withdrawal time. Extracellular DA levels were increased throughout the session on both days; however, the increases at day 7 were significantly less than day 1 for both contingent and noncontingent conditions. DA overflow on day 1 did not differ between animals receiving chronic yoked cocaine or saline. These results suggest that tolerance to the DA-elevating effects of cocaine is not apparent early in withdrawal, but does develop by later time points. DA release in the NAcc may not be directly related to cocaine craving, since DA levels were attenuated after 7 days of withdrawal while responding for cocaine was unaltered.

664.10

RESPONSES OF NUCLEUS ACCUMBENS CELLS TO DOPAMINE AND METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ARE ENHANCED FOLLOWING CHRONIC COCAINE SELF-ADMINISTRATION IN RATS. S.R. White*, G.C. Harris, K.M. Imel and M.J. Wheaton. Dept. of Vet. & Comp. Anat., Pharm. and Physiol., Washington State Univ., Pullman, WA 99164.

Cells in the nucleus accumbens are sensitized to the inhibitory effects of dopamine for several weeks following chronic exposure to noncontingent injections of cocaine (Henry & White, J. Pharm. Exp. Ther., 1991, 258:882). The present study examined the effects of cocaine self-administration on responses of nucleus accumbens cells to dopamine and to methylenedioxymethamphetamine (MDMA). Rats pressed a lever in an operant chamber to receive cocaine infusions through cannulae implanted in the jugular vein. The average daily cocaine dose obtained by the cocaine self-administering rats was 31.4 $\pm$ 1.2 mg/kg, iv. Following 15 daily sessions of cocaine self-administration, access to cocaine was terminated. Electrophysiological experiments were conducted 1-11 days following termination of cocaine self-administration. Dopamine and MDMA produced dose-dependent inhibition of glutamate-evoked firing in the nucleus accumbens of both the cocaine-pretreated group and a saline-control group of animals. However, dopamine and MDMA had significantly greater inhibitory effects in the cocaine self-administration group than in controls. It is concluded that repeated cocaine exposure at doses that are self-administered sensitizes nucleus accumbens cells to the inhibitory effects of dopamine and of other drugs of abuse that elevate extracellular levels of dopamine in the accumbens. (Supported by DA-08116.)

664.11

ACUTE INTRAVENOUS ADMINISTRATION OF COCAINE AND AMPHETAMINE PREFERENTIALLY INCREASES GLUCOSE UTILIZATION IN THE SHELL OF THE NUCLEUS ACCUMBENS OF FREELY MOVING RATS.

F.E. Pontieri*, M. La Riccia, J.V. Faroni, V. Colangelo and F. Orzi. Dept. Neurosciences, University "La Sapienza", Roma (Italy).

The effects of the administration of psychomotor stimulant drugs in experimental animals depend on a series of factors. It has been previously demonstrated that intravenous (i.v.) but not intraperitoneal administration of cocaine in the rat produces increases in glucose utilization in portions of the mesocorticolimbic system, such as the nucleus accumbens (NACC) and the medial prefrontal cortex. Anatomical and biochemical differences have been reported within the NACC, with regard to the density of dopamine nerve terminals and the functional connections. In this study, the 2-[^{14}C]deoxyglucose method was applied to measure the effects of the acute i.v. administration of cocaine or amphetamine on glucose utilization in the shell and core of the NACC in freely moving rats. After catheterization of the femoral vessels, animals were treated with either cocaine (1 mg/kg), amphetamine (0.5 mg/kg) or saline. Computer-assisted image overlay was performed in order to identify the different portions of the NACC in histological sections. Administration of either cocaine or amphetamine produced increases in glucose metabolism in the shell of the NACC, with respect to control animals. These results provide an useful basis for the understanding of the neural substrates of the effects of reinforcing doses of psychomotor stimulant drugs.

664.12

ALTERATIONS IN DOPAMINE UPTAKE TRANSPORTER DENSITY IN COCAINE SELF-ADMINISTERING RATS APPEAR TRANSIENT.

J. H. Graham*, S. I. Dworkin and L. J. Porrino. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

The *in vitro* autoradiographic distribution of dopaminergic uptake transporter sites (DAT) was determined, using [^{3}H]mazindol (MAZ) as a ligand, in brain sections from Fisher 344 rats chronically self-administering (SA) cocaine (COC) (0.33mg/kg/inf) i.v., in daily 3hr sessions. Rats were sacrificed either immediately following a SA session, or after 4 days of withdrawal. Values for DMI insensitive [^{3}H]MAZ binding were compared to those from yoked saline controls similarly sacrificed. Although the distribution of MAZ binding sites in SA rats was decreased in both mesolimbic and nigrostriatal systems, DAT densities returned to levels at or above those found in untreated controls, following 4 days withdrawal. DAT levels in the accumbens (shell and core) and olfactory tubercle of withdrawal rats were elevated above control levels, whereas levels in other areas, e.g. caudate, SNc, rostral pole of accumbens, did not differ from controls. Therefore, adaptations in response to chronic COC SA within both the nigrostriatal and mesocorticolimbic dopaminergic systems may be transient in nature, and only persist as long as COC SA continues. *(Supported by NIDA grants P50 DA 06634 and DA 07522).*

664.13

POSSIBLE MECHANISMS OF BIPHASIC FLUCTUATIONS IN MESOLIMBIC DOPAMINE ACCOMPANYING COCAINE SELF-INJECTIONS: A VOLTAMMETRIC STUDY

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Cocaine is known to have a complex action on the mesolimbic dopamine (DA) system enhancing, due to reuptake inhibition, accumulation of pre-released DA and inhibiting, due to depression of DA cells, impulse-dependent DA release. Rapid biphasic fluctuations in nucleus accumbens DA-dependent signal have been reported in our previous electrochemical studies to accompany individual cocaine self-injections (Kiyatkin et al., 1992; Kiyatkin and Stein, 1994). Electrochemical signal gradually increased preceding the lever-press for cocaine, and abruptly (<20 sec) transiently (~2 min) decreased after drug injection. Although inhibiting action of cocaine on reuptake of released DA appears to determine the gradual signal increase preceding self-injections, the mechanisms of post-cocaine signal decreases are unknown. To test the contribution of presynaptic autoreceptor activation and local anesthetic action to the post-cocaine signal decreases, electrochemical signal changes were studied in cocaine-experienced rats during self-injections of the direct DA agonist apomorphine (APO; 25 µg/kg) and the local anesthetic procaine (PRO; 0.8-3.6 mg/kg). Signal decreases were found after APO self-injections, but they occured with a definite latency (90-100 sec) that is not compatible with the immediate signal decreases seen after cocaine. Gradual signal increases seen preceding cocaine self-injections were absent in this case. Thus, although activation of presynaptic DA autoreceptors may contribute to inhibiting action of cocaine on DA release, this relatively slow and long-term effect seems unlikely to mediate the immediate post-cocaine signal decreases. In contrast, an abrupt (<20 sec) signal decrease found after PRO self-injections implicate cocaine's anesthetic action as a possible contributor to the immediate post-drug signal depression. The combination of local anesthetic and reuptake inhibiting properties may underlie the unique abuse potential of cocaine in contrast to agents with only DA uptake inbiting properties (mazindol, nomifensine) or local anesthetic properties (lidocaine, procaine).

664.14

TRANSLOCATION OF DOPAMINE AND BINDING OF WIN 35,428 MEASURED UNDER IDENTICAL CONDITIONS IN RAT STRIATAL SYNAPTOSOMES.

C. Xu, L.L. Coffey, S.M. Lasley* and M.E.A. Reith, Dept. of Basic Sciences, University of Illinois College of Medicine, Peoria, IL 61656.

One strategy of searching for a cocaine antagonist is to look for a compound that potently inhibits binding of a cocaine-related radioligand to the dopamine transporter but does not affect dopamine translocation. The conditions commonly used for these assays are not the same, confounding data interpretation. In this study, identical conditions have been adopted for the parallel measurement of the binding of [^{3}H]WIN 35,428 and the translocation of [^{3}H]dopamine in crude synaptosomal preparations from rat striatum: 8-min incubation at 25°C with radioligand and inhibitor in phosphate uptake buffer containing Na^+, K^+, Mg^{2+}, Ca^{2+}, glucose, and nialamide. Binding IC_{50} values for a series of different uptake blockers (cocaine, WIN 35,428, benztropine, nomifensine, mazindol, methylphenidate, BTCP, Lu 19-005, GBR 12909, GBR 12935 and CGS 12066B) were on the average 1.4-fold higher than the uptake IC_{50} values. For slowly equilibrating inhibitors, uptake IC_{50}'s decreased by monitoring [^{3}H]dopamine uptake for 1 min only during the last min of the 8-min presence of inhibitor, and under these conditions the average binding over uptake IC_{50} ratio increased to 2.3. Kinetic calculations, taking into account both radioligand and inhibitor equilibration kinetics, indicated that the latter comparison between binding and uptake measurements was most relevant, and suggested the involvement of complexities beyond simple competitive inhibition of dopamine transport such as different binding domains for substrate and blocker recognition, or spare receptors for blockers. The present results indicate that binding over uptake IC_{50} ratios should be interpreted with caution depending on the experimental conditions used to measure these ratios. Supported by NIDA 03025.

664.15

LOCOMOTOR ACTIVATING AND REWARDING EFFECTS OF DOPAMINERGIC AGONISTS IN VENTRAL PALLIDUM OF RATS.

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The ventral pallidum (VP) is an output structure of nuc. accumbens. This anatomy suggests that VP may share functions with nuc. accumbens. Our research investigated whether a DA input to VP from ventral tegmental area has a role in locomotor activity and reward. We prepared rats with chronically indwelling cannulae in VP. Drug injections of 0.5 µl were bilateral. Locomotor activity was tested in 1 hr tests in Digiscan activity chambers. Amphetamine SO_4 (10 µg) and dopamine HCL (25 µg) increased activity. Activity was initially suppressed and subsequently elevated by cocaine HCL (12.5-100 µg); the initially suppressed activity was similar to that induced by procaine (50 µg). Place preference conditioning was conducted using a counterbalanced design pairing drug injection in VP with a distinct chamber for two 30 minute sessions. Amphetamine SO_4 (10 µg) produced a strong place preference conditioning. Cocaine HCL (50 µg) also increased time spent on the cocaine-paired side. These data indicate that dopaminergic transmission in VP may be involved in the locomotor and reward effects of psychostimulants, and suggest some effects of intracranially administered cocaine are due to local anesthesia.

664.16

OSCILLATORY EFFECTS OF COCAINE ON DOPAMINE EFFLUX IN RATS

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Variability in responsiveness to therapeutic and addictive drugs is a major problem in the replicability of pharmacological research involving both humans and laboratory animals. Recent data from our laboratory (Antelman et al, 1992) suggest that one source of variability may be the tendency of physiological systems to change their response to repeated drug treatment from a unidirectional to an oscillatory pattern. In the present study one injection of cocaine HCl (COC; 15 mg/kg, i.p.), 30 min. before sacrifice, increased in vitro amphetamine (AM; 10µM)-induced DA efflux from striatal slices, compared to controls. This increase was prevented when COC treatments were given 4 d and 30 min before sacrifice. This oscillatory pattern continued through 6 COC treatments (4 d apart). As each COC treatment was added, it reversed the effects of the preceding one. Most importantly, the direction of COC's effects was completely reversed over the 6 treatments. COC increased DA efflux by 53% when given only once but decreased it by 32% after 6 treatments. In a subset of rats, this oscillatory pattern also was seen for DA-efflux in the nucleus accumbens in response to 4 COC pretreatments. Supported by MH 24114 and DA 07546.

664.17

EFFECTS OF CHRONIC COCAINE ADMINISTRATION ON DOPAMINE TRANSPORTER mRNA LEVELS. S.R. Letchworth*, A. Hedgecock, L.J. Porrino. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157 USA.

Repeated administration of cocaine produces sensitization, as seen in increased locomotor behavior. Changes in the density of dopamine transporter protein have been reported in rat striatum after chronic cocaine exposure. The purpose of this study was to investigate possible changes in dopamine transporter mRNA as a result of chronic cocaine treatment. Male Sprague-Dawley rats were treated with 0, 10, 15, or 25 mg/kg cocaine, ip once daily for 8 days, and locomotor activity recorded. The levels of mRNA for the dopamine transporter were determined autoradiographically using *in situ* hybridization with an oligonucleotide probe specific for the transporter. The mean density/area was measured in the substantia nigra and ventral tegmental area. *In vitro* autoradiography using ^{3}H-mazindol to measure the density of the dopamine transporter protein will be carried out on tissue from the same animals. Cocaine treatment resulted in decreased levels of dopamine transporter mRNA by approximately 20% in both midbrain regions as compared to control levels. In contrast to the dose-dependent effects of repeated cocaine treatment on locomotor activity, however, the changes in dopamine transporter mRNA were not dose-dependent in either the substantia nigra compacta or ventral tegmental area. The lack of correlation between these two measures suggests that regulation of the dopamine transporter may not be critical for the expression of cocaine-induced sensitization. *Supported by NIDA Grant DA07522.*

664.18

RATS SELF-ADMINISTER PHENCYCLIDINE (PCP) AND MK-801, BUT NOT NOMIFENSINE, DIRECTLY INTO PREFRONTAL CORTEX. W. A. Carlezon, Jr.* and R. A. Wise Center For Studies in Behavioral Neurobiology, Concordia Univ., Montréal, Que, CANADA H3G 1M8

We have previously reported that rats will self-administer microinjections of MK-801 (a non-competitive NMDA receptor antagonist), nomifensine (a dopamine [DA] reuptake inhibitor), or PCP (a drug with both of these properties) directly into nucleus accumbens septi (NAS). Because others have reported that the DA reuptake inhibitor cocaine is self-administered into prefrontal cortex (PFC) but not NAS, we explored the possibility that these drugs would also be reinforcing when microinfused into PFC. Rats learned to lever press when given response-contingent microinfusions of PCP or MK-801 directly into PFC at the same concentration of each drug that was effective in NAS (12 nmoles and 1.2 nmoles per 120 nl infusion, respectively). However, when lever-pressing was reinforced with microinfusions of NOM into PFC at a concentration that increased responding when given into NAS (1.7 nmoles/inf), rates of responding were not higher than those of animals that received response-contingent microinfusions of vehicle. Higher concentrations of NOM were not tested due to poor solubility. These findings confirm that drug actions in the PFC can be rewarding but, taken by themselves, suggest that it is the action of PCP at the NMDA receptor rather than PCP-induced blockade of DA reuptake that accounts for its rewarding effects in this brain region. This suggestion—and the fact that NOM was not self-administered into PFC—remains to be reconciled with the previous report that cocaine is self-administered into PFC but not NAS.

664.19

BEHAVIORAL EFFECTS OF NOVEL 4'- and 4',4"-SUBSTITUTED-3σ-DIPHENYLMETHOXYTROPANE ANALOGS. J.L. Katz*, S. Izenwasser, A.C. Allen, A.H. Newman. Drug Development Group, Psychobiology Section, NIDA Intramural Research Program, NIH, Baltimore, MD 21224, U.S.A.

Cocaine-like behavioral effects of a series of 4'- and 4',4"-substituted-3σ-diphenylmethoxy-1αH,5αH-tropane analogs were assessed. These analogs of benztropine share structural features with cocaine and the dopamine uptake inhibitor, GBR 12909; they bind with high affinity to the dopamine transporter and inhibit dopamine uptake. Cocaine and GBR 12909 produce dose-related increases in locomotor activity of Swiss-Webster mice, whereas, of the diphenylmethoxytropane analogs tested, only the 4',4"-difluoro-substituted compound (AHN 1-055) produced increases in this activity that approached those of the reference drugs. In another study, rats were trained to discriminate saline from cocaine (10 mg/kg, ip). After cocaine injections, 20 consecutive responses on one of two levers produced food; after saline, responses on the other lever produced food. Once performances were stable, effects of various doses of cocaine, and of the diphenylmethoxytropane analogs were assessed during test sessions in which 20 consecutive responses on either lever produced food. Cocaine produced a dose-related increase in responding on the cocaine lever, reaching 100% at 10 mg/kg. Only AHN 1-055 (17 mg/kg) and the 4',4"-dimethoxy analog, AHN 1-057 (5.6 mg/kg), produced effects different from saline. These compounds produced maximum percentages of cocaine responding of 65 and 35, respectively. With AHN 1- 057, doses higher than 5.6 mg/kg produced less cocaine responding. With AHN 1-055, doses higher than 17 mg/kg could not be tested because they virtually eliminated behavior. These compounds represent the first tropane analogs that have structural and neurochemical similarities to cocaine, but are behaviorally distinct from all other known compounds that share this profile.

664.20

NOVEL 4'- and 4',4"-SUBSTITUTED-3α-DIPHENYLMETHOXYTROPANE ANALOGS ARE POTENT AND SELECTIVE DOPAMINE UPTAKE INHIBITORS. A.H. Newman*, R.H. Kline, A.C. Allen, S. Izenwasser, J.L. Katz. Drug Development Group, Psychobiology Section, NIDA Intramural Research Program, NIH, Baltimore, MD 21224.

A series of 4'- and 4',4"-substituted-3α-diphenylmethoxy-1αH,5αH-tropane analogs have been prepared as probes for the dopamine transporter. Initial studies showed that 4'-chloro-3α-diphenylmethoxytropane displaced [^{3}H]WIN 35,428 binding (K_i=30 nM) more potently than cocaine, inhibited dopamine uptake (IC_{50}=115 nM) and yet did not display a cocaine-like behavioral profile in locomotor activity or drug discrimination studies. Sensitivity to phenyl ring substitution in this series suggested an additional binding domain that might be exploited for the identification of potential cocaine antagonists. In order to further explore the structure activity relationships in this series of compounds, for binding to the dopamine transporter, inhibiting dopamine uptake and producing cocaine-like behavioral effects, an extended series of analogs, substituted in the *para*-position of one or both phenyl rings was prepared. These compounds were evaluated in radiolabeled binding assays for the dopamine transporter ([^{3}H]WIN 35,428), norepinephrine transporter ([^{3}H]desmethylimipramine), and the serotonin transporter ([^{3}H]citalopram). All of these compounds monophasically displaced [^{3}H]WIN 35, 428 binding in rat caudate-putamen with K_i values ranging from 11-2000 nM. None of the compounds displaced >60% of [^{3}H]desmethylimipramine binding (10 μM) or >66% of [^{3}H]citalopram binding (10 μM). Therefore, the most potent dopamine uptake inhibitors are highly selective for the dopamine transporter. Furthermore, since the parent drug benztropine is a potent muscarinic antagonist, these compounds were also evaluated for binding to muscarinic-m_1 receptors ([^{3}H]pirenzepine) and muscarinic-m_2 receptors ([^{3}H]AF-DX 384). All of the the analogs displayed moderate to high affinity for the muscarinic-m_1 sites (K_i range =1-100 nM) and variable affinities for the muscarinic-m_2 sites (K_i range =2-1500 nM). Structure activity relationship trends are evident and distinct for each of the binding sites and suggest that it will be possible to prepare more selective ligands in the future.

664.21

LOCOMOTOR STIMULANT ACTION OF THE DOPAMINE UPTAKE INHIBITORS GBR-12935 AND GBR-12909: ADDITIVE AND NON-ADDITIVE INTERACTIONS WITH COCAINE. D. Krug*, M.J. Forster, M. Stokely, and J. Ebe. Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

A study was conducted to compare the locomotor stimulant properties of cocaine with the dopamine uptake inhibitors GBR 12909 and GBR-12935. Horizontal locomotor activity of Swiss Webster mice was recorded for 1 h in a Digiscan apparatus following i.p. injection of each drug alone or in combination with 5 to 20 mg/kg cocaine. By themselves, both compounds produced stimulation of locomotor activity with ED_{50} values of 5.8- and 5.4-mg/kg for GBR-12909 and GBR-12935, respectively. Maximal motor stimulation produced by these drugs was nearly equivalent to that produced by cocaine, and persisted for at least 1 hour following injection. In interaction studies, GBR-12935 produced a dose-related leftward shift in the inverted U-shaped cocaine dose effect curve, whereas, GBR-12909 produced a leftward shift of only the ascending portion. Maximal doses of GBR-12909 failed to influence the response to 20 mg/kg cocaine. These results indicate a non-additive influence of GBR-12909 and cocaine in the modulation of locomotor activity. [Supported by U.S.D.H.H.S.-P.H.S. contract N01-DA-2-9305.]

664.22

THE RELATIONSHIP OF COCAINE CONCENTRATION IN BRAIN TO BEHAVIOR: EVIDENCE FOR THE PREFRONTAL CORTEX AS THE INITIATING SITE FOR THE COCAINE LOCOMOTOR STIMULANT EFFECT. Robert J. Carey*, Ernest N. Damianopoulos and Gail P. De Palma. Psychiatry, SUNY Health Science Center and Research and Development Service -151, VA Medical Center, Syracuse, NY 13210

The behavioral stimulatory effects of cocaine have been linked to cocaine inhibition of dopamine transporter mechanisms in the prefrontal cortex, neostriatum, and limbic areas. In the context of behavioral studies, however, rarely have the cocaine concentrations been measured in these brain structures. Using a recently developed assay procedure, we examined the effects of IP cocaine (10 mg/kg) administration upon locomotor behavior and the concentration of cocaine in the medial prefrontal cortex, neostriatum and limbic-olfactory brain areas. Cocaine concentrations were found to be highest in the cortex followed by the neostriatum and limbic area. Significantly, however, locomotor stimulation effects were reliably correlated only with cocaine concentration in the medial prefrontal cortex. This observation suggests that the medial prefrontal cortex may be the critical initiating site for the mechanisms mediating the cocaine stimulatory effects on locomotor behavior.

664.23

COMPARISON OF COCAINE AND COCAETHYLENE IN THE VERVET MONKEY: PLASMA PHARMACOKINETICS AND EFFECTS ON EXTRACELLULAR DA. C.W. Bradberry*, J.B. Nobiletti, R. Iyer, P. Jatlow. Yale Univ. Sch. of Med., Depts. of Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, West Haven, CT 06516.

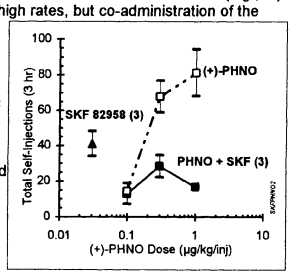
Cocaethylene is a psychoactive metabolite resulting from concurrent cocaine and ethanol consumption, during which a transesterification converts the methyl ester of cocaine to an ethyl ester. It is more selective than cocaine in that it has a lower affinity for the 5-HT and noradrenergic uptake sites than cocaine. This, and the fact that it can be administered to humans in a clinical setting, makes it a unique tool for investigating drug reward mechanisms. Previous work has demonstrated equivalent actions of the two drugs at the dopamine uptake site in the rat both in vivo and in vitro. We have used microdialysis to compare the effect of 1.5 μmol/kg (equivalent to 0.5 mg/kg cocaine-HCl) cocaine and cocaethylene on extracellular DA in the caudate of the anesthetized vervet monkey following i.v. administration. Samples were collected at 5 minute intervals following drug administration. Concurrent plasma samples were collected for pharmacokinetic analysis. Data were fit to an open two compartment model using weighted non-linear fitting (by PC Nonlin ver. 4). Microdialysis results indicated that both drugs caused an identical four-fold increase in extracellular DA following i.v. administration which peaked in the sample collected during the 5-10min post drug period. Pharmacokinetic analysis indicated the terminal elimination half-lives were 31 ± 3 for cocaine, and 53 ± 4 for cocaethylene, $p < 0.01$. However, because of a larger steady state volume of distribution for cocaethylene (3.7 ± 0.2 l/kg vs 2.5 ± 0.4, $p < 0.03$), the clearances were not different. Supported by DA 08073, DA 08227, DA 04060, The Yale VA Alcoholism Research Center, and a NARSAD Young Investigator Award to CWB.

DRUGS OF ABUSE: COCAINE—DOPAMINE RECEPTORS

665.1

COCAINE SELF-ADMINISTRATION PATTERNS MAINLY REFLECT D_1 NOT D_2 OR D_3 RECEPTOR ACTIVATION. J.D. Belluzzi*, S. Kossuth, O. Chan, N. Khamamkar, Q. Nguyen and L. Stein, Dept. of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

Cocaine self-administration (S-A) is characterized by precisely-spaced response patterns and an "inverted-U" dose-response curve. These characteristics may be duplicated in S-A of D_1 agonists (Self & Stein, 1992), but not in D_2 agonist S-A which is characterized by erratically spaced responding and a positively accelerating dose-response curve (Belluzzi *et al.* 1993). Here we report that co-administration of a low dose of a D_1 agonist converts erratic D_2-agonist S-A into cocaine-like and D_1-like response rates and patterns. Rats were trained to bar press for i.v. cocaine (750 μg/kg/inj) in daily 3-hr sessions. After baseline rates had stabilized, different doses of PHNO were substituted for cocaine. PHNO alone (Fig., □) was self-administered erratically at high rates, but co-administration of the D_1 agonist SKF 82958 (3 μg/kg/inj) with PHNO (■) induced low-rate responding similar to that seen with the D_1 agonist alone (▲). When combined with SKF 82958, other D_2 (quinpirole, N-0923) or D_3 (7-OH-DPAT) agonists gave similar results. Our data indicate (1) that cocaine S-A patterns are reproduced by S-A of D_1 but not D_2 or D_3 agonists, and (2) that D_1 patterns predominate when D_1 and D_2-like agonists are co-administered.



(Supported by NIDA grant DA07747)

665.2

EFFECT OF COCAINE ON cAMP-ADENYLATE CYCLASE SYSTEM IN RAT STRIATUM. S.K. Sahni, J.M. Davis and J.I. Javaid*. Illinois State Psychiatric Institute, University of Illinois at Chicago, Chicago, IL 60612.

Repeated low doses or a single high dose of cocaine administration produces behavioral sensitization in rats. Also, repeated cocaine administration in rats has been reported to increase forskolin-stimulated adenylate cyclase (AC) activity in nucleus accumbens. In the present study, we examined the effects of cocaine on the formation of cAMP induced by dopamine and a D_1-selective agonist (through D_1 receptors), NaF (via G protein) and forskolin (acting directly on catalytic unit of AC). Adenylate cyclase activity in striatal membranes was measured by [^{32}P]-cAMP formation using [^{32}P]-ATP as a substrate. Acute administration of cocaine (10 mg/kg or 40 mg/kg) did not significantly affect cAMP formation 1 hr or 24 hrs post injection. However, when rats were pretreated with a single injection of high dose (40 mg/kg) and then administered a cocaine challenge (10 mg/kg), a significant decrease (about 30%) was observed in the formation of cAMP in the striatum induced by stimulation with D_1 agonist. Similarly, a trend towards decreased formation of cAMP was observed in dopamine-sensitive activity, although it did not reach statistical significance. Since under these conditions of cocaine treatment behavioral sensitization is also manifested in rats, present findings raise the possibility that an inhibition of D_1-mediated AC activity in the striatum may play a role in cocaine-induced behavioral sensitization.

665.3

SYSTEMIC ADMINISTRATION OF SCH-23390 ATTENUATES LOCOMOTION ELICITED BY INTRA-ACCUMBENS COCAINE. L.E. O'Dell*, R. A. Fuchs, T.V. Khroyan, and J.L. Neisewander, Dept. Psychology, Arizona State University, Tempe, AZ 85287-1104.

We have previously demonstrated that intra-accumbens injections of the D2-like receptor antagonist sulpiride potently block locomotion induced by systemic administration of cocaine, whereas intra-accumbens injections of the D1-like receptor antagonist SCH-23390 only block this effect at a high dose. The present study further assessed the involvement of D1-like receptors by examining the effects of systemic SCH-23390 administration on locomotion elicited by intra-accumbens cocaine. Rats received systemic injections of SCH-23390 (0, 0.03, 0.3, 1.0 mg/kg, i.p.) and 10 min later received bilateral injections of either saline or cocaine (100 μg/0.5 μl/side) into the NAc. Locomotion and stereotypic behaviors were then measured for 60 min. This procedure was repeated twice at 6-day intervals. The two highest doses of SCH-23390 decreased cocaine-induced locomotion, as well as baseline locomotion. The magnitude of cocaine-induced locomotion did not change across repeated intra-accumbens administrations. Stereotypic behaviors were not observed following acute or repeated intra-accumbens cocaine administration. In summary, although intra-accumbens SCH-23390 does not potently block locomotion produced by systemic cocaine administration, systemic SCH-23390 potently blocks locomotion produced by intra-accumbens cocaine administration. These findings suggest that D1-like receptors in brain regions other than the NAc are involved in mediating cocaine-induced locomotion. (Supported by DAO7730.)

665.4

EFFECT OF D_2 RECEPTOR ANTISENSE IN THE NUCLEUS ACCUMBENS ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN THE RAT. A. McGregor and D.C.S. Roberts*. Life Sciences Research Centre, Carleton University, Ottawa, K1S 5B6, Canada.

These experiments examined whether an antisense oligonucleotide directed against D_2 receptor mRNA in the nucleus accumbens of the rat could disrupt intravenous cocaine self-administration behaviour. Rats were trained to self-administer cocaine (1.5 mg/kg/inj IV) under a progressive ratio (PR) schedule of reinforcement and then implanted with intracerebral cannulae above the nucleus accumbens. Following re-establishment of stable self-administration behaviour, three injections of the antisense or randomised antisense sequence were administered at 12 hour intervals. Phosphorothioated at all positions, the antisense and randomised antisense sequences were delivered in a saline vehicle (2.5 nmol/1.0 μl/ injection). A large and significant decrease in break point (BP) was produced following antisense treatment, which took five to six days to return to baseline BP. In contrast, treatment with randomised antisense produced a much smaller decrease in BP, which showed a rapid return to pretreatment BP. Furthermore, antisense treatments had no effect on operant responding for food reinforcement under a fixed ratio (FR20) schedule. These results suggest that injection of D_2 antisense into the nucleus accumbens caused large reductions in the reinforcing properties of cocaine as measured under the PR self-administration schedule. The lesser effect of the randomised sequence may be due to nonspecific behavioural deficits following such treatment, and indicates that attention must also be given to the nonspecific effects of such oligonucleotide treatments. Supported by NIDA Contract No. NO1DA-3-7302 and MRC of Canada.

665.5

DIFFERENTIAL EFFECTS OF INTRA-ACCUMBENS SULPIRIDE ON COCAINE-INDUCED LOCOMOTION AND CONDITIONED PLACE PREFERENCE. D.A. Baker*, L.E. O'Dell, T.V. Khroyan, J.L. Neisewander, Department of Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

The effects of intra-accumbens administration of sulpiride on the stimulant and reinforcing effects of intravenous (IV) cocaine were investigated using the conditioned place preference (CPP) paradigm. Adult male Sprague-Dawley rats received 3 conditioning trials which consisted of 30-min exposures to two distinctive compartments on consecutive days. On one day of the trial, the rats received bilateral injections of saline or sulpiride (0.05 or 0.2 µg/ 0.5 µl/ side) into the nucleus accumbens (NAc). Fifteen min later, the rats were placed into a compartment and immediately injected with either saline or cocaine (4.2 mg/kg, IV). On the alternate day, rats received sham intra-accumbens injections and 15 min later were placed into the other compartment. Locomotion, stereotypies, and grooming were measured on the first and last trial. Cocaine produced an increase in locomotion which was attenuated by both doses of sulpiride. Cocaine also produced an increase in sniffing and headbobbing, and a decrease in grooming. These behaviors were not affected by either dose of sulpiride. Headbobbing was the only behavior sensitized by repeated administration of cocaine, and this sensitization was blocked by the high dose of sulpiride. Cocaine produced a robust CPP that was not altered by either dose of sulpiride. Conditioned locomotion was evident following saline injections in the drug-paired environment, and acquisition of this response was blocked by the high dose of sulpiride. These findings suggest that D2-like receptors in the NAc mediate cocaine-induced locomotion, but not CPP. This research was supported by DAO7730.

665.6

EXAMINATION OF THE NOVEL BENZAMIDE ANALOGS IN THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE IN RATS. A.K. Singha* and L.L. Hernandez. V.A. Medical Center & University of South Carolina, Columbia, S.C. 29201.

The present study examined the effects of two novel benzamide analogs, MABN and MBP, on the discriminative stimulus effects of cocaine. Both MABN and MBP are D_2 receptor antagonists. Rats were trained to discriminate i.p. injections of cocaine (10 mg/kg) from saline (1 ml/kg) in a two-lever drug discrimination paradigm. When rats (n=5) were given MABN (0.1 mg/kg)in combination with cocaine, responding on the drug lever was attenuated to 65%, while a higher dose of MABN (0.4 mg/kg) produced 73% responding on the cocaine associated lever. Likewise, rats (n=7) given MBP (0.1 mg/kg) displayed a partial blockade (50% drug-lever responding) of the cocaine cue. At the doses tested, animals' rate of response was not significantly reduced. These data support the role of dopamine (D_2 receptors) in mediating the subjective effects of cocaine.

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665.7

THE EFFECTS OF PARTIAL DOPAMINE D_2 AGONISTS ON SELF-ADMINISTRATION OF COCAINE ON A PROGRESSIVE RATIO SCHEDULE IN RATS. R. Ranaldi*, G. Vickers and D.C.S. Roberts. Life Sciences Research Center, Carleton University, Ottawa , Canada K1S 5B6

There now exists considerable evidence showing that the rewarding effects of cocaine depend on dopamine (DA) neurotransmission. Thus, drugs that interfere with DA function may potentially be beneficial in the treatment of cocaine addiction. Electrophysiological and biochemical studies have demonstrated that the intrinsic efficacy of partial D_2 receptor agonists can be rank-ordered from greatest to least as follows: B-HT 920, SND 919, (-)-3-PPP, SDZ 208-912, SDZ MAR 327. We investigated the effects of these partial D_2 receptor agonists on cocaine self-administration in rats. Subjects were trained to intravenously self-administer each of four unit doses of cocaine (0.075, 0.15, 0.3 and 0.6 mg/injection) on a progressive ratio (PR) schedule and the effects of B-HT 920 (0.25 mg/kg), SND 919 (0.1 mg/kg), (-)-3-PPP (1.25 mg/kg), SDZ 208-912 (0.25 mg/kg) and SDZ MAR 327 (0.25 mg/kg) on the breaking points at each unit dose of cocaine were investigated. The results demonstrated that drugs with the least intrinsic activity (SDZ 208-912 and SDZ MAR 327) decreased breaking points for cocaine. These drugs also disrupted food-rewarded responding on a PR schedule. The drugs with greatest intrinsic activity (B-HT 920 and SND 919) did not affect or **increased** breaking points for cocaine reward. Thus, the effect of these partial agonists on breaking points for cocaine reward is positively correlated with their intrinsic activity at the D_2 receptor. The data suggest that there may be a point on the continuum where agonists may decrease breaking points for cocaine but not disrupt food-rewarded responding. (Supported by NIDA Contract NO1DA-3-7302).

665.8

THE EFFECTS OF THE D-3 RECEPTOR AGONIST 7-OHDPAT ON COCAINE SELF-ADMINISTRATION MAY BE MEDIATED POST-SYNAPTICALLY. L. H. Parsons*, S.B. Caine, G.F. Koob and F. Weiss, Department of Neuropharmacology (CVN-15), The Scripps Research Institute, La Jolla, CA 92037.

Recent work from this laboratory has demonstrated that the dopamine (DA) D-3 receptor-selective agonist 7-OHDPAT potentently decreases cocaine self-administration in the rat at doses that are not themselves reinforcing (Caine et al., 1993, Science, 260, 1814-1816). In the present experiment, the effects of 7-OHDPAT on extracellular DA in the nucleus accumbens during cocaine self-administration were examined using in vivo microdialysis. Rats were trained to self-administer cocaine (0.25 mg/inf) on an FR-5 schedule of reinforcement until stable baseline responding was achieved. On the test day, nucleus accumbens dialysate samples (0.3 ul/min flowrate) were collected during a self-administration session in which rats were allowed to self-administer cocaine (0.25 mg/inf), a combination of cocaine (0.25 mg/inf) and 7-OHDPAT (4.0 ug/inf), 7-OHDPAT alone (4.0 ug/inf), and saline during four consecutive 1.5 hour periods. The baseline dialysate DA concentration was 4.9±0.7 nM (n = 5). During cocaine self-administration, DA levels rose to 373±18% of baseline, with an average of 2.3±0.2 cocaine infusions per ten minutes. When the drug solution was switched to cocaine and 7-OHDPAT, the number of self-administered drug infusions decreased to 1.1±0.3 infusions per ten minutes. During this period dialysate DA concentrations decreased to a stable level of 134±8% of baseline. Almost immediately after switching the drug solution to 7-OHDPAT alone, the number of self-administered infusions increased to an average of 9.1±0.3 infusions per ten minutes. Despite this increased responding for drug, dialysate DA levels decreased to a stable level of 37±6% of baseline. Operant responding extinguished within 90 minutes of switching the drug solution to saline, during which time dialysate DA did not rise above 40% of baseline. The decrease in nucleus accumbens DA efflux coupled with the increased responding during the self-administration of 7-OHDPAT alone suggest that the effects of 7-OHDPAT on cocaine self-administration may be mediated post-synaptically.

665.9

DOPAMINE D_3 RECEPTOR AGONISTS PARTIALLY REPRODUCE THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE. Roger D. Spealman*, Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772-9102

Dopamine agonists with reported selectivity for the D_3 subtype of dopamine receptor were studied for their capacity to reproduce the discriminative stimulus effects of cocaine in squirrel monkeys. Monkeys were trained to discriminate cocaine (0.3 mg/kg) from vehicle using a conventional two-lever choice procedure and subsequently were tested with a range of doses of 7-hydroxy-dipropylaminotetralin (7-OH-DPAT; 0.0001 - 0.1 mg/kg), 2-(N-phenylethyl-N-propyl) amino-5-hydroxytetralin (N-0434; 0.001 - 0.18 mg/kg), quinpirole (0.003 - 0.3 mg/kg) and quinelorane (0.01 - 0.3 mg/kg). Each drug engendered dose-related partial substitution for cocaine, reaching average maximums of 60 - 80% cocaine-appropriate responses after the highest doses tested. The degree of cocaine substitution engendered by the D_3 agonists was comparable to that reported previously for D_1 and D_2 agonists. The results are consistent with the view that D_3 receptors in addition to other dopamine receptor subtypes play a significant role in mediating the discriminative stimulus effects of cocaine in monkeys. Supported by DA00499, DA03774 and RR00168.

665.10

EFFECTS OF DOPAMINE ANTAGONISTS ON CONDITIONED AND UNCONDITIONED RESPONDING DURING A MULTIPLE SCHEDULE OF FOOD AND COCAINE SELF-ADMINISTRATION IN RATS R. Weissenborn*, G.F. Koob and F. Weiss Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037

The present series of experiments sought to examine the effects of selective D1 and D2 dopamine receptor antagonists (SCH 23390 and raclopride) and a D2 partial agonist (SDZ 208-911) on food and cocaine self-administration, as well as on responding for environmental cues associated with the primary reinforcers. Rats were trained on a multiple schedule during which lever-pressing was reinforced with a food pellet and simultaneous presentation of a tone, or with a cocaine infusion and presentation of a stimulus light. Each session was preceded by a 5 min component during which lever presses resulted in presentation of the respective cues only. Non-contingent delivery of food or cocaine prior to this initial component resulted in a significant shift in preference for the tone or light respectively, suggesting that the cues had taken on the role of conditioned reinforcers with incentive-motivational value. Systemic administration of SCH 23390 (10 ug/kg) and raclopride (100 and 200 ug/kg) blocked, while SDZ 208-911 (0.025 - 1.6 mg/kg) enhanced the preference shift induced by non-contingent delivery of cocaine. SCH 23390 and raclopride non-selectively suppressed responding following non-contingent food. During the multiple schedule, SCH 23390, raclopride and SDZ 208-911 decreased the inter-reinforcer intervals for cocaine, and at higher doses increased those for food. These data suggest that low doses of D1 and D2 antagonists and a D2 partial agonist selectively block the reinforcing effects of cocaine but not food under the schedule parameters used here. In addition, selective dopamine antagonists can attenuate cocaine-induced responding for a conditioned reinforcer, implicating both D1 and D2 receptor mechanisms in mediating conditioned stimulus-reward associations. This work was supported by NIDA grants DA 07348, 08467, 04398 (FW and GFK).

665.11

REDUCTION OF THE REINFORCING PROPERTIES OF COCAINE BY A PARTIAL DOPAMINE AGONIST. L. Pulvirenti*, D. Smith and G.F. Koob Dept. of Neuropharmacol., Scripps Res. Inst., La Jolla, Ca 92037 and **Mondino-Tor Vergata" Ctr for Exp Neurobiol, Un. of Rome "Tor Vergata", Rome, Italy

Partial dopamine agonists are a recently characterized novel class of compounds acting at the dopamine receptor site with high affinity and low intrinsic activity. These drugs act as functional antagonists in conditions of high dopamine tone, while they show an agonistic profile in conditions of dopamine depletion (e.g. denervation). Since the reinforcing properties of cocaine seem to depend upon activation of forebrain dopamine neurotransmission, the aim of the present study was to evaluate the effects of acute pretreatment with SDZ 208-911, an aminoergoline with partial dopamine agonistic activity, in rats trained to self-administer cocaine IV (0.75 mg/kg/injection). SDZ 208-911 (0.025-1.6 mg/kg IP) dose-dependently reduced the reinforcing properties of cocaine as shown by a reduction of the inter-reinforcement interval in rats self-administering cocaine IV with limited daily access. The pattern of responding was similar to that of animals self-administering a lower dose of cocaine (0.37 mg/kg/injection) and to that of animals pretreated with a dopamine receptor antagonist. Cocaine abuse in humans is characterized by intake of high amount of drug followed by withdrawal. Since partial dopamine agonists also show an agonistic profile in conditions of low dopamine tone (i.e. cocaine abstinence), the potential use of these compounds for effective pharmacological intervention during the various phases of cocaine addiction is a hypothesis worth testing.

665.12

INCREASED SENSITIVITY TO THE LOCOMOTOR DEPRESSANT EFFECTS OF A DOPAMINE ANTAGONIST DURING COCAINE WITHDRAWAL IN THE RAT B.A. Baldo* and G.F.Koob Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Clinical studies have identified a behavioral syndrome, occurring in stimulant abusers after prolonged cocaine binges, which is characterized by psychomotor depression and anhedonia. This "cocaine withdrawal syndrome" has been hypothesized to contribute to recidivism in cocaine users; accordingly, much effort has been dedicated to the development of animal models of cocaine withdrawal in order to elucidate its underlying mechanisms. Recent studies in this laboratory have determined that thresholds for rewarding brain stimulation are elevated following prolonged self-administration sessions in the rat. The post-cocaine threshold elevation was reversible by the dopamine receptor agonist bromocriptine, suggesting that impaired dopaminergic function may contribute to cocaine withdrawal. In order to determine additional behavioral correlates of cocaine withdrawal, and to further study the role of the dopamine system in cocaine withdrawal, spontaneous locomotor activity was measured in photocell testing cages 4 h following 12 h cocaine self-administration sessions. Prior to locomotor activity testing, cocaine-exposed and control animals were injected with saline, or 0.05 mg/kg cis-flupenthixol, a dopamine receptor antagonist. Locomotor activity was found to be significantly depressed in cocaine-exposed animals, but not control animals, treated with cis-flupenthixol. In a separate experiment, doses of cis-flupenthixol as high as 0.4 mg/kg failed to produce significant locomotor depression in drug-naive animals. It is hypothesized that dopamine receptor blockade by a low dose of cis-flupenthixol may interact with cocaine-induced neurochemical changes to produce locomotor depression during cocaine withdrawal. Supported by DA04398 (GFK) and an NSF Predoctoral Fellowship (BAB).

665.13

SENSITIZATION TO COCAINE SELF-ADMINISTRATION FOLLOWING CHRONIC *cis* (Z)-FLUPENTIXOL R.L. Peltier* and M.W. Emmett-Oglesby. Dept. of Pharmacology and S.A.I.N.T, UNTHSC, Fort Worth, TX 76107.

Acute pretreatment with the D_2/D_1 antagonist, *cis* (Z)-flupentixol (FLU), results in an increase in the rate of cocaine self-administration (e.g. Ettenberg *et al., Psychopharmacology.* **78**: 204, 1982). The present study investigated the effects of acute as well as chronic administration of FLU on the dose-effect curve for cocaine self-administration. Rats were implanted with chronic indwelling jugular catheters. Following implantation, they were allowed to self-administer cocaine (0.25 mg/injection) on a fixed-ratio two (FR2) schedule of reinforcement, 15 reinforcers a day, until baseline responding was stable. Using a multi-dose procedure, a dose-response curve for each rat was then obtained. Rats were then assigned to one of two groups. One group received a 3 hr pretreatment with FLU (0.032, 0.1, or 0.32 mg/kg, i.p.), followed by a multi-dose test. The other group was treated with FLU chronically (0.32 mg/kg/12 hr; s.c.). The chronic regimen lasted for five days, during which rats did not have access to cocaine self-administration. Seventy-two hours after the last chronic injection, cocaine dose-response curves were re-obtained. Acute treatment with FLU produced a dose-dependent shift to the right of the cocaine dose-response curve. In contrast, three days after terminating chronic FLU, a 2-fold shift to the left of the dose-response curve was obtained. The present data suggest that acute dopamine blockade results in a decrease in the reinforcing properties of cocaine as observed by a shift to the right of the cocaine dose-response curve. In contrast, three days after terminating the chronic blockade of dopamine receptors by FLU, there was an increase in the reinforcing properties of cocaine as observed by a shift to the left of the cocaine dose-response curve. Supported by NIDA RO1 4137.

665.14

DOPAMINE RECEPTOR BLOCKADE ALTERS PREPRODYNORPHIN AND ZIF/268 mRNAs IN RATS THAT "BINGE" ON COCAINE J.B. Daunais* J.Q. Wang, W.T.Bohler, D.C. Mayer, and J.F. McGinty Dept. Anat. & Cell Bio., East Carolina University School of Medicine, Greenville, NC 27858-4354

A single injection of cocaine increases *c-fos and zif/268* mRNAs whereas repeated cocaine increases dynorphin immunoreactivity (Dyn-ir) and preprodynorphin (PPD), but not preproenkephalin (PPE), mRNA in rat striatum. Cocaine's effects on *c-fos* and *zif/268* mRNAs, and Dyn-ir, are blocked by the dopamine (DA) D_1 receptor antagonist, SCH23390. However, the effects of cocaine "bingeing" on *c-fos* and *zif/268* mRNAs, or of DA D_2 receptor blockade on *c-fos, zif/268,* and PPD mRNAs after "bingeing" are unknown. In an effort to answer these questions, thirty adult, male Wistar rats were injected i.p. with SCH23390 (0.5 mg/kg) or the D_2 receptor antagonist, sulpiride (50 mg/kg) 30 min prior to 3 hourly injections of i.p. saline or 20 mg/kg cocaine for 1 day. One hr after the final injection, the rats were anesthetized and decapitated. Sections were collected through the anterior striatum at the level of the nucleus accumbens, and hybridized with 40mer oligonucleotides coding for *c-fos* or *zif/268*, or 48mers coding for PPD or PPE. Quantitative image analysis of autoradiograms revealed that SCH23390 completely blocked the cocaine-induced increase in striatal *zif/268* and PPD mRNAs. Furthermore, SCH23390 alone significantly decreased the basal levels of *zif/268* and PPD mRNAs. Sulpiride completely blocked cocaine-induced PPD mRNA and significantly decreased the induction of *zif/268* mRNA in the striatum. Sulpiride alone decreased basal levels of PPD mRNA but enhanced the expression of *zif/268* mRNA as compared to basal levels in saline-treated rats. C-fos mRNA was undetectable and PPE mRNA remained unchanged by the repeated administration of cocaine. These data demonstrate that 1) the D_2 receptor plays a substantial role in modulating the tonic expression of PPD mRNA in the striatum, a role attributed primarily to the D_1 receptor, 2) PPD and *zif/268* gene expression is modulated by both D_1 and D_2 receptors following a cocaine "binge". Supported by DA 03982.

665.15

COCAINE INDUCED LOCOMOTOR HYPERACTIVITY IN THE RAT IS REDUCED BY QUINACRINE. Kevin H. Souza, Aaron J. Janowsky*+, Malcolm S. Reid , S. Paul Berger. UCSF/VAMC, Psychiatry Services 127, 4150 Clement St., San Francisco, CA 94121; + Res Svc 151-PP, VAMC, 3710 SW Veterans Hosp. Rd., Portland, OR 97201

Quinacrine is a phospholipase A2 (PLA2) inhibitor which is clinically used for the treatment of malaria. As a PLA2 inhibitor one of its main effects is the reduction of arachidonic acid levels. The release of arachidonic acid has been suggested to play a role in dopamine D2 receptor signal transduction mechanisms. In the present study we have investigated whether quinacrine has any effects on the acute locomotor and stereotypic behavioral responses to cocaine in Sprague-Dawley rats. Animals were pretreated with quinacrine (16 mg/kg, i.p.) 15 min prior to cocaine (30 mg/kg, i.p.) administration and locomotor activity and stereotypy were scored every 10 min for 1 hr in an Actimex box. Pretreatment with quinacrine reduced the cocaine stimulation of locomotor activity by approximately 30%, but had no effect on the stereotypic behavioral response. Quinacrine alone had no effect on locomotion and stereotypy. The ability of quinacrine to modulate caffeine induced locomotor stimulation was also tested, and it was found that quinacrine had no effect on caffeine. These findings suggest that the ability of quinacrine to reduce drug induced stimulation of locomotion is mediated via the dopamine system. Further studies on the behavioral effects of selective D1 and D2 receptor agonists are underway.

666.1

COMBINED SPECT AND QUANTITATIVE EEG STUDIES IN COCAINE ABUSERS. J.W. Crayton*, L.M. Konopka, T. Milo, E. Barnes, and P. Shirazi. Biological Psychiatry Section and Nuclear Medicine Service, Hines VA Hospital, Hines, IL 60141.

To examine the relationship between cerebral blood flow as measured by HMPAO-SPECT and brain electrical activity as assessed by quantitative EEG (qEEG), we studied the pattern of distribution of blood flow and EEG activity in 9 chronic cocaine abusers and compared the results with nine normal controls. HMPAO was injected for SPECT while the subjects were continuously monitored for EEG activity under carefully controlled conditions. Using a novel data-reduction algorithm, SPECT data from the nine subjects could be reduced to a single set of group images for comparison with the normal control group. All of the cocaine abusers had abnormal SPECT scans. Using subtraction topographic maps, cocaine abusers showed areas of both hyperperfusion and hypoperfusion. Hyper-perfusion was particularly notable in frontal regions. This hyperperfusion contrasts with other studies in which only areas of hypoperfusion were noted. In addition, there appear to be small right-left asymmetries in the perfusion pattern.

Quantitative EEG studies of the cocaine abusers showed increased total alpha power compared to the controls. The increase in alpha activity came from two sources: first, a widening of the alpha peak indicating a spreading out of the distribution curve of alpha frequencies within the alpha band; and second, a significant penetration of the normally occipital alpha activity into more frontal regions.

Combining SPECT and qEEG revealed a strong correlation between increased frontal alpha activity and frontal hyperperfusion as assessed by SPECT.

666.2

REGIONAL BRAIN BLOOD FLOW DURING INDUCED COCAINE CRAVING A.R. Childress, D. Mozley, J. Fitzgerald, M. Reivich, J. Jaggi, and C.P. O'Brien*. Depts. of Psychiatry and Radiology, Univ. of Penn. School of Medicine, and Philadelphia VA Medical Center, Philadelphia, PA 19104

Human cocaine users can experience profound drug desire when they encounter cues (other drug users, drug-buying or drug-using locations, drug paraphernalia, etc.) which remind them of cocaine, but little is known about the brain correlates of this state. Clinically, cue-induced cocaine craving is often accompanied by a number of signs and symptoms similar to the effects of cocaine itself, including generalized arousal, palpitations, light-headedness, ear-ringing, chest-tightness, the 'taste' of cocaine in back of the throat, and even mild euphoria. The drug-like nature of these responses suggests that brain structures activated during cocaine craving may be among those activated by cocaine itself, including particularly the limbic regions implicated in cocaine's pleasurable effects.

We are testing whether limbic regions may be differentially activated during cocaine craving by measuring regional cerebral blood flow (rCBF) with Positron Emission Tomography (PET) and radioactively-labeled water (H_2O^{15}) as the flow tracer. Cocaine patients (n=5 thus far) are imaged during exposure to ambient room stimuli (Baseline), and to videos of Neutral (non-drug) and Cocaine-related scenes. Each subject's functional PET images are co-registered with an MRI for anatomical localization of imaged radioactivity. Change in rCBF from Baseline is calculated for both Neutral and Cocaine cue conditions across selected brain reigons. Initial results show the Cocaine video triggered craving, and rCBF during the Cocaine video increased in several limbic regions. Systematic increases in rCBF did not occur in reference areas (eg., whole brain or hemispheres) or in response to the Neutral Video.These preliminary data suggest limbic activation may be one component of drug craving.

666.3

PHYSIOLOGICAL ACTIONS OF COCAINE IN SENSORY CIRCUITS: DRUG INFLUENCES ON SIGNAL TRANSMISSION THROUGH RAT POm AND VPM THALAMIC NUCLEI. I. Bekavac*, J.J. Rutter and B.D. Waterhouse. Dept. of Physiol. and Biophys., MCP/HU, Phila. PA 19102

Previous reports from our laboratory have described a preferential potentiating effect of systemically administered cocaine (0.25-1.0 mg/kg) on long latency excitatory responses (E2) of rat "barrel field" cortical neurons to threshold level stimulation of individual whiskers on the contralateral face. Such long latency responses are relayed to cortex via the POm nucleus of thalamus, whereas short latency responses are relayed to cortex via the VPM thalamus. We postulated that systemic cocaine might produce changes in response properties of neurons in primary somatosensory cortex by exerting differential effects on signal transmission through POm versus VPM thalamus. Thus, the goal of the present study was to characterize the effects of cocaine on somatosensory thalamic neuron responsiveness to peripheral activation of afferent synaptic pathways. Extracellular recordings were obtained from spontaneously active single units in POm and VPM thalamic nuclei of halothane-anesthetized rats. Spontaneous firing rate and cellular responses to mechanical displacement of a single whisker were monitored before and after systemic administration of cocaine (1.0 mg/kg, i.v.). VPM neurons responded to whisker stimulation with a short latency (4-10 msec) burst of action potentials. Responses of POm neurons to whisker stimulation occurred at longer latencies (16-85 msec), and were less robust than those recorded from VPM, appearing as a slightly elevated discharge occurring over a period of 30-100 msec. After cocaine injection, stimulus evoked responses of VPM (n=7) neurons were generally unchanged or within (+/-) 4% of control, whereas, in 5 of 6 cases POm responses were increased 60% above control levels. Cocaine's effects were rapid in onset with peak effects occurring at 6 min post-injection and recovery to control patterns of discharge observed by 20 min. These results provide preliminary support for the idea that cocaine exerts a preferential facilitating effect on POm neuronal responses to whisker displacement. Since the POm confers a qualitative aspect to stimulus discrimination, drug actions at this site may underlie cocaine's influence on sensory perception. (Supported by NIDA DA 05117 to BDW)

666.4

COCAINE ACTIONS ON SOMATOSENSORY CORTICAL CIRCUITS: COMPARISON WITH PROCAINE AND AMPHETAMINE. F.M. Sessler*, T.N. Felder, R.D. Mouradian, R.C-S. Lin, J. Lehmann, J.P. Utz and B.D. Waterhouse. Depts. of Physiol.& Biophys., and Neurosurg., Hahnemann U., Philadelphia, PA 19102.

In studies of the action of cocaine on local circuits and intrinsic membrane properties of rat somatosensory cortical neurons, we have observed differential actions of cocaine on identified subpopulations of layer V neurons ("regular spiking" vs "doublet cell"). To distinguish between the local anesthetic vs psychostimulant actions of cocaine, we tested the effects of procaine and amphetamine, respectively, on somatosensory cortical circuits. Experiments were conducted using an *in vitro* brain slice preparation from rat somatosensory cortex. Microelectrodes containing either neurobiotin (2-3%) or lucifer yellow (5%) were used for characterization of individual layer V neurons. Postsynaptic potentials (EPSP and IPSP) were evoked by stimulating the cortical white matter. Drugs were applied at various concentrations (0.3-100uM) to examine the full spectrum of the drug action. Bath application of procaine at high concentrations (10-100uM) produced a dose dependent decrease in EPSP amplitude, area, and duration, as well as spike firing probability in both regular and "doublet" spiking layer V neurons. At lower concentrations (0.3-1uM), procaine did not mimic the cocaine-mediated enhancement of EPSP amplitude and spike firing probability which were routinely observed in "doublet" cells. On the other hand, amphetamine (0.3-10uM) increased both spike firing probability and EPSP amplitude of cortical neurons. These results suggest that cocaine exerts both local anesthetic and complex influences on cells within the cerebral cortical circuitry that can be mimicked by procaine and amphetamine, respectively. Some of these effects may involve monoamine release and may depend on cell type and monoamine terminals reaching these neurons. Thus, to assess the impact of cocaine on signal processing within a sensory cortical network, it may be necessary not only to obtain specific information about the effect of the drug on different cell types but also to describe the type of monoaminergic inputs they receive. (Supported by NIDA DA08405 to FMS and DA05117 to BDW).

666.5

GLUCOCORTICOIDS & DRUG ABUSE (I): INFLUENCES OF CHRONIC INHIBITION OF CORTICOSTERONE SYNTHESIS BY METYRAPONE ON COCAINE-INDUCED LOCOMOTION AND RELAPSE OF COCAINE SELF-ADMINISTRATION. P.V. Piazza*, M. Marinelli, C. Jodogne, V. Deroche, F. Rougé-Pont, S. Maccari, M. Le Moal and H. Simon. Psychobiologie des Comportements Adaptatifs, INSERM U.259, Univ. de Bordeaux II, 33077 Bordeaux Cedex, France.

It has been shown that basal and stress-induced secretion of corticosterone enhances vulnerability to drugs of abuse. In this report, we studied the effects of metyrapone, an inhibitor of corticosterone-synthesis, on cocaine-induced locomotion and intravenous self-administration. Locomotor response to cocaine was studied because psychomotor and reinforcing effects of drugs are related. Self-administration (SA) was studied in the relapse phase because blockade of relapse is central to the therapy of addiction. Before any other test, animals were submitted to 8 days of food-restriction during which they were progressively reduced to 90% of their initial body weight. Food-restricted animals were used in order to test for non-specific effects of metyrapone on motivational behavior. For the SA experiment animals were first trained for cocaine SA for 10 days, they then received a drug-free period of 4 days after which they were treated with either metyrapone or vehicle. After 8 days of this treatment, rats were again allowed to self-administer cocaine, and the metyrapone-treatment was continued. For all other experiments, animals were submitted to an 8-day treatment with either metyrapone (100mg/kg twice a day) or vehicle. Metyrapone-treatment totally blocked stress-induced corticosterone-secretion. Metyrapone-treatment also reduced cocaine-induced locomotor activity and the reinstatement of cocaine SA, without inducing non-specific disruptions of behaviors such as: i) locomotor response to a saline injection; ii) hole exploration in the SA cage, that is the response required during SA; iii) performance in reaching food in a straight alley test. In conclusi on these results confirm the involvement of glucococorticoids in determining vulnerability to drugs, and may open new therapeutic strategies of addiction.

666.6

GLUCOCORTICOIDS & DRUG ABUSE (II): INFLUENCES OF ACUTE INHIBITION OF CORTICOSTERONE SYNTHESIS AND ADMINISTRATION OF CORTICOSTEROID RECEPTOR ANTAGONISTS ON COCAINE -INDUCED LOCOMOTION. M. Marinelli, P.V. Piazza, M. Barrot, F. Rougé-Pont, M. Kharouby, M. Le Moal and H. Simon*. Psychobiologie des Comportements Adaptatifs, INSERM U.259, Université de Bordeaux II, 33077 Bordeaux Cedex, France.

Previous results have shown that chronic suppression of corticosterone reduces the behavioral effects of cocaine. In the present experiments we studied the effects of acute pharmacological manipulations of the hypothalamo-pituitary-adrenal (HPA) axis on the locomotor response to cocaine (15 mg/kg i.p.). In a first experiment we investigated the effects of a single injection of the blocker of corticosterone-synthesis metyrapone (50 mg/kg), injected subcutaneously 3h before the cocaine injection. In these conditions, animals pre-treated with metyrapone showed a lower locomotor response to cocaine than control rats. This effect was corticosterone-dependent since it was abolished if metyrapone-treated animals were concomitantly injected with corticosterone (20 mg/kg s.c. in oil). In a second experiment we studied the effects of an acute administration of type I and type II corticosteroid receptors antagonists on the locomotor response to cocaine. These antagonists (100 ng in 3µl) were injected i.c.v. 2h before the cocaine injection. Animals receiving the type I antagonist spironolactone showed an increase in the locomotor response to cocaine, whereas the administration of the type II antagonist RU 38486 had no significant effect. Concomitant administration of both antagonists reduced the locomotor response to the drug. Since administration of corticosteroid antagonists increases corticosterone levels, the increase in the locomotor response to cocaine observed after the administration of the type I antagonist was probably mediated by an over stimulation of type II receptors. Even though, type II antagonist alone had no effects, blockade of both receptors seemed necessary to reduce the locomotor response to cocaine. In conclusion, these results show that behavioral effects of drugs can be modulated by acute pharmacological manipulations of the activity of the HPA axis. These findings may have implications for new therapeutic strategies of addiction.

666.7

GLUCOCORTICOIDS & DRUG ABUSE (III): INFLUENCES OF BASAL CORTICOSTERONE SECRETION ON THE EFFECTS OF COCAINE AND MORPHINE ON ACCUMBENS DOPAMINE. M. Barrot, F. Rougé-Pont, S. Maccari, M. Marinelli, M. Le Moal*, H. Simon and P.V. Piazza. Psychobiologie des Comportements Adaptatifs, INSERM U.259, Univ. Bordeaux II, Bordeaux, France.

It has been previously shown that basal corticosterone-secretion facilitates behavioral effects of cocaine and morphine. In these experiments we investigated if changes in the response of mesolimbic dopaminergic (DA) neurons to these drugs may account for the behavioral effects of corticosterone. Morphine- and cocaine-induced changes in extracellular concentration of dopamine were studied in three groups of rats: i) sham operated (Sham); ii) adrenalectomized (ADX); iii) ADX receiving a substitutive treatment which reproduced the corticosterone circadian secretion (ADX+Cort). Dopamine extracellular concentrations were recorded in the nucleus accumbens by means of microdialysis in freely moving animals. We also investigated the functional state of the postsynaptic DA receptors by studying the locomotor response to the direct DA agonist apomorphine (1.5 mg/kg s.c.). Adrenalectomy reduced the locomotor response to both morphine (2mg/kg) and cocaine (15mg/kg) whereas it differentially modified the increase in dopamine concentrations induced by the two drugs. Morphine-induced increase in dopamine levels was reduced by adrenalectomy, in contrast adrenalectomy enhanced the increase in dopamine levels induced by cocaine. For both drugs the effects were corticosterone-dependent since ADX+Cort animals did not differ from the sham group. These results suggest that the ADX-induced decrease in locomotor response to morphine may be mediated by a decrease in the release of dopamine, whereas ADX-effects on locomotor response to cocaine should involve postsynaptic modifications. Indeed, suppression of corticosterone-secretion also reduced DA postsynaptic transmission. ADX animals showed a lower locomotor response to apomorphine and this effect was not present in ADX+cort rats. In conclusion, corticosterone can modulate dopaminergic reactivity to drugs by acting on both the presynaptic and postsynaptic side.

666.8

GLUCOCORTICOIDS & DRUG ABUSE (IV): INFLUENCE OF STRESS-INDUCED CORTICOSTERONE SECRETION ON STRESS-INDUCED INCREASE IN COCAINE'S EFFECTS ON ACCUMBENS DOPAMINE. F. Rougé Pont, V. Deroche, M. Marinelli, M. Kharouby, M. Le Moal, H. Simon and P.V. Piazza. (SPONS: Europ. Brain Behav. Soc.) Psychobiologie des Comportements Adaptatifs, INSERM U.259, Univ. de Bordeaux II, 33077 Bordeaux Cedex, France.

Several data indicate that stressful experiences induce a parallel increase in behavioral and dopaminergic responses to psychostimulants. Such may be at the basis of the enhanced vulnerability to psychostimulants induced by stressors, since mesolimbic dopaminergic neurons are one of the principal substrates of the reinforcing effects of drugs. We have previously shown that stress-induced corticosterone-secretion mediates the stress-induced increase in the behavioral effects of psychostimulants. In this experiment we have investigated if stress-induced corticosterone-secretion could also modulate the enhancement of the dopaminergic response to drugs induced by stress. For this purpose, we have studied the effects of the inhibition of corticosterone-synthesis by metyrapone (100 mg/kg s.c., twice a day for 8 days) on the increase in dopaminergic and locomotor response to cocaine (10 mg/kg i.p.) induced by food-restriction. Food-restriction, 8 days during which animals were progressively brought to 90% of their initial body weight, was used as a stressor because it induces a clear-cut increase in corticosterone levels and in the stimulant and reinforcing effects of drugs. Dopamine was studied by means of microdialysis in the nucleus accumbens of freely moving rats. Food-restriction induced a significant enhancement of cocaine-induced increase in both dopamine and locomotion. Metyrapone-treatment, during the food-restriction regimen, blocked the development of these effects. Metyrapone-treatment was also able to reverse the effects of food-restriction once these had established. Locomotor and dopaminergic response to cocaine in metyrapone-treated animals did not differ from *ad libitum*-fed controls also when the treatment was started after 8 days of food-restriction. In conclusion, stress-induced corticosterone-secretion may modify behavioral effects of drugs by acting on dopaminergic neurons.

666.9

DIFFERENTIAL EFFECTS OF CORTICOSTERONE ON LOCOMOTOR SENSITIZATION TO COCAINE IN FISCHER AND LEWIS RATS: POSSIBLE BIOCHEMICAL MECHANISMS J. Ortiz*, T. A. Kosten, J. DeCaprio and E.J. Nestler. Lab. of Molecular Psychiatry, Dept. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

The Fischer and Lewis inbred rat strains differ in their preferences for opiates, alcohol, and cocaine. They also differ markedly in hypothalamic-pituitary-adrenal axis function, which seems to contribute to cocaine-induced behaviors in other strains. Here we report that corticosterone (CORT) administration by slow-release pellets (giving plasma levels around 150 ng/ml) was necessary for the Fischer rats to initiate locomotor sensitization to cocaine (7.5 mg/kg i.p. for 5 consecutive days). In contrast, Lewis rats developed cocaine sensitization independently of CORT administration. Similarly, CORT administration to Fischer rats, but not to Lewis rats, increased levels of tyrosine hydroxylase (TH) in the ventral tegmental area (VTA) (+75%), abolishing previously described differences between the Fischer and Lewis strains. This supports a possible link between TH levels (and dopamine function) in the VTA and the initiation of locomotor sensitization.

Since TH expression is regulated in cell lines by cAMP and glucocorticoids, we further explored CORT action at the level of glucocorticoid receptor (GR) and cAMP response-element binding protein (CREB) immunoreactivity in the VTA. The drug-naive Fischer strain had higher levels of CREB (+49%) than the Lewis strain, but comparable levels of GR. Strikingly, CORT down-regulated GR levels in the VTA of Lewis rats (-47%), but not in Fischer rats. These observations could account for the differential responsiveness of the two strains to glucocorticoids: 1) In Lewis rats, GR down-regulation could decrease responsiveness to CORT; 2) In Fischer rats, the lack of GR down-regulation, together with the higher basal CREB levels, could increase the transcriptional actions of glucocorticoids at the TH and other genes.

666.10

EFFECTS OF 17-β ESTRADIOL ON INTRAVENOUS COCAINE SELF-ADMINISTRATION BY CASTRATED FEMALE RATS. J. W. Grimm* and R. E. See. Department of Psychology, Washington State University, Pullman, WA 99164-4820.

Previous studies of female rats self-administering cocaine have indicated differences across the estrous cycle. The present experiments were conducted to further the understanding of the motivation to self-administer cocaine by females and help discern the role of estrogen in cocaine-mediated reinforcement in females. Female Sprague-Dawley rats were castrated, sub-cutaneously implanted with Silastic capsules containing 17-β estradiol or cholesterol, and allowed to self-administer intravenous cocaine (0.78 mg/kg/infusion) on fixed interval and progressive ratio schedules of reinforcement. Animals receiving chronic 17-β estradiol showed increased self-administration of cocaine indicating that estrogen may modulate cocaine craving in female rats. These results may also contribute to an understanding of possible sex-differences in cocaine self-administration. The effects of chronic vs. acute treatment with 17-β estradiol on cocaine self-administration will be discussed. (Supported by the State of Washington Initiative Measure No. 171.)

666.11

AN EFFECT SALIENT TO COCAINE'S REINFORCEMENT IS MODIFIED BY CENTRAL ADMINISTRATION OF DELTA SPECIFIC OPIOIDERGIC AGENTS.
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Lab. for Psychopharm., RPI, Troy, NY 12180 and Dept. of Pharm., U. of Arizona, Tucson, AZ 85724

Some rats were implanted with bilateral, chronically indwelling cannulae for administration of agents to the accumbens n. Others were implanted with a cannula for intraventricular administrations. All rats were implanted with chronically indwelling bipolar electrodes for intracranial stimulation (ICS) of the lateral hypothalamus. Rats were trained to press a lever for ICS and then allowed to press daily for a fixed intensity of ICS. After pressing rates were stable, rats were given IP injections of cocaine at doses producing clear enhancement of pressing. Then, naltrindole and other agents influencing delta opioid receptors were centrally administered in addition to cocaine. Agents blocking delta opioid receptor processes blocked cocaine's effects. These data confirm that effects salient to cocaine's reinforcement are sensitive to delta opioidergic manipulations and extend them by showing that the critical effect is a central neural effect.

666.12

EFFECTS OF COCAINE AND HEROIN, ALONE AND IN COMBINATION, ON MILK DRINKING IN RATS. J. K. Rowlett* and W. L. Woolverton. Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216.

The use of drug combinations (e.g., cocaine and heroin "speedball") is common among drug abusers; however, relatively few studies have assessed the pharmacological basis of this phenomenon. In the present experiment, the behavioral effects of combined cocaine and heroin were examined using a milk drinking procedure in rats. Rats (n=8) were given access to a sweetened milk solution for 15 minutes daily until intake had stabilized (3 days with less than 10% variation across days and between subjects). Rats then were administered saline or one of four doses of either cocaine (4.0-32 mg/kg, i.p.) or heroin (0.4-3.2 mg/kg, i.p.) 15 minutes prior to milk access. Both cocaine and heroin produced a dose-dependent decrease in milk drinking, with ED_{50}s (mean mg/kg ± SEM) of 7.7 ± 1.1 and 1.9 ± 0.14, respectively. The dose-response function for cocaine then was redetermined in combination with either 0.8 mg/kg or 1.6 mg/kg heroin. Isobolographic analysis of these dose-response functions revealed that the combination of cocaine and heroin was dose-additive. These results suggest that the combination of cocaine and heroin is additive in disruption of ongoing behavior. (Supported by NIDA grant DA-05951).

666.13

MODULATION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE BY MORPHINE IN RATS. K.M. Kantak*, R.D. Spealman, A. Riberdy and P. Clutton. Boston Univ., Boston, MA 02215 and New England Regional Primate Research Center, Southborough, MA 01772.

Modulation of the discriminative stimulus (DS) effects of cocaine by morphine was investigated in rats trained to discriminate a relatively low (3 mg/kg) or a relatively high (10 mg/kg) dose of cocaine from vehicle. When tested alone, cocaine (0.3 - 18 mg/kg) engendered dose-related increases in cocaine-appropriate responding under both training conditions, with ED_{50} values being about 3-fold greater under the high-dose training condition than under the low-dose training condition. Morphine (0.3-5.6 mg/kg) did not engender substantial cocaine-appropriate responding under either training condition. Pretreatment with morphine did, however, enhance the DS effects of cocaine (0.3 - 3.0 mg/kg) under both training conditions, with the degree of enhancement dependent on the dose of morphine (3.0 or 5.6 mg/kg) and the pretreatment time (15 or 30 min). The greatest enhancement was observed when 5.6 mg/kg morphine was administered 30 min before cocaine. At these parameters, morphine produced a leftward shift of the cocaine dose-effect curve under both training conditions and a concomitant reduction in ED_{50} of 8- to 12-fold. The results are consistent with those previously reported in squirrel monkeys and provide relevant information about the boundary conditions under which morphine-cocaine interactions are observed.

666.14

U50,488, A KAPPA AGONIST, ATTENUATES COCAINE-INDUCED INCREASES IN EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS. *I.M. Maisonneuve* and S.D. Glick, Dept. Pharm/Tox, Albany Medical College, Albany, NY 12208 and the Capital District Center for Drug Abuse Research and Treatment, Albany, NY 12208.*

Recent observations suggest that manipulation of endogenous opioid systems may modify the dopamine-dependent effects of cocaine. The combination of cocaine and a mu opioid agonist produces a mutual enhancement of their reinforcing effects and is commonly referred to as "speed ball" by abusers (Masukawa et al., 1993). In contrast, kappa opioid agonists have been shown to attenuate the discriminative stimulus effects of cocaine (Spealman and Bergman, 1992). Using *in vivo* microdialysis in awake and freely moving female Sprague Dawley rats, we investigated whether U50,488, a selective kappa agonist, would alter cocaine-induced dopamine (DA) increases in the shell of the nucleus accumbens. Cocaine (20 mg/kg, i.p.) produced a ten-fold increase in extracellular DA levels that peaked 20-40 minutes after administration. Pretreatment (20 minutes beforehand) with the selective kappa agonist U50,488 (10 mg/kg, i.p.) produced a 50% decrease in the effect of cocaine on DA levels. This attenuation was completely reversed by administration of nor-binaltorphimine (10 mg/kg, s.c.), a kappa antagonist, 20 minutes before the agonist challenge. These findings indicate that activation of kappa receptors attenuate cocaine's effects and thus kappa agonists may have a potential role in the pharmacological management of cocaine addiction (supported by DA03817 and by the Aaron Diamond Foundation).

666.15

Choline acetyltransferase activity is reduced in rat nucleus accumbens after unlimited access to self-administration of cocaine. J.M. Wilson*, M.E. Carroll, S.T. Lac, L.M. DiStefano and S.J. Kish. Clarke Institute of Psychiatry, Toronto, Ontario and University of Minnesota, Minneapolis.

Activity of choline acetyltransferase (ChAT), the acetylcholine synthesising enzyme, was measured in discrete areas of rat brain after chronic, unlimited access to self-administration of cocaine. The duration of drug exposure was 6 weeks, during which time the rats self-administered a mean daily dose of approximately 90 mg/kg. Rats were sacrificed either on the last day of cocaine access ($n=10$) or after three weeks drug withdrawal ($n=8$). As compared with the controls ($n=15$), ChAT activity was slightly reduced in striatum (-10%, $P>0.05$) and moderately reduced in nucleus accumbens (-26%, $P<0.05$) on the last day of cocaine access. After three weeks withdrawal from cocaine, ChAT activity was still reduced (striatum -18%, $P<0.05$; nucleus accumbens -32%, $P<0.05$) relative to controls. These data suggest that self-administration of cocaine is associated with a long-lasting reduction in ChAT activity. Such a reduction in ChAT activity suggests reduced activity of dopamine receptive cholinergic neurones in the basal forebrain, which could underlie some of the behavioral effects of cocaine. (Supported by NIDA grant DA07182).

666.16

AMPEROZIDE REDUCES ORAL CONSUMPTION OF COCAINE AND COCAINE-CONDITIONED PLACE PREFERENCE IN RATS. E.A. Jones*, H.L. Williams, R.D. Myers & B.A. McMillen, Dept. of Pharm., Sch. of Med., East Carolina Univ., Greenville, NC 27858.

Amperozide is a novel serotonin$_2$ ($5HT_2$) receptor antagonist with a low affinity for dopamine receptors. Previously, amperozide (0.5, 1.0 or 2.5 mg/kg b.i.d.) given for 3 days reduced oral consumption of cocaine or alcohol during and after treatment with the drug. In this study, rats were induced to drink a cocaine-saccharin solution, a concentration which produced maximal consumption was used in a two-choice paradigm with 0.03% saccharin solution in a second drinking tube. Amperozide (2.5 mg/kg b.i.d. X 7 days) decreased cocaine consumption by 42% (21.7 to 12.6 mg/kg/day; $p<0.01$). A decrease in food consumption during the 7 day treatment period was recorded. The $5HT_2$ antagonist, trazodone (2.5 mg/kg b.i.d.), but not ritanserin (1.0 mg/kg b.i.d.), reduced the volitional intake of cocaine by 26% ($p<0.05$). Continuous s.c. infusion by mini-pump of 2.0 mg/kg/day amperozide for 2 weeks decreased intake of cocaine by 42% and 30% during the week after. To test the effect of amperozide on a cocaine-conditioned response, a conditioned place preference (CPP) was induced using 2.5, 5.0 or 10 mg/kg i.p. cocaine. Amperozide (0.1 mg/kg) administered s.c. 60 min. prior to testing did not affect a 5.0 mg/kg cocaine-induced CPP. However, amperozide (0.5, 1.0 or 2.5 mg/kg) reduced the time spent in the cocaine-paired environment. Thus, amperozide potently reduces both the oral consumption of cocaine and cocaine-seeking behavior. (Supported by NIAAA grant AA-04200-11)

666.17

PROFOUND EFFECT OF COCAINE ON NEUROPEPTIDE Y (NPY) AND NPY-Y1-RECEPTOR mRNA LEVELS IN THE RAT BRAIN. C. Bjenning* and C. Wahlestedt. Div. Neurobiol., Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Considerable evidence, obtained by studies in man and experimental animals, indicates that NPY plays a role in behavior. For example, NPY appears to be an endogenous anticonflict/anxiolytic agent, whose action depends on activation of NPY-Y1-receptors (Wahlestedt *et al., Science* **259**, 528-31, 1993). We have hypothesized that the reduction of forebrain NPY levels (and reduced NPY-Y1-receptor occupancy) resulting from repeated cocaine treatment may be associated with severe anxiety and depression-like states that often follow cocaine withdrawal (Wahlestedt *et al., PNAS*, **88**, 2078-82, 1991).

The NPY mRNA and NPY-Y1-receptor mRNA levels in frontal cortex, peripheral cortex, nucleus accumbens, hippocampus and in the hypothalamus were measured with a solution hybridization assay using 300 bp fragments of respective rat cDNA. The hybridization was linear to 500 pg for both NPY and NPY-Y1-receptor sense transcripts.

After 24-hour treatment with cocaine (10mg/kg bw i.p., twice daily), NPY mRNA levels decreased between 20 and 70% in the assayed brain regions. Cocaine treatment for 72 hours, and up to 7 days, further decreased NPY mRNA levels in the sampled regions. NPY-Y1-receptor mRNA levels, on the other hand, peaked (125 to 600% increase in the different regions) after 24 hours and stabilized at 70-185% increase after 72 hours.

Our data thus indicate that treatment with cocaine causes profound alterations in NPY and NPY-Y1-receptor mRNA levels. However, the temporal patterns of these effects are distinct for the two mRNA species. (Supported by DA06805).

666.18

REPEATED COCAINE INJECTIONS DECREASE THE FUNCTION OF STRIATAL $GABA_A$ RECEPTORS. J. Peris* & U. Geiss. Dept. Pharmacodynamics, Univ. Florida, Gainesville, FL 32610.

Behavioral sensitization to cocaine is correlated with increased striatal dopamine (DA) neurotransmission which may occur via upregulation of DA receptors or DA release. It is also possible that alterations in other striatal neurotransmitters contribute to these cocaine-induced changes. For example, cocaine decreases ^{35}S-TBPS binding to $GABA_A$ receptors in striatum (Pecins-Thompson & Peris, Psychopharmacology, 110: 443-450, 1993). A decrease in GABA-mediated inhibition in striatum might cause a greater increase in striatal DA output caused directly by cocaine. The function of $GABA_A$ receptors was assessed in cortex, substantia nigra and striatum of rats that had received 8 daily injections of cocaine (15 mg/kg. i.p.) or saline. GABA-stimulated ^{36}Cl uptake in microsacs was dose-related in all three regions and was decreased significantly in cocaine-treated rats ($F(1,8) = 9.2$; $p < 0.05$). Since repeated ethanol treatment also affects $GABA_A$ receptor function, we next measured ^{36}Cl uptake in striatal microsacs from rats exposed to 14 daily cocaine (10 mg/kg, i.p.) or ethanol (1.0 g/kg, i.p.) injections given alone or simultaneously. Muscimol stimulation of uptake was dose-related in saline-treated rats and was decreased significantly in all cocaine-treated rats ($F(1,16) = 7.4$; $p < 0.05$) regardless of ethanol treatment. Picrotoxin and bicuculline inhibition of muscimol-stimulated uptake were not affected by drug exposure. Thus, $GABA_A$ receptor function in striatum may be selectively decreased by cocaine treatment which may contribute to changes in DA transmission and behavioral sensitization. Supported by AA00135 and a grant from the Alcohol Beverage Medical Research Foundation.

666.19

Glycine/NMDA antagonist, (+)-HA-966, prevents behavioral sensitization to cocaine and subsequent effects on aversive conditioning.

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The noncompetitive NMDA receptor antagonist, MK-801, has been shown to prevent locomotor sensitization to repeated cocaine. However, MK-801 has been shown to cause locomotor sensitization to itself. We tested (+)-HA-966, 1-hydroxy-3-aminopyrrolidone-2, an antagonist at the glycine site of the NMDA receptor complex, on locomotor sensitization to repeated cocaine administration. Vehicle or (+)-HA-966, 15 mg/kg i.p., was given 30 min prior to vehicle or cocaine, 15 mg/kg i.p., daily for 5 consecutive days. Seven days later, all rats were challenged with cocaine, 15 mg/kg. (+)-HA-966 prevented sensitization to the cocaine challenge and did not cause a sensitization to itself. Three weeks later, rats were subjected to aversive conditioning: 10 randomly presented tones terminated with 0.5 sec, 400 mA footshocks over 30 min. The following day the rats were returned to the chamber, given 10 tones without footshock, and sacrificed after 30 min. The medial prefrontal cortex (mPFC) and nucleus accumbens (NAS) were harvested and assayed by HPLC-EC for serotonin and dopamine along with their metabolites. As expected, the conditioned rats had significantly greater immobility (freezing) during and after the tone, increased fecal boli and elevated dopamine turnover (DOPAC/DA) in the mPFC and NAS. Prior exposure to cocaine attenuated the increase in behavioral (i.e. freezing and fecal boli) and blocked some of the biochemical (NAS dopamine turnover) correlates of the aversive conditioning. Preexposure to (+)-HA-966 with cocaine prevented these effects. The role of the NMDA receptor with regards to cocaine sensitization and subsequent cross-tolerance to aversive conditioning will be discussed.

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666.20

EFFECT OF NMDA RECEPTOR ACTIVATION IN THE VENTRAL TEGMENTAL AREA ON COCAINE-INDUCED LOCOMOTOR ACTIVITY.

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Repeated exposure to cocaine causes an enhanced response to subsequent challenges with this drug, a phenomenon called behavioral sensitization. The augmented behavioral response is primarily mediated by enhanced dopamine transmission in neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens. Glutamatergic input to VTA dopaminergic neurons acting through NMDA receptors has been implicated in the development of the sensitization phenomenon. The present study is an effort to further characterize the role of NMDA receptor activation in behavioral sensitization. Male Sprague Dawley rats were injected with cocaine (15 mg/kg, ip) or saline (1 ml/kg, ip) for 5 consecutive days. After a week of withdrawal, the expression of sensitized behavior was tested with bilateral microinjection of an NMDA agonist, cis-ACDA, into the VTA. Locomotor activity in response to cis-ACDA injection was elevated in rats sensitized to cocaine compared to that of saline injected controls. In a separate experiment, the ability of repeated NMDA receptor activation in the VTA to initiate sensitization was evaluated. Rats were injected with either saline or different doses of cis-ACDA on three days with a 72 hour interval between injections and their locomotor response was monitored. Cis-ACDA injection caused enhanced locomotor activity with a clear dose-response relationship on the first day of administration. On two subsequent cis-ACDA injections, rats appeared to develop tolerance to the locomotor activating effects. A week after the last microinjection, the locomotor response to ip cocaine challenge (15 mg/kg) was measured. Rats that received cis-ACDA injections showed a significantly higher locomotor activity in response to the cocaine challenge, suggesting that repeated NMDA receptor activation may be at least partially responsible for the initiation of behavioral sensitization.

666.21

EFFECTS OF INTRAVENOUS COCAINE ADMINISTRATION ON GLUTAMATE-INDUCED EXCITATION AND SPONTANEOUS ACTIVITY IN RAT'S CEREBELLAR PURKINJE CELLS.

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Cocaine is a well known substance of abuse with central psychostimulant effects and local anesthetic properties. Biochemical studies indicate that cocaine's main action is to increase central synaptic levels of monoamines through blockade of reuptake mechanisms. The present study investigated the effects of intravenous (i.v.) cocaine administration on Purkinje cells response to glutamate, a putative neurotransmitter with clear actions in the cerebellum. Excitatory responses of individual neurons to microiontophoretic pulses (10 sec.) of glutamate (1-45 nA) were examined, before, during and after cocaine injections (0.25 and 1mg/kg, i.v.). At doses of 0.25 mg/kg cocaine induced a significant reduction in spontaneous activity (spikes/sec $\pm$ SE; 20.49 $\pm$ 4.63 to 5.56 $\pm$, n = 6) and glutamate-evoked response (37.61 $\pm$ 7.22 to 23.39 $\pm$ 10.97). Similar effects were observed at doses of 1 mg/kg, however, they were lower in magnitude (SA - 24.69 $\pm$ 2.77 to 17.96 $\pm$ 4.20; evoked - 43.7 $\pm$ 4.25 to 29.15 $\pm$ 5.47 n = 12). The major effect was observed within the first 2 min. after cocaine administration. Animals treated with procaine showed no significant changes in either spontaneous or glutamate-evoked excitation. These data suggest that cocaine can modify spontaneous neuronal activity and glutamate-induced excitation in the cerebellum via a mechanism independent of its local anesthetic properties. Studies are underway to elucidate the role of monoamines in this action (Supported by DA 07175 and RCMI RR03035 to C.A.J.R.).

666.22

THE ROLE OF NUCLEUS ACCUMBENS DOPAMINE AND EXCITATORY AMINO ACIDS IN THE EXPRESSION OF COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN RATS.

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We monitored the behavioral response to intra-accumbally administered dopamine and excitatory amino acid agonists (amphetamine and AMPA, respectively) following 7 daily ip cocaine or saline injections. Our results indicated that both of these drugs dose-dependently enhanced behavioral hyperactivity in cocaine-relative to saline-pretreated rats. In separate animals, we performed *in vivo* microdialysis in order to determine if changes in accumbal dopamine release might underlie these behavioral effects. The neurochemical data revealed that the local administration of both amphetamine and AMPA (through the probe) produced a significant concentration-dependent increase in accumbal dopamine in saline pretreated animals. Among cocaine pretreated rats, however, there was a potentiation of accumbal dopamine release only following amphetamine administration. Taken together, these results suggest at least two distinct mechanisms that contribute to the expression of cocaine-induced behavioral sensitization: 1) a dopamine-mediated mechanism that is expressed through the potentiated ability of psychostimulants to increase accumbal dopamine and 2) an increased ability of non-NMDA excitatory amino acid agonists to induce behavioral hyperactivity that is independent from the accumbal dopamine system.

666.23

THE ROLE OF GLUTAMATERGIC ACTIVITY THROUGH NUCLEUS ACCUMBENS AMPA RECEPTORS IN THE EXPRESSION OF BEHAVIORAL SENSITIZATION TO COCAINE.

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Through two behavioral experiments in the rat, we examined the role of glutamatergic activity through nucleus accumbens (NA) AMPA receptors in the expression of cocaine sensitization. In experiment 1, one group received seven daily IP cocaine treatments, while a control group received an identical treatment schedule with IP saline. For all subjects, open-field locomotor activity was monitored for two hours following first and last treatments to assess the extent of sensitization. On days 20, 21 and 22 of withdrawal from treatment, each subject received bilateral intra-NA microinfusion of 0, .03, or .1 nmol/side of the glutamate agonist AMPA. All subjects received each microinjection dose once, in sequences balanced for order effects. Subjects receiving cocaine and displaying sensitization to criterion (>20% increase in cocaine-induced locomotion, last cocaine versus first cocaine, 15 mg/kg each) showed significantly greater locomotor activity in response to .1 nmol/side intra-NA AMPA than either subjects administered saline or cocaine-administered subjects which failed to sensitize. In experiment 2, all subjects received the chronic cocaine treatment schedule used in experiment 1. On days 14, 17, 20 and 23 of withdrawal from daily treatment, each subject received 0, .01, .1 or 1 nmol/side intra-NA microinfusion of the non-NMDA receptor antagonist CNQX immediately prior to 15 mg/kg IP cocaine. In subjects which met the sensitization criterion, pre-treatment with .1 nmol/side intra-NA CNQX reduced cocaine-induced hypermotility to the level seen in response to the first cocaine administration (p=.06). Taken together, the results of these experiments indicate that the expression of behavioral sensitization to cocaine depends, in part, upon sensitization-specific, AMPA receptor-mediated changes in glutamate transmission within the NA.

666.24

ELECTROPHYSIOLOGICAL ANALYSIS OF EXCITATORY AMINO ACID RECEPTOR SUBTYPES INVOLVED IN GLUTAMATE-INDUCED ACTIVATION OF RAT NUCLEUS ACCUMBENS NEURONS *IN VIVO*.

X.-T. Hu* and F.J. White. Neuropsychopharmacology Lab., Dept. of Neuroscience, Finch Univ. of Health Sci./The Chicago Medical School, North Chicago, IL 60064.

We have previously demonstrated that repeated administration of psychomotor stimulants (amphetamine and cocaine) leads to reduced responsiveness of nucleus accumbens neurons to glutamate, administered iontophoretically. However, to date, there have been no studies regarding the subtypes of excitatory amino acid (EAA) receptors which mediate the effects of iontophoretically administered glutamate on accumbens neurons *in vivo*. The present study used extracellular single cell recording and microiontophoresis to study the contributions of various EAA receptors to the regulation of accumbens neuronal activity. Pulse-ejected glutamate (1-128 nA) caused a current-dependent increase in the firing of accumbens neurons. A stronger excitatory response to AMPA was observed at much lower ejection currents (0.1-6.4 nA). Compared to AMPA and glutamate, NMDA induced a much less potent excitation in a narrow current range (1-4 nA) usually after accumbens neurons had been "primed" by previous glutamate iontophoresis. Higher ejection currents of all three EAA agonists drove accumbens cells into a state of apparent depolarization block. AMPA-evoked firing was selectively blocked by the AMPA receptor antagonist DNQX whereas NMDA-induced activity was selectively prevented by the NMDA receptor antagonist D-AP5. DNQX, but not D-AP5, significantly attenuated glutamate-evoked activity. The metabotropic glutamate receptor agonist 1S,3R-*t*-ACPD failed to evoke excitatory response of accumbens neurons, but significantly reduced the excitatory effects of the other EAA agonists. These results suggest that the excitatory effects of glutamate on rat accumbens neurons *in vivo* are primary mediated by AMPA receptors and that metabotropic glutamate receptors may function to dampen excessive transmission through ionotropic EAA receptors. Supported by USPHS grants DA 04093 and DA 00207 (FJW).

666.25

EFFECTS OF THE METABOTROPIC GLUTAMATE RECEPTOR BLOCKER AP-3 ON COCAINE-INDUCED DOPAMINE RELEASE AND LOCOMOTOR BEHAVIOR. P.A. Vincent* and E..L. Gardner. Dept. of Psychiatry, Albert Einstein Col. Med., Bronx, NY 10461

Administration of Cocaine (C) has been previously shown to induce dopamine (Da) release in the nucleus accumbens (Nac) using microdialysis-HPLC. To identify the neural substrates that modulate C-induced Da release, we have studied the glutamatergic system because the Nac receives Glu projections from the hippocampus, amygdala and prefrontal cortex, and Glu has been shown to interact with Da functioning. To study this interaction, we administered the Glu metabotropic receptor blocker L(+)-2-Amino-3-phosphonopropionic acid (AP-3) through a microdialysis probe placed in the Nac while monitoring Da release. 0.1mM AP-3 (2 μl/min) or veh was administered to Sprague-Dawley rats treated with C or veh, ip. Dialysates were collected from animals every 10 min for 10 samples. Animals treated with veh and C had an increase in peak Da release of 484% above baseline. Animals receiving AP-3 and plus showed a 160% increase in Da. Animals receiving AP-3 plus C exhibited a 491% increase in Da, similar to C alone. In a second experiment, 0.1, 0.5, and 1.0 mM AP-3 or veh was infused through bilateral cannulae placed in the Nac 15 min prior to receiving 20 mg/kg C, ip. Locomotor behavior was recorded for the first 2 min of 10 successive 10 min periods. Locomotor activity was not significantly diminished in animals given AP-3 plus C compared to veh plus C. These data suggest that Glu neurons may regulate the same pool of Da that C releases. Further work is needed to determine whether C-induced motor output is modulated by Glu metabotropic receptors. Supported by the Aaron Diamond Foundation.

666.26

IDENTIFICATION OF A NOVEL [^{125}I]RTI-121 BINDING SITE IN RAT CEREBRAL CORTEX: A POSSIBLE SITE OF ANTIDEPRESSANT ACTION. J.W. Boja*, J.L Cadet, F.I. Carroll, A.H. Lewin, P. Abraham and M.J. Kuhar. Molecular Pharmacology Section, NIH-NIDA Intramural Research Program, P.O. Box 5180, Baltimore, MD 21224.

Previously we have identified a cocaine analog (RTI-121) that exhibits nanomolar affinity and high selectivity for the dopamine transporter in rat striatum. There was a high correlation ($p < 0.0001$) between the potencies of drugs to inhibit specific [^{125}I]RTI-121 binding in the striatum (STR) and the potencies of these drugs to inhibit specific [^{3}H]dopamine uptake. No correlation was found with the potencies of these compounds to inhibit specific [^{125}I]RTI-121 binding with either [^{3}H]serotonin or [^{3}H]norepinephrine uptake.

When [^{125}I]RTI-121 binding was conducted in the frontal pole (FP) of the rat cerebral cortex several differences were apparent. While [^{125}I]RTI-121 still bound to a high- and low-affinity site, the number of sites labeled by [^{125}I]RTI-121 was approximately 1% of that observed in the striatum. However, while the binding of [^{125}I]RTI-121 observed in the FP was similar to that observed in the STR, several compounds displayed a significantly lower IC_{50}s in the FP than in the STR. These included several of the tricyclic antidepressants (imipramine, chlorimipramine, amytriptyline, nortriptyline and desipramine), trazadone, nisoxetine, tomoxetine, paroxetine and (-)cocaine. There were no differences in the STR or FP IC_{50}s for either mazindol, WIN 35,428, RTI-121, nomifensine, GBR 12909, talsupram, desipramine, citalopram, or fluoxetine. In addition, these sites showed less sensitivity to the effects of 6-OHDA which unlike striatal binding sites were almost completely eliminated by that toxin. These results suggest that [^{125}I]RTI-121 may bind to a yet uncharacterized site in the cerebral cortex that may recognize cocaine and many antidepressant drugs with high affinity.

666.27

AFFINITY PURIFICATION OF COCAINE RECEPTORS FROM RAT BRAIN. **L.P. Raymon*, X.S. He, S. Kim, J. Wright and M.E. Eldefrawi. Pharmacol., Univ. of MD, School of Med., Baltimore, MD 21201.**

Dopamine, serotonin and norepinephrine transporters are recognized as the main target for cocaine reinforcement and addiction. 1% digitonin solubilized rat forebrain proteins exhibited cocaine-sensitive [^{3}H]citalopram (CTL), [^{3}H]BTCP and [^{3}H]GBR12935 binding. A BTCP analog, reduced citalopram and an amino-cocaine analog were linked to AffiGel 10 for affinity purification. They all yielded low μg quantities of proteins that retained cocaine-sensitive binding activity after fast dialysis through ultrafiltration membranes. [^{3}H]CTL gave the largest binding signal of various transporter ligands tested. Its affinity for the serotonin transporter was not altered by solubilization and it was always recovered in part in the three affinity fractions. The proteins eluted from the aminococaine affinity column exhibited cocaine-sensitive specific binding of [^{3}H]BTCP, [^{3}H]GBR12935 and [^{3}H]CTL. SDS-PAGE electrophoresis of the isolated proteins will also be presented.

(Supported in part by NIDA grant # DA06830)

666.28

EFFECTS OF DIETARY FAT AND PROTEIN ON COCAINE-INDUCED STEREOTYPY IN RATS. J.S. Shumsky*, P.L. Shultz, J.R. Galler, and J. Tonkiss. Center for Behavioral Development & Mental Retardation, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

Dietary fat and protein produced progressive alterations in cocaine-induced stereotypy that may be associated with changes in drug metabolism. Female Sprague-Dawley rats were fed one of three diets over a three week period: low protein (5%), high fat (15%); high protein (22%), high fat (15%); or high protein (23%), low fat (5%), i.e. rat chow. Following one week of adaptation to the diets, the rats were injected every 3-4 days with either cocaine (30 mg/kg, IP) or saline and total amount of stereotypy was measured. Following each injection, the total amount of cocaine-induced stereotypy increased, peaked within the 90 min observation interval, and then declined for all diet groups. With repeated injections, intensity of stereotypy increased in all diet groups; however, the two high fat diet groups exhibited significantly more stereotypy across injections than the low fat diet group. By the fourth injection, the low protein diet group exhibited the slowest onset and the longest duration of cocaine-induced stereotypy. These data cannot be explained by differences in body weight, and are therefore likely to be related to differences in body composition or alterations in drug metabolism. Supported by NIH grant DA 07934.

PSYCHOTHERAPEUTIC DRUGS: ATYPICAL ANTIPSYCHOTICS

667.1

ANTI-GLUTAMATERGIC INFLUENCES OF CLOZAPINE: EFFECTS AT CLINICALLY RELEVANT CONCENTRATIONS. T.I.Lidsky*, L.Zuck, S.P.Banerjee Inst.Basic Res., S.I., N.Y.

The bases of clozapine's (Cz) enhanced antipsychotic potency and reduced incidence of motor side effects is unknown. It is conceivable that glutamate, because of its influences on dopamine release and its participation in motor side effects, is a target of Cz's actions. The present work assessed this possibility.

Glutamatergically-mediated field potentials were monitored in striatal slices perfused with Mg^{2+}-free artificial CSF (aCSF). These potentials were suppressed by kynurenic acid or MK-801 and were thus presumed to be mediated, in large part, by NMDA receptors. Cz, added to the aCSF, suppressed these responses in concentrations as low as 10 nM.

Since previous work showed displacement of [^{3}H]MK-801 at much higher concentrations (K_D=17μM), Cz has at least two sites of action on the NMDA receptor. The Cz concentration that suppressed responses in striatal slices was similar to that in CSF of patients receiving Cz for treatment of psychosis. Thus, activity at one of these sites suggests that anti-glutamatergic effects may enter into this drug's clinical profile.

667.2

TYPICAL AND ATYPICAL NEUROLEPTICS DIFFERENTLY AFFECT FOS PROTEIN EXPRESSION IN THE RAT FOREBRAIN. A. Fink-Jensen* and P. Kristensen. Pharmaceuticals Division and Biopharmaceuticals Division, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark.

The cellular synthesis of the transcription factor protein Fos is regarded as a biochemical marker of neuronal activity and previous studies suggest that the effect of the atypical neuroleptic clozapine on Fos protein expression in the prefrontal cortex (PFC) may be related to its unique effects on negative symptoms in schizophrenia (Neurosci. (1992) 46:315-328). In order to investigate if the Fos protein expression pattern induced by clozapine applies to other compounds with an atypical profile in preclinical or clinical trials, we investigated the acute effect of the atypical neuroleptics clozapine, risperidone, sertindole and NNC 22-0031 (4-(6-flouro-1,2-benzisoxazol-3-yl)-1-(3-(3,4-methylenedioxyphenylcarboyloxy)propyl)piperidine) as well as the prototypical neuroleptic haloperidol. The Fos protein expression was assessed by use of immunohistochemistry. The present study shows that atypical and prototypical neuroleptics can be differentiated on the basis of their ability to induce Fos protein since the ratio between Fos protein expression in the limbic PFC and in the non-limbic dorsolateral striatum (DLSt) was higher for the atypical neuroleptics than observed with the prototypical neuroleptic.

667.3

THE ATYPICAL ANTIPSYCHOTIC CLOZAPINE IS A POTENT AGONIST AT HUMAN CHOLINERGIC MUSCARINIC m4 RECEPTORS AND AN ANTAGONIST AT m1, m2, m3, & m5 RECEPTORS. K.M. Ward, D. Liston, S.B. Jones, and S.H. Zorn*. Pfizer Inc., Central Research Division, Department of Neuroscience, Groton, CT 06340.

Clozapine is an atypical antipsychotic drug that is more efficacious than conventional neuroleptics and does not produce EPS. Previous reports have shown that clozapine binds with high affinity to all 5 muscarinic receptor subtypes (Eur. J. Pharmacol. 1921:205,1991). Clozapine is an antagonist at some of these subtypes, and has been widely assumed to be an antagonist at all of them. Clinically, one of clozapine's notable side effects is hypersalivation. However, since hypersalivation and clozapine-induced dopamine turnover in rat striatum are blocked by muscarinic antagonists, clozapine may have direct cholinomimetic activity (JPET 268:1452, 1994). In the present study the functional interaction of clozapine with human m1-m5 receptors expressed in CHO cells was characterized. It was discovered that clozapine is a potent (EC50 = 7.5 nM) and full agonist at only the m4 muscarinic receptor subtype, producing concentration dependent inhibition of forskolin (FSK)-stimulated cAMP accumulation. This effect could be blocked by N-methyl-scopolamine and pertussis toxin. In contrast, clozapine potently antagonized agonist-induced responses at the other four muscarinic receptors. Thus, clozapine was found to block the inhibitory effect of carbachol on FSK-stimulated cAMP accumulation in m2 expressing cells as well as carbachol-induced PI turnover in cells expressing the m1, m3 & m5 receptor subtypes. Since the m4 receptor is found in salivary glands and is enriched in striatum, the present findings suggest that clozapine-induced hypersalivation as well as its effects on striatal dopamine turnover may be due to direct stimulation of m4 muscarinic receptors in these tissues. This agonist action at the m4 receptor may contribute to clozapine's atypical antipsychotic efficacy.

667.4

FURTHER EVIDENCE FOR MUSCARINIC ANTAGONISM AS CLOZAPINE'S DISCRIMINATIVE STIMULUS. B.M. Kelley, K.A. Nuti, J.H. Porter*. Psychology, Virginia Commonwealth Univ. Richmond, VA 23284.

Although clozapine (CLZ) has been shown to be superior to conventional neurolep-tics in the treatment of schizophrenia, CLZ's mechanism of action has yet to be comp-letely delineated. A two-lever drug discrimination procedure was employed in order to more precisely characterize the effect of drugs interacting with the cholinergic system on the discriminative stimulus properties of clozapine. Evidence for both muscarinic and\ or serotonergic mediation of CLZ's cue has been demonstrated in previous studies. Because many of these serotonergic drugs show high binding affinity for cholinergic receptors, two discrimination groups were trained. One group was trained to discriminate CLZ (5.0 mg/kg, i.p.) from vehicle, and another group was trained to discriminate scopolamine (0.125 mg/kg, i.p.) (SCP) from saline. Male Sprague-Dawley rats (85% B.W.) were tested in 15 min. sessions under a FR30 schedule of food reinforcement. After generalization testing with CLZ-trained rats (ED_{50}=0.1296) and SCP-trained rats (ED_{50}=0.0136), promethazine (PRO) and cyproheptadine (CYP) were tested in both groups. Mean percent drug-lever responding (DLR) with PRO peaked at the 10.0 mg/kg dose (99.3%) in the SCP group (ED_{50} = 0.609) and peaked at 2.5 mg/kg (97.0%) in the CLZ group (ED_{50} = 0.751). Complete substitution with CYP also was observed in both groups. In the SCP group, the 10.0 mg/kg dose produced peak substitution (ED_{50} = 3.513). In the CLZ group, the 1.25 mg/kg dose produced maximal DLR (98.2%) (ED_{50} = 0.114). Methylscopolamine (MSCP) substitution testing and antagonism of the CLZ-cue with oxotremorine (OXO) also were assessed in the CLZ-group. MSCP did not substitute for CLZ at any dose. MSCP (1.5 mg/kg) was coadministered (i.e., to block the peripheral effects of OXO) with OXO (0.0325, 0.0625, and 0.125 mg/kg), and the highest dose of OXO caused CLZ-DLR to decrease from 96.1% to 61.7%. Results from this study support the notion that CLZ's discriminative stimulus properties in rats are mediated by antagonism at muscarinic receptors.

667.5

EFFECTS OF SUBCHRONIC CLOZAPINE AND HALOPERIDOL ON RATS TRAINED IN A FORELIMB TREMOR TASK. S.C. Fowler*, J.A. Stanford and S. Das. University of Mississippi, University, MS 38677.

Rats trained to extend the forelimb through a rectangular hole and exert downward pressure on a force transducer received either the atypical neuroleptic clozapine or the typical neuroleptic haloperidol for 11 consecutive days. Doses were individually titrated daily for each rat in an attempt to achieve a 50% reduction in time on task (TOT). Clozapine treated rats exhibited dramatic tolerance to the drug's suppressive effect on TOT. In contrast, haloperidol rats displayed little tolerance. Despite the accentuated tolerance reflected by TOT in the face of escalating doses of clozapine, no tolerance was seen in clozapine's marked slowing of the dominant frequency of oscillations in forelimb force as measured by Fourier analysis of the force-time recordings. Haloperidol did not produce the oscillation slowing. The dissociation between the tendency to respond (TOT) and the oscillator slowing observed for clozapine may reflect effects at different neurotransmitter receptor sites. Supported by MH43429.

667.6

ASSESSMENT OF CLOZAPINE'S SUBCHRONIC DOSE EFFECTS ON FORCE, DURATION, AND RHYTHM OF LICKING IN RATS. Shyamal Das* and S.C. Fowler. University of Miss., University, MS 38677.

Three doses (1.5, 3.0, 4.5 mg/kg, ip, 45 min) of the atypical antipsychotic clozapine were studied in a subchronic dosing paradigm (at least 7 consecutive days at each dose with 4 or more vehicle only days separate dosing periods) in order to evaluate potential tolerance or sensitization effects in rats. Thirsty rats (n=20) licked water from a force-sensing disk while force-time waveforms of licking were recorded for a 2-min session. Lick rhythm was quantitated by applying Fourier methods to the force-time records. Other behavioral measures were peak force and duration of individual licks and number of licks. At 3.0 and 4.5 mg/kg, modest, but significant, tolerance effects emerged. 1.5 mg/kg produced sensitization effects, and the lick rhythm was the variable most affected by the drug as measured by omega squared. Clozapine's powerful effects on lick rhythm are unlike those of haloperidol (Fowler & Das, PBB, in press) and may reflect effects at serotonin receptors on the hypoglossal nucleus. Supported by MH43429.

667.7

LIKE CLOZAPINE, OLANZAPINE SLOWS RATS' FORELIMB FREQUENCY OSCILLATIONS IN A PRESS-WHILE-LICKING BEHAVIORAL TASK. J.A. Stanford* and S.C. Fowler. Univ. of Mississippi, University, MS 38677.

Rats were trained to press a force-sensing transducer with one forelimb in a press-while-licking (water fountain) task while initial peak force (PF), hold force (HF), force frequency oscillations (FREQ), and time-on-task (TOT) were measured. Following extensive experience on the task, subjects were administered olanzapine (OL, 0.5, 1.0, 2.0 mg/kg), a candidate atypical neuroleptic. OL significantly decreased TOT dose-dependently and had a significant slowing effect upon FREQ as quantified by Fourier analysis. These results were compared to clozapine's (CL) similar effects on the same measures previously reported in this task and contrasted with haloperidol's (HAL) FREQ acceleration (Fowler, et al., Psychopharmacology, in press). Since typical neuroleptics such as HAL tend to induce motor side effects such as tremor, these findings may reflect the antitremor and atypical properties of OL and CL. Supported by MH43429.

667.8

SELF DESTRUCTIVE BEHAVIOR AND CLOZAPINE DISCONTINUATION.

F.G. Moeller[1]*, Y.W. Chen,[1] Joel L. Steinberg, M.D.[2,3] M. Fulton,[2,3] F. Petty,[2,3] G.W. Ripper,[2] D.L. Garver.[2,3] 1. University of Texas Houston Health Science Center 1300 Moursund, Houston TX 77030 2. Dallas VAMC 3. Univ. of Texas Southwestern Med. School at Dallas. The atypical antipsychotic clozapine has effects as a serotonin antagonist as well as a dopamine antagonist. There have been reports that clozapine increases depressive symptoms in some patients and it has been speculated that this increase is due to the serotonin antagonist effects of clozapine. In order to determine if patients with preexisting suicidal or self mutilative ideation or behavior were more likely to have a poor outcome with clozapine than patients without this behavior, logistic regression was performed on data obtained from 729 patients treated with clozapine in the VA hospital system. Variables analyzed in the regression included age, sex, race, prior history of treatment failure, presence of suicidal behavior in the past month or ever in the past, and presence of self mutilative behavior in the past month or ever in the past. Of the 729 patients in the study 32 (4.3%) had suicidal ideation or behavior in the month prior to starting clozapine, 337 (46.2%) had suicidal ideation or behavior ever in the past, 32 (4.4%) had self mutilation in the past month, 137 (18.8%) had self mutilation ever in the past. There was no significant effect of suicidality or self mutilatory behavior on clozapine discontinuation in the logistic regression model. There was an effect of race and prior history of treatment failure which have been reported previously. Our study does not support suicidality or self mutilatory behavior as increased risk factors for poor outcome with clozapine.

667.9

D3 AND D4 DOPAMINE RECEPTORS MEDIATE CLOZAPINE-INDUCED C-FOS EXPRESSION IN THE FOREBRAIN. N. Guo* and H.C. Fibiger, Division of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. Canada V6T 1Z3

The receptor mechanisms underlying clozapine-induced *c-fos* expression in the forebrain are unknown. The regional disparity between clozapine- and haloperidol-induced *c-fos* expression in the brain suggested that D2 receptor antagonism is not sufficient to account for the unique pattern produced by clozapine. In addition, previous studies have demonstrated that neither serotonergic nor noradrenergic mechanisms are involved in this effect of clozapine. To determine whether blockade of D3 and/or D4 dopamine (DA) receptors may contribute to clozapine's effects, the DA receptor agonists quinpirole and 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OHDPAT) were co-administered with clozapine.

Quinpirole, which has approximately equal affinity for D3 and D4 receptors and about 100-fold lower affinity for D2 receptors, completely blocked clozapine-incuced *c-fos* expression in the medial prefrontal cortex (mPFC), nucleus accumbens (NAc) and lateral septal nucleus (septum). Co-administration of 7-OHDPAT , which has about 75-fold higher affinity for D3 than D2 receptors, and very low affinity for D4 receptors, reduced the number of clozapine-incuced Fos positive neurons by 79% in the NAc and by 52% in the septum but had no effect in the mPFC. These results suggest that D4 receptors are involved in clozapine-induced *c-fos* expression in the mPFC, while blockade of D3 receptors mediates clozapine-induced *c-fos* expression in the NAc and septum. These results are consistent with the view that the contrasting clinical profiles of haloperidol and clozapine are due to the fact that these compounds target different populations of neurons in the forebrain and that the atypical properties of clozapine may be related in part to its antagonist effects at D3 and D4 receptors.

667.10

EFFECT OF CHRONIC NEUROLEPTIC ADMINISTRATION ON RAT ORAL DYSKINESIA AND THE mRNA EXPRESSION OF GAD, ENKEPHALIN AND SUBSTANCE P. T. Hashimoto*, X.-M. Gao, O. Shirakawa, T. Kakigi, A.J. Tobin[1], C.A. Tamminga. M.P.R.C., Department of Psychiatry, U.M.A.B., Baltimore, MD 21228, and Department of Biology, University of California, Los Angeles, CA 90024[1].

Chronic neuroleptic treatment causes tardive dyskinesia in humans. Vacuous chewing movements (VCM) in rats has been used as a model of tardive dyskinesia in humans. We examined VCMs and GAD_{67}, GAD_{65}, enkephalin, and substance P mRNA expression in rats treated chronically with neuroleptics. Rats were treated with different dose levels of haloperidol (1.5 or 3.0 mg/kg/day) and clozapine (10, 20 or 30 mg/kg/day). After 3 months of drug treatment, haloperidol produced significant rates of VCMs: 15.8 ± 2.2 for haloperidol; 1.2 ± 0.5 for water (mean ± S.E./5 min.). Clozapine did induce VCMs (6.4 ± 1.5) but at significantly lower rate than with haloperidol. There was no apparent difference in VCMs between different dose groups. In the basal ganglia of rats treated chronically (6 months) with haloperidol (1.5 mg/kg/day), in situ hybridization revealed a significant increase in GAD_{67} mRNA in striatum of 11% and a decrease in globus pallidus of 15%, with no change in GAD_{67} mRNA in SNR. GAD_{65} mRNA did not change in striatum or SNR. Enkephalin mRNA increased 23% in striatum, while substance P mRNA remained unchanged. Changes in GAD_{67} mRNAs did not correlate with the rates of VCMs. The results indicate that chronic blockade of D_2 receptor might not induce VCMs in dose dependent manner. Moreover, the other neural mechanisms, in addition to the activation of striatopallidal GABA/enkephalin containing neurons with its D_2 receptor blockade, such as D_1 receptor mediated transmission in striatonigral neurons, may be involved in the expression of tardive dyskinesia.

667.11

SELECTIVE ACTIONS OF THE NOVEL ATYPICAL ANTIPSYCHOTIC DRUG AMPEROZIDE IN THE LIMBIC FOREBRAIN. G.G.Nomikos*, M.M.Marcus, C.S.Tham, H.C.Fibiger and T.H.Svensson. Karolinska Institutet, Dept. of Pharmacology, S-17177 Stockholm, Sweden

Amperozide (APZ) exhibits a multireceptor profile with high affinity for 5-HT_2 receptors, relatively low affinity for dopamine (DA) receptors, and a rather marked limbic selectivity of action. Recently, *in vivo* microdialysis studies have revealed that APZ induces a more pronounced increase in DA concentrations in the medial prefrontal cortex (MPC) than in the nucleus accumbens (NAC) or the striatum, analogously with clozapine but not with classical antipsychotics. This effect of APZ may be due to its combined 5-HT_2 and DA-D_2 receptor antagonistic properties. By employing *in vivo* voltammetry and Fos immunohistochemistry, we examined the effects of APZ on DA concentrations in two subsets of the NAC, the shell and core, that are associated with limbic and striatal functions, respectively, and on *c-fos* expression in the limbic forebrain and the basal ganglia. APZ (1.0 and 2.0 mg/kg, iv) almost exclusively increased DA concentrations in the NAC-shell compared to the core. Similar results were obtained with ritanserin but not with raclopride, which instead increased DA equipotently in both parts. The number of Fos-positive neurons was significantly increased by APZ (5.0 and 10 mg/kg, sc) in the MPC and the lateral septum but not in the striatum or the NAC. Thus, APZ shows a somewhat clozapine-like profile as regards also its effects on c-fos expression in the forebrain. These results emphasize amperozide's limbic selectivity of action, the importance of 5-HT_2 receptor antagonism for such an effect, and strengthen the hypothesis that the therapeutic profile of atypical antipsychotics may be related to their distinctive effects in the mesolimbocortical dopaminergic system.

667.12

RITANSERIN POTENTIATES THE STIMULATORY EFFECTS OF RACLOPRIDE ON DOPAMINE ACTIVITY AND RELEASE PREFERENTIALLY IN THE MESOLIMBOCORTICAL DOPAMINERGIC SYSTEM. J.L.Andersson, G.G.Nomikos, M.M.Marcus and T.H.Svensson*. Karolinska Institutet, Dept. of Pharmacology, S-17177 Stockholm, Sweden

The limbic selectivity of action of some atypical antipsychotic drugs has been attributed to potent 5-HT_2 receptor antagonism and a high ratio of 5-HT_2 to dopamine (DA)-D_2 receptor affinities. By employing *in vivo* single unit electrophysiology, dialysis and voltammetry, we examined the effects of the DA-D_2 receptor antagonist raclopride in combination with the 5-HT_2 receptor antagonist, ritanserin, on DA transmission. Raclopride (10-2560 ug/kg, iv) dose-dependently increased firing rate and burst firing of midbrain DA neurons; this effect was more pronounced in the ventral tegmental area (VTA) than in the substantia nigra-zona compacta (SN-ZC). Ritanserin (1.0 mg/kg, iv) enhanced firing rate and burst firing of midbrain DA neurons. Ritanserin pretreatment (30 min) potentiated the stimulating effects of low doses of raclopride on burst firing, preferentially in VTA DA neurons. Raclopride (50 ug/kg, sc) increased dialysate DA concentrations in the striatum and the medial prefrontal cortex (MPC) to 220 and 175% of baseline, respectively, while ritanserin (1.5 mg/kg, sc) elevated striatal and cortical DA levels by about 30%. Ritanserin pretreatment (40 min) potentiated the raclopride-induced DA increase in the MPC to 255% without affecting the action of raclopride on striatal DA levels. Similar results were obtained in the nucleus accumbens where the raclopride-induced increase in the voltammetric DA signal was enhanced by ritanserin pretreatment. These data indicate that 5-HT_2 receptor antagonism in conjunction with some degree of DA-D_2 receptor antagonism facilitates DA transmission selectively in the mesolimbocortical dopaminergic projection.

667.13

DIFFERENTIAL EFFECTS OF ANTIPSYCHOTIC DRUGS ON EXTRACELLULAR GABA LEVELS IN THE VENTRAL PALLIDUM AND GLOBUS PALLIDUS OF RATS. M.A. Chapman*and R.E. See. Department of Psychology, Washington State University, Pullman, WA 99164-4820. Antipsychotic drugs (APDs) are believed to treat schizophrenia through their effects on mesolimbic dopamine (DA) pathways and produce motor side effects via effects on nigrostriatal DA pathways. In order for these APD-induced changes to affect behavior, neurotransmitter levels must be altered in the output nuclei of these two systems. To test this hypothesis, female, Sprague-Dawley rats were given a subcutaneous injection of haloperidol (1.0 mg/kg), clozapine (30.0 mg/kg), or metoclopramide (10.0 mg/kg). Intracranial microdialysis was used to assess GABA levels simultaneously in the ventral pallidum and globus pallidus, regions which receive major GABAergic projections from the mesolimbic and nigrostriatal dopamine systems, respectively. GABA was analyzed using o-phthaldialdehyde derivatization followed by high-performance liquid chromatography with electrochemical detection. Both haloperidol and metoclopramide increased extracellular GABA levels in the globus pallidus, while clozapine decreased GABA in this region. Clozapine also decreased extracellular GABA levels in the ventral pallidum, whereas haloperidol and metoclopramide had no effect. These results suggest that increased extracellular GABA levels in the globus pallidus may be related to the motor side effects of antipsychotic drugs, while decreased GABA levels in the ventral pallidum may be related to the unique clinical profile of clozapine. (Supported by NIH grant MH10644 to M.A.C. and DE09678 to R.E.S.).

667.14

AMPHETAMINE-INDUCED STEREOTYPY AS A RODENT MODEL FOR THE EXTRAPYRAMIDAL SIDE EFFECT (EPS) LIABILITY OF ANTIPSYCHOTIC DRUGS. H. Donovan, C.A. Bankoski, S.H. Lang, L. Rajachandran* and D.C. Hoffman. Behavioral Biology, Neurogen Corp., Branford, CT 06405.

Typical antipsychotics block amphetamine-induced stereotypy in rodents. It is unclear whether this behavioral effect predicts therapeutic efficacy or EPS liability. Because the atypical antipsychotic clozapine is efficacious in treating schizophrenia while producing minimal EPS, we decided to re-evaluate it's effects on amphetamine (10 mg/kg)-induced stereotypy, and compare them to the effects of the typical antipsychotic haloperidol and the novel antipsychotics raclopride, risperidone and savoxepine in male Sprague-Dawley rats. With the exception of clozapine, all drugs produced a significant and dose-related antagonism of stereotypy. Clozapine significantly reduced stereotypy at several doses but the inhibition was never greater than 50 percent. For all drugs, the antagonism of amphetamine-induced stereotypy was accompanied by a significant increase in locomotor activity (measured in Omnitech activity monitors) at the intermediate doses. This reflects the emergence of hyperactivity normally seen with lower doses of amphetamine. Thus, according to two measures (stereotypy ratings and activity scores), clozapine is effective in antagonizing amphetamine-induced stereotypy but it is considerably less efficacious than haloperidol and the novel antipsychotics. This latter finding may be related to the few EPS associated with clozapine treatment in humans, and suggests that blockade of amphetamine stereotypy may predict EPS liability more so than therapeutic efficacy.

667.15

CATALEPSY AS A RODENT MODEL FOR DETECTING ANTIPSYCHOTIC DRUGS WITH EXTRAPYRAMIDAL SIDE EFFECTS (EPS) IN HUMANS: A RE-EVALUATION OF SAVOXEPINE. D.C. Hoffman* and H. Donovan. Behavioral Biology, Neurogen Corporation, Branford CT 06405.

The predictive validity of catalepsy as a rodent model for detecting the EPS of antipsychotic drugs was recently questioned when the novel antipsychotic savoxepine produced little catalepsy in rodents while producing significant EPS in schizophrenic patients. Because catalepsy is viewed as an important model for predicting EPS, we decided to re-evaluate the effects of savoxepine. Savoxepine, haloperidol, clozapine, raclopride, risperidone and ORG 5222 were examined in two tests for catalepsy (Grid and Bar Tests) in male Sprague-Dawley rats. The ability to antagonize amphetamine-induced hypermotility was also examined since this measure is believed to predict clinical efficacy. With the exception of clozapine, all drugs produced dose-dependent catalepsy in both tests. For each drug, the MEDs for producing catalepsy were greater than those necessary for antagonizing amphetamine-induced activity. Clozapine demonstrated the widest separation of MEDs in the catalepsy and activity models while haloperidol showed the narrowest separation. This is consistent with the clinical effects of haloperidol (severe EPS) and clozapine (mild EPS). The ratios of MEDs in catalepsy and activity for the remaining novel drugs fell between haloperidol and clozapine; this is consistent with the preliminary clinical findings indicating some EPS with each of these compounds. Thus, catalepsy remains a suitable rodent model for detecting compounds with EPS liability in humans.

667.16

PRECLINICAL PHARMACOLOGY OF ZD3638: A NOVEL AGENT WITH ATYPICAL ANTIPSYCHOTIC ACTIONS. Jeffrey M. Goldstein* and Michael T. Klimas. Departments of CNS Pharmacology and Medicinal Chemistry, ZENECA Pharmaceuticals Group, Wilmington DE 19897.

ZD3638 is a new putative antipsychotic agent that was evaluated through a series of in vitro and in vivo tests in a variety of species including mice, rats, and monkeys. ZD3638 displays high affinity for dopamine D1, D2 and serotonin 5HT2 receptors (Kis, nM, 13,42,39, respectively). ZD3638 also has high affinity for α1-adrenergic receptors (Ki=63 nM) and is devoid of activity at muscarinic M2 and M3 cholinergic receptors. ZD3638 is active orally in classical tests for antipsychotic activity, eg conditioned avoidance in primates and behavioral or electrophysiological tests involving the reversal of dopamine agonist-induced effects. It possesses considerable selectivity for the limbic system. Both dopamine D1 and D2 functional antagonism, eg reversal of D1 and D2 agonist-induced rotation in rats and blinking in squirrel monkeys, as well as serotonin 5HT2 functional antagonism, eg reversal of quipazine-induced head twitch in rats, can be demonstrated. Additionally, ZD3638, unlike classical antipsychotic agents, has a reduced liability for sensitizing drug-naive Cebus monkeys after chronic administration. When administered chronically to rats, ZD3638 produces selective depolarization inactivation of mesolimbic (A10) dopamine neurons. These last two properties are like those of clozapine, and are believed predictive of reduced extrapyramidal side effects. On the basis of these overall results, ZD3638 is predicted to have enhanced antipsychotic efficacy with a superior side effect profile as compared to classical antipsychotic agents.

667.17

ATYPICAL NEUROLEPTICS SHOW REGIONAL SELECTIVITY: ROLE OF OLFACTORY TUBERCLE. A.R. Cools*, E.P.M. Prinssen and B.A. Ellenbroek. Dept.Psychoneuropharmacology, University of Nijmegen, P.O 9101, 6500 HB Nijmegen, The Netherlands.

In order to validate the hypothesis that atypical neuroleptics are far more potent in the olfactory tubercle (OT) than in the nucleus accumbens (ACC; Behav. Pharmacol. 3, s18, 1992), we investigated to what extent risperidone, sertindole, olanzapine, ORG 5222 and prothipendyl, viz. all compounds claimed to act as atypical neuroleptics, differentially attenuate locomotor activity elicited from the OT and the ACC respectively. First, we used the so-called paw-test to identify the atypical profile of these compounds (Life Sci. 42, 1205, 1988): in all cases, the dose required for enhancing hindlimb retraction time (HRT) was lower than that required for enhancing forelimb retraction time (FRT), underscoring the atypical profile of the tested compounds. Second, the ability of intracerebral administration of the drug (0.5 μl) to attenuate locomotor activity elicited either by dopamine injections (10 μg/0.5 μl) into the OT or by ergometrine injections (1 μg/0.5 μl) into the ACC was compared. Following normal habituation procedures to standard locomotor cages, dose-effect curves (1 ng - 10 μg) were made, using computerized recordings. Using the ratio of the minimum effective doses (OT : ACC), it was found that all compounds were far more potent in the OT than in the ACC. It is concluded that neuroleptics with an atypical profile are far more potent in the olfactory tubercle than the nucleus accumbens; the reverse holds true for classical neuroleptics.

667.18

BEHAVIORAL PHARMACOLOGY OF BW 1205U90, A SATURATED PHTHALAZINONE WITH MIXED DOPAMINE AND SEROTONIN ANTAGONIST ACTIVITY. G.C. Rigdon* and M.H. Norman. Divisions of Pharmacology and Organic Chemistry, Burroughs Wellcome Co, Research Triangle Park, NC 27709.

BW 1205U90 ((+/-)-*cis*-2-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)-4A, 5, 6, 7, 8, 8A-hexahydro-1(2H)-phthalazinone hydrochloride) was synthesized as part of a program to develop antipsychotics with reduced liability for extrapyramidal side effects (EPS). It bound to 5-HT_2 receptors (IC_{50} = 0.8 nM) more potently than to dopamine D_2 receptors (IC_{50} = 1.6 nM) and also bound to 5-HT_{1a} and adrenergic α_1 receptors potently. In mouse, BW 1205U90 antagonized climbing (efficacy) and stereotyped behavior (EPS) induced by apomorphine with ED_{50}s = 16 and 74 mg/kg p.o., and 0.04 and 0.6 mg/kg s.c., respectively. The ED_{50} for induction of catalepsy (which also predicts EPS) was 58 mg/kg p.o. Head twitches in mice induced by the serotonin agonist 5-methoxydimethyltryptamine were antagonized with ED_{50}s = 2.0 mg/kg p.o. and 0.03 mg/kg s.c.

In rat, BW 1205U90 blocked apomorphine-induced circling in subjects with unilateral 6-hydroxydopamine lesions of the striatum (ED_{50} = 1.1 mg/kg p.o.) and antagonized apomorphine-induced stereotypy (ED_{50} = 3.3 mg/kg p.o.). Apomorphine-induced disruption of prepulse inhibition of acoustic startle reflex was antagonized at 0.2 mg/kg s.c. and conditioned avoidance responding was inhibited with an ED_{50} = 0.05 mg/kg s.c. Shaking behavior induced by the serotonin precursor 5-hydroxytryptophan, was antagonized (ED_{50} = 0.6 mg/kg p.o.).

BW 1205U90 antagonized 5-HT_2-mediated effects more potently than D_2-mediated effects. It was potent in tests predictive of antipsychotic efficacy, but much less potent in tests for EPS.

EPILEPSY: ANTICONVULSANT DRUGS—OTHER NEUROTRANSMITTER RECEPTORS

668.1

EFFECT OF LAMOTRIGINE ON VOLTAGE DEPENDENT SODIUM CURRENTS AND ON SPONTANEOUS ACTIVITY IN IN VITRO EPILEPTOGENESIS. M. Caeser, K.D. Keegan*, M.L. Evans and C.D. Benham. SmithKline Beecham, Harlow, Essex CM19 5AD UK.

The recently launched anticonvulsant lamotrigine was investigated on TTX-sensitive Na^+ currents (I_{Na}) and on spontaneous activity in *in vitro* models of epileptogenesis relative to phenytoin, carbamazepine and pentobarbital. All recordings were performed on hippocampal cell cultures using whole cell patch clamp. Sodium currents were activated by membrane depolarizations of 20 ms duration to potentials greater than -60 mV. I_{Na} was reversibly depressed in a voltage dependent manner by bath application of lamotrigine. The block of I_{Na} -evoked by voltage command pulses to -20mV- by lamotrigine (50 μM) was increased from 12±2% (n=16) to 57±4% (n=20) upon depolarizing the membrane holding potential from -90 mV to -57mV. Similar observations were made when carbamazepine and phenytoin was tested at 50μM concentration. Starting from a membrane holding potential of -57mV, lamotrigine (1 and 250μM) reduced I_{Na} to 92±2% (n=8) and 18±4% (n=7) of control values, respectively. The EC_{50} value was 28±5μM at a stimulus frequency of 0.1Hz. The depression of sodium currents by lamotrigine (50μM) was use dependent, in that raising the stimulus frequency from 0.1 to 5 Hz reduced the peak amplitude of I_{Na} by additional 12±2% (n=7). In current clamp experiments, the shift in resting membrane potential, high spontaneous activity of neurones as well as paroxymal discharge pattern observed in the presence of high potassium (7.5 mM) or zero Mg^{2+} was significantly reduced by lamotrigine, phenytoin and pentobarbital (all tested at 50μM). This study demonstrates, that similar to phenytoin and carbamazepine, lamotrigine acts as a voltage dependent sodium chennel blocker at therapeutic concentration, which might contribute to the reported reduction of glutamate release.

668.2

ANTICONVULSANT EFFECTS OF CHRONIC MELATONIN AT DIFFERENT TIMES OF THE DAY IN MALE GERBILS. TH Champney*, WH Hanneman, ME Legare and JC Champney. Depts Human Anat and Vet Anat, Texas A&M Univ, College Station, TX 77843.

Recently, melatonin's anticonvulsant action has been demonstrated in gerbils (Neuroreport 3:1152-1154, 1992). The present study explored the effects of melatonin (MEL) administration at one of two times of the day on seizure severity after pentylenetetrazol (PTZ) administration. Also, changes in γ-aminobutyric acid (GABA) and amine content from selected brain regions of these animals was determined. Male Mongolian gerbils (Meriones unguiculatus) received daily injections of melatonin (MEL, 25 μg/inj, sc) at 0800 or 1700 h, saline at the same two time points or no treatment for 12 weeks. At the end of the treatment period, the gerbils were challenged with PTZ (60 mg/kg, sc) to produce convulsions and were monitored for convulsive behavior for 50 minutes. Three minutes prior to death, they received 3-mercaptopropionic acid (100 mg/kg, ip) to prevent GABA degradation. The gerbils were anesthetized with metofane and killed by decapitation. Cortex, hippocampus and hypothalamus were collected. These regions were assayed for GABA, dopamine, serotonin and 5-hydroxyindoleacetic acid content (J Chromat Biomed Appl 579:334-339, 1992). MEL injected gerbils were able to survive and respond to seizures better than control animals. Total convulsion scores were reduced in MEL injected gerbils at 0800 or 1700 h compared to saline injected animals. Only one MEL injected gerbil died during seizure-induction (1/12 compared to 5/12 in saline or no treatment animals). Interestingly, the animals receiving no treatment had lower convulsion scores than saline injected gerbils. GABA levels and amine levels were similar in all gerbils with no significant differences between any of the treatment groups. These results suggest that MEL's anticonvulsant activity does not correlate with changes in neurochemicals in the regions studied. The ability of melatonin to prevent convulsions was not time of day dependent as has been observed when examining the reproductive effects of melatonin.

668.3

FURTHER EVIDENCE THAT SEROTONIN PLAYS AN ANTICONVULSANT ROLE IN GENETICALLY EPILEPSY-PRONE RATS (GEPRS). Q-S. Yan*, P. C. Jobe and J. W. Dailey. Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61656.

Previous reports from this laboratory indicate that fluoxetine, a selective serotonin uptake inhibitor, has anticonvulsant effects in genetically epilepsy-prone rats (GEPRs). Also, the protection against audiogenic seizure following the administration of fluoxetine appears to be selectively correlated with enhanced serotonergic transmission (Dailey et al., 1992; Yan et al., 1994). An important question raised by these findings is whether the anticonvulsant action following the administration of the serotonin uptake inhibitor in GEPRs is peculiar to fluoxetine. This study was designed to evaluate further the role of serotonin in regulating susceptability and/or severity of audiogenic seizures in GEPRs. For this purpose, sertraline, another highly selective and potent inhibitor of serotonin uptake, was studied. Severe seizure genetically epilepsy-prone rats (GEPR-9s) were used as experimental subjects in the investigation. Sertraline (7.5, 15 and 30 mg/kg, i.p.) produced a dose-dependent reduction in the audiogenic seizure severity. Brain microdialysis studies showed that the same doses of sertraline also caused dose-dependent increases in the extracellular serotonin concentration in the thalamus. Interestingly, the peak anticonvulsant effect correlated temporally with the peak increases in the extracellular serotonin concentration for this drug. These findings provide further support for the concept that serotonin plays an anticonvulsant role in GEPRs.

668.4

WAY100135 ANTAGONIZES THE SEROTONIN-MEDIATED INHIBITION OF EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL CA1 NEURONS. Delanthi Salgado* and Karim A. Alkadhi. Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77204-5515 USA.

Previous studies conducted in our laboratory have shown that serotonin inhibits epileptiform activity (Salgado and Alkadhi, Soc. Neurosc. Abstr., 764.4, 1993). In addition, experiments done thus far suggest that the serotonin-mediated inhibition of epileptiform discharge is due to activation of the 5-HT_{1A} receptor subtype. The purpose of this study was to provide more conclusive evidence regarding the involvement of the 5-HT_{1A} receptor in the inhibition of epileptiform activity. Thus we used the new, selective 5-HT_{1A} receptor antagonist, WAY100135. We employed conventional electrophysiological techniques for intracellular recording from CA1 neurons in the rat brain slice preparation. Initial experiments demonstrate that WAY100135 (10 μM) prevents the serotonin-mediated hyperpolarization, decrease in membrane resistance and current-induced action potential bursts. Furthermore, the serotonin-mediated suppression of bicuculline-induced epileptiform bursts was also antagonized in the presence of WAY100135. The results obtained thus far strongly suggest that activation of 5-HT_{1A} receptors in hippocampal CA1 neurons can suppress epileptiform activity. This study suggests that treatment of epilepsy may be possible with agents selective for serotonin 5-HT_{1A} receptor sites on hippocampal CA1 neurons.

668.5

THE NOVEL ANTIEPILEPTIC GABAPENTIN ENHANCES PROMOTED RELEASE OF GABA IN HIPPOCAMPUS. O. Honmou*, A. Oyelese, and J. D. Kocsis. Dept. of Neurology, Yale Med. Sch., New Haven, CT. 06510, and Neuroscience Res. Ctr., VAMC, West Haven, CT. 06516.

Gabapentin (GBP), 1-(aminomethyl) cyclohexane-acetic acid, is a new anticonvulsant drug which is used clinically in the United Kingdom and the United States, but its mode of action is unknown. The antiepileptic properties of GBP were initially predicted because it is a structural analogue of the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA). However, it lacks agonist and antagonist properties at both $GABA_A$ and $GABA_B$ receptor sites, and does not appear to block GABA uptake. GBP has been shown to increase the apparent rate of synthesis of GABA in several brain regions, and enhanced promoted release of GABA following GBP treatment has been observed in neonatal optic nerve. Here we provide evidence from electrophysiological experiments in the rat hippocampal slice preparation that GBP treatment enhances the reduction in excitatory postsynaptic potentials (EPSPs) elicited by promoted GABA release with nipecotic acid (NPA). NPA both blocks uptake of GABA and promotes release by heteroexchange through the GABA transporter. GBP had no effect on the normal excitability properties of the hippocampus. We suggest a novel mechanism for the antiepileptic properties of GBP whereby there is enhanced nonvesicular release of GABA possibly through reverse operation of the GABA transporter at times of intense electrical activity.

668.6

CHRONIC GAMMA-VINYLGABA REDUCES RAT BRAIN GLUTAMINE SYNTHETASE ACTIVITY. R. A. Waniewski*, K. Rimvall and D. L. Martin, Wadsworth Center for Labs and Research, New York State Department of Health, Albany, NY 12201.

The effect of elevated GABA levels on the glial enzyme glutamine synthetase (GS) was examined in rat brain. Repeated daily s.c. injections of the GABA-transaminase inhibitor, gamma-vinylGABA (GVG), an anti-epileptic medication, produced a gradual decline in brain GS activity. After 21 days, cortical GS activity was reduced by 36% with 150 mg/kg per day and 9.5% with 30 mg/kg per day compared to saline-injected rats. Cerebellar GS activity was reduced by 22% with 150 mg/kg and unchanged with 30 mg/kg. Skeletal muscle and liver GS activities were unaffected. Cortical GABA levels were increased by 170% after 150 mg/kg for 21 days but were unaffected at 30 mg/kg. Glutamine and glutamate levels were significantly reduced but only at the 150 mg/kg dose. GS activity was inversely correlated with brain GABA levels in the cortex. Shorter dosing periods produced smaller effects on brain GS activity. After 4 days, cortical GS was reduced by 13% and cerebellum was unaffected by 200 mg/kg GVG. GABA levels are maximally elevated even after this short dosing interval. The delay in the effect of GVG on GS may be related to the slow turnover of this enzyme in vivo. The glutamine cycle, which provides renewed substrate for neuronal GABA and glutamate synthesis, may be down-regulated by chronically elevated GABA. The reduction in GS activity appears to decrease brain glutamine production and thereby may limit transmitter glutamate synthesis and contribute to the anti-epileptic effect of GVG.

668.7

THE EFFECTS OF D-23129, A NEW EXPERIMENTAL ANTICONVULSANT DRUG, ON NEUROTRANSMITTER AMINO ACIDS (AA) IN THE RAT HIPPOCAMPUS *IN VITRO*. I.M. Kapetanovic*, W.D. Yonekawa and H.J. Kupferberg. Epilepsy Branch, NINDS, Bethesda, MD 20892.

D-23129 (N-[2-amino-4-(4-fluorobenzylamino)-phenyl]carbamic acid ethyl ester; Asta Medica AG, Germany; ADD230001) is a promising new anticonvulsant compound under investigation in the Antiepileptic Drug Development (ADD) Program. Recently, this compound was also shown to have some unique properties in suppressing *in vitro* epileptiform activity in rat hippocampal slices (W.D. Yonekawa *et al., Soc. Neurosci. Abst. 19*:1632, 1993). The balance between the effects of inhibitory (GABA) and excitatory [glutamate (GLU) and aspartate (ASP)] neurotransmitter AA is thought to be important in normal neurological function and also may play a crucial role in the pathogenesis and potential treatment of epilepsy. This study examined the effects of D-23129 and its dihydrochloride (D-20443; ADD172014) on total tissue and newly synthesized (NEW) levels of neurotransmitter AA. NEW AA may be more closely related to the neuronally active pools than their total tissue levels. Isotopic enrichment, after incubation with stable-isotopically labeled precursor ($^{13}C_6$-glucose), was used to measure NEW AA. Total tissue and NEW AA were determined by GC-MS. The primary effect of ADD172014 (5-40 μM) was a dose-related increase in NEW GABA. ADD172014 was unique in this behavior. Several clinically effective or experimental anticonvulsants either had no effect on total tissue and NEW AA or decreased NEW AA. This effect of ADD172014 was not tetrodotoxin-sensitive and suggests that it is not voltage dependent. While itself increasing NEW GABA, ADD172014 antagonized the TTX-sensitive 4-aminopyridine-induced elevation of NEW GABA, NEW ASP, and NEW GLU. This latter effect was similar to that of phenytoin, carbamazepine, lamotrigine, and U54494A, whose mechanism of action may involve inhibition of use-dependent voltage-sensitive sodium channels (I.M. Kapetanovic *et al., Soc. Neurosci. Abst. 18*:379, 1992). Therefore, ADD172014 exhibits two different effects on neurotransmitter amino acids which may contribute to its anticonvulsant action.

668.8

NOVEL MECHANISM OF ACTION OF ANTICONVULSANT ALKYL-SUBSTITUTED THIOBUTYROLACTONES: REDUCTION OF NEURONAL Ca CURRENTS. J.A. Ferrendelli*[1], R.A. Gross[2], D.F. Covey[3], & R.W. Subiaga, Jr.[2] Depts. of Neurology[1,2] & Pharmacology[1,3], [1,3]Washington University, St. Louis, MO, [2]University of Minnesota, Minneapolis, MN.

Alkyl-substituted thiobutyrolactones (TBLs) produce either convulsant or anticonvulsant effects by modulating the picrotoxin site of the $GABA_A$ receptor. Because of structural similarity to ethosuximide, an antiepileptic drug that blocks T-type Ca currents, we tested the effects of the anticonvulsant α-ethyl,α-methyl-thiobutyrolactone (α-EMTBL) on Ca currents of cultured DRG and neocortical neurons.

Whole cell Ca currents were evoked at +10-20 mV in media which blocked Na and K currents, with 5 mM Ca^{2+} as the charge carrier. Effects on Ca channel subtypes were assessed using nifedipine (L-type) and ω-conotoxin GVIA (N-type) and ω-agatoxin IVA (P-, Q-type). α-EMTBL (500 μM) had a voltage-dependent effect: high-threshold DRG currents were reduced ≤ 10% when evoked from -80 mV, but by 30-50% when evoked from -40 mV. Current inactivation was increased, with a greater effect on currents evoked from more polarized potentials. Neocortical currents were similarly affected. In DRG neurons, 10 μM ω-conotoxin GVIA reduced currents ~50% and 500 nM ω-agatoxin IVA reduced currents ~15%; 10 μM nifedipine reduced currents 10-15% at -80 mV and 45% at -40 mV. None of these, used alone, significantly affected the reduction induced by α-EMTBL.

These data suggest that α-EMTBL may have a second mechanism of action, reduction of neuronal Ca currents. This is relatively non-specific for channel subtypes and may occur by open channel block.

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668.9

INHIBITION OF MAXIMAL ELECTROSHOCK (MES) SEIZURES BY A1 ADENOSINE RECEPTOR AGONISTS. J.B. Wiesner* and S. Zimring. Gensia, Inc., San Diego, CA 92121.

Adenosine receptor agonists exert anticonvulsant activity in a variety of animal models, including those involving chemically-induced seizures, audiogenic seizures, and kindled seizures. However, little information exists concerning their efficacy in maximal electroshock (MES) seizures. Since the efficacy profile in such seizure models are often believed to predict efficacy in clinical seizure types, we investigated the effect of adenosine agonists in MES seizures, as well as seizures induced by s.c. pentylenetetrazol (PTZ); we also investigated effects on blood pressure, body temperature, and motor performance. Electrical current (60 Hz, 0.2 sec) was delivered via corneal electrodes at 150 mA in rats and 50 mA in mice at 20 min after i.p. injection of each agonist. The A1 receptor agonists cyclopentyladenosine, R-phenylisopropyladenosine, cyclohexyladenosine, 2-chloroadenosine, and N-ethylcarboxamidoadenosine, dose-dependently inhibited MES seizures in both species. Respective ED_{50} values (mg/kg) for MES in rats were 2.3, 3.7, 3.6, 5.7 and 10.4. Respective ED_{50} values for MES in mice were 1.7, 3.6, 13.4, 8.2, and 73.8. The A2 selective agonist CV1808 was ineffective at doses of up to 10-30 mg/kg. The A1 agonists also inhibited PTZ seizures with respective ED_{50} values of 11.2, 3.1, 4.5, 11.0, and 9.1 (rats) and 1.7, 0.6, 6.7, 3.2, and 0.1 (mice). All of the A1 agonists induced substantial akinetic or ataxic effects at anticonvulsant doses. In addition, the ED_{50} dose (2.3 mg/kg) of cyclopentyladenosine in conscious rats decreased blood pressure and heart rate by approximately 60% and decreased body temperature by 4-6°C.

These results show that stimulation of A1 adenosinergic receptors exerts an anticonvulsant effect in the MES seizure model in rats and mice, indicating potential utility of an adenosinergic approach in generalized tonic-clonic seizures in man. However, the clinical utility of direct A1 agonists will be limited if similar side effects are found in man.

668.10

THE PHENYLCARBAMIC ACID ESTER D-23129 IS HIGHLY EFFECTIVE IN EPILEPSY MODELS FOR GENERALISED AND FOCAL SEIZURES AT NONTOXIC DOSES. C. Tober, C. Rundfeldt*, A. Rostock and R. Bartsch. Arzneimittelwerk Dresden GmbH, Department of Pharmacology, Meißner Str. 191, 01445 Radebeul, FRG.

Complex partial seizures comprise the major uncontrolled seizure type in adult patients with epilepsy. New anticonvulsants should not only reduce seizure severity but also suppress focal seizure activity for full seizure protection. The goal of this study was to demonstrate that D-23129 is capable to suppress focal seizure activity at nontoxic doses. The broad effectiveness of D-20443, the hydrochloride of D-23129 (N-[2-Amino-4-(4-fluorbenzylamino)-phenyl]carbamic acid ester), was shown previously (Nickel et al, Epilepsia 34, Suppl. 2, 95, 1993). We could now demonstrate that D-23129 and D-20443 are equipotent in suppressing the tonic extension of hind limb in the maximal electroshock test in mice (ED_{50} 18,2 and 17,4 mg/kg i.p. respectively); both compounds also nearly doubled the threshold for clonic seizures in the i.v.-metrazole test after administration of 16 mg/kg i.p. and suppressed audiogenic seizures in DBA/2 mice at 3 mg/kg. D-23129 reduced not only kindled seizure parameters after supramaximal stimulation but also increased the threshold for induction of afterdischarges indicating a suppression of focal seizure activity in a dose dependent manner. After oral administration of 10 mg/kg 60 min prior to testing all animals were seizure protected if stimulated with double the individual seizure threshold. The afterdischarge threshold was increased to 408 and 597% of control threshold after 10 and 15 mg/kg respectively. After 15 mg/kg all seizure parameters were significantly reduced after suprathreshold stimulation and no motor impairment was present. First electrophysiological data indicate that part of the effects of D-23129 is mediated through effects on voltage gated Na^+ and Ca^{++} channels. The results make D-23129 a promising candidate for treatment of generalised and complex partial seizures.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID XI

669.1

TRANSGENIC MOUSE AND RAT STUDIES OF ALZHEIMER AMYLOID PRECURSOR PROTEIN.

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Deposition of the 4 kD Aß protein, derived by proteolytic cleavage from the amyloid precursor protein (APP), in brain is a neuropathological hallmark of Alzheimer's disease (AD). Expression of full length human APP in brains of animals should provide a model system for studying proteolytic processing of APP *in vivo*. These animals also serve as a basis to address parameters required for the development of ß-amyloid-related neuropathologies in brain. We have generated transgenic mouse and rat lines harboring full length human APP transgenes containing native sequence and genetic mutations linked to familial AD (ΔI_{717}, $\Delta NL_{670/671}$) and hereditary cerebral hemorrhage with amyloidosis-Dutch type (ΔQ_{693}). Human APP transgene RNA and protein occurs predominantly in brain. *In situ* hybridization shows human APP RNA enriched in neurons. Evidence for proteolytic cleavage of human APP in mouse brain which is required for Aß production has been obtained using antibodies specific for human APP fragments. Recent evidence has implicated apolipoprotein E in the pathogenesis of AD. Apolipoprotein allele E4 cosegregates at high frequency with late-onset and sporadic AD. To study the role of apoE in amyloid deposition we have generated transgenic lines expressing both human apoE4 and APP. Characterization of these lines and results from our ongoing search for amyloid-related neuropathologies in APP transgenic mice and rats will be presented.

669.2

TRANSCRIPTIONAL REGULATORY ELEMENTS OF THE RAT AMYLOID PRECURSOR PROTEIN (APP) GENE PROMOTER. P. W. Hoffman* and J. M. Chernak. National Institute on Aging, Lab. of Cellular and Molecular Biology, MPGS, Baltimore, MD 21224

Levels of some amyloid precursor protein (APP) mRNAs are selectively increased in Alzheimer's disease and Down's syndrome (DS) brains relative to age-matched control brains, and overexpression of the APP gene in DS could be related to gene dosage effects. Therefore, transcriptional regulation of the APP gene may play a role in the pathophysiology of several neurodegenerative diseases. In order to study transcriptional regulation of the APP gene, we have begun characterizing the rat APP promoter. The 375 bp directly upstream of the start codon contains all of the transcriptional start sites and drives transcription in rat PC12 cells and human SHSY5Y cells. The promoter activity appears to be dependent upon two small regulatory elements. Deletion of one element at position -260 to -248 reduced activity by 85% in PC12 cells while deletion of a second element at position -223 to -192 reduced activity by 30%. A double mutant which internally deletes both sites reduced transcription to nearly background levels. Additional experiments have suggested that deletion of the element at -260 to -248 may be responsible for uniformly large effects in several rodent and human cell lines, while deletion of the element at -223 to -192 may produce cell line-specific effects. Gel mobility shift assays with nuclear extracts derived from rat brains or rat cell lines suggest that one or more proteins may interact with the element at -223 to -192 but not with the one at -260 to -248. We are currently investigating the role of these regulatory elements using site-specific mutagenesis, further gel shift assays and gel super-shift analysis.

669.3

APOLIPOPROTEIN E INDUCES EXPRESSION OF THE AMYLOID ß-PROTEIN PRECURSOR IN CULTURED CEREBROVASCULAR SMOOTH MUSCLE CELLS. S.M. Saporito-Irwin*, M. Wessel, W.E. Van Nostrand*. Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine CA 92717-4025.

Recent studies have implicated apolipoprotein E (apoE) in the pathogenesis of Alzheimer's disease (AD). The E4 allele of apoE has been shown to be linked to the occurrence of late onset familial and sporadic AD. In addition, apoE can bind the amyloid ß-protein (Aß) and has been found tightly associated with amyloid deposits in both senile plaques and cerebral blood vessels. Immunohistochemical studies showed apoE reactivity in the smooth muscle cell medial layer of intracortical and leptomeningeal blood vessels of patients with AD. Furthermore, smooth muscle cells have also been implicated in the cerebrovascular expression of the amyloid ß-protein precursor (AßPP) and the production of Aß peptide. In view of these findings, we examined the effect of purified apoE from normal pooled plasma on the expression of AßPP in cultured cerebrovascular smooth muscle cells. Cells were incubated with increasing concentrations of purified apoE for 72 hr and then the levels of secreted and cell-associated AßPP were measured by quantitative immunoblotting. Cerebrovascular smooth muscle cells incubated with 1 µM purified apoE expressed five-fold higher levels of both secreted and cell-associated AßPP compared to untreated cells. The effect of apoE was specific in that purified apoA or apoC had no effect on the levels of secreted nor cell-associated AßPP in the cerebrovascular smooth muscle cells. These findings suggest that in the cerebrovasculature apoE may be involved with increased expression of AßPP and, potentially, the subsequent production of higher amounts of Aß peptide. Soluble Aß peptide produced by smooth muscle cells may interact with apoE leading to aggregation and deposition in the cerebrovasculature.

669.4

APOLIPOPROTEIN E REGULATES APP LEVELS. Benjamin Wolozin*, Robert Canter, Yongquan Luo, Dale VanderPutten, Trey Sunderland. Lab. of Clinical Science and Lab. of Biochemical Genetics, National Institute of Mental Health, Bethesda, MD 20892.

Recent studies have shown that the apolipoprotein E allele, apo ε4, is an important risk factor for Alzheimer's disease. Previous studies showing that Apo E binds to ß-amyloid, *in vitro*, and associates with neuritic plaques, *in vivo*, suggest the risk may be due to an association between ß-amyloid and apo E (W. J. Strittmatter, et al, 1993 PNAS 90:8098). However, the mechanism for this association is unclear because the affinity of Apo E for ß-amyloid is low. We now show that apolipoprotein E is a potent regulator of APP levels in cell culture, being active at levels as low as 30 nM. Addition of apolipoprotein E or HDL to PC12 cells grown in cell culture induces a decrease in the levels of both secreted and cellular amyloid precursor protein (APP) in a dose-dependent manner by up to 63%, with an IC50 of 30 nM. Blockade of protein processing with 1 µM brifeldin A, 60 µM chloroquine or 10 µg/ml leupeptin prevents this, which suggests that internalization and catabolism of APP or Apo E is necessary for this process. These data point to a physiologic role for apo E in the regulation of APP and suggest that binding of apo E to APP may be the first step in the association between Apo E and ß-amyloid.

669.5

TRANSPORT OF ALZHEIMER'S AMYLOID BETA AND APOLIPOPROTEINS E AND J AT THE BLOOD-BRAIN BARRIER. B.V. Zlokovic,* J.B. Mackic, C.J. Martell, T. Wisniewski, B. Frangione, J. Ghiso. Dept. Neurosurgery, USC School of Medicine, Los Angeles, CA 90033

Two apolipoproteins, apoE and apoJ, have been recently shown to be associated with Alzheimer's amyloid ß (Aß). It has been proposed that apoE is an Aß pathological chaperone protein, acting to modulate and/or stabilize a ß-pleated sheet structure in Aß. Consistent with this hypothesis, there is a linkage between apoE4 isotype and late onset Alzheimer's disease. On the other hand, apoJ has been shown to be complexed to Aß in cerebrospinal fluid. Aß, apoE and apoJ are present in senile plaques; however, their origin is not known. We have studied the behavior of these three components at the BBB level. An "in vivo" brain perfusion technique and capillary depletion method were used to determine cerebral capillary binding and BBB transport of [^{125}I] labeled apoE, apoJ and synthetic Aß1-40 in guinea pigs. A saturable specific binding and transcellular BBB transport were demonstrated for all components although both apolipoproteins exhibit different BBB transport behavior. After 10 min perfusion, the volume of distribution for apoJ was 4-5 times higher than for apoE. Pre-perfusion with a very low dose of cold apoJ completely blocks the transport, indicating a high affinity interaction.

669.6

STUDIES OF TRANSCELLULAR TRANSPORT OF Aβ ACROSS MDCK MONOLAYERS. D. J. Watson and D. J. Selkoe*. Program in Neuroscience, Harvard Medical School and Center for Neurologic Disease, Brigham and Women's Hospital, Boston, MA 02115.

The discovery of soluble amyloid β (Aβ) protein in CSF and plasma raises the possibility that one source of some amyloid deposits in Alzheimer brain may be the circulation. We have used a cell culture model to test whether Aβ can be transported across cellular monolayers which form tight physiological barriers. Madin-Darby canine kidney (MDCK) epithelial cells form polarized monolayers with intercellular tight junctions when grown on polycarbonate filters in Transwell chambers. MDCK cells were grown to post-confluence in DMEM/10% FCS. Either ^{3}H-inulin (a marker for intact tight junctions) or physiological concentrations (0.1 to 1.0 nM) of ^{125}I-Aβ were added to the apical or basal chambers in either serum-free DMEM, fresh DMEM/10% FCS or 16-hour MDCK-conditioned DMEM/10% FCS. After incubation for 3 or 6 hr at 37°C, medium from both chambers was sampled and counted. Under these conditions, transcellular transport of ^{125}I-Aβ was observed to be no greater than the background inulin transport in both the apical-to-basal and basal-to-apical directions. We are therefore examining molecules which are known to be transported across MDCK monolayers by a receptor-mediated mechanism, and asking whether these molecules are able to act as carriers for Aβ during transcellular transport. The results of such *in vitro* studies can be coupled with intravascular Aβ injection studies in animals to determine whether Aβ is able to cross the blood-brain barrier.

669.7

BRAIN CLUSTERIN (APO-J) AND ITS INTERACTION WITH ß-AMYLOID (Aß) G.M. Pasinetti*, T.Oda, S.A. Johnson, C.E. Finch. Andrus Gerontology Center Neurogerontology Division and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

The senile plaque contains aggregations of ß-amyloid (Aß). In addition to aggregated Aß, senile plaque contains other proteins that may be pertinent to neurodegeneration, particularly complement proteins and the putative complement inhibitor, clusterin. We purified clusterin from Alzheimer's disease (AD) cerebral cortex by column chromatography (DEAE, antibody affinity and HPLC size-exclusion). Brain clusterin presented slightly smaller species about 35-40 kDa vs. purified serum clusterin (39-42 kDa), under reducing conditions. In the inhibition of complement-mediated hemolysis, brain and serum clusterin were indistinguishable. Aggregation of Aß(1-42) was assayed by centrifugation with [^{125}I]-Aß1-42 and gave 30% sedimentable Aß (48 hrs, 25°C, pH 7.0). Clusterin showed a concentration-dependent inhibition of Aß sedimentation. Supported by Alzheimer's Association grant to GMP and by grants to CEF from NIA AG-7909 and Sankyo Co.

669.8

ALZHEIMER'S Aß AND APOLIPOPROTEIN J INTERACTION. E Matsubara, B Frangione, J Ghiso*. Dept. of Pathology, NYU Medical Center, New York, NY, 10016.

Peptides with the same amino acid sequence as Alzheimer's amyloid ß (Aß) exist as a normal soluble protein (sAß) in biological fluids. Hence, a key question is what factors alter sAß in the disease state, promoting conformational changes, aggregation, amyloid fibril formation and cell death. Biochemical and immunohistochemical studies have shown i) apoE and apoJ bind sAß under different conditions in vitro, and ii) sAß is complexed to apoJ in vivo. We have further characterized the interaction ApoJ-Aß using purified ApoJ and immobilized Aß1-40 on a solid phase on capture ELISA experiments. The binding ApoJ-Aß1-40 was saturable and specific. When the preformed complex was offered to Aß1-40 complete binding inhibition was achieved, indicating complex stability in physiologic solutions. Solid phase competitive inhibition assay using other plasma/cerebrospinal fluid proteins with demonstrated avidity for Aß (ApoE3, ApoE4, α1-antichymo-trypsin, Transthyretin, Vitronectin) showed that none of these completely displaced apoJ from the complex up to 100 molar excess. Only apoE3 at high molarity (approximately 60 molar excess over apoJ) partially competes with apoJ for Aß. The data support the notion that the association ApoJ-Aß may play a role in the transport and delivery of sAß.

669.9

THE ROLE OF APOLIPOPROTEIN E IN AMYLOID FIBRIL FORMATION. F. Prelli, E. M. Castaño, M. Pras, S.F. Ferris,* B. Frangione Dept. of Pathology, NYU Medical Center, New York, NY 10016

Amyloidosis comprises a heterogeneous group of diseases of diverse etiology characterized by the systemic or localized extracellular and intracellular deposition of proteinaceous β-pleated fibrils, which are biochemically and immunohistologically distinguishable. Amyloid P-component, α1-antichymotrypsin, glycosaminoglyans, vitronectin, apolipoprotein J (apoJ) and apolipoprotein E (apoE) are significant components of these amyloid deposits, although their role in amyloidogenesis is not defined. Recent genetic studies suggest an association of apoE type4 allele with sporadic and familial late onset Alzheimer's Disease (AD). In vitro studies have shown apoE binding to synthetic Aβ and tau protein, the main constituents of paired helical filaments in neurofibrillary tangles (NFT), an AD hallmark. These data have led to the hypothesis that Apo E plays a pathogenetic role in AD. However apoE immunoreactivity is present in all cerebral and systemic amyloidoses tested.

In order to study the role of apoE, we purified amyloid AA and AL fibrils from patients with familial Mediterranean fever and primary amyloidosis, respectively. In each case, apoE fragments, detected by Western immunoblot, copurified with the amyloid fibrils. Following further separation techniques, (HPLC, SDS-PAGE and Western blot) we were able to characterize by microsequencing analysis these apoE fragments as the C-terminus fragment of apoE, similar to the 10 kd fragment produced by thrombin digestion. This is the purported binding region of apoE to Aβ. We propose that this binding is not specific for Aβ or tau, but rather that apoE plays a more general role in amyloid formation in acting as a "pathological chaperone" which may modulate the aggregation of amyloidogenic proteins into the β sheets that typically constitute insoluble amyloid fibrils.

669.10

CHONDROITIN SULFATE PROTEOGLYCAN FORM OF ALZHEIMER'S AMYLOID PRECURSOR PROTEIN IS PRODUCED MOSTLY IN ASTROCYTES BUT NOT IN NEURONS. J. Shioi[1], M. N. Pangalos[1], C. Mytilineou[2], N. K. Robakis[1]*. Dept. [1]Psychiatry and Fishberg Ctr. Neurobiol., and [2]Neurology, Mount Sinai Med. Ctr., New York, NY 10029.

C6 glioma cells and rat and human brain produce the chondroitin sulfate proteoglycan form of Amyloid Precursor Protein (CSPG-APP). In this report we show that CSPG-APP production in Neuro 2A neuroblastoma cells is markedly reduced upon differentiation with cAMP. Using primary cell cultures from rat brain, we examined which cell types in the brain synthesize the CSPG-APP. Neuronal and glial cultures were prepared from cerebral cortices of prenatal and neonatal rats, respectively. Purity of the cell populations in each culture was determined by immunocytochemistry using cell specific markers. Cells were labelled with ^{35}S-sulfate and both media and cell extracts were immunoprecipitated with various anti-APP antibodies. Immunopurified molecules were further analyzed by SDS/PAGE and fluorography. The PG nature fo APP was demonstrated by glycosaminoglycan degradation by chondroitinase. Neuronal cultures produced high levels of APP_{695} but very little CSPG-APP. Astroglial cultures produced mainly $APP_{751/770}$ and high levels of the CSPG form, whereas oligodendrocytes produced only APP but no CSPG-APP. Microglial cultures showed only low levels of APP and CSPG-APP production. Since astrocytes have unique functions such as neuronal guidance, our results suggest that CSPG-APP may play a role in such a cell-specific fuction, and may contribute to neuronal survival.

669.11

ENDOTHELIAL CELL SURFACE PROTEOGLYCANS BIND TO Beta-AMYLOID PEPTIDE. M.Matic,B. Leveugle, H.M.Fillit*, Department of Geriatrics, Mt. Sinai Medical Center, New York, NY

Proteoglycans (PGs) codeposit with Beta-amyloid peptide (BAP) and are thought to play fundamental role in all form of amyloidosis. Amyloid precursor protein (APP) has at least two distinct processing pathways: a secretory pathway and endosomal-lysosomal pathway which involves reinternalization of cell surface APP. We have previously demonstrated binding of secreted neuroblastoma and vascular PGs to APP and to BAP. We have also reported binding of cytoplasmic PGs to BAP. In order to examine if PGs might associate with APP at the cell surface, we purified the cell surface pool (trypsin releasable) of metabolically labeled mouse endothelial cells on DEAE-sepharose. Enzymatic digestion with heparitinase II and chondroitinase ABC, as well as nitrous acid degradation of isolated cell surface PGs demonstrated presence of heparan sulfate PGs and indicated presence of chondroitinase ABC sensitive material. Interaction of isolated PGs with BAP (1-28AA) was examined by affinity chromatography. The largest fraction of PGs eluted from the BAP column at 0.65 M NaCl. TNF-alpha treatment of the cells did not alter binding of the cell surface PGs to BAP. Binding of the cell surface PGs to BAP may indicate interaction of PGs and APP at the cell surface and point to the role of PGs in endosomal-lysosomal APP processing and/or APP secretion by APP secretase.

669.12

BINDING OF SOLUBLE β-AMYLOID IN VITRO AND IN VIVO. A.Golabek, M.Marques, I.Koji, J.Ghiso, J.Herbert*, B.Frangione, T.Wisniewski. Dept.of Pathology and Neurology, New York Medical Center University, N.Y., 10016.

β-amyloid (Aβ) is the major constituent of amyloid deposits in the senile plaques and vessels of Alzheimer's Disease (AD). This same peptide exists as a normal component of biological fluids; hence, the Aβ peptide can have the β-pleated sheet structure of amyloid as well as being a soluble protein. Several proteins are reported to bind sAβ including transthyretin, α_1-antichymotrypsin, apolipoprotein(apo) E and apo J. An allele of one of these proteins, apo E4, has been linked to late onset AD. Using synthetic peptides homologous to Aβ1-40 we have done direct binding experiments with the above proteins and found that SDS-stable complexes can be formed with each of them. To evaluate whether such complexes may also exist in vivo immunoprecipitation of these binding proteins was done in CSF and co-precipitation of sAβ was determined by Western blotting. In addition an affinity column with 4G8 antibodies (recognizing Aβ17-24) was used to extract sAβ from pooled normal CSF and associated binding proteins were identified by Western blotting and N-terminal sequencing. These experiments of in vivo binding suggest that in normal CSF the major binding protein is apo J; although, other proteins also bind including apoE. These carrier or chaperone proteins of soluble Aβ(sAβ) may influence its conformational state. Experiments are underway to determine if the carrier proteins differ in AD and normal patients.

669.13

THE AMYLOID-ASSOCIATED PROTEINS α_1-ANTICHYMOTRYPSIN AND APOLIPOPROTEIN E CATALYZE THE ASSEMBLY OF THE ALZHEIMER β-PROTEIN INTO FILAMENTS AND CO-LOCALIZE WITH APP AT THE NEUROMUSCULAR JUNCTION. J. Ma*, A. Yee, &H. Bryan Brewer, U. Kayyali, and H Potter. Department of Neurobiology, Harvard Medical School, Boston, MA 02115; &National Heart and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

The protease inhibitor α_1-antichymotrypsin (ACT) and the lipid transport protein apolipoprotein E (apoE) are intimately associated with the 42-amino acid β-peptide (Aβ) in the filamentous amyloid deposits of Alzheimer's disease. We have found that these two amyloid-associated proteins serve a strong stimulatory function in the polymerization of Aβ into amyloid filaments. Addition of either ACT or apoE to the Aβ peptide promoted filament formation 10 to 20-fold. ApoE4, the isoform recently identified as a risk factor for Alzheimer's disease, showed the highest catalytic activity. We are currently testing the effect of ACT and apoE on the neurotoxicity of Aβ peptide in cell culture. These components of the Alzheimer amyloid deposits—APP, ACT, and apoE—can also be found to be co-localized at the neuromuscular junction together with the acetylcholine receptor. Two proteins are important in the formation of the neuromuscular junction: ARIA, which induces expression of the acetylcholine receptor, and Agrin, which induces its aggregation. Experiments are underway to determine whether, like the acetylcholine receptor, APP, ACT, and apoE mRNAs are up-regulated by ARIA, and whether the proteins are induced to aggregate under the influence of Agrin both at the neuromuscular junction and at neuronal synapses more generally. In sum, our results suggest that Alzheimer amyloid filaments arise when Aβ and amyloid promoting factors (pathological chaperones) are expressed together in certain brain regions.

669.14

REGULATION OF ß-AMYLOID PEPTIDE AGGREGATION BY RECOMBINANT TRANSTHYRETIN AND ITS ANALOGS.

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Amyloid plaques are found in the brains of patients with Alzheimer's disease and contain fibrillar aggregates of non-covalently linked ßAP. *In vitro*, synthetic ßAP spontaneously aggregates into morphologically identical fibrillar aggregates which we find are toxic to neurons in culture. The spontaneous transition from soluble ßAP to insoluble ß-amyloid is inhibited by the presence of μM levels of transthyretin (TTR, Schwarzman et al., PNAS, 1994, in press). The mechanism whereby TTR inhibits ßAP aggregation is unknown, but may be mediated by specific interactions between TTR and ßAP (Schwarzman et al., ibid and Soc. Neurosci. Abs. 1994). To define the domains of TTR which function to inhibit ßAP aggregation, we have produced recombinant human TTR in bacteria using the pET system. Following a multi-step purification protocol, we find that rTTR inhibits ßAP aggregation and binds thyroxine in contrast to protein purified from vector controls lacking TTR. Natural TTR purified from cerebral spinal fluid and rTTR inhibited ßAP aggregation with equal potencies. Based on our model of ßAP-TTR interactions (Schwarzman et al. ibid.), we have mutated the residues of TTR which comprise the putative ßAP binding site. The potency with which these TTR analogs inhibit ßAP aggregation and modulate neurotoxicity will be discussed.

DEGENERATIVE DISEASE: PARKINSON'S—RODENT AND PRIMATE STUDIES

670.1

AN ELISA ASSAY TO DETECT A DOPAMINE RELEASING PROTEIN (DARP) AND POTENTIAL CLINICAL APPLICATIONS. S.Kuhananthan* and V.D Ramirez. Dept of Molecular and Integrative Physiology, Univ of Illinois Urbana, IL 61801

A dopamine releasing (DARP) protein was first purified from rat adrenal gland (Chang and Ramirez 1988) and subsequently characterized as a multiunit glycoprotein of 200 Kd. Monoclonal antibodies of IgM isotype were produced against DARP to study this protein further. These antibodies were shown to arrest fetal development and deplete brain dopamine levels when injected into rat fetuses and newborn pups, respectively (Kuhananthan et al 1991).

We developed a direct ELISA assay for DARP utilizing the MAb. A synthetic peptide (DARP 36) consisting of the first 36 N-terminal aminoacids of DARP is specifically detected by the ELISA assay in a dose dependent manner. The presence of DARP in human serum was also specifically demonstrated in ELISA assay. Affinity purified serum DARP releases DA from in vitro corpus striatal fragments and is detected in ELISA assay in 1-10 ng protein. Furthermore the crude serum and affinity purified DARP compete for DARP 36 in a competitive ELISA assay, whereas several other proteins did not compete.

The DARP is present in high concentration in the rat brain, liver and adrenal gland. ELISA assay using three different antibodies indicate that the tissue form of DARP is different from circulating DARP.

Interestingly Parkinson disease (PD) patients have higher levels of serum DARP compared to age matched control subjects. The DARP levels in human serum may serve as a biochemical marker and aid in the diagnosis of PD.

670.2

TREATMENT WITH A SYNTHETIC 36aa PEPTIDE FROM THE N-TERMINAL SEQUENCE OF DARP PARTIALLY RESTORED DA LEVELS IN MPP+ LESIONED RATS AND DRASTICALLY REDUCED AMPHETAMINE-INDUCED ROTATION. Ramirez VD*, Phelps S, Kim S. Department of Molecular and Integrative Physiology, Univ. Illinois, Urbana, IL 61801, USA.

Dopamine releasing protein (DARP) is a complex multisubunit protein originally discovered in the adrenal gland. To examine the role of DARP-36 on a rat model of Parkinson's disease, adult male rats were lesioned with MPP+(84μg/2μl) in the left corpus striatum (L-CS) through a Push-Pull cannulae implanted 5-6 d before the lesion (local infusion group, LIG) or by direct infusion of the neurotoxin on the day of the operation (systemic injection group, SIG). The LIG received 24 h after the lesion a daily infusion of DARP (1μg/10μl/10min for 10d) or BSA as control. In the 11d they received an amphetamine challenge (2mg/kg,ip) and rotations were recorded for the last 30 min of a 45 min duration test. Rats were sacrificed next day and DA levels in both CS were determined by HPLC-EC. The SIG was divided in 4 subgroups: vehicle-treated and 5, 15 and 30μg DARP-treated. All rats received a subcutaneous injection for 10 d. The data indicate a dose response in the SIG with a modest recover of the DA levels in the 30 μg subgroup to 20% of the contralateral levels but without effect on the AMPH-induced rotations (1.85±.31 vs 3.1±.42, vehicle (n=12) and DARP-treated (n=9) p<.01 L-CS, respectively. The local infusion of DARP in the lesioned side led to a 50% recovery of DA levels compared to the contralateral side (7.91±.34 vs 3.3±.45 DARP, n=9 vs vehicle, n=11, L-CS, respectively) and interestingly to a near complete recovery of the response in the rotation test (vehicle=87±25 vs DARP=12±16 rotations/30 min). The data indicate that DARP-36 may be a potential beneficial peptide in the treatment of Parkinsons patients. (Partially supported by Chiron).

670.3

METHYL β-CARBOLINIUM (BC^+) ANALOGS OF MPP^+: SUBSTANTIA NIGRA (SN) INJECTIONS IN RATS CAUSE NIGROSTRIATAL TOXICITY.
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Eleven BC^+s and MPP^+ were stereotaxically injected (40–200 nmoles in 5μl of vehicle) unilaterally into the SN of anesthetized adult male Sprague-Dawley rats. The rats were sacrificed after three weeks. The ipsilateral striatum was analyzed for dopamine and DOPAC levels with HPLC. The brainstem injection site was fixed and cut coronally. The largest lesion area in each animal was measured using NIH *Image*. Three BC^+s approached MPP^+'s effectiveness (defined as 100%) in producing lesions at the injection site: 2,9-Me_2-harman (94%), 2-Me-harmol (74%), and 2,9-Me_2-norharman (57%). Three other compounds were somewhat less effective: 2-Me-harmaline (34%), 6-MeO-2-Me-harman (29%), and 2-Me-harmine (25%). The remaining compounds were ineffective (≤ 12%): norharman, 2-Me-norharman, 2-Me-harman, harmine, and 2-Me-6-MeO-harmalan. A 40 nmole dose of MPP^+ reduced ipsilateral dopamine to 0.6% of control. None of the β-carbolines approached this, although several did significantly reduce striatal dopamine at doses of either 40 nmoles (2,9-Me_2-harman (37%), 2,9-Me_2-norharman (42%), and 2-Me-harman (63%)) or 200 nmoles (2-Me-harmaline (23%), norharman (63%), and 2-Me-norharman (64%). There was a strong negative correlation between lesion size and dopamine level ($r=-.77$). There were also moderately strong correlations ($r=.55-.74$) between the BC^+ lesion area or dopamine level potencies and their previously described IC50 values for inhibiting mitochondrial respiration or their toxicity to PC12 cells in culture. Although BC^+s appear less specific than MPP^+, their levels in human SN are 8–20 fold higher than in cortex, making their role as relatively selective SN toxins in Parkinson's disease plausible. (NIH grant NS 23891)

670.4

CORRELATION BETWEEN AGE AND DOSE OF MPTP IN HEMIPARKINSONIAN RHESUS MONKEYS
A. Ovadia, Z. Zhang*, M. Smith and D.M. Gash. Department of Anatomy and Neurobiology, University of Kentucky, Lexington, Ky. 40536-0084.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) administered to nonhuman primates induces Parkinsonian-like features. However, the vulnerability of these animals to this neurotoxin appears to be age dependant. To test this hypothesis, 27 female Rhesus monkeys, ranging in age from 5 to 23 years old, were given sufficient MPTP via the right carotid artery to induce moderate unilateral Parkinsonian features. Cumulative doses ranged from 0.4 mg/kg to 1.8 mg/kg. In order to produce similar Parkinsonian features in all animals, one or two additional doses of MPTP were needed in most younger monkeys. Horizontal home cage activity was recorded continuously (24 hours) by photocells as well as behavior rated from bimonthly video tapes. After achieving similar scores on the hemiparkinsonian clinical rating scale, most monkeys showed a decrease in home cage activity. However, to achieve similar scores, the younger Rhesus monkeys ($n=12$, <10 years old) required a total of 1.0-1.8 mg/kg MPTP, while the older Rhesus monkeys ($n=15$, >14 years old) required only 0.4 mg/kg MPTP, with the exception of one which received 0.6 mg/kg. The correlation between age and dose was $r^2=0.81$ ($p<0.01$) with a significant difference ($p<0.01$) in dose between younger and older monkeys. This data suggests that older Rhesus monkeys are more vulnerable to MPTP toxicity than younger monkeys. Therefore, when producing primate models for Parkinson's disease, age should be a major contributing factor in determining the appropriate MPTP dose to administer. Supported by NIH NS25778

670.5

UNILATERAL INTRACAROTID ADMINISTRATION OF MPTP IN NONHUMAN PRIMATES: EFFECT ON DIFFERENTIAL SUBSTANTIA NIGRAL CELL LOSS
R Assietti, KD Terry, NM Oyesiku*, RAE Bakay Department of Neurosurgery, Emory University, Atlanta, GA 30322.

Behaviorly, the similarities between the MPTP non human primates model and Parkinson's disease (PD) are quite striking. MPTP's neuropathological effects (specifically, the quantification of cell loss) in the substantia nigra have not been studied in a manner similar to that used to study human PD, and meaningful comparisons have been difficult to obtain. In this study, rhesus (*Macaca mulatta*) monkeys (N=13) with ages ranging from 1 to 20 years were given single or multiple intracarotid left-sided injections of MPTP (0.5mg/kg over a 20 minute period). The monkeys were parkinsonian before sacrifice for a mean period of 1 year (range: 5 months to 2 years). Normal monkeys (N=9) with ages ranging 1 to 24 years of age were examined in a similar fashion. Serial sections of the midbrains were obtained and immunocytochemically stained for tyrosine hydroxylase (TH). Cell number, cell and nuclear diameters and cell volume were measured in A8, A9 (dorsolateral, dorsomedial, ventrolateral, ventromedial), A10 paranigral nucleus and A10 total areas using a computerized image analyzer and corrected according to the Abercrombie formula. No difference in cell counts was observed in comparing the left and right sides of the normal monkeys. A trend in cell loss according to age was observed in both normal (dorsolateral A9) and MPTP treated (ventrolateral A9) animals. The most consistent results were obtained at the level of the third cranial nerve. The relative mean TH cell loss in the A9 area (pars compacta) compared to the mean values observed in the normal monkeys was: dorsolateral A9: -82.3%; dorsomedial A9: -73%; ventrolateral A9: -90.1%; ventromedial A9: -81.6%; total A9: -84.2% ($p<0.005$). Relative cell loss in A8 was -68% and in A10 (ventral tegmental area) total:-74% with a ($p<.05$). There was no significant cell loss observed in A10 paranigral nucleus areas. The analysis of our data demonstrates that the greatest amount of TH cell loss caused by the neurotoxin, MPTP, occurs in A9, and most severely in the ventrolateral area of A9. Importantly, it is this area that has been shown to be most affected in human parkinson's disease. Supported by USPHS Grant RR00165 and NINCDS N2NS4340.

670.6

SUBCHRONIC MPTP TREATMENT WITH TETRABENAZINE CHALLENGE: EFFECTS ON NIGROSTRIATAL MARKERS IN THE PRIMATE.
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We hypothesized that MPP^+, accumulated in dopaminergic synaptic vesicles during repeated treatment with subtoxic doses of MPTP, could be released by tetrabenazine (TBZ), causing nigrostriatal toxicity. Rhesus monkeys were dosed with MPTP i.v. 3 times/wk at 0.1 mg/kg for 8 wks (low dose; total dose 2.4 mg/kg) or at 0.1 mg/kg for 4 wks, followed by 0.2 mg/kg for 4 wks (high dose; total dose 3.6 mg/kg). At the end of dosing, one set of the high dose group was sacrificed. Remaining high and low dose animals were challenged with saline or TBZ (15 mg/kg) and sacrificed 4 wks later. Striatal and adrenal markers were assessed. Controls got 8 wks saline, TBZ, then 4 wks recovery.

The repeated MPTP treatment caused a dose-related decrease in striatal dopamine (DA) 4 wk after dosing. The low dose group sustained a 29% loss of striatal DA. The substantia nigra pars compacta (SNc) remained largely intact, but swollen, degenerating cells were evident. The high dose animals showed >90% reduction in striatal DA and marked SNc cellular pathology (e.g. swollen, distorted or pyknotic neurons with disintegrating processes). Animals in the high dose group sacrificed immediately after the 2 month dosing period showed a modest reduction (38%) in striatal DA and minimal damage to the SNc. Challenge with TBZ following subchronic low dose MPTP caused ptosis, but did not enhance the toxicity of MPTP. These observations suggest that degeneration progressed following the end of MPTP dosing, but was not enhanced by TBZ treatment.

Adrenal MPP+ levels were not related to cumulative MPTP dose or survival time. While all treatment groups receiving MPTP had high levels of adrenal MPP+, there were no differences between groups, suggesting that 1) accumulation was limited, 2) clearance of accumulated MPP^+ from adrenal was very slow and 3) TBZ treatment was not sufficient to cause release of MPP^+ from chromaffin granules, its presumptive storage site.

670.7

[^{3}H]FLUNITRAZEPAM BINDING TO $GABA_A$ RECEPTORS IN THE EFFERENT PATHWAYS OF THE STRIATUM OF MPTP-MONKEYS TREATED CHRONICALLY WITH A D2 AGONIST (U-91356A) OR L-DOPA: RELATIONSHIP WITH DEVELOPMENT OF DYSKINESIA.
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Dyskinesia is one of the main side effects following chronic dopaminomimetic treatment in Parkinsonians and in MPTP monkeys. Many of the basal ganglia neurons are gabaergic and are probably implicated in the development of dyskinesia. Thus, the $GABA_A$/benzodiazepine receptor complex was quantified by autoradiography with [^{3}H]Flunitrazepam in the brain of MPTP monkeys following continuous or intermittent D2 agonist treatment with U-91356A (Upjohn) or intermittent L-DOPA treatment. Animals treated intermittently with U-91356A or levodopa developed dyskinesia whereas animals treated continuously with U-91356A did not. [^{3}H]Flunitrazepam binding in the striatum, internal (GPi) and external segments of globus pallidus, subthalamic nucleus and substantia nigra were investigated. MPTP and dopaminergic treatments affected differently [^{3}H]Flunitrazepam binding in these regions suggesting different gabaergic input levels in the various nuclei of the basal ganglia. The most interesting change was an elevation of [^{3}H]Flunitrazepam binding in the GPi of animals treated intermittently with U-91356A or levodopa and concomitantly developing dyskinesia (+38% and +36% vs MPTP untreated, respectively; $p<0.05$). These results suggest supersensitivity of the GPi to gabaergic inputs which may be involved in the triggering of dyskinesia. Supported by the MRC of Canada and the Parkinson Foundation of Canada.

670.8

EFFECT OF DOPAMINERGIC DENERVATION AND LEVODOPA THERAPY ON TYROSINE HYDROXYLASE mRNA EXPRESSION IN THE MONKEY SUBSTANTIA NIGRA
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Loss of midbrain dopaminergic neurons is the pathological characteristic of Parkinson's disease (PD) in human and MPTP neurotoxicity in monkeys. A decrease in tyrosine hydroxylase (TH) protein and mRNA content has been reported *post-mortem* in the surviving neurons in the midbrain from PD patients. Yet, because all patients are treated by levodopa therapy, it is difficult to determine if this decrease is due to dopaminergic denervation or to levodopa therapy.

The present study was aimed to analyze by quantitative *in situ* hybridization the effect of MPTP intoxication and levodopa therapy on the mRNA encoding for TH in four groups of *Macaca fascicularis* monkeys: normal control animals, MPTP-treated animals with moderate or severe parkinsonian symptoms, and levodopa-treated parkinsonian monkeys. Midbrain sections were hybridized with antisense ^{35}S-labelled riboprobes for TH mRNA. Quantification of mRNA expression at the cellular level using computerized image analysis revealed a decrease in TH mRNA expression in dopaminergic neurons from moderate, severe and levodopa-treated parkinsonian monkeys as compared to control monkeys without statistically significant differences between the three groups. Similarly, TH protein content measured in the same animals also decreased.

These results suggest that dopaminergic denervation provokes a decrease in TH mRNA and protein expression which is not reversed by levodopa therapy.

670.9

DO GLUTAMATE ANTAGONISTS REVERSE HALOPERIDOL-INDUCED EXTRAPYRAMIDAL SIDE-EFFECTS IN PRIMATES? C.L. Christoffersen* and L.T. Meltzer. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Acute administration of almost all antipsychotic (AP) drugs produces a syndrome of extrapyramidal side-effects (EPS) characterized by dystonia, akathisia and Parkinson-like tremor and rigidity. In both humans and non-human primates, AP-induced EPS is reversed or prevented by treatment with anti-Parkinson drugs. Recent data have suggested that glutamate antagonists may be useful in the treatment of Parkinson's disease (PD). Thus, glutamate antagonists may be effective in reversing AP-induced EPS. We tested, in cebus monkeys, the ability of CPP, a competitive NMDA antagonist, and NBQX, a competitive AMPA (non-NMDA) antagonist, when administered alone or in combination with a sub-threshold dose of apomorphine (APO), to reverse HAL-induced EPS.

Subjects were cebus apella monkeys that were sensitized to acute EPS by once weekly administration of haloperidol (HAL). Individual EPS signs were rated by non-blinded observers on a three point scale, totaled at each time point for each monkey, and group means derived. Test agents were administered 3 hr after administration of HAL, a time at which EPS signs were well established and steady. Ratings were made every 15 min for 1 hr after drug challenge and then at 30-60 min intervals for the next 2 hr. APO, given i.m., produced a dose-related reversal of all EPS signs. No reversal of the HAL-induced EPS was produced by NBQX, CPP or the combination of NBQX and CPP, given s.c. Neither NBQX nor CPP potentiated the ability of a sub-threshold dose of APO to reverse the HAL-induced EPS. These data suggest that glutamate antagonists would not be effective in ameliorating AP-induced EPS and question their role in the treatment of PD. Further experiments are evaluating the effects of treatments designed to increase the brain levels of NBQX on these interactions.

670.10

DIFFUSION TENSOR BRAIN IMAGING OF MPTP-LESIONED MONKEYS.
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Magnetic resonance imaging(MRI) have been used to map the scalar diffusivity (D) of water in tissues. In brain ischemia, D is decreased when conventional MRI is still uninformative. It has been recently proposed that the trace of the effective diffusion tensor ($Tr(D^{eff})$), measured by diffusion tensor imaging, more accurately reflects the mobility of water in tissue (1). We evaluated the utility of $Tr(D^{eff})$ to detect selective neuronal lesions such as the degeneration of the substantia nigra pars compacta (SNc) neurons and their projections to the neostriatum following MPTP systemic administration. Cynomologus monkeys were administered with MPTP (0.5 to 1.5 mg/kg i.v. weekly) and scanned with MRI before, immediately after their first MPTP administration and than at 1 week after each MPTP injection. Each scan consisted of the acquisition of 14 diffusion weighted coronal images from which both $Tr(D^{eff})$ and T2 weighted signal intensity maps were calculated. In the 2 stable parkinsonian animals (cumulative dose of 3-3.5 mg/kg of MPTP) studied so far, $Tr(D^{eff})$ was decreased by 22% in the dorso-lateral putamen and by 6% in the caudate nucleus. No changes were observed in other brain structures. $Tr(D^{eff})$ alteration persisted in one animal scanned 44 days after the last MPTP injection. These findings are consistent with previous immunohistochemical studies. The data of this ongoing study suggest that $Tr(D^{eff})$ could provide "in vivo" information on selective brain damage.

1)Basser, PJ et al., *Jour. Magn. Res.* , Series B, 247-254 (1994).

670.11

ALTERATIONS OF PALLIDAL CHOLINERGIC ACTIVITY IN MPTP TREATED MONKEYS: EFFECT OF DIHYDRO-α-ERGOCRYPTINE (DEK). D. Curti*, L. Brambilla E. Izzo and R. Ferrari. Institute of Pharmacology, University of Pavia, 27100 Pavia, Italy.

The neurotoxin MPTP induces Parkinson(PD)-like symptoms in human and non-human primates (Burns et al., PNAS, 80,1983; Gerlach et al., Eur.J.Pharmac., 208, 1991). The effect of nigrostriatal denervation on cholinergic activity outside the neostriatum is not well understood and it is likely to play a role in PD-associated motor disturbances (Campbell and Dill, Exp.Neurol., 42, 1974). The aim of this investigation was to evaluate in some striatal and extrastriatal brain regions, the short term consequences of MPTP administration on the cholinergic enzymes (choline acetyltransferase, ChAt, and acetylcholinesterase, AchE) and on pyruvate dehydrogenase complex (PDHc) which catalyzes the oxidative decarboxylation of pyruvate to acetylcoenzyme A and links cholinergic and energy metabolism. A treatment with dihydro-α-ergocryptine (DEK), a D_2 receptor agonist was also performed. This ergot derivative improves motor performances PD patients (Martignoni et al., Clin.Neuropharmac., 14, 1991). Monkeys, intravenously administered with MPTP at the dose of 0.3 mg/kg for 5 consecutive days, develop a severe PD-like syndrome. Cholinergic enzyme activities are increased in the internal segment of the globus pallidus (GPi) and into a lesser extent in the external globus pallidus (GPe). Cholinergic activities are not significantly affected in the caudate and putamen nor in the frontal, parietotemporal, occipital cortices and cerebellum. The treatment of the animals twice daily for two weeks with DEK starting 5 days before the first MPTP administration counteracts the neurotoxin-induced alteration in the internal pallidum and ameliorates some motor related parkinsonian symptoms.

670.12

DRAMATIC DESENSITIZATION OF THE BEHAVIORAL RESPONSE TO A LONG-ACTING D1 BUT NOT D2 AGONIST IN MPTP MONKEYS. R. Grondin*, P.J. Blanchet, P.J. Bédard, D.R. Britton[2], K. Shiosaki[2] and J.W. Kebabian[3]. Neurobiol. Res. Center., Enfant-Jesus Hosp., Quebec, Canada, [2]Neurosci. Res., Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Ill, USA, [3]Res. Biochemicals International, Natick, Mass., USA.

The effects of repeated administration of selective long-acting D1 and D2 dopamine (DA) agonists were examined in monkeys (*Macaca fascicularis*) previously rendered parkinsonian by MPTP. The animals were rated to quantify the antiparkinsonian response and the motor activity was monitored by photocells mounted on each cage. All drugs were administered subcutaneously. In a first experiment, all animals treated once daily with the long-acting D1 DA agonist A-77636 (2 mg/kg), initially showed improvement of their parkinsonian features and a marked increase of their locomotion. However, this effect decreased rapidly in the following days to reach more or less control level in a week. In an another group of MPTP monkeys, the long-acting D2 DA agonist cabergoline given every 48 hours at a dose of 0.25mg/kg, also relieved all parkinsonian features and greatly stimulated locomotion. In this group, the locomotion response decreased somewhat, but was thereafter maintained at a high level (more or less 6 times higher than control values). The dramatic desensitization seen with A-77636 was selective for the D1 receptors since, in the same animals, the response to an acute challenge dose of the D1 agonist SKF-82958 (1 mg/kg) was virtually abolished but not the response to the D2 agonists quinpirole (0.1 mg/kg) and (+)-PHNO (6ug/kg).

These results confirm that it is possible to relieve parkinsonian features with either D1 or D2 DA agonists and suggest that the D1 system can be desensitized more completely and independently from the D2 system under a continuous stimulation. The desensitization mechanisms will be explored further. [Supported by Parkinson Foundation and MRC of Canada].

670.13

RILUZOLE PREVENTS MPTP-INDUCED PARKINSONISM IN RHESUS MONKEYS : A PILOT STUDY. A.Benazzouz[1], Th.Boraud[1], P.Dubédat[2], A.Boireau[2], J.M.Stutzmann[2]*, and Ch.Gross[1]. CNRS URA 1200, [1] Université de Bordeaux 2, 33076 Bordeaux Cedex France, [2] Département Biologie, Rhône Poulenc Rorer, CRVA, Vitry Cedex, France.

MPTP provokes selective destruction of dopaminergic (DA) neurones in the substantia nigra (SN). Previous studies have shown that riluzole (2-amino-6-trifluoromethoxy-benzothiazole), a drug which interferes with glutamate neurotransmission, has a neuroprotective action in rodent models of cerebral ischemia. Experiments were performed on two rhesus monkeys (RI and RII). Both monkeys received a single injection of MPTP (0.4 mg/kg) in the right internal carotid. RI was injected with saline one hour before and six hours after the injection of MPTP. From days 2 to 30, it received further administration of saline. From days 31 to 45, it received a single daily injection of riluzole (4 mg/kg i.p.). RII received riluzole (4 mg/kg) one hour before and 6 hours after the injection of MPTP. From day 2 to 30, this monkey received a single daily injection of riluzole. Both monkeys were examined clinically and muscular rigidity was quantified by electromyography. When riluzole was administered before MPTP, bradykinesia and rigidity were absent. When injected daily in RI, riluzole significantly reduced bradykinesia and rigidity. HVA/DA ratio, currently assimilated to an index of DA use, increase of 81% after MPTP treatment in RI. Interestingly, the pretreatment with riluzole in RII increased more markedly this ratio (982%). Thus, a neuroprotection coupled to a facilitation of DA release may explain the behavioural effects reported with riluzole treatment. These results show that riluzole would possess both neuroprotective and palliative effects in a primate model of Parkinson's disease.

670.14

EFFECTS OF THE FULL DOPAMINE D-1 RECEPTOR AGONIST DIHYDREXIDINE ON COGNITIVE FUNCTION AND MOTOR ACTIVITY IN MPTP-TREATED MONKEYS. Z.-Q. Sun*, B.J. Johnson, and J.S. Schneider. Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102.

Dopamine D-2 receptors have been assigned a major role in regulating motor function, based primarily on rodent and human studies with D-2 agonists and the lack of effect of the partial D-1 agonist SKF 38393 on parkinsonian monkeys and humans. However, there are still questions concerning the potential role of D-1 agonists in treating parkinsonism. The discovery of full efficacy D-1 agonists should help to answer questions concerning the therapeutic potential of D-1 receptor stimulation. In the present studies, we used the D-1 agonist dihydrexidine to assess the potential of this drug to induce rotational asymmetry in hemiparkinsonian monkeys and to correct cognitive deficits resulting from chronic low dose MPTP exposure. Dihydrexidine (0.3 to 0.9 mg/kg), administered to 5 hemiparkinsonian monkeys, caused a dose-dependent increase in contralateral rotations that could be blocked by the D-1 antagonist SCH 23390 but not by the D-2 antagonist raclopride. Dihydrexidine also caused a dose dependent reversal of the number of cognitive errors made by 3 chronic low dose MPTP-treated monkeys during delayed response performance. This effect could also be blocked by the D-1 antagonist SCH 23390. These results suggest that D-1 receptor stimulation may be therapeutically effective in treating parkinsonism. Additional studies are needed to further compare the therapeutic and side effect profiles of selective D-1 and D-2 receptor agonists in primate parkinson models. Supported by NIH grant MH-46531.

670.15

CHRONIC GM1 GANGLIOSIDE ADMINISTRATION REVERSES COGNITIVE DEFICITS AND DELAYS ONSET OF MOTOR SYMPTOMS IN A SLOWLY PROGRESSING PARKINSON MODEL IN MONKEYS. Anne Coleman*, D.P. Roeltgen and J.S. Schneider. Dept. of Neurology, Hahnemann University, Phila., PA 19102.

We have previously shown that GM1 ganglioside, administered for 6 to 8 weeks after the last of several MPTP injections, can reverse motor deficits and partially restore striatal dopamine levels in monkeys with severe parkinsonism (Science, 256 (1992) 843-846). We suggested that GM1 might be most effective as an anti-Parkinson therapy if administered early after diagnosis, while there is still considerable host dopaminergic function. This study was conducted to assess the potential of long-term GM1 treatment as a means of slowing the progression of parkinsonism. Five macaques were exposed to low doses of MPTP for 24 weeks. These monkeys developed cognitive deficits, followed by mild motor deficits. At this stage, judged to be analagous to early parkinsonism, monkeys were randomly assigned to receive either daily saline or GM1 injections (30 mg/kg) while all monkeys continued to receive the same doses of MPTP. The monkeys receiving GM1 showed significant improvement in cognitive task performance by the end of the first 8 weeks of treatment that has been maintained over time while the saline-treated animals have continued to show increasing task performance deficits. Saline-treated animals are also showing a trend toward a progressive decrease in motor ability not apparent in GM1-treated animals. These results suggest that early intervention with GM1 ganglioside treatment may exert both symptomatic and protective effects in Parkinson's disease patients. Supported by the F.M. Kirby Fdn. and Fidia Pharmaceuticals.

670.16

EFFECTS OF GM1 GANGLIOSIDE TREATMENT ON DOPAMINE RELEASE AND REUPTAKE IN MPTP-TREATED MICE. D.S. Rothblat* and J.S. Schneider Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102.

Both *in vivo* and *in vitro* studies have suggested that GM1 ganglioside treatment may enhance rescue and survival of MPTP/MPP+-damaged dopamine (DA) neurons. Recent data also suggest that GM1 may enhance DA synthesis in residual neurons. This study measured the extent to which GM1 treatment may cause an increase in the releasable pool of striatal DA. Young (8 wk old) C57 Bl6J mice were administered MPTP (20 mg/kg, 2x day for 5 days) and then saline or GM1 (30 mg/kg, 1x day) for 3 weeks. DA release and reuptake were measured electrochemically using Nafion-coated carbon fiber electrodes. DA release was stimulated by pulses of 120mM KCl pressure ejected from a micropipette attached to the recording electrode, at 0.5 mm intervals through the dorsal-ventral and medial-lateral extents of the mid-striatum. Maximal DA release and clearance time were determined at each location. In MPTP/saline-treated mice, the greatest decrease in DA release was in the lateral striatum (39% of normal) and GM1 treatment increased release by almost 25%. The clearance time for released DA increased the most in the dorsolateral striatum in MPTP/saline-treated mice and decreased by almost 30% after GM1 treatment. These changes coincided with a 92% decrease in striatal ^{3}H mazindol binding in MPTP/saline-treated mice vs. a 67% decrease in ^{3}H mazindol binding in MPTP/GM1-treated mice. These results further demonstrate the ability of GM1 ganglioside to enhance dopaminergic neurotransmission after a DA-depleting lesion. Supported by NIA grant AG 10280.

670.17

GM1 GANGLIOSIDE TREATMENT INCREASES DOPAMINE SYNTHESIS IN RESIDUAL NIGROSTRIATAL NEURONS IN MPTP-TREATED MICE. J.S. Schneider* and L. DiStefano. Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102.

GM1 ganglioside has been shown to increase striatal dopamine (DA) levels after various types of lesions to the nigrostriatal DA system. Both *in vivo* and *in vitro* studies have suggested that GM1 ganglioside treatment may have rescue and survival effects on damaged DA neurons and may stimulate a sprouting response in striatal DAergic terminals. The present study was conducted to assess the extent to which GM1 treatment may also cause a biochemical upregulation in residual DAergic neurons after an MPTP-induced lesion. Young (8 week old) C57 Bl6J mice were administered MPTP (20 mg/kg, twice daily for 5 days) and then saline or GM1 ganglioside (30 mg/kg, once daily) for 3 weeks. Some animals received injections of diethyldithiocarbamate (DDC, 400 mg/kg) prior to MPTP injections and then received 3 weeks of saline or GM1 ganglioside treatment. Some animals were injected with NSD-1015 (100 mg/kg) 30 min. prior to sacrifice to allow measurement of striatal dopa accumulation consequent to aromatic amino acid decarboxylase activity. MPTP/saline-treated animals had 46% less accumulated dopa than normal animals whereas MPTP/GM1-treated animals had only 17% less dopa than normal. DDC+MPTP-treated animals had more severe dopamine depletions than MPTP-treated animals and striatal dopa levels 41% of normal. GM1 treatment had no effect on DA or dopa levels in these animals. These data suggest that under certain conditions, GM1 treatment can enhance the function of residual DA neurons by increasing dopa synthesis. Supported by NIA grant AG-10280.

670.18

INTRANIGRAL INFUSION OF CNTF, BUT NOT bFGF, EGF OR TGF-ß1, RESTORES STRIATAL DOPAC BUT NOT DOPAMINE LEVELS IN MPTP-TREATED MICE. T. W. Farris*, L. DiStefano and J. S. Schneider. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA 19102.

Growth factors (GFs) may rescue or support damaged dopaminergic neurons *in vivo* raising hope for their therapeutic potential in Parkinson's disease. However, the degree of tissue trauma from surgery and CNS delivery of GFs has varied greatly across experiments. Mechanical damage to the CNS can induce considerable release of GFs, complicating the interpretation of results following their administration. In this study, we have minimized non-specific tissue trauma to better isolate GF effects on recovery of DA-ergic markers following an MPTP-induced lesion. 8-week old male C57/BL6J mice were administered MPTP (20 mg/kg, ip.) or saline twice daily for 5 days. Two days later mice were implanted with 30 ga. cannulae connected to low flow rate osmotic pumps (0.2 µl/h) for delivery of rh-CNTF (20 µg/14 d), rh-EGF (4 µg/14 d), rh-bFGF (4 µg/14 d), rh-TGF-ß1 (2 µg/14 d) or vehicle to the substantia nigra (SN) or striatum (ST). Infusion of CNTF into SN of MPTP-lesioned mice restored ipsilateral striatal DOPAC levels to 88.5% of vehicle-injected/vehicle-infused controls (α=.01, Dunnett's test) while DA levels were unaffected (34% of control). EGF, bFGF and TGF-ß1 had no effects on striatal DA (27.4%, 33.4% and 30.7% of control, respectively) or DOPAC (34.7%, 43.7% and 39.2% of control). Striatal infusions of GFs had no effect. Similar experiments with these GFs in our lab employing more traumatic delivery methods have produced more positive results. We speculate that trophic substances released by trauma may be crucial for some GFs to alter DA-ergic markers. Supported by NIA AG-10280.

DEGENERATIVE DISEASE: OTHER II

671.1

USE OF IN SITU LABELING OF DNA FRAGMENTATION AS AN INDICATOR OF CELL DEATH IN VARIOUS DISEASE PROCESSES IN THE RODENT AND HUMAN CNS. L.B. Thomas*, D.J. Gates and D.A. Steindler. Dept. of Anat. & Neurobiology and Neurosurgery, Univ. of Tenn., Memphis, TN 38163

Cell death occurs by either apoptosis (programmed cell death) or necrosis. Apoptosis occurs during embryogenesis, at the end of the normal life span of differentiated cells, in tumors, and many other physiologic and pathologic conditions. Biochemically, the DNA of apoptotic cells undergoes cleavage via endonuclease into oligonucleosomal-sized fragments. The abundant 3'-OH ends of these fragments can be identified in situ by the addition of labeled nucleotides with the enzyme terminal deoxynucleotidyl transferase. Few studies have investigated this technique in the study of cell death in disease processes of the CNS. We describe the application of this technique to various disease processes in human and rodent CNS, including grades 0-4 of Huntington's disease (HD), human gliomas, and traumatic brain injury. Labeled cells were present in brain areas where degenerated cells would be expected, e.g., associated with the known medial to lateral gradient of cell death in the neostriatum with advancing stages of HD, around necrotic areas in glioblastoma, and in periwound areas following traumatic brain injury. Labeling occurred in control tissues as would be expected, e.g., associated with blood vessels where normal cell turnover occurs. GFAP, OX-42, and PCNA immunohistochemistry was also performed, and these correlative studies demonstrate that death of various types of CNS cells can be detected and distinguished from, e.g., injury-induced cell proliferation. Studies are underway to investigate labeling in ischemic tissue, which is considered to undergo cell death by necrosis. It appears that this method can be used to label dying CNS cells in a variety of disease states. However, it is unclear at this time if it identifies cell death by necrosis, or if it can be used as an early marker of cells at risk of dying. Supported by NIH NS20856 and the Hereditary Disease Foundation.

671.2

OVEREXPRESSION OF THE S-100ß GENE IN DOWN'S SYNDROME. A STUDY IN LYMPHOCYTES. H. Riol*[1], G. Gaudreault[2], J. Cloutier[2] and M.R.V. Murthy[1]. [1]Dept. of biochemistry, Fac. of Medicine, Laval University G1K 7P4 and [2]Centre hospitalier de Charlevoix, Baie-St-Paul G0A 1B0; Québec, Canada.

The S-100ß gene which is localised on chromosome 21 and is mainly expressed in astrocytes, may be implicated in the neurologic abnormalities observed in trisomy 21 which leads to Down's syndrome (DS). However, overexpression of this gene has not been consistently found in DS. S-100ß is also expressed in lymphocytes. We have measured the level of S-100ß mRNA in lymphocytes of 12 patients with DS (25 to 47 years) and of 16 controls (27 to 53 years), using semi-quantitative reverse transcription combined with polymerase chain reaction (PCR).

The average level of S-100ß mRNA was 2.3 fold higher in the DS group as compared to the controls (p=0.001). However, two DS subgroups were clearly distinguishables. The first (n=5) did not overexpress S-100ß mRNA, while the second (n=7) overexpressed this mRNA by 2.4 to 5.3 fold (p=0.001) when compared to age-matched controls. A negative correlation of the S-100ß mRNA level with age was found in normal individuals (r=-0.600; p=0.007). The DS individuals of the first subgroup showed similar negative correlation with age in their S-100ß mRNA expression (r=-1.000; p=0.009), but in the second subgroup, there was no significant correlation of the S-100ß mRNA level with age. Using genomic DNA amplification of the S-100ß gene, preliminary results indicated that all the DS patients were in fact trisomic for this gene. Therefore, these data suggest that a proportion of DS patients express S-100ß gene as do normal individuals whereas another proportion overexpress this gene in a manner which is higher than the 1.5 fold expected.

The degree of mental deficiency of the DS individuals, based on their learning capabilities, was estimated in a blind study. This was found to be negatively correlated with the S-100ß mRNA level (r=-0.700; p=0.001). These data suggest that S-100ß expression may be implicated in controlling the severity of the mental deficiency in adults with DS.

671.3

TAU PATHOLOGY IN NEURODEGENERATIVE DISORDERS: BIOCHEMICAL ANALYSIS. V. Buée-Scherrer, L. Buée, P. Vermersch, P.R. Hof, B. Leveugle, D.P. Perl and A. Delacourte*. INSERM U156, Lille, France; Mount Sinai Medical Center, New York, USA.

Neurofibrillary tangles (NFT) are found in a number of neurodegenerative disorders including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), amyotrophic lateral sclerosis/parkinsonism-dementia of Guam (ALS/PDC), post-encephalitic parkinsonism (PEP), Down syndrome (DS), and in the hippocampal formation of elderly non-demented individuals. NFT result from the aggregation of abnormally phosphorylated tau proteins into paired helical filaments or straight filaments. In this study, we used Western blotting and several immunological probes directed against phosphorylated and non-phospho-rylated tau proteins, to investigate whether these neurodegenerative disorders could be distinguished by differences in tau profile. Using specific antibodies to abnormally phosphorylated tau proteins, a triplet of proteins referred to as tau 55, 64 and 69 (or A68), was consistently detected in brain homogenates from AD cases and DS patients older than 30 years. This tau triplet was never found in any brain regions of non-demented elderly individuals, with the exception of the hippocampal formation. In PSP brains, a doublet of abnormally phosphorylated tau proteins (tau 64 and 69) was systematically found in both cortical and subcortical regions. In ALS/PDC cases, the characteristic tau triplet was found in both cortical and subcortical areas. In all PEP cases, a triplet of tau proteins was also found, but their isocortical distribution was quantitatively different among cases. As expected, in Huntington disease (HD) cases younger than 60 years, abnormal tau proteins were not found since no NFT are observed. In all of these neurodegenerative disorders, a very close relationship was observed between the tau pathology in the association cortical areas and intellectual impairment. With this approach, we were able to distinguish at least two types of neurodegenerative disorders: the Alzheimer type (DS, AD, ALS/PDC, PEP) and the PSP type.

This work is supported by the French Research Ministry, the France Alzheimer Association, NIH Grants AG05138, AG08802, and the Brookdale Foundation.

671.4

BIOCHEMICAL MAPPING OF NEUROFIBRILLARY PATHOLOGY IN PROGRESSIVE SUPRANUCLEAR PALSY: EVIDENCE FOR A SEVERE CORTICAL INVOLVEMENT.

P. Vermersch*, V. Buée-Scherrer, L. Buée, P.R. Hof, D. Perl, D. Gauvreau, A. Destée, H. Petit, A. Delacourte. INSERM U156 and Depts of Neurology, Lille, France; Fishberg Research Center for Neurobiology and Dept of pathology, Mount Sinai School of Medecine, New York, USA and Projet Image, University of Montréal, Canada.

A study was performed to quantify and map the neurodegenerative process in cortical and subcortical brain areas in 5 cases of progressive supranuclear palsy (PSP). Our approach was based on a western blot analysis of pathological Tau proteins, which are the basic components of neurofibrillary lesions. In two cases, the abnormal Tau proteins were detected in all cortical areas, sometimes in larger amounts than in some subcortical areas. In two cases, posterior cortical areas were spared. In a non demented case, abnormal Tau proteins were not detected in cortical areas while they were present in basal ganglia and thalamus. These abnormal Tau proteins consist of a doublet called Tau 64 and 69, except in the entorhinal cortex where a Tau triplet (Tau 55, 64, 69) similar to that found in Alzheimer's disease, was detected in the older cases. The amounts of abnormal Tau proteins were higher in some neocortical regions, such as area 4, as compared with the hippocampal formation.

Our results show that the neocortical pathology in PSP is more extensive than usually thought, and may play a role in the pathogenesis of the cognitive changes associated with this disease. This biochemical approach clearly distinguishes two types of neurofibrillary pathology, the Alzheimer type with a triplet of abnormal Tau proteins and the PSP type with a characteristic doublet.

This study is supported by ADERMA, CH&U de Lille, NIH grants AG05138, AG 08802, the Brookdale Foundation and Fondation de l'âge d'or du Québec.

671.5

NEURONAL DEGENERATION IN THE PRIMATE DORSAL LATERAL GENICULATE NUCLEUS (LGN) FOLLOWING ELEVATION OF INTRAOCULAR PRESSURE. A.J. Weber*[1], P.L. Kaufman[2], W.C. Hubbard[3], and L.R. Stanford[4]. The Waisman Center[1,4], Department of Ophthalmology and Visual Sciences[2,3], Department of Comparative Biosciences[4], and Wisconsin Regional Primate Research Center[1,2], University of Wisconsin, Madison, WI 53706.

Elevation of intraocular pressure (IOP) is considered to be an important causative factor associated with glaucoma, a leading cause of blindness. Using intracellular staining techniques, we have described previously the degenerative effects that increased IOP has on single ganglion cells in the primate retina (Invest. Ophth. and Vis. Sci. suppl. ('94) 35:1573). Here we describe the changes that elevated IOP has on neurons in the LGN, the primary target of retinal ganglion cell axons. IOP was elevated experimentally in one eye of five rhesus monkeys by focal scarification of the trabecular meshwork using an argon laser. Two additional cynomologus monkeys with similar, but non-laser induced, elevations of IOP also were included in the study. After 1-6 months of elevated IOP (mean IOP: 40-50mm Hg) each animal was deeply anesthetized and perfused transcardially with a 10% formol saline solution. Brains were embedded in low viscosity nitrocellulose, blocked in the frontal plane, sectioned at 25um, and counter-stained with thionine. Cell area measurements were made for both the magno- and parvocellular layers of the LGN. While as little as 3 weeks of elevated IOP results in neuronal changes in the retina, no differences in soma size are seen for LGN neurons receiving input from either the normal or glaucomatous eye. By contrast, 12 and 24 weeks of elevated IOP produce mean decreases in the soma sizes of LGN neurons receiving input from the damaged eye of 19% and 44%, respectively. Thus, in addition to producing direct degenerative effects in the retina, prolonged elevation of IOP also has a dramatic effect on the target neurons of retinal ganglion cells in both the parvo- and magnocellular layers of the LGN. Supported by NIH-NEI grants EYO2698 (PLK) and EYO4977 (LRS), and National Glaucoma Research/American Health Assistance Foundation (AJW).

671.6

EXPRESSION STUDIES OF THE SPINOCEREBELLAR ATAXIA TYPE 1 GENE. A. Servadio, M.-y. Chung, R.P. Elde*, S. Banfi, L. Duvick, H.Y. Zoghbi, and H.T. Orr. Baylor College of Medicine, Houston, TX 77030 and University of Minnesota, Minneapolis, MN 55455.

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant disorder characterized by progressive degeneration of the cerebellum, spinal cord and brainstem. The mutation causing the disease is an expansion of a trinucleotide repeat (CAG) whose size correlates with the severity and age of onset of the symptoms. Recent cloning and characterization of the *SCA1* gene revealed that the transcript is 10,660bp and that the CAG repeat resides within a coding region of 2448bp and codes for a glutamine tract. Sequence analysis did not reveal any homology with known proteins, so that the *SCA1* gene appears to code for a novel protein (ataxin-1) of 816aa with an estimated molecular weight of 87kd. To begin to understand the biology of the *SCA1* gene, we initiated studies aimed at characterizing the expression patterns of both the transcript and the protein. Northern blot analysis using mRNAs from 16 different human tissues revealed that the *SCA1* transcript is expressed in all tissues examined. Analysis of *SCA1* expression in adult mouse brain by *in situ* hybridization revealed that this gene is expressed widely throughout the central nervous system. Regions of enhanced *SCA1* expression include the cerebellum (Purkinje and granule cells, as well as neurons within the deep cerebellar nuclei) and thalamus. Analysis of RNA expression from normal and SCA1 alleles demonstrated that the allele with the expanded CAG repeat is transcribed. This argues against a pathogenetic mechanism involving loss of function at least at the RNA level. Polyclonal antibodies have been generated using either synthetic peptides or a fusion protein as an immunogen. Western blotting analysis revealed that the protein expression pattern is similar to that of the RNA. Detailed characterization of the antibodies is currently in progress to confirm their specificity.

671.7

APOLIPOPROTEIN E (APOE) ISOFORMS IN PATIENTS WITH LEWY BODIES (LB). M.G. Martinoli*[1], J.Q. Trojanowski[2], V. M-Y Lee[2], M.L. Schmidt[2], H.I. Hurtig[2], J-P. Julien[1] and C. Clark[2]. 1: Centre for Research in Neuroscience, McGill Univ. & Montréal General Hospital, Montréal, Qué., H3G 1A4, Canada, Department of Pathology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104, USA.

Lewy bodies (LB) are small round eosinophilic inclusions found mainly in brainstem nuclei and cerebral cortex. The presence of (LB) in the pigmented neurons of the substantia nigra is a hallmark of idiopathic Parkinson's disease. The presence of cortical LB and dementia is often called diffuse Lewy body disease (DLBD). When sufficient plaques and tangles are also present the condition may be called Lewy body variant of Alzheimer's disease (LBVAD). Brainstem LB are seen in about 20% of patients with Alzheimer's disease (AD). Apolipoprotein E (APOE) is a lipoprotein expressed in liver and brain as one of 3 isoforms (APOE 2, APOE 3 and APOE 4). Recent findings suggest that the presence of APOE 4 with an increased risk for late onset AD. We examined the frequency of APOE isoforms in a cohort of patients with brainstem LB, some of whom had cortical LB with or without coexisting plaques and tangles. APOE genotype was determind by PCR techniques using DNA isolated from frozen cerebellar cortex. Anonymously donated blood samples were used for the control group genotype. Our preliminary results show that while there is a slight increase in the frequency of APOE 4 in subjects with LB, the correlation is strongest for those patients with co-existing plaques and tangles.

671.8

OXIDATIVE STRESS AND GLUTAMATERGIC HYPOTHESES OF TARDIVE DYSKINESIA *G. Tsai*, D. Goff, J. T. Coyle*, Dept. Psychiat., Mass. General Hospital and Harvard Med. School, Boston, MA

The pathophysiologic basis of neuroleptic-induced tardive dyskinesia (TD) remains unclear. The dopamine supersensitivity hypothesis can not account for the time course of the onset of TD nor for the persistence of TD and the associated structural changes after neuroleptics are discontinued. Neuroleptics can induce a vicious cycle by triggering a mutually reinforcing cascade of oxidative stress and glutamatergic activation in animals. Haloperidol increases glutamate (Glu) concentration in the striatum and Glu agonists can enhance the rate of reactive oxygen species generation. The activity of the glutamatergic pathway is increased during long term neuroleptic exposure. The hyperglutamatergic state can have excitotoxic effects on the the GABAergic pathways from basal ganglia. On the other hand, neuroleptics increase the turnover of catecholamine which can be metabolized to produce cytotoxic free radicals. Free-radical formation causes release of Glu from rat hippocampal slices. Conventional neuroleptics have recently been shown to inhibit complex I of the mitochondrial electron transport chain. Failure of the electron transport chain causes the formation of free radicals and may make neurons more vulnerable to excitoxicity. We have studied 13 patients with TD and 13 patients on neuroleptics without TD to determine whether the oxidative stress is related to change in glutamatergic transmission in human TD. Our data support the hypotheses that TD patients have higher indices of oxidative stress and of glutamatergic transmission. TD patients have lower superoxide dismutase (SOD) activities ($p<0.05$) and higher Glu, Aspartate, and N-acetylaspartylglutamate concentrations ($p<0.01$) in their CSF compared to non-TD patients. There is a significant correlation between SOD and aspartate, SOD and glycine. It is plausible that the pathophysiology of TD can be better explained by the interaction between oxidative stress and hyperglutamatergic state.

671.9

EXPRESSION OF mRNA FOR DRPLA GENE (ATROPHIN-1) AND HD GENE (IT15) IN DEVELOPMENTAL AND ADULT BRAIN. S-H. Li, R.L. Margolis, X-J. Li, C.A. Ross*. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave., Ross 615, Baltimore, MD 21205.

Dentatorubral pallidoluysian atrophy (DRPLA or Smith's disease) is an autosomal dominant neurodegenerative disease. It shares many clinical, genetic and pathological features with Huntington's disease (HD). The molecular basis of DRPLA is an unstable expansion of a CAG repeat in the coding region of a gene originally cloned in our laboratory (CTG-B37, Li et al, Genomics 16: 572-579, 1993, Nagafuchi et al and Koide et al, Nature Genetics, 1994). We are now cloning the full length cDNA for this gene which we have termed atrophin-1 (Margolis et al, see abstract). Since DRPLA and Huntington's disease are similar and little is known about atrophin-1 mRNA expression, we compared the mRNA expression of the DRPLA gene and the HD gene. Northern analysis revealed that atrophin-1, a 5kb transcript, is widely expressed in human tissues, as is IT15. In human brain regions, the greatest atrophin-1 mRNA expression is in the cerebellum, though it is present in a variety of regions within the brain. We also compared the expression of these two genes in embryonic and adult rat brain. Atrophin-1 was predominantly expressed in early rat embryo brain (E16), whereas the greatest expression of the HD gene was in the adult rat brain. These results indicate the differential developmental regulation of IT-15 and atrophin-1.

671.10

PROTECTION OF CELL INJURY AGAINST OXIDATIVE STRESS BY RESVERATROL. S.J. Lee, Y. Cheng and A.Y. Sun*, Dept. of Pharmacology, Univ. of Missouri, Columbia MO 65212.

There is a general consensus that free radical generation leads to alteration of biochemical and biophysical properties of cell membranes and in turn, these changes may lead to pathological manifestations in aging and other degenerative diseases. Recent epidemiological studies have indicated an inverse relationship between moderate consumption of wine and incidence of coronary heart disease. This "French paradox" has drawn considerable attention and analysis has identified the presence of trans-resveratrol (R) in wine. The antioxidant properties of R may contribute greatly to protection of the endothelial cells lining the arteriole walls from oxidative damage. We have investigated the possible protective effect of R in the nervous system after various oxidative insults. PC12 cells were used because of their resemblance to catecholamine neurons. An increase in cell death, as indicated by the extent of intracellular LDH released to the incubation medium, was observed after PC12 cells were exposed to Fe^{2+}/DETAPAC (0.1mM) for 36 hrs. R was able to exert a dose-dependent protective effect on the Fe^{2+}/DETAPAC-induced cell death. Furthermore, the combined action of R plus Vitamins C and E was far better than the use of each individual antioxidant alone. The result indicated that R may be a potential therapeutic agent for the prevention and treatment of neurodegenerative disease (Supported in part by NIH Grant #AA02054).

671.11

PREFERENTIAL EXPRESSION OF SUPEROXIDE DISMUTASE BY CHOLINERGIC AND PARVALBUMIN NEURONS IN THE MONKEY STRIATUM. A. Reiner*, G. Figueredo-Cardenas, and L. Medina. Dept. Anat. & Neurobiol., Coll. of Medicine, Univ. of Tennessee, Memphis, TN 38163.

Superoxide radicals may be involved in neurodegenerative processes occurring in aging or in brain ischemia (1). Previous studies showed that global brain ischemia produces a very selective degenerative pattern in the striatum, with loss of projection neurons and sparing of interneurons (2). In the present study, we have investigated the expression of superoxide free radical scavengers by the striatal neurons of the rhesus monkey. We have used antibodies against two enzymes that are involved in the scavenging of superoxide free radicals in cells: copper, zinc superoxide dismutase (SOD1), located in the cytosol; and manganese superoxide dismutase (SOD2), located in mitochondria. Our results indicate that striatal neurons generally express low levels of SOD2 immunoreactivity, except a population of large neurons that were strongly immunoreactive for both SOD1 (SOD1+) and SOD2 (SOD2+), and a small population of medium-sized neurons that were strongly SOD2+. Double label immunofluorescence showed that the large neurons that are strongly SOD1+ or SOD2+ were also labeled for choline acetyltransferase (ChAT). In addition, most of the medium-sized neurons that were strongly SOD2+ were also labeled for parvalbumin (PV). Since monkey striatal neurons expressing low levels of SOD2 were not labeled for either ChAT or PV, we concluded that the majority of them were striatal projection neurons. Our results indicate that the cholinergic and parvalbumin striatal neurons express higher levels of superoxide free radical scavengers than striatal projection neurons, which may help to explain why these interneurons survive striatal degeneration observed following global brain ischemia or in Huntington's disease. (1) Ann.Neurol.,32:522-527(1992). (2). Soc. Neurosci.Abstr.,19:1652(1993). Supported by NS-19620, NS-28721, Hereditary DiseaseFoundation (A.R.), and Neurosci. Center of Excellence at U.T. Memphis (L.M.).

671.12

ABNORMAL PHOSPHATASE ACTIVITY IN THE PATHOGENESIS OF ALS. R. Wagey*, C. Krieger, and C.A. Shaw, Depts. of Medicine and Ophthalmology, c/o Dept. of Anatomy, Univ. British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

Earlier studies have shown that [^{3}H]MK-801 binding to the NMDA receptor ion channel is decreased in spinal cord tissue from ALS patients compared to controls. This reduction could be completely reversed by the activation of protein kinase C by phorbol ester (PB) (Krieger et al., 1993). These studies suggested that NMDA receptors in ALS spinal cord may be altered by abnormal regulation of enzyme activity. [^{3}H]MK-801 binding was increased after exposure to PB (15 μM) in both control and ALS spinal cord sections. In the present study, a return to PB-free media resulted in a gradual decrease in specific [^{3}H]MK-801 binding to the original levels with time, where t1/2 = 60 min. for control and 15 min. for ALS. The rate constants for PB-induced effects on [^{3}H]MK-801 in control and ALS were 0.01/min. and 0.05/min., respectively. In control tissue the decay to the original binding level could be blocked by phosphatase blockers, NAV (sodium orthovanadate, a tyr-selective inhibitor) and NABD (Sodium B-D-glycerophosphate, a ser/thr inhibitor). For NABD, half maximal block was achieved at the concentration of 10^{-7} M for control and 10^{-3} M for ALS. Almost complete block (80%) was achieved at 10^{-3} M for control whereas in ALS a complete block could not be achieved. These differences between ALS and control spinal cord may indicate fundamental alterations in the activity, amount or type of endogenous phosphatase in spinal cord tissue in ALS patients.

671.13

FAC1 INTERACTS WITH THE 160 KD NEUROFILAMENT PROTEIN DURING DEVELOPMENT AND IS RE-EXPRESSED IN AMYOTROPHIC LATERAL SCLEROSIS. R. Bowser*, J.L. Seeburger[2], J. Springer[2], and P. Davies. Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461 and [2]Dept. Neurology, Hahnemann Univ., Philadelphia, PA 19102.

FAC1 is a developmentally regulated protein that is localized throughout the cell cytoplasm during human brain development but found primarily in neuronal nuclei after birth. We have isolated soluble FAC1 protein from fetal brain by affinity chromatography and report that FAC1 interacts with the 160 kD neurofilament protein (NF-M). Other cytoskeletal elements such as NF-L, tubulin or MAP's do not co-elute with FAC1. Therefore FAC1 represents a novel neurofilament associated protein. Neurofilament abnormalities are present in many neurologic diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). FAC1 abnormalities have also been found in these diseases. Within the neocortex of AD brain FAC1 is present in a subset of neuritic plaques. We investigated FAC1 protein expression in fetal, adult and ALS spinal cord by immunocytochemistry. In the developing lumbar cord FAC1 resides in nuclei of multiple cell types, with highest protein levels in anterior horn cells. In adult spinal cord little FAC1 protein is seen. However in ALS lumbar spinal cord FAC1 protein is observed throughout the motoneurons and in axon tracts. We are currently determining if FAC1 re-expression occurs only in cells with NF abnormalities or if FAC1 expression precedes NF changes. Results of *in situ* hybridization studies using probes for FAC1 mRNA will also be presented. Re-expression of FAC1 may be characteristic of cells responding to early cellular abnormalities that occur during AD and ALS.

671.14

OXIDATIVE MARKERS, ELECTRON TRANSPORT CHAIN ACTIVITY, AND SUPEROXIDE DISMUTASE ACTIVITY IN FAMILIAL AND SPORADIC AMYOTROPHIC LATERAL SCLEROSIS. A.C. Bowling*, E.E. Barkowski, J.B. Schulz, U. MacGarvey, R.H. Brown, and M.F. Beal. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

We investigated superoxide dismutase (SOD) activity, markers of oxidative damage, and electron transport chain activity in patients with familial amyotrophic lateral sclerosis (FALS) and sporadic amyotrophic lateral sclerosis (SALS). Studies were conducted with erythrocytes from 30 controls, 31 SALS patients, and 13 FALS patients with six different *SOD1* mutations. In addition, motor cortex (Brodmann area 4) was obtained from postmortem brain samples from 19 controls, 16 SALS patients, and four FALS patients with a codon 4 mutation (ala -> val). In the SALS patients, SOD activity in erythrocyte lysates and brain cytosol was not significantly different from that of controls. In the FALS patients, erythrocyte SOD activity was decreased by 49% ($p<0.001$, range = 25-69%) and brain cytosol SOD activity was decreased by 52% ($p<0.001$). In brain tissue from the SALS patients, the carbonyl content of cytosolic proteins was increased by 47% ($p<0.001$) and the 8-hydroxy-2-deoxyguanosine content of nuclear DNA was increased by 32% ($p<0.05$). Also, complex I activity in brain mitochondrial preparations from the FALS patients was increased by 75% ($p<0.001$). These findings indicate that oxidative stress may be increased in FALS and SALS patients. Further studies will examine additional brain areas and other markers of oxidative damage.

671.15

AMYOTROPHIC LATERAL SCLEROSIS IMMUNOGLOBULINS INCREASE INTRACELLULAR CALCIUM IN A MOTONEURON CELL LINE. L.V. Colom*, M.E. Alexianu, R.G. Smith and S.H. Appel. Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Amyotrophic Lateral Sclerosis (ALS) is a devastating motoneuron disease of unknown cause. Recent evidence suggests that autoimmune mechanisms are involved in the origin and progression of the disease. We have previously demonstrated the presence of antibodies to voltage-gated calcium channels in ALS patient sera, and shown that ALS IgG induce calcium-dependent cytotoxicity in an hybrid motoneuron cell line, VSC4.1 (Smith *et al.*, PNAS 91:3393-97, 1994). These ALS IgG also increase the amplitude of voltage-dependent calcium currents in VSC4.1 cells (Mosier *et al.*, Soc. Neurosci. Abstr. 19:196, 1993). To determine whether ALS IgG can increase intracellular calcium in VSC4.1 cells, we have employed confocal scanning microscopy and the calcium-sensitive dye Fluo-3. In VSC4.1 cells, basal intracellular calcium levels were 74±32 nM. Increases in intracellular calcium were observed in 29% of cells, following bath addition of IgG from 6 ALS patients. Two types of intracellular calcium changes were observed: 1) an early fast transient (364±233 nM), appearing 15-120 sec after adding ALS IgG to the bath, and 2) a slower, progressive increase appearing 30-120 min later. Of the cells that showed a fast transient, 1/3 developed a slower progressive calcium increase (compared to less than 10% of the cells that lacked a fast transient). Addition of IgG from 4 control patients produced calcium increases in only 1% of cells. ALS IgG failed to produce fluorescence changes in calcium free media. These findings suggest that ALS IgG induces an early calcium influx through the cell membrane which, despite its short duration, may initiate a cascade of events leading to a later progressive increase in calcium and subsequent cell death. This work was supported by M.D.A. and Cephalon, Inc.

671.16

MARIANAS DEMENTIA, A PURELY DEMENTING FORM OF ALS/PARKINSONISM-DEMENTIA COMPLEX OF GUAM. D.P. Perl*, P.R. Hof, J.C. Steele, D.P. Purohit, C. Esteban-Santillan, R. Peterson, L.T. Kurland. Mt. Sinai Med. Ctr., Dept. of Neurobiology, New York, NY 10029; Marianas Health Study, Guam 96923; Mayo Clinic, Rochester, MN 55905.

Among the Chamorro people of Guam, the high incidence and prevalence of ALS has decreased in the past decade, while parkinsonism-dementia complex (PDC) continues to be frequently encountered. We have recently noted the frequent occurrence of cases of dementia who remain free of amyotrophy or parkinsonian signs through the entire course of their illness. Pending further characterization of this disorder, we have referred to such cases as Marianas dementia. We now report neuropathologic findings on 7 pure dementia cases (73 ± 8.5 years old, duration of illness 3.5 ± 1.6 years) among inhabitants of Guam. None of these cases showed evidence of amyotrophy or extrapyramidal features despite careful neurologic surveillance. Five of these cases were of Chamorro extraction and had been life-long inhabitants of Guam. These cases showed evidence of severe cerebral cortical atrophy with widespread neurofibrillary tangles (NFT), in the absence of neuritic plaque formation. The NFT heavily involved limbic structures and predominated in layers II/III (as opposed to layer V) of the neocortex. There was loss of neurons in the substantia nigra with NFT in the upper brainstem. These 5 Chamorro cases were virtually indistinguishable neuropathologically from PDC. The remaining 2 cases were migrants to Guam (82 year old Caucasian, 83 year old Filipino) and both showed typical neuropathologic features of Alzheimer's disease. This study demonstrates that a purely dementing syndrome, in the absence of amyotrophic or parkinsonian manifestations represents an additional form of ALS/PDC among at-risk Chamorro natives of Guam with chronic exposure to unknown environmental factors present on the island.

671.17

POTENTIATION OF CYCAD TOXIN-INDUCED DNA DAMAGE IN BRAIN TISSUE BY DNA-REPAIR INHIBITORS. G.E. Kisby*†, C. Sweatt, S. McEvoy, P.S. Spencer$^{\Delta}$. Center for Res. on Occup. and Environ. Toxicol.; and Depts. †Pharmacology and $^{\Delta}$Neurol., Oregon Health Sci. Univ., Portland, OR 97201.

Cycasin, the β–D-glucoside of methylazoxymethanol (MAM), is a possible etiological factor for western Pacific amyotrophic lateral sclerosis/Parkinsonism dementia complex. We have hypothesized that these genotoxins act as 'slow toxins' by permanently altering post-mitotic neural tissue DNA . In previous studies, we demonstrated that MAM-induced neurotoxicity *in vitro* is potentiated by O^6-benzylguanine (O^6-BG), an inhibitor of the DNA-repair protein methylguanine methyltransferase (MGMT). To further examine the relationship between DNA damage and MAM-induced neurotoxicity, MGMT levels and DNA damage were determined in mature mouse cortical explant tissue, primary rat cortical astrocytes, and primary rat cerebellar granule cell cultures treated for 1-3 days in the presence or absence of 5.0 μM O^6-BG with 0.1 mM of the genotoxins MAM, *N*-methylnitrosourea or procarbazine. Western blots demonstrated MGMT in protein extracts of all three tissue types, with particularly low levels in granule cell cultures. MGMT levels were reduced in explants and astrocytes treated with O^6-BG. The level of apurinic/apyrimidinic endonuclease, an excision-repair protein, was also reduced in protein extracts from explants treated with O^6-BG. DNA damage, as determined by immuno-slot-blot using the monoclonal antibody EM-21 to the DNA adduct O^6-methydeoxyguanosine (O^6-mdG), was not detected in cultures treated for 24 h with MAM or other genotoxins. However, O^6-mdG was detected in all cultures treated for 3 days with MAM. O^6-mdG levels increased ~2x when explants were treated with MAM and 5.0 μM O^6-BG. Taken together, the present studies indicate that modulation of DNA repair can increase the susceptibility of nervous tissue DNA to damage by genotoxins that may lead ultimately to cell death. [Supported by a grant from the Medical Research Foundation of Oregon and NS19611]

NEUROTOXICITY: DRUGS

672.1

The Organophosphate Insecticide - Chlorpyrifos: Proconvulsant & Behavioral Effects in Adult Rats. John N.D. Wurpel* and Jesse H. Bidanset. Department of Pharmaceutical Sciences, College of Pharmacy. St. John's University, Jamaica, NY 11439

Chlorpyrifos (CPF) has been shown to have proconvulsant effects in immature rats (Wurpel et al, 1993) and elicits behavioral effects when administered pre- or post-natally (Muto et al,1992). These early studies also demonstrated an interaction of CPF and xylene (XYL). Experiments were performed to determine both the effects of CPF on kindling and behavioral neurotoxicity in adult rats. Xylene was administered alone or incombination with CPF. Rats (Taconic Farms) weighing 175-225 grams recieved amygdalar electrodes 1 week prior to initiation of kindling paradigms. CPF or XYL, alone or in combination, was administered subcutaneously in 10% peanut oil/saline (vehicle - VEH). Dose range: CPF 0.3-100 mg/kg; XYL 0.2, 0.5 or 1%. In all cases N ≥ 5. The afterdischarge threshold (ADT) was determined in rats treated with CPF, XYL, CPF+XYL or VEH. No differences were noted between the groups, ADT was not effected by treatments. Kindling was performed (1 stimuli/hour, 400 μA, 1 sec duration, 60 Hz, square wave pulses) in CPF, XYL, CPF+XYL or VEH treated rats. CPF accelerated kindling in a dose dependent manner. XYL also accelerated kindling within the dose range used. CPF and XYL treatment displayed additivity (kindling rate accelerated). CPF displayed neurotoxicity in adult rats as determined by the rotorod test. XYL appeared to be without effect in this test. The combination of CPF and XYL displayed addivity in this test. Spontaneous Motor Activity (SMA) displayed a biphasic effect of CPF; at 2 hours post-CPF activity increased modestly in a dose-dependent manner. At 36 hours CPF elicited a modest decrease in SMA at the highest doses used. XYL caused an increase in SMA at 2 hours, yet at 36 hours SMA did not differ from VEH-treated controls CPF +XYL demonstrated additivity at 2 hours post dose. Brain cholinesterase activity was determined by a spectrophotometric method. CPF caused reductions in cholinesterase activity in a dose dependent manner from 2 hours up to 72 hours following CPF administration. Studies supported by St. John's University, Coll. Pharmacy.

672.2

HYDROGEN PEROXIDE HYPERPOLARIZES RAT HIPPOCAMPAL PYRAMIDAL NEURONS BY OPENING K^+ CHANNELS. **V. Seutin*, J. Scuvée-Moreau, L. Massotte, A. Dresse. Lab. of Pharmacology, Univ. of Liège (B-4000), Belgium.**

Hydrogen peroxide (H_2O_2) may be involved in a number of pathological conditions affecting neural cells, but its electrophysiological effects have not yet been studied in detail in the rat.

Using intracellular recordings of presumed CA1 pyramidal neurons in the slice preparation, we observed that H_2O_2 (0.3-3 mM) induces in these cells a reversible and reproducible hyperpolarization (-12 ± 1 mV, Mean ± SEM, N=13, by 3 mM) from resting potential (-65 ± 2 mV), as well as a decrease in input resistance. This effect persisted in the presence of TTX (0.5 μM) and was mainly due to a K^+ channel opening because (1) in 2.5 mM K_e, it had the same reversal potential (-97 ± 3 mV, N=3) as the one of baclofen (10 μM) (-99 ± 1 mV), a known K^+ channel opener; (2) its reversal potential shifted according to the Nernst equation for K^+ when $[K]_e$ was changed (from -96 ± 3 mV in 2.5 mM to -63 ± 2 mV in 10.5 mM (N=3); (3) it was reduced by a K^+ channel blocker (100 μM Ba^{2+}, N=4).

The precise identity of the channels that are affected, as well as the mechanism by which they are opened, remain to be determined.

672.3

NEUROTOXICITY OF HYDROGEN PEROXIDE. P.A. Boxer*, O. Ben-Yoseph[1], N. Dujovny, B.D. Ross[1]. Neuroscience Pharmacology, Parke-Davis Research, Div. of Warner-Lambert Co., Ann Arbor, MI 48105 and [1]Depts. of Radiology and Biological Chemistry, Univ. of Michigan, Ann Arbor, MI 48109.

Free radical induced neuronal injury has been implicated in a number of neurological disorders and has been linked to excitotoxic cell death. The current experiments evaluated the toxicity produced by the direct application of the progenitor of hydroxyl radicals, hydrogen peroxide (H_2O_2). Experiments were performed on 17-day old cerebrocortical primary cultures under conditions similar to those employed in excitotoxicity experiments. Cell death was assessed both visually and quantitatively by the measurement of lactate dehydrogenase (LDH) released into the medium. Following a 30 min exposure to H_2O_2 there was a concentration dependent cell death assessed 24 hr later (EC_{50}=102 μM). Shorter exposure times to H_2O_2 were less effective. LDH release did not occur until >8 hrs after removal of H_2O_2 indicating a delayed cell death, similar to that seen following exposure to glutamate. Pretreatment with the iron chelator deferoxamine (2 mM) produced a rightward shift in the H_2O_2-induced toxicity curve (EC_{50}=458 μM), while inhibition of catalase with aminotriazole (30 mM) enhanced the H_2O_2 induced toxicity (EC_{50}=54 μM). This neurotoxicity was not mediated by NMDA receptors, since the NMDA antagonist MK-801 did not prevent the cell death. These results are consistent with the assumption that the generation of hydroxyl radicals is necessary for cell death. As with excitotoxicity, a rise in $[Ca^{2+}]_i$ may play a critical role, since there was an abrupt increase in $[Ca^{2+}]_i$ about 40 min following continual exposure to H_2O_2. Removal of extracellular Ca^{2+} prevented the increase in $[Ca^{2+}]_i$, suggesting that the increase was not the result of the liberation of intracellular stores of calcium. These data demonstrate that H_2O_2-induced toxicity has many of the same features as excitotoxic cell death, suggesting that there may be a causal link between these forms of neurotoxicity.

672.4

CHARACTERIZATION OF HUMAN SERUM-INDUCED TOXICITY IN RAT PRIMARY HIPPOCAMPAL CULTURES. P.S. Puttfarcken*, A.M. Manelli, and D.E. Frail, Neuroscience Research, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL. 60064.

Previous studies have reported the presence of complement proteins in senile plaques of Alzheimer's (AD) brains. Since the hippocampus is a region of the brain severely affected in AD, the effect of complement on primary cultures of rat hippocampal neurons was evaluated. Both morphological and biochemical changes were examined following treatment with either human or rat serum. 3 day old cultures (days in culture, DIC) were not affected by treatment with either serum. In contrast, a significant amount of cell death was observed in 6 DIC treated with 15% human serum for 24 hours. Under these conditions, the magnitude of cell toxicity varied between the lots of serum tested and LDH release did not always correlate with activation of the complement cascade, as measured by iC3b formation. A 24 hour treatment with 15% rat serum had no significant effect on these cultures. To determine if the activation of complement was responsible for the cell death observed following treatment with human serum, cells were treated with serum deficient in C9, the component of the classical complement cascade responsible for completing the assembly of the lytic membrane attack complex. The addition of C9 to 3 DIC treated with C9-deficient serum was not toxic. In contrast, the addition of C9 induced toxicity in 6 DIC treated with C9-deficient serum. These experiments suggest that human serum-induced toxicity in hippocampal neurons is, in part, through the activation of the complement cascade, dependent on the age of the culture, and subject to homologous restriction.

672.5

POLYCHLORINATED BIPHENYLS REDUCE TYROSINE HYDROXYLASE ACTIVITY IN RAT BRAIN. R.F. Seegal* and W. Shain. Wadsworth Center, NYSDOH, Albany, NY 12201.

Polychlorinated biphenyls (PCBs) reduce dopamine (DA) concentrations in adult rat and non-human primate brain and in pheochromocytoma (PC12) cells. Using neuroblastoma (N1E-N115) cells that do not express the enzyme dopa decarboxylase (DDC), required for the conversion of *l*-Dopa to DA, we have shown that PCBs reduce media *l*-Dopa concentrations, suggesting that PCBs reduce the ability of tyrosine hydroxylase (TH) to convert tyrosine to *l*-Dopa. To determine if reductions in brain DA concentrations are also due to inhibition of TH we exposed adult Wistar-derived rats to either Aroclor 1254 (1,000 ppm in chow) or control chow for sixty days and sacrificed them thirty minutes after they received intra-peritoneal injections of either 100 mg/kg of 3-hydroxybenzylhydrazine (NSD-1015), an inhibitor of DDC or saline. DA, *l*-Dopa, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations were determined in the striatum and nucleus accumbens by HPLC. PCBs significantly reduced DA concentrations in the striatum and DOPAC and HVA concentrations in both brain regions. Co-treatment with NSD-1015 resulted in further decreases in DOPAC and HVA as well as reductions in *l*-Dopa concentrations compared to control/NSD-1015 treated animals. These reductions in *l*-Dopa and DA metabolite concentrations strongly suggest that PCBs, *in-vivo*, inhibit production of newly synthesized DA at the level of tyrosine hydroxylase.

Supported by NIH Grant #ESO491304.

672.6

NEUROTOXICITY OF IBOGAINE IN CD MICE S.F. ALI*, X. M. MENG AND J.P. O'CALLAGHAN. Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079, Neurotoxicology Division, US EPA, Research Triangle Park, NC 27710.

Ibogaine is an indolealkylamine known to have CNS stimulant and hallucinogenic properties in animals. Recently, ibogaine has been proposed for use as a treatment for drug addiction. The neurochemical basis for such therapy, however, remains unclear. The present study was designed to evaluate the neurotoxic effects of ibogaine in mice. Ibogaine was administered to adult female CD-mice (0, 50, 100, or 200 mg/kg, ip) once daily for three days. On day six, animals were sacrificed and brains were removed, and ten different regions were dissected for neurochemical analysis. Dopamine (DA), serotonin (5-HT) and their metabolites were measured by HPLC/EC. Astrogliosis, a common response to neural injury, was quantified by assaying the major intermediate filament protein of astrocytes, GFAP. Ibogaine produced a dose-related decrease of 5-HT in caudate nucleus (CN), frontal cortex (FC) and brain stem (BS). Ibogaine caused a significant increase of 5-HT in hippocampus (HIPP). There were no significant change in DA, however, DOPAC was elevated in CN and FC. Ibogaine increased GFAP in olfactory bulb, HIPP and BS. These data suggest that ibogaine has a complex neurotoxicity profile in female mice, as assessed by changes in monoamine concentrations and astrogliosis.

672.7

CHRONIC EXPOSURE TO IBOGAINE RESULTS IN SEX-DEPENDENT ASTROGLIOSIS IN THE RAT. J.P. O'Callaghan*, L.E. Rodman, T.S. Rogers, J.B. Terrill and J.G. Page. U.S. Environmental Protection Agency, Res. Tri. Pk., NC 27711; Southern Res. Inst., Birmingham, AL 35255; NIDA, Rockville, MD 20857.

Ibogaine (IBG) is a naturally occurring alkaloid derived from the root of the African shrub, *Tabernanthe iboga*. Evidence exists showing that IBG interrupts the physiological and psychological aspects of drug addiction, both in experimental animals and in man. Recent reports (e.g. O'Hearn and Molliver, Neurosci. 55: 303, 1993) also indicate that IBG has the potential to induce neurotoxicity because, in male rats, it causes degeneration of cerebellar Purkinje cells with an attendant gliosis. Here we evaluated the potential of chronic exposure to IBG to cause neurotoxicity in male and female rats as evidenced by the presence of astrogliosis, a generic response to CNS injury. IBG was administered to male and female Sprague-Dawley rats (0, 25, 75, or 150 mg/kg, p.o) once daily for 14 days. Rats were sacrificed on day 15 and 31. Brains were dissected into olfactory bulb, hippocampus, striatum, cortex, brain stem and cerebellum. IBG-induced astrogliosis was quantified by assaying the content of the astrocyte intermediate filament protein, GFAP, in each brain region, using a sandwich ELISA. In male rats, GFAP was not elevated as a function of IBG treatment at any dose or in any region on day 15. In female rats, large (as much as 200% of control), dose-, time-, and region-dependent increases in GFAP were observed on day 15. The greatest increases were seen in olfactory bulb and brain stem; notably, the cerebellum was not affected. In both male and female rats GFAP was unaffected on day 31, a finding consistent with the time-dependent nature of chemical-induced astrogliosis. The data indicate that chronic exposure to IBG causes a pronounced sex-dependent astrogliosis, findings suggestive of underlying brain damage. Supported by NIDA Contract-1-9302 & NIDA IAG-1Y01DA-30063.

672.8

A CYTOTOXIC EFFECT OF ESTROGEN ON AMYGDALA NEURONS. S.Kitot J.Semba, R.Miyoshi , M. Kanazawa, A.Ando, S.Chin and L.Shimada. Division of Health Sciences, The University of the Air, Chiba Japan, *Division of Basic Medical Sciences, Royal Free Hospital School of Medicine, London.

It has been described that estrogen exerts cytotoxic effect on β-endorphin containing neurons in the rat hypothalamus.

In this paper, effects of estrogen on cultured rat amygdala neurons were studied. Primary cultures of rat fetal amygdala neurons were used for the experiment. After adding various concentrations of estradiol, cells were incubated for 24 hours. Then the percent survival of cells was calculated by assaying *intracellular LDH activity*. *Effect of estradiol on intracellular* Ca^{2+} concentration in the cultured neurons were also examined by fura-2 fluorometry.

As results, estradiol had a cytoprotective effect on amygdala neurons at concentrations of 10^{-9} ~10^{-5} M and cytotoxic effect at higher concentrations. On the other hand, estradiol caused long-lasting Ca^{2+} elavation within cultured amygdala neurons.

Mechanisms of this Ca^{2+} elevation was analyzed by adding dihydropiridine, ω-conotoxin and mRNA inhibitors. Currently, in situ hybridization experiments of hepatocytic growth factor on rat amygdala before and after estrogen administrations are in progress.

672.9

NEUROCHEMICAL CHANGES FOLLOWING L-CHLOROPROPIONIC ACID-INDUCED DAMAGE P.S. Widdowson, I. Wyatt, E.P. Christian* and E.A. Lock, Zeneca Central Toxicology Laboratory, Macclesfield, UK and Zeneca Inc, Wilmington, DE 19897, USA.

L-Chloropropionic acid (CPA) when administered systemically to rats produces selective destruction of cerebellar granule cells. We examined the neurochemical changes in rat cerebellum and forebrain during the development of the granule cell necrosis. Groups of rats were administered with CPA (250 mg/kg/day) for 3 days. At various time points groups of rats were killed. Concentrations of amino acids were measured in cerebellum and forebrain using HPLC. Densities of NMDA, kainate, adenosine-A_1 and $GABA_A$ receptors were also measured in brain regions by autoradiography. By day 3, concentrations of the excitatory amino acid transmitters, glutamate and aspartate began to fall and remain low (- 55 to 70%). Concentrations of GABA and glutamine increased transiently (+300%) but fell back to control levels by day 5. Concentrations of taurine fell by day 3 (-60% of controls) and remained low. Glycine concentrations increased at day 3 (+200% of control values) and remained elevated. The densities of the glutamate receptors, NMDA and kainate were reduced in cerebellar cortex from CPA treated rats. There was no change in the densities of NMDA or kainate receptors in any other brain region examined. Densities of adenosine A_1 and $GABA_A$ receptors in cerebellar and forebrain regions were not changed following CPA adminstration. In conclusion, we suggest that reduced glutamate and aspartate levels and NMDA and kainate receptor densities in the cerebellum are related to loss in cerebellar granule cells. Changes in other amino acids may reflect compensatory events in response to the toxic insult.

672.10

HIPPOCAMPUS-HPA-AXIS FUNCTIONS IN RATS INTOXICATED WITH ORGANOTIN COMPOUNDS. H.Imai[1], M.Kabuto*[1], K.Yan[1], T.Suzuki[1], M.Akaike[2], and N.Kato[3]. [1]Natl. Inst. Environ. Studies, 16-2 Onogawa, Tsukuba City, Ibaraki, 305 JAPAN, [2]Hoechst Japan Ltd., 1-3-2 Minamidai, Kawagoe City, Saitama, 350 JAPAN, [3]Shiga Univ. of Med. Sci., Seta-Tsukinowa, Ohtsu City, Shiga, 520-21 JAPAN.

To investigate whether the hippocampal damage caused by thimethyltin (TMT) involves corticosterone (CORT)-receptors, effects of a single oral dose of TMT-chloride (TMT-c) administration (8, 4 and 0 mg/kg, p.o.) on 1) plasma CORT levels on Day 0, 3, 4 and 5 after the TMT-c and 2) response of the level at 0, 30, 60, 90 and 120 min after corticotropin-releasing-factor (CRF) injection (28 µg/kg, i.v.) on Day 4 were examined using male SD rats aged 6 wks. An apparent hippocampal damage especially in CA3 and CA4 pyramidal cells were observed 4 to 5 days after an administration of TMT-hydroxide of 9 mg/kg (p.o.) in our previous studies. A significant enhancement of the CORT level on Day 3 (mean: 124 ng/ml) and 4 (mean: 187 ng/ml) after the TMT-c of 8 mg/kg compared to the vehicle (distilled water) controls (mean: 24 ng/ml) but no more increase with CRF injection was observed. The enhanced CORT levels on Day 4 were shown to be almost completely suppressed at 90, 180 and 300 min after an administration of dexamethasone of 100 µg/kg (s.c.), indicating that the CORT-receptors both in the hypothalamus and pituitary may not be affected by the TMT-c, whereas alterations of the hippocampal CORT-receptors could not be denied. On the other hand, the animals treated in the same manner with triethyltin-chloride (TET-c) of 4 mg/ml (i.p.) showed significant increases of the CORT level on Day 3 and 4 but no more increase in response to the CRF on Day 4, but those with triphenyltin-chloride (TPT-c) of 4mg/kg (i.p.) did not show significant increase on Day 3 or 4, but their responses of the CORT level to the CRF on Day 4 were exaggerated compared to those among the vehicle (corn oil) controls, suggesting that actions of TPT-c on the hippocampus-HPA axis may be different from those by TMT-c and TET-c.

672.11

GLIAL FIBRILLARY ACIDIC PROTEIN IN THE RAT BRAIN RESPONDS BIPHASICALLY TO INHALED TOLUENE. AR Little, ZL Gong, HAN El-Fawal*, HL Evans . Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY, 10987

GFAP concentration in the hippocampus and cerebellum was evaluated as a biomarker of the neurotoxicity of inhaled toluene. Fisher-344 rats were exposed to toluene at 100ppm or 1000ppm by inhalation for 6 hours/day, 5 days/week. Days 1,3,7,21,and 42 were evaluated. GFAP response in the hippocampus was biphasic at both doses, initially decreasing but later increasing above control by day21 to day42. In the cerebellum GFAP levels declined significantly by day 3, increasing thereafter, never quite returning to control levels. These data are consistent with a hypothesis emerging from this laboratory concerning the mechanism by which exposure to low doses of toxic chemicals affect regulation of GFAP. The most familiar finding is an increase in GFAP typical of reactive gliosis. A less frequently observed change may involve decreased GFAP levels, observed with very low level exposures. Our studies also indicate a biphasic response with exposure to Pb (Gong et al., 1994). Relative- ly low level exposures administered chronically may be necessary to observe this early phase of the glial response, a transient decrease followed by an increase if given enough time or a large enough dose. Low level or short term exposure to toluene may induce changes in glial cell function that precede overt toxicity characterized by gliosis Supported by the American Petroleum Institute and grant ES-04895.

672.12

SEXUAL DIMORPHIC EFFECTS ON BEHAVIOR FOLLOWING IN UTERO EXPOSURE TO PHENOBARBITAL.
W. Pizzi*, S. Baranski, A. Newman, & R. Andrews, Northeastern Illinois Univ. Chicago, IL. 60625

Pregnant rats were administered phenobarbital (PHB, 40 mg/kg, sc) on gestation days 12-18, while controls received equal amounts of saline. PHB-exposed pups showed a hyperexcitability resulting in earlier attainment of a surface righting task ($p<0.01$). In adult behavioral testing PHB-exposed male rats showed reduced performance on a DRL-20 operant conditioning schedule, & a significant reduction in performance on a FR-10 schedule ($p<0.05$). Male PHB-exposed rats showed a reduced auditory startle response, but a prepulse inhibition pattern similar to controls. Female PHB-exposed rats showed no significant differences on these tasks. In a two-bottle taste preference for a sweet solution the usual sex dimorphism was seen with females drinking greater amounts than males; however, PHB-exposed males drank more of the sweet solution than control males. In these experiments, prenatal exposure to PHB differentially affected male versus female offspring. These data suggest that PHB-exposure may reduce testosterone levels at a critical period of brain development leading to feminized behavior in male offspring.

672.13

THE PEPTIDIC SUBSTANCE P ANTAGONIST [D-PRO^2, D-TRP^{7,9}]-SUBSTANCE P BUT NOT THE NONPEPTIDIC ANTAGONIST CP-96,345 INDUCES HIPPOCAMPAL NEURODEGENERATION. D. K. Rush*, S. Aschmies and G. M. Bores. Dept. Biol. Res., Neuroscience Strategic Business Unit, Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876.

Direct injection (1 µl) of the peptidic Substance P (SP) antagonist [D-Pro^2,D-Trp^{7,9}]-Substance P (DPDT-SP, 1-3 nmol) induced neurodegeneration in the dorsal hippocampus of rats. Co-injecting SP (30 nmol) reduced the neurotoxicity induced by 3 nmol of DPDT-SP. Another SP antagonist [Arg^6, D-Trp^{7,9},MePhe^8]-SP(6-11) (10 nmol) was also neurotoxic after intrahippocampal injection. Surprisingly, the nonpeptidic SP antagonist CP-96,345 was not neurotoxic at a dose of 10 nmol. The reduced neurotoxicity of the nonpeptide antagonist was apparently not due to a lower affinity for the SP receptor; CP-96,345 displaced the NK-1 agonist [^{3}H][Sar^9,Met(O$_2$)^{11}]-SP with higher affinity (IC_{50}=0.34 µM) than DPDT-SP (IC_{50}=8.9 µM) in rat brain membranes. Binding of the peptidic and nonpeptidic antagonists at different receptor subtypes or different epitopes of the same subtype, both of which have been reported for SP receptor ligands, might account for the differential neurotoxicity observed in the present experiments.

672.14

DYNORPHIN A INDUCES RELEASE OF LDH IN PC-12 CELLS THROUGH A NON-OPIOID MECHANISM. A.G.Mukhin* and A.I. Faden, Georgetown University Medical Center, Washington, DC 20007.

Dynorphin A (Dyn A) has been implicated in the pathophysiology of both traumatic brain and spinal cord injuries. Both opioid and non-opioid actions of Dyn A seem to be involved. However, the basic mechanisms underlying the damaging effects of Dyn A remain speculative. In order to better address this issue, we have studied the effects of Dyn A and related peptides *in vitro*, using PC-12 cells. PC-12 cells were cultured in minimal essential medium (MEM) supplemented 7.5% fetal bovine serum and 7.5% horse serum. On day 3, cells were washed twice by serum-free MEM, and coincubated with studied drugs in serum-free MEM. LDH release has been used as a marker of cell injury in many *in vitro* studies and has been shown to correlate with subsequent cell death in experiments examining glutamate toxicity. Dyn A, in the micromolar concentration range, induced release of LDH in culture medium, with an ED_{50} of approximately 20 µM. This effect was maximal by 10 min. Pretreatment with the opioid antagonists naloxone, WIN-44,441-3, or nor-binaltorphimine in concentrations up to 1 mM failed to significantly attenuate the effect of 10 µM of Dyn A. Moreover, Dyn A-(2-17), which is inactive at opioid receptors, was similar in potency to Dyn A in its ability to induce LDH release. Structure activity studies showed lack of effect of dynorphin-related peptides Dyn A-(1-8) and shorter. This profile of activity is similar to that found with spinal cord injury induced by intrathecal dynorphin administration. Taken together, these studies suggest that PC-12 cells may provide a useful *in vitro* model for studying certain pathophysiological actions of dynorphin, particularly its non-opioid actions.

672.15

NEUROPROTECTIVE EFFECTS OF MONOAMINE OXIDASE-B INHIBITORS ON DSP-4 INDUCED DEGENERATION OF NORADRENERGIC AXONS IN THE RAT BRAIN. X. Zhang, P. H. Yu and A. A. Boulton*. Neuropsychiatry Research Unit, University of Saskatchewan, Saskatoon, Canada, S7N OWO.

DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] is a highly selective neurotoxin toward the locus coeruleus noradrenergic (NA) system. Previous biochemical studies have shown that the monoamine oxidase-B (MAO-B) inhibitors, R(-)-deprenyl and 2-HxMP [N-(2-hexyl)-N-methylpropargylamine], are able to prevent DSP-4 induced NA depletion in the mouse hippocampus. It is, however, unknown whether this represents a neuroprotection of NA axons. Employing dopamine-β-hydroxylase immunohistochemical and imaging analysis methods, we have found that 92% and 84% respectively NA nerve fibers in the rat hippocampus are spared from DSP-4 neurotoxicity by a single pretreatment dose of R(-)-deprenyl or 2-HxMP. Similar neuroprotective effects are also observed in the cerebral cortex, thalamus, amygdaloid complex and cerebellum. This is the first morphological evidence demonstrating that deprenyl and 2-HxMP can indeed protect NA axons against DSP-4 neurotoxicity. We are currently also investigating the neuroprotective and neurorescue effects of R(-)-deprenyl and 2-HxMP on DSP-4 induced chronic degeneration of NA neurons in the locus coeruleus.

672.16

DELAYED CONSEQUENCES OF PARENTERAL DOMOIC ACID TREATMENT IN RAT BRAIN. Nathan M. Appel*, Stanley I. Rapoport and James P. O'Callaghan. FDA CDER ORR DRT, Laurel, MD 20708; NIH NIA LNS, Bethesda, MD 20852 and USEPA, Research Triangle Park, NC 27711.

Domoic acid (Dom) is a neurotoxic excitatory amino acid (EAA) which appears to act as an agonist at the kainic acid subtype of EAA receptors. Dom has been identified as the contaminant in mussels causing adverse neurological and gastrointestinal effects, and in some cases death, to people who consumed them. Following i.p. Dom, 2.25 mg/kg, some rats displayed seizures and stereotypy (scratching). Seven days later brains from rats manifesting seizures and stereotypy had intense argyrophilia in anterior olfactory nucleus; CA1 hippocampus; lateral septum; and parietal (layer IV), piriform and entorhinal cortices. Brains from rats which did not manifest seizures or stereotypy did not. This pattern of agryophilia is reminiscent of that seen after kainic acid and of *c-fos* expression shortly after dosing with kainic acid or another convulsant, pentylenetetrazole. Microglia, detected using lectin histochemistry, appeared to be increased in regions having argyrophilia. Glial fibrillary acidic protein expression (GFAP) was more diffuse, however. [^{14}C]Arachidonic acid incorporation, which labels mainly *sn*-2 positions of brain phosphatidylcholine and phosphatidylinositol was increased in all Dom-treated rats. [^{3}H]Palmiticic acid incorporation, which labels the *sn*-1 position of brain phosphatidylcholine, appeared unaffected. Local cerebral glucose utilization ([^{14}C]2-deoxyglucose incorporation) was also determined. These data suggest that acute activation of discrete brain circuits by Dom and subsequent seizures induce delayed alterations in brain homeostasis which is reflected by gliosis and altered metabolism.

672.17

THE EFFECT OF ACUTE ETHANOL TREATMENT ON CALCIUM HOMEOSTASIS IN CULTURED HIPPOCAMPAL NEURONS BEFORE AND DURING DEPOLARIZATION. B.Webb*, S.S. Suarez, M.B. Heaton, and D.W. Walker. University of Florida, V.A. Medical Center, Gainesville, Fl 32610.

The neurotoxic effect of acute ethanol treatment (AET) may lead to an alteration in the regulation of calcium homeostasis in hippocampal neurons. We cultured E18 rat hippocampal neurons for 2 weeks. The cultures were loaded with 2μm indo-1/AM for 1 h at room temperature, rinsed with buffer, and incubated for 1/2 h at 37°C. The cultures were divided into 4 groups: control, 100,400,800 mg/dl ethanol. Control readings were taken, and then the buffer was changed for either control buffer or buffer containing 100,400,800 mg/dl ethanol. Fast responses were recorded for the first 6.4 sec and then at 10,30,45,60 min. To determine the effect of AET on calcium homeostasis in cultured hippocampal neurons during depolarization, the buffer was changed after 60 min to either control buffer and 30mM KCL or buffer with ethanol and 30mM KCL. Fast responses were recorded for the first 3.2 sec and then at 5,10 min. In cultures treated with 400 or 800 mg/dl ethanol, $[Ca2+]_i$ was decreased ($p<0.01$) at later time points before depolarization but increased after depolarization ($p<0.0001$). When AET cultures were compared to control, both 100 and 400 mg/dl ethanol increased resting $[Ca2+]_i$ at 10 min ($p<0.05$). At later time points, 400 and 800 mg/dl treatment decreased $[Ca2+]_i$ in a dose-dependent manner. AET inhibited the rise in $[Ca2+]_i$ after depolarization with 30mM KCL ($p<0.01$) in a dose-dependent manner. The results of this study indicate that AET results in the alteration of calcium homeostasis in cultured hippocampal neurons at resting membrane potentials and during depolarization. These changes in $[Ca2+]_i$ may disrupt normal cellular function and contribute to cell death. Therefore, the alteration of calcium homeostasis may be an underlying mechanism of ethanol neurotoxicity. Supported by NIAAA grants AA00200, AA09128, and Fellowship #1F31AA05372-01; and Medical Research Service Deptartment of Veterans Affairs.

672.18

ETHANOL DECREASES INTRACELLULAR MYO-INOSITOL IN C_6 CELLS. K. E. Isenberg*, S. G. Holstad, B. W. Moore and W. R. Sherman. Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Ethanol produces a dose-dependent decrease in the growth of C_6 glioma cells. We hypothesized that the etiology of this phenomena could be similar to effects observed when the cells are grown in hyperosmolar sodium chloride (NaCl). C_6 cells were grown in Dulbecco's Modified Eagles Medium and 10% fetal calf serum (controls) or grown in the same media supplemented with ethanol or NaCl in chronic exposure (7 days) or acute exposure (up to 2 days) experiments. Chronic exposure reveals that ethanol has a more potent inhibitory effect on growth than equiosmolar additional NaCl. Unlike exposure to hyperosmolar NaCl, chronic ethanol exposure does not alter intracellular concentrations of protein. Acute exposure of C_6 cells to ethanol is associated with a 50% decrease in intracellular concentrations of myo-inositol, the opposite of effects observed upon exposure of the cells to hyperosmolar NaCl. The effects of ethanol upon intracellular concentrations of myo-inositol occur in a dose dependent fashion at concentrations of ethanol (8.5 mM to 130 mM) commonly encountered in human intoxication. Growing the cells in media supplemented with myo-inositol then switching the cells to media with added deuterated myo-inositol and ethanol suggests that ethanol alters myo-inositol influx and efflux. The effects of ethanol on influx of myo-inositol appear to be the opposite of the effects of NaCl upon myo-inositol transport. Ethanol induced reductions in the intracellular concentration of myo-inositol may impair the growth of these cells.

NEUROTOXICITY: METALS

673.1

SUBCHRONIC METHYLMERCURY (MeHg) EXPOSURE ALTERS Ca^{2+} RELEASE FROM AN IP_3-SENSITIVE STORE IN NG108-15 CELLS. J.E. Sirois* and W.D. Atchison Dept. Pharm./Tox., Mich. State Univ., E. Lansing, MI 48824.

MeHg is a neurotoxic organomercurial which exhibits complex effects on intracellular ion homeostasis. Acute exposure to MeHg initially releases Ca^{2+} from an IP_3-sensitive store, increases the concentration of an endogenous non-Ca^{2+} ion and ultimately leads to influx of Ca^{2+} across the plasma membrane. The present study was undertaken to determine if subchronic exposure to low μM concentrations of MeHg could also alter ion homeostasis and/or affect Ca^{2+} release from IP_3-sensitive pools. 24 Hour exposure to 1μM MeHg did not alter the apparent resting $[Ca^{2+}]_i$ but did cause an increase in the response to bradykinin (Bk; 1μM). 2μM MeHg for 24 hr also increased the response to Bk in addition to slightly increasing the apparent resting $[Ca^{2+}]_i$. These results suggest that NG108-15 cells exposed subchronically to MeHg are able to overcome an initial loss of Ca^{2+} from the IP_3-sensitive pool. The enhanced response to Bk may reflect an ability of MeHg either to facilitate release from the IP_3-sensitive pool or possibly to inhibit the uptake of Ca^{2+} following its release from this pool. Supported by NIH grant ES03299.

673.2

NEUROPSYCHOLOGICAL AND PSYCHIATRIC COMPLICATIONS ASSOCIATED WITH HIGH LEVELS OF SERUM COPPER OF UNKNOWN ETIOLOGY. W.J. Coughlin†‡, B.D. Fantie*†, and J. Joyce~. †Human Neuropsychology Laboratory, The American University, Washington, DC 20016, ‡Mt. Vernon Center for Community Mental Health, Alexandria, VA 22309, and ~Psychiatric Associates of Fredricksburg, Fredricksburg, VA 22401.

Chronic copper toxicosis is characteristically associated with Wilson's disease, a rare genetic disorder that usually manifests as either a hepatic or neurological syndrome. Wilson's disease is fatal unless treated with chelating agents. Death results from the direct effects of copper toxicity on the brain and liver. Although the subjects in this investigation do not meet diagnostic criteria for Wilson's disease and are without gross hepatic or neurological symptoms, laboratory blood tests have indicated toxic levels of serum copper of unknown etiology that are as high as those typically reported in Wilson's patients. The subjects in the present study suffer from psychiatric disorders and approximately 20% of Wilson's patients initially exhibit psychiatric symptoms prior to the onset of the full clinical entity. Using a comprehensive battery of neuropsychological tests, we assessed two female psychiatric patients with chronic hypercupremia. Preliminary results include deficits in verbal and nonverbal fluency, verbal and nonverbal recall, motor speed, and motor sequencing. The few neuropsychological studies on Wilson's disease in the literature, to date, have found comparable deficits in verbal recall and motor speed. Explanations for the presence of hypercupremia in these subjects are explored including the possible existence of a previously unknown subtype of Wilson's disease. We suggest it is possible, in some rare instances, that patients may present with psychiatric, cognitive, or motor symptoms that may, in fact, be related to undiagnosed hypercupremia.

673.3

MOUSE BRAIN UPKEEPS ^{3}H-SPIROPERIDOL RECEPTOR BINDING PATTERN IN AN EARLY STAGE OF MANGANESE POISONING. V. Villalobos, J. Estévez, J.O. Davila, E. Bonilla*. INBIOMED, FUNDACITE-ZULIA, Apartado 376, Maracaibo - Edo. Zulia, Venezuela.

Manganese (Mn) poisoning is an occupational disease whose symptoms resembles the Parkinson's. This study is aimed to asses the effects of Mn in the ^{3}H-Spiroperidol receptor pattern of different regions in mice. Male adult mice received a daily intraperitoneal (i.p.) Mn dose of 5mg/kg for eight weeks. Control mice were injected with saline. At the end of assay, the mice were killed by cervical dislocation, and the brains were removed on ice. Olfactory bulb, striatum and hyppothalamus were dissected out and frozen until the assay was performed. After that, samples of each region were taken for Mn determination. ^{3}H-Spiroperidol binding assays were realized according to the method described by Bhargava. Mn concentrations were determined by atomic absorption spectrophotometric. Mn-treated mice showed a significant increase of Mn levels in all regions. The Bmax and Kd values for ^{3}H-Spiroperidol binding were unaltered by the treatment. Our investigation reinforce previous finding which seem to point out that mice submitted to this conditions are in an early stage of Mn poisoning, where cell membranes damage has not occurred.

673.4

ALTERED LEVELS OF NERVE GROWTH FACTOR AND ITS LOW-AFFINITY p75 RECEPTOR IN THE DEVELOPING BRAIN FOLLOWING BY PRENATAL EXPOSURE TO MERCURY VAPOUR.
Stine Söderström and Ted Ebendal*. Dept. of Developmental Biology, Uppsala University, Box 587, S-751 23 Uppsala, Sweden.

Pregnant rats were exposed to low levels of mercury vapour. The levels of mercury (5-10 ng/g wet weight) found postnatally in the brains of their offspring are comparable with those found in human brains after average mercury exposure. The brains of the pups show an approximately 50% increase of NGF protein in the hippocampus and the cortex at postnatal day 21 with a concomitant decrease to 55% of normal levels in the medial septal area whith NGF responsive cholinergic neurons. The levels of mRNA for NGF, p75 LNGFR, p140 trk and ChAT were examined by *in situ* hybridization histochemistry. There were no significant changes of NGF mRNA measured at P22 in the dentate gyrus following the prenatal mercury vapour exposure. The mRNA level of the NGF high affinity receptor p140 trk was sligthly increased in the diagonal band and the medial septum at P22, while the expression of mRNA for the low affinity receptor p75 was significantly reduced to approximately 30% of normal in both the medial septal area and in the diagonal band nucleus. ChAT mRNA was slightly reduced in the diagonal band and the medial septum and was significantly reduced in the striatum. It is suggested that the retrograde transport of NGF from the target to the basal forebrain was interrupted due to the accumulated mercury. Moreover, NGF produced by the fibroblast cell line 3T3 in the presence of organic mercury ($MeHgCl_2$) was found to be doubled when methyl mercury was added at concentrations of 0.1 μM-0.5 μM.

673.5

METHYL MERCURY INDUCED NEUROTOXICITY IS BLOCKED BY ANTI-OXIDANTS.
S.T. Park[1,2], K.T. Lim[1], Y.T. Chung[2] and S.U. Kim[1]*. [1]Div. of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, BC, V6T 2B5, Canada. [2]Dept. of Anatomy, Wonkwang Univ. School of Medicine, Iri 570, Korea.

Methyl mercury compounds are known to induce neurotoxic changes in the mammalian central nervous system (CNS). In order to characterize the mechanism of methyl mercury neurotoxicity, neonatal mouse cerebral neuron cultures were exposed to graded concentrations of methyl mercruy chloride (0-100 μM) for 2-48 hours. Cell viability ws determined using MTT assay and neurofilament ELISA assay.

Methyl mercury was toxic to mouse cerebral neuron cultures (LD50 = 20 μM) after 24 hours of exposure, and neurotoxicity was blocked in a dose-dependent manner by catalase (10-100 μg/ml) and glutathione (0.2-2 mM). Other reagents tested for neuroprotective effects against methyl mercury toxicity such as selenium (1-10 μM), ascorbic acid (20-200 μg/ml), sodium thiosulfate (0.1-1 mM) and cysteine (50-500 μg/ml) were not effective.

These results indicate that anti-oxidants such as catalase and glutathione are effective in blocking methyl mercury neurotoxicity in the CNS.

673.6

METALLOTHIONEIN: ISOFORM PURIFICATION IN HUMAN FRONTAL CORTEX.
K. Kiningham, E.J. Kasarskis*. Graduate Center for Toxicology and Department of Neurology, Veteran's Administration Medical Center and University of Kentucky, Lexington, KY 40536.

Metallothioneins (MTs) are a family of ubiquitous cysteine-rich, 6-7 kD proteins which detoxify heavy metals, participate in zinc homeostasis, and may function as an intracellular antioxidant. Six MT isoforms have been sequenced from human liver. Immunocytochemical data suggest multiple isoforms in the central nervous system; however, only one isoform, MT-III, has been sequenced from human brain.

MTs were purified from cortex by homogenizing in 5 mM Tris-HCl, followed by ultracentrifugation. After heat precipitation, the soluble fraction was treated with chymotrypsin. Residual proteins and metals were removed by acid treatment. The MTs were then derivitized with 4-vinylpyridine and the S-pyridylethylated derivatives were separated by reverse phase HPLC.

Sequencing data suggest the presence of multiple isoforms in the cortex in addition to MT-III. Alterations in the proportions of the various isoforms might suggest an increased sensitivity to oxidative stress, metal accumulation and/or aberrant metal homeostasis. Studies in patients with Alzheimer's disease and Amyotrophic Lateral Sclerosis are ongoing to identify the status of the individual MT isoforms. This work was supported by VA Research Service and NIEHS 5T32ES07266 to K.K.

673.7

EFFECTS OF ALUMINUM ON NEURONAL ION CHANNELS.
C.H. Wu* and I. Seyama. Dept. of Pharmacology, Northwestern University Medical School, Chicago, IL 60611-3008, and Dept. of Physiology, Hiroshima University School of Medicine, Hiroshima 734, Japan.

Aluminum accumulation in the body during long-term kidney dialysis has been implicated in the pathogenesis of seizure in dialysis encephalopathy. However, the mechanism of seizure induction is unknown. Previously, we have shown that external application of aluminum caused a dose-dependent depolarization of axon membranes, prolongation of the action potential duration, and repetitive firings of action potentials (Wu, FASEB J. 7:A698, 1993). The aim of this study was to elucidate the mechanism of aluminum-induced neuronal hyperexcitability at the ion channel level. Voltage clamp experiments were conducted on giant axons of squid and crayfish. In intact axons, external application of aluminum acetylacetonate (1-2 mM) caused a decrease of the potassium current without significant effects on the sodium current. The reduction of K conductance was dose-dependent, and the effect had a slow onset. In internally perfused axons, aluminum had no effects whether it was applied externally or internally. The results suggest that neuronal hyperexcitability seen in dialysis encephalopathy is due to inhibition of potassium channels by aluminum and the inhibition is probably mediated by cytoplasmic constituents. (Supported by NIH grant NS30101).

673.8

METHYLMERCURY POISONING LEADS TO ENHANCED UPTAKE OF ^{35}S-L-CYSTEINE AND ^{3}H-L-GLUCOSE IN C57BL/6J MOUSE BRAIN.
David Park, Simon Yee and Ben. H. Choi*. Neuropathology, University of California, Irvine, College of Medicine, Irvine, CA 92717

Previous studies in our laboratory demonstrated that methylmercury (MeHg) poisoning in mice leads to a significant reduction in glutathione (GSH) contents in both brain and liver. We have also shown that an uptake of ^{35}S-L-cysteine was significantly enhanced in the brains of C57BL/6J mice following MeHg intoxication. To examine whether or not a reduction in GSH may underlie enhanced ^{35}S-L-cysteine uptake in the brain of MeHg-intoxicated animals, a group of C57BL/6J mice were injected with 5 mg/kg of methylmercuric chloride (MMC), and another group with 1.0 mM/kg of buthionine sulfoximine (BSO), γ-glutamyl-cysteine synthetase inhibitor, for 4 consecutive days. The control animals received physiological saline in place of MMC. Four hours after the last injection, ^{35}S-L-cysteine (1.0 μCi/g) and ^{3}H-L-glucose (1.0 μCi/g) were injected intraperitoneally. Forty five minutes thereafter, the brain and liver were obtained following perfusion with saline. Both MMC and BSO groups showed marked GSH reduction in the brain and liver. However, brain uptake of both ^{35}S-L-cysteine and ^{3}H-L-glucose was significantly enhanced only in MMC group and not in BSO group as compared to those in control. These results suggest that the enhanced uptake of ^{35}S-L-cysteine and ^{3}H-L-glucose in the brain of MMC-poisoned animals may be primarily related to a dysfunction of the blood-brain barrier brought about by MeHg intoxication. (Supported in part by NIEHS grant ES 02928)

673.9

COMPARISON OF THE TOXICITY OF TRIMETHYLTIN FOR FOUR INBRED STRAINS OF MICE. J.E. Ekuta, M. Tharp and J.C. Matthews. Dept. of Pharmacology, University of Mississippi, University, MS 38677.

The central nervous system toxicity of trimethyltin (TMT) is characterized by selective nerve and glial cell destruction, particularly granule cells in the hippocampal facia dentata, associated with performance deficits in behavioral tasks used to assess learning and memory. In addition to these rather selective effects, TMT also causes systemic toxicity manifested by malaise, cessation of eating and drinking with associated weight loss, convulsions, and death in severe cases. Male mice at 4 months of age from four inbred strains, AKR/J, BALB/cByJ, C57BL/6J and DBA/2J, were compared for their relative sensitivities to the toxicity of TMT following a single intraperitoneal injection. Body weight and mortality were used as indices for assessing systemic toxicity. The average time taken for the mice from each strain to find the submerged platform (swim time) in the Morris water maze was used to assess their learning abilities. Mice of all strains, except BALB/cByJ, died within 36 hours of 3.0 mg/kg TMT administration. At lower doses, the order of sensitivities of the strains based on severity of weight loss was C57BL/6J > AKR/J > DBA/2J > BALB/cByJ. The swim time ranking for the mouse strains was BALB/cByJ > DBA/2J > AKR/J > C57BL/6J. These results demonstrate that different inbred strains of mice exhibit genetically determined differences in their levels of sensitivity to the toxicities of TMT. This finding is an important first step in our quest to understand the role of neurotoxicity in neurodegenerative disease.

673.10

TRIMETHYLTIN-STIMULATED INOSITOL TRISPHOSPHATE ACCUMULATION IN PC12 CELLS. C.-W. Yang, M. D. Kane, R.P. Maickel* and G. E. Isom. Dept. of Pharmacol. & Toxicol., Sch. of Pharm. & Pharmacal Sci, Purdue Univ., West Lafayette, IN 47907.

Trimethyltin (TMT) is a selective CNS neurotoxicant which produces behavioral, biochemical and histological changes in rodents and man; yet, the primary mechanism(s) underlying its toxicity remain unclear. Since changes in the phosphatidylinositol-mediated second messenger system may play a role in the development of brain injury, it was of interest to determine if TMT could alter phosphatidylinositol turnover. In this study, the effect of TMT on IP_3 formation and the underlying mechanism was studied in rat pheochromocytoma (PC12) cells. As determined by fura-2 microfluorescence, there were no significant changes in cytosolic free calcium in TMT exposed PC12 cells. IP_3 generation was determined by loading cells with [^{3}H] *myo*-inositol and measuring cellular concentration of [^{3}H] IP_3 following treatment. Glutamate or (trans)-1-amino-cyclopentyl-1,3-dicarboxylate-stimulated IP_3 accumulation was blocked by the selective metabotropic glutamate receptor antagonists, (+)-a-methyl-4-carboxyphenylglycine (+)-MCPG) and L-amino-3-phosphonopropionate (L-AP3), indicating PC12 cells express functional metabotropic receptors. IP_3 production was stimulated by TMT in a concentration (1-100 μM) dependent manner and increased levels were detected within 30 sec of exposure. The maximal increase was observed within 2 min following the treatment. Additionally, TMT-stimulated IP_3 production was partially inhibited by atropine and (+)-MCPG. These results indicate that TMT-induced IP_3 accumulation was mediated in part by the cholinergic and metabotropic receptors.

673.11

COMPARISON OF INTRACRANIAL AND SYSTEMIC ADMINISTRATION OF TRIMETHYLTIN ON HIPPOCAMPAL DAMAGE. V. Luevano, E. Rasmussen and E. Castañeda*. Behavioral Neuroscience Laboratory, Department of Psychology, Arizona State University, Tempe, AZ 85287-1104.

It has been suggested that1) accidental exposure to the organometallic toxin trimethyltin (TMT) produces behavioral deficits that are a result of hippocampal damage, and 2) hyperactivity observed in rats treated systemically with TMT may be useful as a model for attention deficit hyperactive disorder. In order to evaluate further these postulates, the effects of various doses of TMT infused into the hippocampus were examined. Adult male Long Evans rats received four bilateral infusions (2/hemisphere) of either vehicle (phosphate buffered saline) or TMT (total of 50, 100, 200, 500 or 1000 μg) into the hippocampus. Two more groups of rats received 8 mg/kg TMT or vehicle systemically (i.p.). At least two weeks later, animals were decapitated and the left hemispheres underwent histological preparation for Nissl staining. The hippocampi were dissected from the right hemispheres and prepared for assay of dopamine and serotonin using HPLC-EC. It was found that there was a dose-dependent ablation produced by intrahippocampal application of TMT that was not evident in rats treated systemically. In addition, only the two highest intrahippocampal doses produced serotonin depletions relative to systemically treated rats. These results have important implications for understanding the central mechanism of action by which TMT produces behavioral deficits in human and nonhuman animals. Currently, behavioral measures are being examined for changes induced by intrahippocampal infusions of TMT.

673.12

TRIMETHYLTIN STIMULATES PROTEIN KINASE C TRANSLOCATION IN DIFFERENTIATED PC12 CELLS. M.D. Kane, G. Pavlaković, and G E Isom*. Dept. of Pharmacol. & Toxicol., Purdue Univ., W. Lafayette, IN.

Trimethyltin (TMT) is a potent neurotoxic agent which causes learning and memory perturbations and culminates in necrotic lesions of the limbic system in rodents and man. Differentiated rat pheochromocytoma (PC12) cells were used to determine the effect of TMT on neuronal signal transduction systems. Treatment of cells with TMT did not increase cytosolic free Ca^{2+}. However, confocal imaging studies of differentiated PC12 cells showed that 10 μM TMT stimulated mobilization of protein kinase C (PKC) to cellular membranes. To characterize this response in more detail, PC12 cells were treated with TMT (0, 5, and 20 μM) or phorbol 12-myristate 13-acetate (PMA; 100 nM) and the extent of PKC translocation to the membrane was determined at 0.5, 4.0, and 24 hrs of treatment; PKC levels were determined in soluble and membrane cell fractions by western blot analysis using anti-PKC antibodies. The control profile showed greater than 95% PKC in the soluble fraction. Both 5 and 20 μM TMT treatments stimulated a partial translocation of PKC (40%-60%) to the membrane fraction at all time points. PMA treatment stimulated greater than 95% PKC translocation at 0.5 and 4 hrs as well as a complete loss of PKC staining in both fractions at 24 hrs indicating PKC down regulation. TMT stimulated translocation did not result in PKC down regulation at 24 hrs. In separate experiments 100 μM TMT inhibited Ca^{2+}/phosphatidylserine stimulated PKC activity to 27% of control activity as determined by the ^{32}P-histone method. These results show that TMT stimulates a partial and sustained translocation of PKC in PC12 cells and inhibits kinase activity. This event appears to be independent of a rise in cytosolic free Ca^{2+}.

673.13

INCREASES IN GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) mRNA PRECEDE INCREASES IN HIPPOCAMPAL GFAP DURING EXPOSURE TO TRIMETHYLTIN (TMT) IN DRINKING WATER. J. S. Duffy*, H. A. N. El-Fawal, H. L. Evans, Z. L. Gong, A. R. Little. NYU Medical Center, Inst. of Env. Med., Tuxedo, NY 10987.

Previous work in this lab demonstrated that GFAP levels in hippocampus of rats increased during TMT exposure in drinking water. In order to evaluate whether the increased levels were due to *de novo* synthesis or some other mechanism, we compared the time course of changes in GFAP mRNA with changes in GFAP in the hippocampus, the brain region most sensitive to TMT.

Forty male Long-Evans rats, 45 days of age, were randomly divided into two groups: a dosed group consisting of 30 animals which were exposed to 3 ppm TMT in drinking water; and a control group consisting of 10 animals given plain drinking water. Sacrifices were on days 14, 25, 26, 27, 28, and 42 (5 animals/day) for the exposed group and days 14 and 28 for the control.

Body weight and water consumption were similar in the dosed and control groups. GFAP mRNA levels in dosed animals were significantly elevated compared to pooled control on days 25, 26, 27, 28 and 42. GFAP levels were significantly greater than control on days 26, 27, 28 and 42. Since there was no difference from control in either GFAP mRNA or GFAP on day 14, we conclude that the message increases preceded protein increases by a time range of between 1 and 11 days. These data suggest *de novo* synthesis of GFAP as an important mechanism by which exposure to TMT increased concentrations of GFAP in hippocampus. These are among the earliest changes which precede clinical signs of TMT toxicity and are thus promising both for understanding the brain's early reaction to toxic metals as well as for screening for neurotoxicity. Supported by the Amer. Petroleum Inst. and Grant ES-04895.

673.14

MANGANESE NEUROTOXICITY: GLYCINE RECEPTORS IN MOUSE BRAIN. G. Cano, E. Bonilla, M.E. Alburges*[1]. Fundacite-Zulia and Inst. Invest. Clínicas, Universidad del Zulia, Maracaibo, Venezuela. [1]Center for Human Toxicology, University of Utah, Salt Lake City.

Glycine (Gly) is an agonist at two neurotransmitter receptor sites, one sensitive to inhibition by Strychnine and linked to an inhibitory Gly-gated chloride channel, and the other a coagonist site on the N-Methyl-D-Aspartate (NMDA) cation channel complex. In previous reports, we have found a reduction of NMDA receptor density in some cerebral areas of manganese intoxicated mice. In the present study, we analyzed the ^{3}H-Gly binding to its receptors by autoradiographic methods. Male albino mice were injected i.p. with manganese chloride (5 mg Mn/kg body weight/day) 5 days per week, during 8 weeks. Control animals were treated with saline. Binding procedures were carried out using 100nM ^{3}H-Gly as a ligand, in the presence or absence of 1mM Strychnine. Non-specific binding was determined using 1 mM Gly.

Twenty brain regions were analyzed. Non-sensitive Strychnine Gly receptors were slightly reduced in all analyzed areas, but this decrease was only significant in Globus Pallidum. Although a reduction similar to that found in NMDA receptor densities was expected, our results suggest that, in manganese intoxication, variations in the expression of NMDA sites are not related with the expression of Strychnine-insensitive Gly sites, or that some Gly sites may not be associated with the NMDA complex. Sensitive Strychnine receptors are markedly absent in brain areas. In the control animals all values were near zero, but treated mice showed a significant increase of these receptors in Globus Pallidum and all areas of Caudate-Putamen. Due to the inhibitory nature of these receptors, these results could be related with motor inhibition detected in late stages of manganese intoxication.

673.15

AN IN *VITRO* MODEL TO STUDY THE RELATIONSHIP BETWEEN IRON AND PROTEIN OXIDATIVE MODIFICATION. S.W. Hulet*[1], B.S. Snyder[1], S. Powers[2], W. Van Gelder[3], J.R. Connor[1]. [1]Depts. of Neuroscience & Anatomy, [2]Neurosurgery, Penn State College of Medicine, Hershey PA and [3]Dept. of Chemical Pathology, Erasmus University, The Netherlands

A relationship between dysfunction of iron homeostatic mechanisms and oxidative damage has been proposed. The basis of iron's ability to induce oxidative damage is through its role in the generation of free radicals. In this study we present an *in vitro* system in which oxidatively modified proteins can be induced and visualized at the cellular level. With this system we test the hypothesis that increasing iron results in oxidative damage. Two human cell lines are used: SK-N-SH (neuroblastoma) and SW-1088 (astrocytoma). The cells are grown to confluence and then exposed to a free radical generating system (1 mM H_2O_2 and 500 uM vanadyl sulfate). Oxidatively modified proteins were demontrated immunohistochemically by modifying the 2,4 dinitrophenylhydrazine (DNPH) method for detection of functional carbonyl groups on proteins. Exposure to the free radical generating system resulted in a visible increase in cell staining of carbonyl groups in the neuroblastoma cell line, but not in the astrocytoma cell line. Exposure to 30 uM $FeCl_3$, for 16 hrs prior to harvesting the cells resulted in a further dramatic increase in the intensity of the immunofluorescence; but again, the increase was specific to the neuroblastoma cells. These results demonstrate that oxidatively modified proteins can be detected in situ and that differences exist among cell types with regards to vulnerability to oxidative damage. Future studies are directed at identifying differences in the two cell types which relate to the variation in vulnerability to oxidative damage.

673.16

IRON-MEDIATED BIOACTIVATION OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP). D.A. Di Monte*, H.M. Schipper[1], P. Chan and J.W. Langston. The Parkinson's Inst., Sunnyvale, CA 94089, and [1]Dept. of Neurology, McGill Univ., Jewish Gen. Hospital, Montreal, Quebec H3T 1E2, Canada.

A process of metabolic activation is thought to be the first step leading to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This conversion forms the toxic 1-methyl-4-phenylpyridinium (MPP^+) metabolite and in the brain is mostly mediated via glial monoamine oxidase (MAO) type B. MAO B inhibition, however, does not completely block MPTP conversion to MPP^+ since MPP^+ is still formed in the presence of MAO inhibitors both *in vitro* and *in vivo*. The purpose of this study was to test the hypothesis that iron may act as a catalyst for the MAO-independent bioactivation of MPTP. Primary cultures of mouse astrocytes were treated with both the MAO A inhibitor clorgyline and the MAO B inhibitor deprenyl prior to the addition of MPTP. Production of MPP^+ was reduced (to approximately 15 %), but not completely blocked by MAO inhibition. This MPP^+ production appeared to be iron-dependent since it was decreased (30 to 50%) by iron chelators, *i.e.* deferoxamine or phenanthroline, and was enhanced (by more than 50%) in the presence of ADP-Fe^{3+}. Data indicate that oxidation via MAO is the primary but not the only pathway of MPTP bioactivation and that transition metals may contribute to the generation of the toxic MPP^+ metabolite in biological systems.

673.17

EFFECT OF MESO-2,3 DIMERCAPTOSUCCINIC ACID (DMSA) ON FORCED-SWIM IMMOBILITY IN LEAD-EXPOSED, PAIR-FED AND CONTROL MICE. P. Stewart, R. Burright and P. Donovick*[1], S.U.N.Y. at Binghamton.

We investigated the effect of DMSA on forced-swim immobility in lead-exposed and control Binghamton Heterogeneous (HET) Stock mice. Forty-one female and 40 male HETs were divided into three groups: 1) lead (seven weeks of 0.5% lead acetate in drinking fluid), 2) pair-fed and 3) water control. One month after the end of lead exposure, half of each group received 50mg/kg DMSA or vehicle for 5 days. Immobility was measured in a variety of situations including: two forced swim tasks and also during the extinction phase of a water maze place-learning task. Lead reduced immobility in the different testing situations relative to both pair-fed and control groups. DMSA treatment attenuated this effect in male mice. Immobility in lead-exposed female mice, however, was unchanged or even further reduced by treatment with DMSA. Blood-lead and possible dopaminergic involvement were also investigated.

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2-This research was supported in part by a grant from HighGate Manor of Cortland & Rehabilitation Services of New York, Inc.

673.18

CHRONIC POST-WEANING LEAD EXPOSURE IMPAIRS PERFORMANCE IN A DELAYED SPATIAL ALTERNATION TASK. B.J. Strupp* and S. Alber. Dept. of Psychology and Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853.

Despite the increasing evidence that low-level lead (Pb) exposure lowers IQ, the specificity of the impairment is unknown. The present study was designed to further elucidate the nature of the impairment, using a delayed spatial alternation task. This type of task is sensitive to dysfunction in both prefrontal cortex and hippocampus, two structures implicated in Pb-induced cognitive dysfunction. Pb acetate (0, 75, & 300 ppm) was administered chronically after weaning, yielding median BPb levels of 0, 19, & 39 μg/dL, respectively. The performance of both Pb-treated groups was significantly inferior to controls but the impairment was constant across the 4 delays, rendering a memory deficit unlikely. Analyses of the types of errors committed, using logistic regression, revealed that the 300 ppm group exhibited a significantly stronger side bias than the two other groups. The outcome of the previous trial (correct or incorrect), although a significant predictor of performance, did not differentially affect the performance of the treatment groups. The present results demonstrate that low-level Pb exposure alters cognition even after the major period of brain development, questioning the current level of concern for occupational exposure, which is 40 μg/dL.

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673.19

CHRONIC LOW-LEVEL LEAD EXPOSURE IMPAIRS SERIAL REVERSAL LEARNING IN THE RAT. J. Hilson, S. Koger*, & B.J. Strupp. Dept. of Psychology and Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, and Dept. of Psychology, Willamette University, Salem, OR 97301.

The present study was designed to further define the nature of the cognitive impairment produced by low-level lead (Pb) exposure and to provide a model system for future studies. A serial reversal task was selected in light of suggestive evidence that functions associated with prefrontal cortex may be particularly vulnerable to developmental low-level Pb exposure. The subjects in the present study had been exposed to Pb chronically since the first day of gestation, with resulting median blood Pb (BPb) levels of <5, 22, and 39 μg/dL in the 3 treatment groups, respectively. Each animal was administered a two-choice olfactory discrimination task, followed by five reversals (intradimensional shifts) and an extradimensional shift. All testing was conducted in automated chambers, with a nose-poke as the critical response. Preliminary analysis reveals a dose-related impairment of reversal learning with increasing BPb. These results add to the growing evidence of cognitive impairment at very low BPb levels, and confirm a previous report of impaired reversal learning in nonhuman primates. These findings suggest an impairment in cognitive flexibility, consistent with prefrontal dysfunction.

Supported by grants from NIEHS (ES05950-03) and the March of Dimes Birth Defects Foundation (12-FY93-0730).

673.20

GENETIC SUSCEPTIBILITY TO LEAD INDUCED BLOOD-BRAIN BARRIER DYSFUNCTION. L. Claudio*, J.G. Wetmur, P.F. Good, J.G. Eisler, P.M. Woolard, H.M. Fillit. Div. of Environmental and Occupational Medicine, Mount Sinai Med. Ctr., New York, NY 10029

Lead intoxication is one of the most widespread environmentally-induced afflictions in the human population, yet many questions remain regarding the mechanisms of lead-induced neurotoxicity. We hypothesize that one mechanism of lead neurotoxicity involves disruption of the blood-brain barrier (BBB). To explore this hypothesis, we have used two mouse strains which differ in the gene coding for δ-aminolevulinate dehydratase (ALAD), an enzyme implicated in susceptibility to lead intoxication in humans. Although the genetic difference we find in these mouse strains appears to be quantitative rather than qualitative, we show that this model parallels observations made in humans. Our findings show that mice with a duplication of the ALAD gene (strain D) accumulate more lead in their blood than mice with a single copy of the gene (strain C) when exposed to the same acute oral doses of lead during adulthood. F1 animals (C x D) show intermediate blood lead levels.

Intracellular localization of lead was assessed by laser microprobe mass analysis (LAMMA) which indicated evidence of lead peaks in perivascular cells. Assessment of BBB function with intravascular tracers and immunocytochemistry for extravasated serum proteins and for basal lamina proteoglycans showed disruption of BBB properties and increased permeability in exposed strain D animals.

The data reported here suggest that lead may cause BBB dysfunction in susceptible animals exposed during adulthood. We hypothesize that a mechanism for these effects of lead may involve a disruption of astrocyte-endothelial cell interactions or of other cellular functions important for maintenance of the BBB.

673.21

LEAD REDUCES CALCIUM ENTRY WITHOUT PASSING THE CELL MEMBRANE OF MAMMALIAN NEURONS: FURA 2 MEASUREMENTS. D. Büsselberg*, R. Domann, L. Wunder, and H. L. Haas. Physiology II and BMFZ, Heinrich Heine-University, POB 101007, D-40001 Düsseldorf, Germany.

Lead is an ubiquitous but neurotoxic element. Recently we have demonstrated that its neurotoxicity is partly due to actions on voltage activated calcium channel currents (Büsselberg et al., 1994, *J. Neurophys.*, **71**). It reduces calcium channel currents with an IC_{50} of ~0.5 µM. Here we prove our prediction that the rise of the intracellular calcium concentration is prevented by lead. Dorsal root ganglion neurons of rat pubs were depolarized by exposure to 50 mM potassium and the rise of internal calcium was measured with Fura 2. Lead reduced this rise in a concentration dependent manner, with a threshold concentration of 0.25 µM and ~5 µM lead reduced the calcium entry by ≥80%. The effect was most likely due to voltage activated channels, since applications of NMDA, an agonist of ligand gated calcium channels did not result in any rise of the internal calcium signal. The effect of lead was partly reversible. While it has been demonstrated that lead itself changes the Fura 2 signal in a typical manner (Tomsig and Suszkiw, 1990, *Am. J. Physiol.* **259**), we should have detected low lead concentrations in the cell. But even with no calcium in the extracellular fluid and lead applications over several minutes at relatively high concentrations (up to 5 µM) we have not seen a change in the fura signal. We conclude that lead prevents calcium entry by reducing the voltage activated calcium channel currents but does not pass through the channel itself.

673.22

EFFECT OF LEAD INTOXICATION ON PYRUVATE DEHYDROGENASE ACTIVITY AND THIAMINE CONTENT IN THE BRAIN OF RAT. K.H.Ko,* J.H.Cheong, H.J.Kim AND J.R.Ryu. Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151, Korea

Thiamine has been reported to reduce the toxic manifestations of lead and lead level in tissues. Conversely, lead intoxication may affect thiamine status and thiamine-related biochemical reactions and physiological responses. In this study, it was tested if lead intoxication could change thiamine content and the activity of pyruvate dehydrogenase in the brain and administration of excessive thiamine can prevent the toxic manifestation of lead.

Five groups of Wistar rats were prepared: 1)control group, 2)lead treated group, 3)lead plus thiamine treated group, 4)thiamine treated group, 5)pyrithiamine treated group. Each group of animals was divided into three subgroups based on ages: 3, 7 and 16 weeks of age subgroups. Lead was administered through drinking water containing 0.2% lead acetate and thiamine through diet containing thiamine tetrahydrofurfuryl disulfide 2mg/kg body weight/day. All animals except pyrithiamine treated ones were received the above treatment from birth. Animals of pyrithiamine treated group received pyrithiamine, 0.5mg/kg/day, via intraperitoneal injection daily. Lead concentrations, thiamine contents and activities of pyruvate dehydrogenase in brain areas; telencephalon, brain stem and cerebellum were measured in each group as well as the seizure threshold to electro-shock. Lead concentrations in all brain regions of lead treated group were significantly higher than those of control group, and those of lead plus thiamine treated group were significantly decreased from those of lead treated group. Thiamine contents in brain regions of lead treated group were significantly lower than those of control group, and those of lead plus thiamine treated group were recovered back to those of control group. Activities of pyruvate dehydrogenase and the seizure threshold to electroshock in lead treated group were significantly lower than those in control group, while those in lead plus thiamine treated group were higher than those in lead treated group.

The results from the present study suggest that neurotoxicity including convulsion following lead intoxication in rats may be mediated at least in part through the changes of thiamine status and thiamine related factors.

673.23

CHRONIC LOW-LEVEL LEAD STIMULATION OF NEURAL CELL SIALYLATION STATE. Fleur D. Hayes & Kieran C. Breen*. Dept. of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital Medical School, Dundee DD1 9SY, Scotland, U.K.

Lead is a neurotoxic agent which acts preferentially on the developing nervous system. Although the molecular mechanisms underlying the actions of lead are poorly understood, it has been suggested that it may act to disrupt the process of neurite elaboration and consequent synapse formation. The neural cell sialylation state is precisely regulated at this stage of development and may thus prove a possible target for lead toxicity.

Hippocampally-derived neuronal cell hybrids were cultured in the presence of low-level lead (10^{-14} to 10^{-8}M) for 72 hours, the cells harvested and the activity of the sialyltransferase enzyme determined. Sialyltransferase activity was stimulated three-fold by very low lead levels in cells derived from both embryonic day 15 and postnatal day 10, the former being more susceptible to lower lead levels. Lectin blot analysis demonstrated an increase in the sialylation state of specific glycoproteins. Although the nerve cell adhesion molecule, NCAM, is a developmentally-regulated sialoglycoprotein, there was no evidence for a lead-induced re-expression of the polysialylated form of the protein.

These results demonstrate that neural cell glycosylation is susceptible to chronic low-level lead exposure, and this may contribute to the upset in synapse formation associated with lead-induced neurotoxicity.

This study was supported by a Wellcome Toxicology Studentship to FDH.

673.24

EFFECTS OF ACUTE ETHANOL *(IN VITRO)* ON SYNAPTIC PLASTICITY IN HIPPOCAMPUS OF ADULT RATS CHRONICALLY EXPOSED TO LEAD *(IN VIVO)*. C. A. Grover* & G.D. Frye, Medical Pharmacology & Toxicology, HSC Texas A&M University, College Station,TX 77843-1114.

Rats chronically exposed to environmentally relevant levels of lead show altered intake and responsiveness to ethanol (Nation et al., *Behav. Neuro. 100:1986; Alcohol 8*: 1990). Lead accumulates in the hippocampus, a CNS area known to be affected by ethanol. Acute ethanol treatment (Sinclair & Lo, *Gen. Pharmacol. 17*: 1986), as well as acute (Altman et al., *Neurosci. Let. 128*:1991) and chronic lead exposure (Lasley et al., *Br. Res. 614*:1993) inhibit long-term potentiation (LTP) in the hippocampus. We examined the effects of acute *in vitro* ethanol treatment on tetanus-induced LTP in the CA1 region of the hippocampus of rats chronically exposed to lead *in vivo*. Adult male Sprague Dawley rats were given 500 ppm lead acetate (Pb) or pair-fed sodium acetate (Na) in the drinking water for 60-70 days prior to *in vitro* electrophysiological experiments. Extracellular recordings of excitatory postsynaptic potentials (EPSPs) were obtained each minute during 15 min bath application of physiological saline, 5 min pre- and 1 min post- tetanus without or with added 60 mM ethanol, an additional 59 min post-tetanus, and again at 90 min post-tetanus. When the same low intensity stimulus was used during tetanus and test pulses, Pb rats showed exaggerated short-term potentiation with LTP comparable to that of Na rats (20-25% > pre-tetanus at 60 & 90 min post-tetanus). Ethanol had no affect on Na slices, but significantly ($p<.05$) blocked LTP in Pb slices (0% > pre-tetanus). When the stimulus intensity during tetanus was increased to produce maximum EPSPs relative to 1/2 maximum EPSPs produced by the test pulse stimulus ethanol had no inhibitory effect on LTP in hippocampal slices from lead exposed rats. The ethanol sensitivity of synaptic plasticity in the hippocampus of chronically exposed lead rats appears to depend on the intensity of the stimulation during tetanus. Supported by ES05639 (CAG) and AA06322 (GDF).

673.25

LEAD INDUCES A BIPHASIC GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) RESPONSE IN RAT BRAIN. Z.L. Gong, A.R. Little, HAN, El-Fawal, H.L. Evans*. Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY 10987.

GFAP was used to test whether repeated exposure to lead (Pb) produces a different pattern of change in astrocytes at early stages of exposure than at later stages; and whether changes at low doses of Pb were different than at higher doses which produce overt toxicity. Male F344 rats (>42 days old) received either lead acetate (Pb: 50 or 1000 ppm) or trimethyl lead (TMPb: 8 or 16 ppm as Pb) in their drinking water for 7 or 14 days. Although TMPb was much more toxic, as shown by decrease in body weight, both Pb and TMPb produced a similar biphasic change in brain GFAP. As predicted, decreases in GFAP occurred at lower levels of exposure (50 ppm Pb) or at early stages of exposure (day 7 of 8 ppm TMPb); increases in GFAP occurred at higher levels of exposure (1000 ppm Pb or 16 ppm TMPb) or at later stages of exposure (day 14 of 8 ppm TMPb). Dose-response was observed in hippocampus of rats exposed to TMPb and in cerebral cortex and cerebellum of rats exposed to Pb. The observed biphasic change in GFAP levels during Pb exposure suggests that astrocytes play a role in the brain's earliest response to neurotoxic metals. We propose a two-stage mechanism in the astrocytic response to Pb. In stage 1 astrocytes sequester and accumulate Pb, which may inhibit GFAP synthesis as suggested by other studies and indicated by GFAP decreases in the present study. In stage 2 astrocytic gliosis occurs in response to neuronal damage as indicated by increased GFAP. Supported by the American Petroleum Institute and Grant ES-04895.

673.26

BICUCULLINE-INDUCED SEIZURE THRESHOLD IN POSTNATALLY LEAD-EXPOSED KITTENS. G.W. Patrick*, J.S. Stewart and A.S. Lloyd. Dept. of Anatomy, Kirksville Coll. of Osteopathic Med. Kirksville, MO 63501.

The purpose of this study was to determine if postnatally lead-treated kittens have a greater susceptibility to bicuculline-induced seizure initiation. Kittens were treated with 20 mg/kg lead acetate solution daily by esophageal intubation for seven days postnatal. Controls were maintained in the same manner but received 20 mg/kg sodium acetate solution. The kittens were allowed to age to one year during which time they were observed for any behavioral abnormalities. No spontaneous seizures were seen in any animals. The kittens were anesthesized with an IM injection of a solution containing 50 mg Ketamine, 5 mg Xylazazine and 1 mg Acepromazine and the femoral vein was cannulated for delivery of drugs. Small holes were drilled into the skull and EEG pin electrodes placed over the frontal and parietal cortex. A tracheotomy was performed, a cuffed pediatric ET tube inserted and the kitten respirated with 95% O_2/5% CO_2. The animals were paralyzed with 150 ug/kg Pavulon. The EEG was recorded during this proceedure to determine baseline neural activity. Bicuculline (1.5 mg/ml) was infused via the femoral cannula by a Harvard infusion pump at a rate of 0.20 mg/min. Seizure initiation as measured by EEG occured with an average of 0.228 mg/kg bicuculline in experimental and 0.369 mg/kg bicuculline in control subjects. The brain of these animals will be processed by the Golgi-Cox method to determine if cerebral cortical neurons are hyperspiny. Hyperspiny neurons in lead-treated kittens have been described previously by our laboratory. It will be of great interest if seizure susceptibility is correlated with the hyperspiny condition. Predilection towards seizure activity may be implicated in neurobehavioral abnormalities seen in lead-exposed humans.

674.1

CYTOCHROME OXIDASE AND ACTIVE GLYCOGEN PHOSPHORYLASE HISTOCHEMICAL CHANGES INDUCED BY INTRASTRIATAL INJECTION OF 6-HYDROXYDOPAMINE OR QUINOLINIC ACID. D. Donaldson, M Levivier, A. Naini*, S. Przedborski. Dept of Neurosurgery, ULB-Hôpital Erasme, Brussels, Belgium; Dept of Neurology, Columbia Univ., New York, NY.

Morphological markers are used to assess the effects of neurotoxins. However, these markers do not reflect the metabolic status of the damaged tissue. We used active glycogen phosphorylase (GP*a*) and cytochrome oxidase (COx) histochemistry to assess metabolic changes following intrastriatal quinolinic acid (QA) or 6-hydroxydopamine (6-OHDA) injection in rats. One week after injection of the neurotoxin, the brain were processed for GP*a* and COx histochemistry as well as for quantitative autoradiography. After 6-OHDA lesion, there were dramatic reductions in striatal binding of [^{3}H]mazindol-labeled dopamine uptake sites while no changes were observed in the binding of [^{3}H]SCH 23390-labeled dopamine D1 receptors or [^{3}H]PK 1119-labeled glial cells. GP*a* and COx staining were not affected by intrastriatal 6-OHDA, except for a weak increase in GP*a* staining along the needle tract and at the injection site. After QA lesion, there was a significant reduction in striatal [^{3}H]SCH 23390 binding and a significant increase in [^{3}H]PK 1119 binding; [^{3}H]mazindol binding was unchanged. Dramatic reduction in COx and increase in GP*a* stainings were observed in the lesioned striatum. Similar but less dramatic changes were observed in the globus pallidus, the subthalamic nucleus and the substantia nigra pars reticulata on the side of the QA intrastriatal injection. Thus, comparison of striatal binding and histochemical findings after 6-OHDA- or QA-induced lesions indicate that both GP*a* and COx reflect the metabolic status of striatal intrinsic elements rather than that of the dopaminergic afferents. The differential striatal changes in these metabolic markers after QA lesion confirm the preferential localization of GP*a* in glia and COx in neurons. Also, QA-induced striatal denervation seems to modify GP*a* and COx staining in its output nuclei.

674.2

6-OHDA NEUROTOXICITY IS PREVENTED BY SELEGILINE AND ITS METABOLITES T. Ahola, A. Haapalinna*, E. Heinonen*, J. Suhonen and A. Hervonen*
Univ. of Tampere, Lab. of Gerontology, Tampere Brain Res. Ctr, FIN-33101, Tampere, Finland, * Orion-Farmos, Department of Neurological Products, Turku, Finland

Selegiline is a selective monoamine B oxidase inhibitor possibly with an antioxidative function. It slows down the progression of Parkinson's disease by rescuing dopaminergic neurons. In animal studies selegiline has significantly increased the life span of rat and protected the dopaminergic neurons from the neurotoxic effect of MPTP, DSP-4 and 6-OHDA. In a previous study we showed that the effect of 6-OHDA on the peripheral sympathetic neurons could be prevented with selegiline. The aim of this study was to investigate which of the metabolites of selegiline (L-amphetamine (L-AMF), L-metamphetamine (L-MAMF) and desmethylselegiline (DMS)) are responsible for the neuroprotection. Two adrenergic histochemical parameters were used: formaldehyde induced fluorescence (FIF) and tyrosine hydroxylase (TH) -immunohistochemistry. In the sympathetic neurons FIF intensity decreased significantly in the saline pretreated 6-OHDA group compared to the control group. In TH staining the saline pretreated 6-OHDA group expressed less positive cells than the control group. In the L-AMF, L-MAMF, DMS or selegiline pretreated groups the number of TH -positive cells was on the same level as in the control group. The submandibular gland (SMG) expressed a dense adrenergic neuronal network, which was significantly reduced after 6-OHDA injection in the saline pretreated group. The pretreatment with L-AMF, DMS or selegiline protected the fibres against the damage, L-MAMF pretreatment showed a less protective effect. All the differences were statistically significant when compared to the saline pretreated 6-OHDA group ($p<0.05$). In conclusion, the pretreatment with selegiline or its main metabolites protects the peripheral adrenergic nerve cells against 6-OHDA induced neurotoxicity. These results indicate that the metabolites of selegiline, at least in part, contribute to the neuroprotection of selegiline against 6-OHDA toxicity.

674.3

MPP$^+$ AS MARKER OF A PHARMACOLOGICAL HETEROGENEITY BETWEEN DOPAMINERGIC AND OTHER MONOAMINERGIC VESICULAR CARRIERS. S. Ruiu, M.P. Piccardi, A. Bocchetta and M. Del Zompo*. Department of Neurosciences "B.B. Brodie", University of Cagliari, I-09124 Cagliari, Italy.

Metabolism of MPTP to MPP$^+$ by the MAO-B and subsequent uptake of this toxic metabolite by dopamine (DA) terminals have been established as critical steps leading to MPTP neurotoxicity. We demonstrated in the mouse DA terminals a vesicular location of a high affinity ^{3}H-MPP$^+$ binding site, which may represent a specific marker of striatal DA storage vesicles. Recently we provided evidence for an uptake of MPP$^+$ in synaptic vesicles from mouse striatum. To define the characteristics of this uptake we compared the vesicular uptake of DA, norepinephrine (NE) and MPP$^+$ in mouse cerebral areas. We found marked differences in the regional distribution of ^{3}H-MPP$^+$, ^{3}H-DA and ^{3}H-NE vesicular uptake in striatum, hippocampus and cerebellum. Kinetic analysis of Lineweaver-Burk plots revealed very similar kinetic values between ^{3}H-DA and ^{3}H-NE in each area tested. We detect an uptake of MPP$^+$ only in the striatum. This study provides an evidence of a heterogeneity of the monoaminergic vesicular carriers.

674.4

ADMINISTRATION OF MPP$^+$ BY *IN VIVO* MICRODIALYSIS IN RAT BRAIN AS A MODEL OF OXIDATIVE STRESS. L. Ste-Marie, L. Vachon* and J.A. Montgomery. Research Centre, Notre-Dame Hospital, Montréal, Québec, Canada.

In the rat striatum, the neurotoxin, 1-methyl-4-phenylpyridinium ion (MPP$^+$), elicits dopamine (DA) release with a concomitant increase in hydroxyl radical ($\cdot$OH) formation. It is not clear if DA release is required for $\cdot$OH production or whether $\cdot$OH is formed directly by redox cycling of MPP$^+$. We have studied $\cdot$OH formation by administering MPP$^+$ either at the level of the anterodorsal striatum or the lateral occipital cortex, the latter as a control for low dopaminergic activity. Microdialysis probes were implanted bilaterally in the striatum and were perfused with salicylate (5 mM; 1 μl/min; 110 min), then with MPP$^+$ unilaterally (5 mM; 20 min), followed by an 80 min recovery period. The probes were then implanted in the cortex. After a 60 min equilibration period with salicylate, MPP$^+$ was perfused (20 min) unilaterally, followed by a 60 min recovery period. DA, dihydroxyphenylacetate (DOPAC) and homovanillate (HVA) were assayed by HPLC-EC, while 2,3- and 2,5-dihydroxybenzoate (DHBZ) (formed by salicylate hydroxylation) were measured either by HPLC-EC or by GC-MS. Basal release (pmol/20 min uncorrected for probe recovery) of DA, DOPAC and HVA was 0.04-0.3, 10-14 and 3-10, respectively. MPP$^+$ produced an increase of DHBZ (2-3 pmol/20 min) and DA (5-10 pmol/20 min), with a concomitant decrease in DOPAC and HVA. MPP$^+$ also induced DHBZ production (1-3 pmol/20 min) in the cortex where DA release was not detectable. While these results are consistent with the hypothesis that $\cdot$OH formation may be induced by MPP$^+$ through the release of DA, the observation that $\cdot$OH is produced in an area displaying no significant DA release suggests that a second mechanism is also involved. The protective potential of MAO-B inhibitors, selegiline and MDL72,974A ([E]-4-fluoro-6-[fluoromethylene]benzene-butanamine), in our model is currently under investigation. (Supported by the Medical Research Council and Heart and Stroke Foundation of Canada.)

674.5

LONG-TERM SURVIVAL OF MPTP-TREATED AGING MICE AND THE ROLE OF GLIA. X.L. Chen* and M. Gupta, Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40292.

Previous studies from this laboratory have shown that MPTP treatment in the young adult mice significantly reduces the number of dopaminergic neurons in substantia nigra (SN). Six months after MPTP treatment, there is a significant recovery of the dopaminergic neurons. The present studies were undertaken to examine plasticity of aging dopaminergic neurons in the SN following toxic insult. Aging male C57BL/6 mice (18 month old) received MPTP injections over a two day period (total dose 60-90 mg/kg i.p.) and were examined 3 days, 2 weeks, 2 months and 6 months after MPTP treatment. Control and MPTP-teated mice were anesthetized and perfused intracardially with 4% paraformaldehyde and 1.5% sucrose in 0.1M PO_4 buffer. Frozen 40 um thick sections were cut through the entire brain. Adjacent sections were stained immunocytochemically tyrosine hydroxylase (TH) and Glial Fibrillary Acidic Protein (GFAP) . The number of TH-positive neurons was quantitated in the SN. The results show a significant reduction of TH-positive neurons in SN at 3 days after MPTP compared to the age-matched controls. In addition, there were a reduced recovery even up to 6 months after MPTP treatment compared to the young adult mice. Since glia convert MPTP into its toxic metabolite MPP+, and there is increased gliosis in the striatum with age as seen with GFAP immunocytochemistry, it is possible that glia play a role in the reduced ability of aging mice to regenerate following toxic insult. Supported by USPHS grant NS 24291 to MG.

674.6

INDUCTION OF C-FOS FOLLOWING MPTP TREATMENT IN THE YOUNG ADULT AND AGING MICE. M. Gupta*, C. Hitchener and X.L. Chen, Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40292.

Previous studies from this laboratory have shown a loss of dopaminergic neurons in the substantia nigra (SN) and decreased levels of dopamine in the striatum following MPTP treatment in the young adult mice. By six months after MPTP, there is a significant recovery in the SN of young adult mice compared to the aging mice, treated in a similar manner. Immediate early genes that are induced by extracellular signals and alter transcription of other genes, are activated in the rat striatum by cocaine and amphetamine treatment. The present studies examined the effects of MPTP treatment on the induction of c-fos in the striatum of young adult and aging mice. Young adult (2-3 month old) and aging (19 month old) male C57BL/6 mice were given a single i.p. injection of MPTP (30 mg/kg body weight) and sacrificed 30 min, 1 hr, 4 hr and 24 hr later. Mice were anesthetized, perfused with fixative and brain sections were immunostained for c-fos. Results show an induction of c-fos in the young adult striatum 30 minuntes after MPTP treatment, paticularly in the dorso-medial striatum, and was maximal at 1 hr after MPTP. At 4 hr, after MPTP, there was a relatively reduced immunostaining for c-fos and by 24hr, it was similar to those in the controls. In the aging mice, induction of c-fos was seen at 30 mt and 1 hr after MPTP and was seen throughout the striatum with a denser distribution in the dorso-medial portion. This increased distribution of c-fos induction in the aging striatum might be due to an increased alteration of protein synthesis in the aging mice treated with MPTP. Supported in part by USPHS Grant NS24291 to MG.

674.7

MPTP INDUCES A PROGRESSIVE HIGH AMPLITUDE SWELLING OF TH-POSITIVE AXONS THAT ARE MYELINATED IN DOG. J.S. Wilson*, B.H. Turner and J.H. Baker. Dept. Anatomy, Coll. Med., Howard Univ., Wash. D.C. 20059

A single systemic injection (3.0 mg/kg; i.v.) of MPTP, a neurotoxin that induces a Parkinsonian-like syndrome, produces a progressive high amplitude swelling of tyrosine hydroxylase (TH) positive neural processes in the proximal third of the nigrostriatal (NS) pathway of dog. This swelling is evident one day after an injection; and by 14 days, many profiles reach a diameter of 30μ or more. In parasagittal sections, the swellings have a club-like appearance with the head pointing rostrally towards the striatum and the handle tapering towards the nigra. The club-like swellings are acidophilic and are immunopositive for the non-phosphorylated neurofilaments. The periphery of the swellings reacts positively for antibodies against myelin basic protein. Examination of the TH-positive swollen profiles with the electron microscope reveal that they are myelinated axons. Thus, unlike rats whose NS-neurons are unmyelinated and survive exposure to MPTP, NS-neurons of the dog are myelinated and are killed by MPTP. We hypothesize that the degree of myelination is positively correlated with a DAminergic neuron's ability to recover from a MPTP insult.

674.8

SPECIFIC MIDBRAIN DOPAMINERGIC NEURONS DEGENERATE FOLLOWING MPTP TREATMENT IN THE FVB MOUSE: RELATIONSHIP TO CALCIUM-BINDING PROTEINS. C.-L. Liang*, E. Nelson, S.G. Speciale, P.K. Sonsalla and D.C. German. Dept. of Psychiat., UT Southwestern Med. Cntr., Dallas, TX 75235, and Dept. of Neurol., Robert Wood Johnson Sch. of Med., Piscataway, NJ 08854.

The present study sought to map and quantify the number and location of midbrain dopaminergic (DA) neurons that are susceptible to degeneration following MPTP treatment, and determine whether the vulnerable cells contain the calcium-binding proteins calbindin-D_{28k} (CALB) or calretinin (CALR). Brain sections from adult male FVB mice were processed for simultaneous two-color fluorescence immunocytochemistry for tyrosine hydroxylase (TH) and CALB, and TH and CALR. Three groups of 3 FVB mice received 4X25 mg/kg MPTP (at 2h intervals) either on Day 1 alone, Days 1 and 3, or Days 1,3,and 5, for cumulative doses of 100, 200, and 300 mg/kg, respectively. Mice were sacrificed 7 days after the last injection. There was over an 80% reduction in striatal dopamine concentrations in all three treatment groups, however, DA cell loss was only observed after the 300 mg/kg dose. There was an average of >40% cell loss that was confined to a mid-rostrocaudal portion of the substantia nigra pars compacta, in a region largely devoid of CALB or CALR in the DA neurons.

674.9

A NONINVASIVE APPROACH TO TEST DOPAMINE OXIDATION BLOCKADE BY MPTP AND MPP^+ NEUROTOXINS IN RATS. S.P. Bagchi*. Nathan Kline Inst.for Psychiatric Res., Orangeburg, NY 10962.

MPTP and MPP^+ may induce Parkinson's disease syndrome and also impair oxidation of dopamine (DA) by mouse striatum. A noninvasive approach based upon urinary DA and DOPAC levels to test oxidation defect induced by MPTP, MPP^+ or other causes may be clinically useful. Neurotoxin was administered i.p. to rats put in metabolic cages for collecting urine over 1 N HCl for subsequent 0-12, 12-24 or 0-24 hr. time periods. Urinary DA and DOPAC were quantitated by published methods employing gas chromatography. A single 5.0 mg/kg dose of MPTP or MPP^+ acutely depressed urinary DOPAC level and DOPAC/DA ratio compared to saline control. Reserpine (RES) (2.5 mg/kg) acutely raised DOPAC level and DOPAC/DA ratio and these effects were reversed by either MPTP, MPP^+, or pargyline (30.0 mg/kg), but not by the MPP^+ congener paraquat (5.0 mg/kg), given 1 hr. before RES. At 13 days after a single MPP^+ dosage, DOPAC level and DOPAC/DA ratio did not differ from the control. RES effects, however, were still blunted at 13 days. Five daily 5.0 mg/kg MPP^+ dosages had powerful effects at 0-24 hrs. following the last dosage but the values reverted to normal by 6 or 13 days; at 27 days, reserpinization revealed impairment of DA oxidation. In conclusion, MPP^+ may cause an acute as well as a long-lasting impairment of peripheral DA oxidation and the latter may become apparent only in reserpinized animals. Also, the RES induced sensitivity is due to an enlargement of the limiting free DA level at MAO site rather than because of the release of stored neurotoxin. Supported by Office of Mental Hygiene, State of New York.

674.10

EVALUATION OF THE EFFECTS OF HEAT STRESS ON THE NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) IN C57/B6N MICE. T.E. Freyaldenhoven*, W. Slikker, Jr. and S.F. Ali. Division of Neurotoxicology, NCTR/FDA, Jefferson, AR 72079 and Dept. of Biochem. & Mol. Biol., UAMS, Little Rock, AR 72205.

MPTP is known to cause neurotoxicity in rodents and nonhuman primates. The present study was designed to determine whether heat stress, with the subsequent induction of stress proteins, 24 hr prior to dosing with MPTP might have an effect on striatal dopamine depletion. Adult male C57/B6N mice were dosed with 4 x 10 mg/kg, ip MPTP at two hr intervals with or without previous heat stress. Animals were sacrificed at 24 hr after the last dose. Striatal dopamine and its metabolites were measured by HPLC/EC and stress proteins by SDS/PAGE. MPTP produced 70% depletion of dopamine in the striatum of non-heat stressed animals, whereas in heat stressed animals striatal dopamine depletion was 83%. Animals subjected to heat stress alone or injected with saline showed no depletion of striatal dopamine. Stress protein induction could not be detected by SDS/PAGE or immunoblotting in either the striatum or the substantia nigra. These results suggest that heat stress has lasting effects which enhance the neurotoxicity of MPTP.

674.11

STRUCTURAL STUDIES OF CONDENSATION PRODUCTS OF BIOGENIC AMINES AS INHIBIOTRS OF TRYPTOPHAN HYDROXYLASE. M. Ota, K. Matsubara[1], W. Maruyama, T. Takahashi, Y. Saito*[2], K. Kimura[1] and M. Naoi, Dept. of Biosci., Nagoya Institute of Technology., Nagoya 466 and Depts. of [1]Legal Med. and [2]Anesthesiol., Shimane Med. Univ., Izumo 693 and Japan.

The effects of condensation products of dopamine and indoleamines on the activity of tryptophan hydroxylase (TPH) were evaluated to determine the structures associated with modulation of this enzyme activity. The compounds having a catechol structure, such as 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6,7-dihydoxy-TIQ), potentially inhibited the activity of the enzyme prepared from the rat brain. The inhibition was non-competitive in terms of both the biopterin cofactor and the substrate L-tryptophan. The substitution on 1 or 2 positions of 6,7-dihydoxy-TIQ did not affect their inhibitory capacities to TPH activities. Among these compounds, a charged substance, 1-methyl-2(N)-methyl-6,7-dihydroxy-isoquinolinium ion, was an extremely potent inhibitor; the Ki values were 0.88 ± 0.17 and 0.64 ± 0.08 μM (mean ± SD) in terms of the substrate and cofactor, respectively. On the contrary, the condensation products of indoleamines scarcely affected TPH activities. 1-Methyl-TIQ, MPTP, and MPP^+ were almost inactive. These results indicated that the catechol structure recognized and combined with TPH at the binding site different from that of the substrate and cofactor, and the positive charged moiety of the dopamine-derived substance enhanced the affinities to TPH. The inhibitory capacities of these compounds would be enhanced by the cyclizing reaction of dopamine. (Supported by a Grant-in-Aid for Scientific Researech on a Priority Area #313 of Japan).

674.12

POTENTIAL BIOACTIVATED NEUROTOXICANTS, N-METHYLATED β-CARBOLINIUM IONS, IN THE LUMBAR CEREBROSPINAL FLUID OF PARKINSON'S PATIENTS. K. Matsubara*, S. Kobayashi[1], A. Akane[2], T. Hatta[3], S. Takahashi, T. Izu and K. Kimura, Depts. of Legal Med., [1]Internal Med. III and [3]Anatomy I, Shimane Med. Univ., Izumo 693 and [2]Dept. of Legal Medicine, Kansai Univ., Moriguchi, Osaka 570, Japan.

Recent studies show that β-carbolinium ions (BC^+s) resemble the synthetic parkinsonian toxicant, MPP^+, with respect to structure and neurotoxic activity (Brain Res. 570, 154, 1992) and are identified in human brain by the GC/MS analysis (Brain Res. 610, 90, 1993). These potential bioactivated neurotoxicants, 2-N-methyl-β-carbolinium (2-MeBC^+s) and 2,9-N,N'-dimethyl-β-carbolinium ions (2,9-Me_2BC^+s), were analyzed in the lumbar cerebrospinal fluid (CSF) of 16 parkinsonian and 16 non-parkinsonian patients using HPLC/fluorescence detection. The levels of BC^+s in the lumbar CSF of parkinsonian patients were significantly higher than those in non-parkinsonian individuals. On the other hand, there was no significant difference in the CSF levels of simple β-carbolines, which are precursores of BC^+s, between parkinonians and non-parkinsonians. These results strongly support the hypothesis that "bioactivated" carbolinium ions, which are 2-MeBC^+s and 2,9-Me_2BC^+s, could be endogenous causative factors in Parkinson's disease. (Supported by a Grant-in-Aid for Scientific Researech on a Priority Area #313 of Japan).

674.13

BEHAVIORAL RECOVERY IN STRIATAL VERSUS SUBSTANTIA NIGRAL 6-HYDROXYDOPAMINE-LESIONED RATS. M.L. Leavitt*, C. Devenyi, K. Klein and J.C. Maroon. Allegheny-Singer Research Inst., Medical College of PA, Pittsburgh, PA 15212.

We are comparing the effects of a single unilateral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the striatum versus the substantia nigra on ipsilateral rotational responses to D-amphetamine injection. Seventeen out of nineteen rats rotated ipsilaterally to D-amphetamine (3.0 mg/kg) when tested at 13-18 days following injection of 25 μg (1.5 μl) of 6-OHDA into the right striatum. Repetition of the 1 hr. rotational test at between 25 and 53 days post-lesion revealed a decreased response in twelve of the fourteen originally positive responders. Ipsilateral rotations were significantly ($P<.005$) reduced to a mean value ($\pm$SEM, 189 $\pm$ 50) which was only 43% of the original response (474 $\pm$ 49). Substantia nigral-lesioned rats (N=6) that tested positive at 10-21 days post-lesion showed a mean response upon retesting at 61-79 days post-lesion (606 $\pm$ 63) which was 160% of their original value (472 $\pm$ 110). These findings demonstrate a limited behavioral recovery in striatal but not substantia nigral 6-OHDA-lesioned rats.

Supported by a BRSG grant to M.L.L.

674.14

AUTORECEPTOR PRESYNAPTIC CONTROL OF DOPAMINE RELEASE FROM STRIATUM IS LOST AT EARLY STAGES OF MANGANESE POISONING. H. Suarez-Roca*, M.C. Cuesta, G. Gomez, Bonilla E. Sec. of Neurochemistry, Instituto de Investigaciones Clínicas, Univ. of Zulia, Apartado 1151, Maracaibo 4001-A, Venezuela.

Manganese (Mn) poisoning in man produces an early psychotic disorder that is later followed by a Parkinson-like syndrome. Alterations in the CNS DA system are thought to be involved. In the present study, we assessed the presynaptic autoreceptor regulation of DA release in the striatum during early and late stages of Mn poisoning. Albino mice were treated i.p. with 5 mgMn/Kg weight/day for 2 and 8 weeks. Controls received equivalent doses of NaCl. Slices preloaded with ^{3}H-DA were superfusated, depolarized with K^+ 25 mM and the radioactive efflux was counted. Apomorphine (APO), a D2 DA receptor agonist, was used to evaluated the presynaptic autoreceptor modulation of DA release from mouse striatum. Mn poisoning, at either 2 or 8 weeks, did not change basal and evoked DA release. In controls, 1 μM APO, produced an inhibition of evoked DA release that was blocked by 1 μM S(-)-Sulpiride, a DA receptor antagonist. APO lost its capacity to inhibit the evoked DA release after 2 weeks of Mn poisoning, although, APO was then able to reduce again evoked DA release after 8 weeks of Mn poisoning. MK-801 (0.3 μM), an NMDA-glutamate receptor antagonist, could restore APO inhibitory control on DA release that had been lost at week 2 of Mn poisoning. These findings suggest a NMDA-glutamate-receptor-mediated loss of autoreceptor presynaptic control of striatal DA release at early Mn poisoning.

674.15

RAPID ATP LOSS CAUSED BY METHAMPHETAMINE IN THE MOUSE STRIATUM: RELATIONSHIP BETWEEN ENERGY IMPAIRMENT AND DOPAMINERGIC NEUROTOXICITY. P. Chan*, D.A. Di Monte, J.J. Luo, L.E. DeLanney, I. Irwin and J. W. Langston. The Parkinson's Institute, Sunnyvale, CA 94089.

There has been considerable interest in the possibility that perturbations of energy metabolism play a role in the nigrostriatal degeneration.*d*-Methamphetamine (METH) has been shown to cause a long-lasting dopaminergic neurotoxicity in the striatum. Although it has been suggested that endogenous dopamine (DA), excitatory amino acids, and oxidative stress are involved in METH toxicity, the relationship between energy impairment and the toxic effects of METH on dopaminergic neurons remains to be explored. In the present study, experiments were conducted to investigate the effects of METH on mouse brain ATP levels and the relationship between METH-induced changes in striatal dopamine and ATP concentrations. C57BL/6 mice were treated with either a single or 4 doses of METH (10 mg/kg, i.p., given at 2 hr intervals) and killed by microwave irradiation of the brain at either 1.5 hr or 1 week after the last injection. ATP levels were measured in the striatum, hippocampus and cerebellar cortex, and striatal DA levels were also determined. Neither striatal ATP nor DA concentrations changed after a single injection of METH, but both were decreased after 4 doses of METH. No changes in ATP were observed in the hippocampus and cerebellar cortex. In a second set of experiments, an intraperitoneal injection of 2-deoxyglucose (2-DG, 1 g/kg), an inhibitor of glucose uptake and utilization, was given 30 min prior to the last 2 injections of the 4 doses of METH. 2-DG significantly potentiated METH-induced striatal ATP loss at 1.5 hr and DA depletions at 1.5 hr and 7 days. These results indicate that a toxic regimen of METH results in a selective energy impairment in striatum, and raise the possibility that energy impairment plays an important role in METH-induced dopaminergic neurotoxicity.

NEUROTOXICITY: OXIDATION

675.1

Excitotoxic mechanisms in cultured neuronal and glial cells overexpressing Cu/Zn-superoxide dismutase (Cu/ZnSOD). O. Bar-Peled*and Y. Groner. Molecular Genetic and Virology Dept., The Weizmann Institute of Science, Rehovot, Israel 76100.

To study the involvement of CuZnSOD overexpression in excitotoxic cell death, susceptibility of cortical and spinal-cord neurons of transgenic-Cu/ZnSOD (TgHS) mice to Kainic acid (KA) was investigated. Glial-free neuronal cultures grown in medium without serum, were exposed to KA for 16-20h and surviving cells monitored by counting the number of Neuron Specific Enolase positive cells. As previously recorded in mixed neuronal and glial cultures, 50 and 100μM KA led to 1.5-2 fold increase in cell death when glial-free cultures from TgHS were compared to control mice. The role of glial cells in this differential vulnerability was studied. Astroglial cultures were prepared and uptake of ^{3}H-glutamate was measured. 50% increase in glutamate uptake per cell was found in control cultures as compared to cultures from TgHS mice. Glutamate uptake is an energy dependent process driven through a sodium gradient maintained by Na^+/K^+ ATPase. To examine whether lower activity of the Na^+ pump in TgHS astrocytes was involved in the reduction of glutamate uptake, ouabain, an inhibitor of Na^+ pump was added. At 10^4M it caused a reduction of glutamate uptake in both TgHS and control astrocytes, however, the inhibition in TgHS cells was 40% larger. When subjected to KA, TgHS spinal-cord neurons were more susceptible than control neurons. Upon incubation with Ouabain (10^{-6}M) for 20h, 60% more spinal neurons died in the TgHS cultures. Electron microscopy analysis of KA-treated neurons revealed condensed chromatin and abundant vacuolization in the cytoplasm, indicating that KA-induced cell death is mediated by apoptosis.

675.2

COMBINATIONS OF OXYGEN DEFICIENCY AND DEXAMETHASONE TREATMENTS: EFFECT ON BEHAVIOR AND BRAIN DEVELOPMENT. E-M. E. Montgomery * and C. R. Almli. Lab of Developmental Neuropsychobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Oxygen deficiency and chronic dexamethasone treatment are known to cause damage to the central nervous system. Cortical regions, such as the hippocampus, are vulnerable to these insults. Several groups were formed based on treatment and timing. Rats received oxygen deficiency treatment on the day of birth (P0), at P7, or at both P0 and P7. Dexamethasone treatment was received by rats with and without oxygen deficiency treatment(s). There were 7 treatment groups including the controls. Oxygen deficiency consisted of 3 treatments each separated by 30 minutes for both the P0 and P7 ages. At P0, oxygen deficiency criterion was reached when the rat demonstrated either 2-15sec or 1-45sec intergasp intervals while in the chamber. At P7, the criteria was 2-10sec or 1-30sec intergasp interval. Dexamethasone treatment was given for 7 consecutive days at .5mg/kg body weight. Openfield activity measures and performance on the holeboard memory task were assessed. Preliminary results indicate that P7 oxygen deprivation caused the greatest behavioral disruption. Rats of groups that received a P7 oxygen deficiency treatment showed a decrease in activity, and were less efficient in completing the memory task. Histological analysis are being conducted. (Conducted under NIH Guide for care and Use of Laboratory Animals).

675.3

RADIO-RESTORATIVE EFFECTS OF RILUZOLE IN THE YOUNG RAT.
J. Pratt[1], F. Alaoui[2], S. Trocherie[2], A. Uzan*[1], L. Count[2] and J-M. Stutzmann[1].
1) Biology, Rhône-Poulenc Rorer S.A., Vitry sur Seine, FRANCE
2)Laboratoire de Neurobiologie de la Division de Radiologie du Service de Santé des Armées, Fontenay aux Roses, FRANCE.

Exposure of the mammalian brain to ionizing radiation, whether as part of a cancer therapy or due to industrial nuclear accidents, causes severe cerebral damage. This study examines the effect of the antiglutamate neuroprotector, riluzole, on the acute structural and behavioural damage inflicted by a dose of 2.5 Gray to the brains of 15 day old rats. Rats were irradiated from a Cobalt 60 source. They received riluzole at up to 8 mg/kg i.p., 20 minutes after the start of the irradiation. Six hours later animals were perfused and brains removed for histological examination. The hippocampus was cut in 1 µM sections and stained with toluidine blue. The proportion of pycnotic cells per 1000 cells in the granular and subgranular layers of the dentate gyrus was counted in three non-serial slides. In other animals behaviour was examined in an open field.

Irradiation at 2.5 Gray caused pycnosis in neurones in the dentate gyrus. Riluzole reduced the number of picnotic cells in irradiated rats, with a dose-dependent and statistically significant protection seen between 1 and 4 mg/kg i.p.(at 1mg/kg 18.0±0.7% of neurones pycnotic compared to vehicle-treated controls with 24.6±0.8% cell death; n=12/group, $p<0.05$). In the open field irradiated animals showed signs of lethargy, unco-ordinated movements and the number of squares crossed in the field fell. Riluzole significantly increased motility to levels of non-irradiated siblings from doses of 2 mg/kg i.p.(squares crossed per 3 minutes: 4.6±0.8, 13.3±2 and 19.7±3.1 for irradiated, irradiated plus riluzole at 2 mg/kg and sham-treated rats). Riluzole may have potential as a neuroprotective agent for use after accidental or deliberate exposure to ionizing irradiation.

675.4

EFFECTS OF IONIZING RADIATION ON ·OH RADICAL GENERATION IN RAT STRIATUM. H.T. Chen, W.R. Prichard, S.B. Kandasamy* and A.H. Harris. Behavioral Sciences Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20889.

Using microdialysis techniques, we applied salicylate hydroxylation as an in vivo trapping procedure to monitor the time course of 2,3- and 2,5-dihydroxybenzoic acid (DHBA) generation in the striatum following whole-body irradiation. Male Sprague-Dawley rats were exposed bilaterally to 10 Gy of ^{60}Co radiation at a dose rate of 10 Gy/min. Microdialysis was performed in anesthetized rats either immediately or on day 1, 2, or 3 after irradiation. The probe was inserted into the right striatum and perfused with artificial cerebrospinal fluid (aCSF) at a flow rate of 1 µl/min. Sample collection every 15 min was started 1 h after probe insertion. After the initial 2 samples were collected, in vivo trapping of ·OH radicals in the striatum was initiated by perfusing 1 mM Na salicylate in aCSF through the microdialysis probe. Formation of 2,3- and 2,5-DHBA was detected by HPLC-EC. The mean concentrations of DHBA in the initial 2 samples were taken as baseline, and their concentrations after salicylate perfusion were expressed as percentage of baseline. The respective baselines for 2,3- and 2,5-DHBA in sham controls were 123.4 ± 17.6 and 250.5 ± 31.7 fmoles/20 µl (n=16). There was no significant difference between the baselines in irradiated rats and sham-irradiated controls. The concentrations of DHBA increased significantly ($p<0.05$) following salicylate perfusion, and reached plateau by 45 min. A significant difference in the striatal 2,5-DHBA levels was observed between irradiated rats and sham controls on day 3 after irradiation. This study demonstrates that there are increased levels of ·OH radicals found in the rat striatum 3 days after exposure to ionizing radiation.

675.5

NEUROTOXICITY ASSOCIATED WITH OXYGEN RADICAL GENERATION IS EXACERBATED BY GLUCOCORTICOIDS. L.J. McIntosh* and R.M. Sapolsky. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305

Glucocorticoids (GCs) are secreted by the adrenals in response to stress, and are also used clinically to control inflammation and autoimmune disorders in millions of people annually. Thus elevated GC levels are not uncommon in populations at risk for neurological injury and it is known that GCs exacerbate various forms of neuronal injury. GCs act on cellular pathways relevant to oxygen radical formation, but it is unclear whether GCs have an effect on oxygen radical toxicity. To test the hypothesis that glucocorticoids may decrease the ability of neurons to protect themselves against reactive oxygen species, we examined the effect of the oxygen radical generator adriamycin in rat primary neuronal cultures. Susceptibility to adriamycin was determined by cell counting after MAP-2 staining, and correlated with biochemical measurements. After establishing a dose-response curve for adriamycin, we determined that the lipophilic antioxidants Trolox and butylated hydroxytoluene were effective in decreasing the adriamycin toxicity, indicating that adriamycin was in fact producing toxicity through reactive oxygen pathways. Increasing GC concentration in the culture media (up to 10^{-6} M) exacerbated the toxicity in the center of the adriamycin dose-response curve. Hippocampal and cortical cultures (brain regions with high or moderate concentrations of corticosteroid receptors, respectively) were compared. Our results indicate that GCs may be directly involved in the pathways that mediate oxygen radical formation, and further study of the connections between oxidative stress and glucocorticoids could indicate useful points at which antioxidant intervention will decrease neuronal death after a neurological insult.

675.6

ASSESSMENT OF OXIDATIVE STRESS IN NEURONS AND GLIA. O. Ben-Yoseph*, P.A. Boxer[1], N. Dujovny[1] and B.D. Ross. Dept. Radiology and Biological Chemistry, University of Michigan Ann Arbor, MI 48109, and [1]Dept. Neuroscience, Parke-Davis Research, Division of Warner-Lambert Co, Ann Arbor, MI 48105.

In view of the importance of oxidative stress in a range of neuropathological conditions, we have characterized the anti-oxidant capacity of primary cerebrocortical cultures. Since the anti-oxidant glutathione pathway is coupled to the pentose phosphate pathway (PPP), monitoring the activation of the PPP could provide an index for cellular oxidative stress. We developed a novel technique to monitor the percentage of glucose metabolized through the PPP relative to glycolysis (Ben-Yoseph *et. al.*, J. Neurochem, 61, 230). Exposure of primary neuronal cultures to H_2O_2 for 30 min stimulated PPP activity in a concentration-dependent fashion from basal levels of 1.3±0.3% to a maximum of 20±1.9% with 100 µM H_2O_2. In primary astrocytic culture, PPP activity increased from basal levels of 5.9±1.2% to 66.7±0.8% with 300 µM H_2O_2. This striking difference in the capacity of these cell types to stimulate the PPP suggests selective neuronal vulnerability to oxidative stress. Indeed, estimated by LDH release, glial cultures exhibited a higher resistance to H_2O_2 (EC_{50}=597 µM) compared to neurons (EC_{50}=102 µM). In the presence of 2 mM deferoxamine (DFX), an iron chelator, maximal PPP activity was increased to 33.4±1.9% while H_2O_2 toxicity decreased (EC_{50}=458 µM). DFX therefore probably reduces H_2O_2 toxicity because it inhibits ·OH formation by the Fenton reaction, thereby maintaining H_2O_2 as a substrate for glutathione peroxidase (GPx). Under these conditions, only when the PPP became saturated did toxicity occur, indicating that ·OH formation from H_2O_2 is more rapid than the ability of GPx to remove H_2O_2. Aminotriazole (AT, 30 mM), a catalase inhibitor, stimulated PPP above 100 µM H_2O_2 to an extent higher than that observed in the absence of AT, suggesting that GPx is the main anti-oxidant enzyme at low [H_2O_2]. We therefore conclude that monitoring PPP activity can provide a useful means to assess the cellular capacity to alleviate oxidative stress.

675.7

PEROXYNITRITE TOXICITY IN CEREBELLAR GRANULE CELLS: REVERSAL BY TIRILAZAD MESYLATE (U-74006F). G.J. Fici, J.S. Althaus, J.R. Zhang, E.D. Hall and P.F. VonVoigtlander*. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

During ischemia, peroxynitrite may be a toxic intermediate which forms *in vivo* when nitric oxide condenses with superoxide. Alone, peroxynitrite appears to directly react with aromatic and sulfhydryl nucleophiles. At physiological pH, peroxynitrite rapidly (within seconds) decomposes to species with ·OH and NO_2^+ character. These reactive species are shown to initiate lipid peroxidation, hydroxylate aromatic residues, and nitrate aromatic residues. This reactivity may contribute to differential toxicity *in vivo* and *in vitro*. Tirilazad mesylate (TM) is a lipid-soluble antioxidant shown to inhibit iron-dependent lipid peroxidation. It is an effective therapy in a variety of neurotraumatic models of CNS injury, and is currently undergoing human clinical evaluation in stroke and head and spinal injury. This study was designed to investigate the cytoprotective properties of TM in a cerebellar granule cell model of toxicity involving treatment with peroxynitrite. Cytoprotective performance of TM was based on viability measurements ([^{3}H]-aminoisobutyrate uptake), blockade of lipid hydroperoxide generation, and blockade of nitrotyrosine formation. Results show that viability measurements associated with peroxynitrite toxicity increased with TM treatment. Interestingly, TM was found to be an effective cytoprotectant (EC_{50} = 100 µM) even as a post-treatment following exposure of cells to peroxynitrite. Similar post-treatment with superoxide dismutase (50 units/ml) or allopurinol (100 µM) failed to produce any cytoprotection. These and other parameters of peroxynitrite-dependent cytotoxicity will be described with respect to TM cytoprotection.

675.8

Effect of regulation of Cu,Zn-Superoxide dismutase (SOD-1) on in vivo toxicity of kainic acid. Paul J. Schwartz* and Joseph T. Coyle. Neuroscience Dept., The Johns Hopkins Medical School, Baltimore MD; Psychiatry Dept., Harvard Medical School, Boston MA.

Over the past 10 years, the importance of copper, zinc superoxide dismutase (SOD-1) in disease states has become recognized. For many years, SOD-1 has been implicated in the etiology of Down's Syndrome (human trisomy 21). In addition, a defect in the SOD-1 gene which both decreases activity and possibly increases the nitrosylation of tyrosine residues is responsible for a familial form of amyotrophic lateral sclerosis (ALS). There is evidence that failure of antioxidant defense mechanisms is in part responsible for neuronal vulnerability to excitatory amino acids (EAAs). Since SOD-1 is one of the most important of the antioxidant enzymes, we have investigated its role in EAA neurotoxicity. We have used molecular and pharmacologic techniques to modify the *in vivo* expression of SOD-1 activity. Mice transgenic for and overexpressing SOD-1 exhibit less damage to a 0.3 µg unilateral intrastriatal injection of kainic acid than their control counterparts (64% vs 53% of contralateral choline acetyl transferase activity, $p<0.05$; and 2.27 vs 4.72 mm^3 lesion volume, $p<0.05$). The toxicity decrease resembles the protection elicited by administration of the antioxidant idebenone (a ubiquinone-10 analog). We have also used intrastriatal injection of both antisense oligonucleotides directed against SOD-1 mRNA and SOD-1 inhibitor diethyldithiocarbamate to decrease SOD-1 activity previous to EAA injection. The inverse relationship between SOD-1 activity and neuronal degeneration, coupled with data suggesting that apoptosis is retarded by antioxidant enzymes, implicates failure of antioxidant defense systems as a generalized mechanism for neuropathologic phenomena.

675.9

INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE (NOS) PROTECTS AGAINST NEUROTOXICITY PRODUCED BY 3-NITROPROPIONIC ACID, MALONATE, AND MPTP. J.B. Schulz*, R.T. Matthews, D.R. Henshaw, M.F. Beal. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Nitric oxide (NO) is synthesized from the guanidino nitrogen of L-arginine and molecular oxygen by nitric oxide synthase (NOS). At least three isoforms of NOS have been identified: constitutive (i) neuronal and (ii) endothelial isoforms, and (iii) an inducible isoform originally isolated from macrophages. 7-Nitroindazole (7-NI) was recently found to be a potent and selective inhibitor of rat cerebellar NOS activity after intraperitoneal administration (Br. J. Pharmacol., 110: 225-228). We examined whether treatment with 7-NI is protective against neuronal injury produced by mitochondrial toxins, which are known to cause secondary excitotoxic cell death. Lesions produced by striatal injections of malonate, a reversible inhibitor of succinate dehydrogenase, were dose-dependently attenuated by 7-NI treatment in rats. 3-Nitropropionic acid is an irreversible inhibitor of succinate dehydrogenase which causes selective striatal lesions closely resembling Huntington's disease. 7-NI treatment significantly delayed the occurence of 3-nitropropionic acid lesions in rats. MPTP induced depletion of dopamine and its metabolites was significantly blocked by 7-NI treatment in mice. Our data indicate that NO plays a role in toxicity of neurotoxins which impair oxidative phosphorylation. Inhibitors of neuronal NOS may be therapeutically useful in neurological diseases hypothesized to be related to an impairment of energy metabolism, such as Parkinson's disease and Huntington's disease.

NEUROTOXICITY: MISCELLANEOUS

676.1

EFFECTS OF COMBINED GESTATIONAL AND LACTATIONAL EXPOSURE TO COPLANAR PCBs OR TCDD ON SPATIAL LEARNING AND MEMORY IN RATS. B.W. Seo, J. Moshtaghian, R.W. Moore, R.E. Peterson, & S.L. Schantz*, Institute for Environmental Studies and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801 and School of Pharmacy, Univ. of Wisconsin, Madison, WI 53706

In a previous study, monkeys showed facilitated spatial learning following combined gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Here, we tested spatial learning and memory of rats following gestational and lactational exposure to coplanar polychlorinated biphenyls (PCBs) or TCDD. Time-mated Sprague-Dawley rat dams were gavaged with 2 or 8 mg/kg PCB 77 (3,3',4,4'-tetrachlorobiphenyl), 0.25 or 1.00 μg/kg PCB 126 (3,3',4,4',5-hexachlorobiphenyl), 0.025 or 0.1 μg/kg TCDD, or corn oil vehicle on days 10-16 of gestation. Litters were culled to eight on day two and weaned on day 21. Beginning on day 80, one male and one female from each litter were tested on an eight-arm radial maze with all arms baited. The animals were tested for 20 sessions, and the data were averaged into blocks of five sessions. All groups had similar numbers of errors (reentries into arms already visited) in the first block of sessions, but the TCDD-exposed males subsequently displayed a pronounced dose dependent decrease in errors relative to controls. The decrease in number of errors was statistically significant at both TCDD doses in the second block and at the higher dose in the third and fourth blocks. The pattern of effects was similar, but less pronounced in the PCB 77- and PCB 126-exposed males. The PCB- and TCDD-exposed females did not show a similar facilitation of spatial learning. Supported by Health & Welfare Canada #4028 to SLS and NIH #ES01332 to REP.

676.2

COMPLEMENT-MEDIATED NEUROTOXICITY IS REGULATED BY HOMOLOGOUS RESTRICTION. Y. Shen, J. Halperin+, M. Brandy, D. Casuto, T. Sullivan, M. Russell and C.-M. Lee*, Neuroscience Research, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064, +Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115

The ability of β-amyloid peptides to activate the classical complement cascade and the presence of various complement proteins including the membrane attack complex (C5b-9) on dystrophic neurites in Alzheimer's disease brains raises the possibility that this neurodegeneration may be complement-mediated. To address this issue, we have studied the effect of complement activation on nerve growth factor (NGF)-differentiated rat pheochromocytoma PC12 cells and retinoic acid (RA)-differentiated human neuroblastoma SH-SY5Y cells. Incubation with complement sufficient human serum (5%) resulted in an activation of complement pathway as monitored by iC3b formation in both differentiated PC12 and SH-SY5Y cells. This treatment can also cause the death of PC12 but not SH-SY5Y cells as shown by a significant increase in the release of lactate dehydrogenase. The killing of PC12 cells by human serum is dose-dependent (3-20%) and time-dependent (1-3 days). Heat-inactivated complement, however, was not neurotoxic. The presence of CD59 (a glycosylphosphatidylinositol-anchored homologous complement cascade inhibitor) was shown in SH-SY5Y cells by both PCR amplification and immunocytochemistry. CD59 could potentially inhibit the action of the membrane attack complex and account for the inability of human complement to lyse the human cells. Indeed, removing glycosylphosphatidylinositol (GPI)-anchored complement inhibitor with a phosphatidylinositol (PI)-specific phospholipase C (PI-PLC) rendered SH-SY5Y cells vulnerable to complement attack and eventually led to cell lysis. Reconstituted C5b-9 was also toxic to PC12 cells and PI-PLC-pretreated SH-SY5Y cells. These observations suggest that complement activation can cause neuronal cell death and this process is regulated by homologous restriction.

676.3

Microtubule-Associated Protein 2 (MAP-2) and Glial Fibrillary Acidic Protein (GFAP) Colocalize in Reactive Astrocytes. M.G. Filbert, H.F. Shafer, B.E. Hackley* and G.P.H. Ballough. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

For more than a decade, MAP-2 immunostaining has been the quintessential marker for neuron identification. Found almost exclusively in the somatodendritic compartment of adult neurons, MAP-2 plays a key role in neuronal growth, differentiation and plasticity. Recent studies by our laboratory demonstrated loss of MAP-2 immunoreactivity that closely paralleled dendritic collapse and neuronal loss resulting from seizure-induced brain damage. However, at the same time, we observed increased MAP-2 immunoreactivity in the penumbral regions bordering necrotic lesions, i.e., regions exhibiting pronounced reactive astrocytosis. The present study was undertaken to examine the possibility that reactive astrocytes express MAP-2. Brain sections, from rats exposed to seizure-inducing doses of soman were double-immunostained with antibodies directed against GFAP and MAP-2; these antigens were visualized by immunofluorescence using FITC and TRITC secondary antisera. Our results demonstrate that MAP-2 and GFAP are colocalized in reactive astrocytes bordering seizure-induced brain damage. Therefore, we conclude that penumbral elevations in MAP-2 immunoreactivity result from expression by reactive astrocytes. Furthermore, these results suggest that caution should be exercised when interpreting MAP-2 immunopositive staining.

676.4

EFFECTS OF REVERSIBLE AND IRREVERSIBLE ANTICHOLINESTERASES ON THE MOLECULAR FORMS OF ACETYLCHOLINESTERASE. M. C. Linternl, M.E. Smith[1] and C.B. Ferry[2]. Spon. by Brain Research Association. [1]Dept. Physiology, The Medical School, University of Birmingham, Birmingham, B15 2TT, UK. [2]School of Pharmaceutical Sciences, University of Aston, Birmingham, UK.

Acetylcholinesterase (AChE) occurs as different molecular forms. The asymmetric (A12) form localised at the neuromuscular junction is probably involved in the hydrolysis of acetylcholine during neuromuscular transmission. In this study the effect of a single dose of a reversible AChE inhibitor, pyridostigmine (PYR) or an irreversible inhibitor echothiophate (ECO) on the activity of the molecular forms in mouse diaphragm was investigated. Adult male BKW mice were given PYR (100μg/kg) or ECO (500nmole/kg) subcutaneously. PYR treated mice were killed by cervical dislocation 3, 6 and 24h post treatment and ECO treated mice were killed 6, 24, 48 and 120h post treatment. The diaphragm was removed and homogenised in high salt solubilising buffer. The molecular forms of AChE were separated on a sucrose gradient. AChE activity was determined by a radio assay. Three peaks which corresponded to the globular monomer (G1), the globular tetramer (G4) and the functional A12 form could be distinguished. At 3h after PYR treatment there was a slight reduction in the A12 form and a slight increase in the G1 form. The effects were more pronounced at 6h with the increase in the G1 form especially prominent. At 24h the profile resembled that of the controls with a return of A12 activity towards normal and a decrease in the G1 form. The total AChE activity did not change significantly throughout the period studied. Therefore any decrease in A12 activity was masked by an increase in G1 activity. After 6h, ECO treatment had reduced the activity of all forms and by 24h the effect was maximum. By 48h the activity of each form had returned to normal with the G1 form returning first. At 120h the activity profile resembled that of the control. The results are consistent with the operation of a feedback mechanism where inhibition of the functional A12 form leads to an increase in the precursor G1 form. This could be via upregulation of the AChE gene in the muscle.

676.5

SCIATIC MOTOR NEURON NEUROFILAMENTOUS INCLUSION FORMATION FOLLOWING INTRACISTERNAL ALUMINUM CHLORIDE EXPOSURE IN AXOTOMIZED RABBITS. MJ Strong*, S Gaytan-Garcia. The Department of Clinical Neurological Sciences, The University of Western Ontario, London, Ontario, Canada

We have previously shown that intracisternal inoculums of $AlCl_3$ to New Zealand white rabbits induces a dose-dependant selective suppression of NFL and NFM mRNA levels while NFH mRNA levels remain unchanged at 48 hours postinoculation. To determine whether alterations in NF mRNA steady state levels are integral to the induction of neurofilamentous inclusions, we examined the response of spinal motor neurons to aluminum exposure *in vivo* 48 hours following a unilateral sciatic axotomy, in which a uniform suppression of all NF mRNA levels occurs. Forty eight hours following either a proximal or distal (mid thigh) axotomy, rabbits received an intracisternal inoculation of either 1000 μg $AlCl_3$ in 100 μl 0.9% NaCl, or 0.9% NaCl alone. At either 48 or 96 hours later, rabbits were killed and the extent of neurofilamentous inclusions quantified. Following proximal axotomy, rabbits developed a complete hindlimb paralysis, while distal axotomy yielded foot drop only. No difference was observed in the number of lumbar spinal motor neurons with inclusions following a distal axotomy at either time interval. Following a proximal axotomy, chromatolytic changes were observed. At 48 hours post $AlCl_3$ inoculation, 29% of contralateral neurons demonstrated inclusions whereas 43% of ipsilateral neurons demonstrated inclusions (Fisher's test, two tailed, $p = 0.0196$). At 96 hours post axotomy, 75% and 83% (resp.) of neurons were involved ($p = 0.0212$). Neurofilamentous inclusions did not form in NaCl-inoculated rabbits. These observations indicate that alterations in steady state NF mRNA levels are not critical to the pathogenesis of Al-induced neurofilamentous inclusions.

676.6

EFFECT OF ANTICHOLINESTERASE TREATMENT ON THE EXPRESSION OF PRO-OPIOMELANOCORTIN IN MOTONEURONES. M. L. Amos[1], M. E. Smith[1] and C. B. Ferry[2], Spon. Brain Research Association*. [1]Dept. of Physiology, Medical School, University of Birmingham, Birmingham, UK. [2]Dept of Pharmaceutical Sciences, Aston University, Birmingham, UK.

The pro-opiomelanocortin (POMC) gene is upregulated in motoneurones in conditions where motor neuropathy is present including inherited muscular dystrophy in C57BL/6J mice and following nerve transection. Increased expression of the POMC-derived peptides beta endorphin and alpha melanotropin have been demonstrated in adult motoneurones in diabetes mellitus, where peripheral neuropathy can be a late complication, and after treatment with a neurotoxin which targets motoneurones. In the present study we investigated the effect of administration of a reversible and an irreversible acetylcholinesterase (AChE) inhibitor on the expression of the POMC gene in motoneurones as a marker for early neuronal damage.

A single submyopathic dose (100μg/kg) of the reversible AChE inhibitor pyridostigmine bromide or a single dose (500nm/kg) of echothiophate (an irreversible AChE inhibtor) which caused myopathic damage, were administered subcutaneously to adult mice of the BKW strain. The animals were killed at different times after the injection, by cervical dislocation. The spinal cords were removed and POMC mRNA expression was detected using *in situ* hybridization histochemistry. cDNA oligonucleotide probes encoding for the adrenocorticotropin (ACTH) 4-11 and beta endorphin 1-8 regions of POMC conjugated to alkaline phosphatase were used. POMC expression was not affected by the sign free dose of pyridostigmine. However treatment with a myopathic dose of echothiophate induced a significant but transient increase in POMC mRNA in motoneurones at 3h and 24h post treatment. The level had returned to normal 5 days following the treatment.It is possible therefore that upregulation of the POMC gene can accompany abnormal changes at the neuromuscular junction.

676.7

PERIODIC HIGH-DOSE METHAMPHETAMINE CAUSES DRL 36-S ACQUISITION DEFICITS IN RATS. K.E. Sabol*, J.B. Richards, L.S. Seiden. Dept. Pharm./Phys. Sci., University of Chicago, Chicago, IL 60637

Large dose regimens of methamphetamine (METH) cause long term depletions of dopamine (DA) and serotonin (5HT), as well as acquisition deficits in reaction time in rats (Richards et al., Brain Res., 627, 254-260, 1993). In the present experiment, rats were administered large doses of METH and evaluated in the acquisition of the DRL 36-s task, subsequent to the METH treatment. Rats were administered 2 METH regimens, at 6 wk intervals. Each regimen consisted of 4 injections of 15 mg/kg METH, at 2 hr intervals, or 4 injections of saline. In one group (cooling group) rats which showed signs of overheating during the drug regimen, were placed on ice for 15-30 min. In a 2nd group, the temporary cooling procedure was not used (no-cooling group). Two wks after treatment, DRL 36-s acquisition began. Four wks later, the 2nd METH regimen was administered and DRL 36-s training began again two wks after the 2nd METH regimen. The cooling group showed acquisition deficits after the 1st METH regimen, but not after the 2nd regimen. However, in this group 4 rats died after the 1st regimen and 6 more died after the 2nd regimen (starting with a total of 16 rats). In contrast, the no-cooling group showed acquisition deficits after the 1st and 2nd regimens; lethality in the cooling group was reduced to 3 rats/regimen. Post-mortem tissue assays (13 wks survival time after the 1st regimen) showed that all METH treated rats had significant reductions in DA and 5HT. The rats with the temporary cooling treatment were more depleted in forebrain structures (frontal, somatosensory, and posterior cortex, and hippocampus) than the rats which were not cooled; however, in basal structures such as hypothalamus and ventral midbrain, the cooled rats were less depleted than the rats which were not cooled. The depletion results in the hypothalamus and ventral midbrain may represent a hyper-reinervation in rats which were more depleted in forebrain regions.

In summary, rats which were temporarily cooled during the METH regimens, showed a better survival rate, greater acquisition deficits on the DRL 36-s schedule, and larger DA and 5HT depletions in comparison to rats which were not temporarily cooled. (Supported by NIDA DA-00085 & RSA 10562, L.S. Seiden).

676.8

COMPLEMENT MEDIATED CYTOTOXICITY OF AMYLOID ß PEPTIDE. J.J. Schultz*, J. Schaller, M. McKinley, and J. Rogers. Sun Health Research Institute, Sun City, AZ 85372.

Cytotoxic effects of amyloid ß peptide (Aß) have previously been observed on cultures of embryonic rodent brain. We investigated the potential of Aß to exert its neurotoxic effects via a complement mediated mechanism. The first component of the classical pathway of complement activation, C1q, binds Aß and thereby activates the classical cascade in vitro. The terminal component of the classical pathway, C5b-9, decorates neurites in the vicinity of Aß deposits in situ. This final component is termed the membrane attack complex due to its lytic action on bound cells. We utilized lactic dehydrogenase release and morphologic evaluation to determine the effect of complement availability on Aß induced lysis of rodent embryonic hippocampal cultures. We demonstrated that Aß required an active source of complement in order to exert its neurotoxic effects in the nM and low μM range. We accomplished this by incubating embryonic brain cultures with increasing amounts of Aß together with either 3% normal human serum or 3% C3 depleted serum. C3 depletion halts complement activation before the lytic components are generated, but otherwise provides normal serum components. We observed a large complement mediated enhancement of cytotoxicity to embryonic cultures over a 800 nM to 50 μM range of Aß. The physiological condition of complement availability rendered even nM concentrations of Aß cytotoxic whereas high μM concentrations are required to induce cytotoxicity when Aß alone is present. These results suggest that the neurodegeneration associated with AD could result from complement mediated cellular destruction induced by Aß.

676.9

EUK-8, A SYNTHETIC CATALYTIC SCAVENGER OF REACTIVE OXYGEN SPECIES, PREVENTS ß-AMYLOID PEPTIDE-INDUCED NEURONAL DEATH IN ORGANOTYPIC HIPPOCAMPAL CULTURES. A. J. Bruce*, B. Malfroy†, and M. Baudry. USC Program in Neurosciences, Los Angeles CA 90089-2520, and †Eukarion, Inc., Bedford MA 01730.

The ß-amyloid peptide (ßAP), the core constituent of senile plaques in Alzheimer's Disease (AD), has been shown (both in vivo and in vitro) to be neurotoxic under certain conditions. To test the hypothesis that oxygen free radicals are involved in ßAP-induced neurotoxicity, we evaluated the effects of EUK-8, a synthetic catalytic superoxide and hydrogen peroxide scavenger, on neuronal injury produced by ßAP in organotypic hippocampal slice cultures. Mature hippocampal cultures were exposed to various doses of ßAP (1-42 or 1-40) in the absense or presence of EUK-8. Neuronal injury was assessed by LDH release into the media, semi-quantitative analysis of neuronal propidium iodide (PI) uptake, and loss of Nissl stain. Treatment with ßAP (50-250 µg/ml) resulted in a reproducible pattern of damage, causing a 200-800 % increase in neuronal PI uptake. ßAP-induced PI upake was observed in both CA1 and CA3 pyramidal neurons, and to a lesser extent, in dentate granule cells. The same treatment also caused a 60-300 % increase in LDH release. In Nissl stained sections, ßAP treatment resulted in widespread neuronal swelling, and in many cases, a complete loss of identifiable neurons.

Slices exposed to ßAP in the presence of EUK-8 were significantly protected from ßAP toxicity. EUK-8 at a concentration as low as 5 µM provided significant protection, and slices co-incubated wih 25 µM EUK-8 and ßAP (50-100 µg/ml) were fully protected from ßAP toxicity. These results support a role for oxygen free radicals in ßAP toxicity, and highlight the therapeutic potential of synthetic catalytic scavengers in AD.

676.10

NEUROTOXICITY OF BETA AMYLOID PEPTIDE (Aβ) AND ITS POTENTIATION BY BLOCKAGE OF CD59 COMPLEMENT PROTEIN IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS. T. Sullivan, C.-M. Lee and Y. Shen* Neuroscience Research Department, Abbott Laboratories, Abbott Park, IL 60064

Accumulation of β-amyloid peptide (Aβ) in the brain is one of the major features in Alzheimer's disease (AD) and is considered to contribute to the neuronal degenerative processes. This neurodegeneration may be mediated by the activation of complement cascade which is found in AD brain. The levels of CD59, a glycoprotein linked to the cell membrane via glycosylphosphatidylinositol anchor that inhibits lysis of the cell by the final complex of the complement cascade (C5b-9), are reported to be increased in AD brain. Using retinoic acid-differentiated human neuroblastoma SH-SY5Y cells as a neuronal *in vitro* model as opposed to mixed primary rat cultures, the toxic effects of freshly prepared and aged different fragments of Aβ peptides (1-40) and (1-42) were compared. The Aβ peptides were aged in serum-free media at 37C for 1, 3 and 7 days. Treatment with aged Aβ(1-42) led to SH-SY5Y cell degeneration characterized by a swollen, chromatic or centric cell body with swollen or shrunken neurites. In addition to morphological changes, cell injury by the peptide was further characterized by LDH release measurements. Aged Aβ(1-42) caused dose- (1-30uM) and time-dependent (1-3 days) increases in LDH release from SH-SY5Y cells. Increased toxic potency was found as the aging period of Aβ(1-42) was extended with 7 days being the most toxic. Little or less cell death was induced by Aβ(1-40) or freshly prepared Aβ(1-42) peptides. To determine if a CD59 deficiency can cause cell vulnerability, we examined the effects of aged Aβ(1-40) and freshly prepared Aβ(1-42) on SH-SY5Y cells following pretreatment with a CD59 antibody. Significant cell death was observed compared to treatment with either of these peptides alone.

676.11

INCREASING INTRACELLULAR MAGNESIUM LEVELS CAUSES A RISE IN FREE INTRACELLULAR CALCIUM LEVELS IN XENOPUS OOCYTES. J.M. Nave * and J.D. Connor. Department of Pharmacology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

It has been shown that zinc (Zn2+) can pass through open AMPA receptors and enter neuronal cells (Weiss, J.H. et al, 1993). Although it is known that Zn2+ and magnesium (Mg2+) can interfere with molecules important in signal transduction, the net effects of altering intracellular concentrations of these divalent cations on corresponding intracellular calcium (Ca2+) levels have not yet been studied. Ionic interactions of this type would be of interest because there are many reports indicating a direct correlation between high intracellular Ca2+ levels and neuronal toxicity caused by excitatory amino acid receptor agonists. The Xenopus oocyte expression system and the fluorescent Ca2+ indicator fura-2 were used to address two questions: 1) What effect does increasing the intracellular Mg2+ concentration have on free intracellular Ca2+ levels in Xenopus oocytes? and 2) If increasing the concentration of Mg2+ intracellularly changes resting levels of intracellular Ca2+, is this effect correlated with cell death? Xenopus oocytes previously injected with water or rat brain poly(A^+) mRNA were injected a second time with either fura-2 free acid (200 nM) or a fura-2 and $MgCl_2$ mixture. The final intracellular concentrations of Mg2+ (i.e. once diluted inside the cell) ranged from 1.5 times (1.33×10^{-7} mM $MgCl_2$ injected) to 6 times (1.33×10^{-6} mM $MgCl_2$ injected) physiological levels in oocytes. Increases in intracellular Mg2+ caused a steep rise in free intracellular Ca2+ levels which could not be increased further by the addition of glutamate and glycine. The high intracellular Ca2+ levels were not directly correlated with cytotoxicity. The Mg2+-induced increases in intracellular Ca2+ levels may be part of a mechanism and/or pathway whereby divalent cations participate in the phenomenon of excitotoxicity.

676.12

CALBINDIN GENE TARGETING AND NEURON-SPECIFIC OVEREXPRESSION OF CALBINDIN AND PARVALBUMIN. M. S. Airaksinen*, M. Meyer and H. Thoenen.
Max-Planck-Institute for Psychiatry, Department of Neurochemistry, D-82152 Martinsried, Germany.

The calcium-binding proteins calbindin and parvalbumin are differentially expressed in many neurons of the central nervous system, but their function is unclear. For example, it has been suggested that they may protect against ischemia and excitotoxic damage. In order to study their physiological roles, loss- and gain-of-function transgenic mice are being generated.

First, the calbindin gene has been disrupted by homologous recombination. Several targeted clones of embryonic stem cells have been injected into blastocysts; this should produce highly chimeric mice and good germline transmission. Preliminary analysis of the phenotype of the mutant animals will be presented in the meeting.

Second, several founder mice were obtained after pronucleus injection of a transgene in which neuron specific enolase promoter drives parvalbumin or calbindin cDNA. These should overexpress the proteins in most neurons; the pattern and levels are being determined by immunostaining. They will be used to test whether these proteins can protect neurons against, e.g., ischemia, kainic acid injection or facial nerve lesion.

676.13

IDENTIFICATION OF UBIQUITINATED CALCIUM BINDING PROTEINS IN ADULT RAT FOREBRAIN. S.J.Smith, A.J.Hunter*, A.P. Chapman, R.Mummery, C.C.Rider, P.W Beesley, Royal Holloway , Dept. Biochemistry, University Of London, Egham , TW20 OEX, UK and SmithKline Beecham, Neurology Research, Coldharbour Road, Harlow, Essex, CM19 5AD UK

We have prepared a calcium binding protein fraction from adult rat forebrain using phenyl Sepharose hydrophobic chromatography. Ubiquitin conjugates were identified in EGTA eluted fractions by SDS PAGE (8%) and Western blotting with a specific anti-ubiquitin monoclonal. Bands of 68, 58, 51, 41, 36 and 28 kDa were present in this fraction. Comparison with Coomassie blue stained gels shows that a subset of these proteins was ubiquitinated. Staining with ruthenium red confirmed that this fraction contained calcium binding proteins (Charuk et al. (1990) Analytical Biochem 188 123-131).

This is the first evidence for ubiquitinated calcium binding proteins in normal brain. Calmodulin from plant sources, and vertebrate tissues, (reticulocytes, RBC, skeletal muscle and testis), has previously been shown to be ubiquitinated.

FRIDAY AM SYMPOSIUM

677

SYMPOSIUM. NEURAL CODING OF VISUAL SPACE: VISUAL MECHANISMS AND MULTIMODAL INTEGRATION. G.Rizzolatti, Univ. of Parma, Italy (Chairperson); J.W. Gnadt, SUNY, Stony Brook; C.L. Colby, NEI, NIH; M.T. Wallace, Bowman Gray Sch. of Med., Wake Forest Univ.

The aim of this symposium is to review recent progress in understanding how cortical areas and subcortical centers code space. Jim Gnadt will discuss visuo-motor integration in area LIP of the parietal lobe and describe how LIP neurons express a premotor signal for directing gaze that is encoded in three dimensional space. Carol Colby will compare how space is represented in LIP and in area VIP. She will present evidence that whilst LIP neurons encode spatial location in oculocentric coordinates, some VIP neurons encode stimulus trajectories in head-centered coordinates. Giacomo Rizzolatti will provide an overview on how space is coded in the premotor cortex (area F4). In this area, which controls arm movements plus head movements, most neurons are bimodal (somatosensory and visual) and code space in head-centered coordinates. Mark Wallace will discuss how multisensory integration takes place in the Superior Colliculus and polysensory cortex of the cat. He will show that the maturation of multisensory integration requires a protracted postnatal period during which time a functional relationship between cortex and midbrain is being established. An important point which results from the different presentations is that the space is represented differently in different areas and that the the representation in a given area reflects the motor output by which a stimulus can be acquired.

678.1

COGNITIVE ESTIMATION AND THE FRONTAL LOBES.
R.E. O'Carroll* and R. Taylor of MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Edinburgh, EH10 5HF, Scotland, U.K.

The Cognitive Estimation Test (CET) was devised by Shallice & Evans (Cortex, 1978, 14, 294-303) in an attempt to quantify the tendency observed in some patients with frontal lobe lesions to produce bizarre estimates in response to questions to which people do not usually know exact answers (e.g. "what is the length of an average man's spine?"), despite performing normally on standard intelligence tests. In the present study, the CET performance of 370 patients suffering from head injury, brain tumour, ruptured aneurysm (anterior communicating artery and other), multiple sclerosis, dementia, encephalitis, Korsakoff's syndrome and anxiety/depression were compared with CET scores from 150 healthy controls. Only the patients with Korsakoff syndrome demonstrated significantly impaired CET performance. This impairment of CET performance in Korsakoff's syndrome is interpreted as possible resulting from: (a) a dysfunctional semantic memory system, and (b) lack of cognitive "error checking", perhaps related to the confabulatory behaviour which is characteristic of the disorder. A sub-group of patients with discrete frontal lesions (N = 15) was compared with a group with localised posterior lesions (N = 17). No significant difference in CET performance was observed between anterior and posterior lesioned patients (frontal CET score (mean, s.d.) = 5.9 (4.3), posterior = 6.3 (3.8), t = 0.34, N.S.). The sensitivity of the CET to anterior brain dysfunction is called into question by the present findings.

678.2

HOW DEEP IS THE "DENIAL" OF PARALYSIS (ANOSAGNOSIA) IN PARIETAL LOBE SYNDROME?
V. S. Ramachandran*, UCSD, Brain and Perception Lab, Psychology, 0109, La Jolla, CA 92093-0109

Patients with right parietal lobe disease often deny their paralysis. Is the denial only at a verbal/semantic level so that the patient is subconsciously aware that the arm is paralyzed? We devised three novel tasks: a) A vertical mirror was placed on the table so that he could see the reflection of his right hand superimposed on his paralyzed hand. When moving his right hand, his paralyzed left hand *appeared* to move. Would this startle the patient as measured by raised eyebrows or a GSR? b) Given a choice between performing an easy bimanual task (e.g., making a "bow knot"; cutting circular shape) vs. a difficult unimanual task (e.g., threading a nut on a bolt), would the patient spontaneously prefer the latter? If so, would the preference be reversed by caloric stimulation? c) If the patient watched his failure to perform in a large mirror, would he admit paralysis?

Two patients (LR & BM) with right parietal lesions were studied. Interestingly, on most tests they showed no evidence of "subconscious knowledge" of paralysis, suggesting that the denial was deep. BM, however, showed a consistent preference for the unimanual task but "didn't know why." Intriguingly, with caloric stimulation she acknowledged having been paralyzed for *several days*; the denial apparently had not prevented memory consolidation! Yet 8 hours later, she denied having admitted the paralysis during stimulation. Far from being merely a bizzare manifestation of cerebral disease, these syndromes may be an "encapsulated" form of the kinds of denials, repressions and rationalizations, that characterize our *normal* mental life. Our tests may therefore provide a novel and sensitive probe for studying these enigmatic effects.

678.3

NEURAL BASIS FOR RETROGRADE KNOWLEDGE RETRIEVAL.
D. Tranel* R.D. Jones, J.P. Brandt, H. Damasio, & A.R. Damasio. Dept. of Neurology, University of Iowa, Iowa City, IA 52242.

As a part of a project to elucidate the neural basis of retrograde memory, we studied 30 patients with focal, stable lesions caused by cerebrovascular disease (n=12), herpes simplex encephalitis (n=5), temporal lobectomy (n=9), and anoxia/ischemia (n=4). Hypothesis-driven cognitive experiments were developed to investigate retrograde memory at different levels (e.g., unique/nonunique), in different domains (e.g., public/personal), along different dimensions (e.g., recent/remote), and for different types of material (e.g., verbal/nonverbal). The procedures were standardized in 60 normal controls. The results indicated that: (1) Severe defects for retrieval of unique personal and public knowledge were associated with bilateral anterolateral temporal damage involving the temporal polar region (area 38) and the anterior sector of the inferotemporal (IT) region (areas 20/21); (2) Mild, albeit significant, defects in retrieval of unique personal and public knowledge were associated with unilateral right-sided damage to these same structures; (3) Damage to more posterior temporal regions, including posterior IT, produced circumscribed, modality- and material-specific defects in conceptual (e.g., in prosopagnosia) or lexical (e.g., in anomia) knowledge retrieval, but not the defects described in #1 and #2; and (4) damage confined to mesial temporal structures (amygdala, entorhinal cortex, hippocampus) produced expected material-specific defects in anterograde memory, but did not affect retrieval of knowledge from the retrograde compartment. The findings implicate nonmesial, anterolateral temporal cortices, especially those on the right, in the retrieval of retrograde knowledge.

Supported by NINDS P01 NS19632.

678.4

IMPLICIT BUT NOT EXPLICIT CONJUNCTION INFORMATION IN A BALINT'S PATIENT.
E. Wojciulik*, L. Robertson, and N. Kanwisher University of California, Los Angeles (EW & NK), University of California, Davis (LR)

This experiment examined a patient (RM) with bilateral parietal damage, testing for the presence of implicit information about feature conjunctions under conditions where the subject was at chance in an explicit conjunction task. In a Stroop display with two color-name words, one word was presented in one of four colors (the target), and the other was presented in white (the distractor). The patient's task was to name the color in which the target word was displayed. The display duration was adjusted for each block to a level at which RM showed chance performance on an interleaved explicit conjunction task. The Stroop results showed that RM's response time was significantly slower when the target only was incompatible with the color (1419 ms) than when the distractor only was incompatible (1148 ms), suggesting that correct conjunction information must have been available at some level in the visual system. These findings show that despite the absence of explicit conjunction information and despite his presumed attentional deficit, RM nonetheless has implicit knowledge of color and shape conjunctions.

678.5

PRAXIC AND NONVERBAL COGNITIVE DEFICITS IN A LARGE FAMILY WITH A GENETICALLY TRANSMITTED SPEECH AND LANGUAGE DISORDER
F. Vargha-Khadem[1], K.E. Watkins[1], K. Alcock[2], P. Fletcher[3], R.E. Passingham[2]. [1]Inst. Child Health and Great Ormond Street Hospital for Children, London, [2]Dept. Exp. Psychol., Uni. Oxford, and [3]Dept. Ling. Sci., Uni. Reading, U.K.

(SPON: European Brain and Behaviour Society).

A severe developmental dysphasia has been described in half of the members of a large family of 4 generations. This suggests that the disorder is transmitted by a dominant gene. Gopnik (*Nature*, **344**, 1990) and Pinker (*The Language Instinct*, 1994) have suggested that the affected members suffer from a selective grammatical defect. Our investigations of the same family indicate that the affected members' disorder transcends the expression of grammar to include grossly impaired articulation of speech sounds, the processing and expression of areas of the grammar unrelated to features, and, further a severe extralinguistic orofacial apraxia. In addition, the affected family members have Verbal and Performance IQ scores that are on average 18-19 points below those of the unaffected members. This psychological profile indicates that the disorder does not affect syntactical-semantic features exclusively, or even primarily; rather it affects cognitive, linguistic and orofacial praxic functions generally. It is therefore erroneous to conclude, as Gopnik and now others, such as Pinker, have done, that the affected members suffer from a genetic disorder that affects only grammar, and indeed only certain aspects of grammar, and that this family provides evidence for the existence of "grammar genes".

678.6

CATEGORY-SPECIFIC ANOMIA: IMPLICATIONS FOR THE NEURAL BASES OF OBJECT KNOWLEDGE.
J. Hart & B. Gordon*. Depts. of Neurology and Cognitive Science, and the Zanvyl Krieger Mind/Brain Institute, The Johns Hopkins University, Balto., MD 21287

Considerable evidence supports a hierarchy of processing modules in human cognition, including featural, object, and supraordinate category levels. The assumed hierarchical organization of these levels has implied that category level knowledge does not modulate processing of subordinate levels. We present evidence from three patients with category-specific anomias suggesting that supraordinate information is a strong influence on object naming and featural processing. Patient 1 had a category-specific anomia for animals, fruits and vegetables, and plants; Patient 2, for small household objects and tools; and Patient 3, for animals only. Patient 1 had a co-existing deficit for identifying features of objects within her impaired category. In each case, processing of object names (and feature identification in Patient 1) that were in the affected category was strongly impaired, while processing of names in different categories was relatively unaffected. These data imply that categorical information strongly influences access to other levels of information within human language and its subsystems. The existence of this highly nonlinear interaction is a strong constraint for theories of processing dynamics within cognitive systems. Moreover, bilateral temporal pathology in each of the three cases strongly implicates this region of the brain as the repository of such types of information and their interactions.

678.7

MODEL ANALYSIS OF STROOP INTERFERENCE.
Kiyohiko Nakamura*. Grad. Sch. of Information Systems. Univ. of Electro-Communications, 1-5-1 Chofugaoka, Chofu Tokyo 182, Japan.

A model of cortical circuitry for processing the Stroop word-color tasks is presented. MODEL: The circuitry consists of the Wernicke area, the color-processing pathway, the Broca area, and the premotor area. The former two project to both the latter two. Each area is assumed to be an array of cortical columns, which are composed of populations of pyramidal cells and inhibitory interneurons. Membrane characteristics are given by the Hodgkin-Huxley circuit. Instruction to subjects controls excitability of the areas by norepinephrine release from the brain stem, which reduce potassium conductance. RESULTS: The model demonstrates: (1) The circuitry of each area performs *temporal competition* of the millisecond range: lateral inhibition between the columns restricts firing to the first activated columns by surpressing the others before firing. This enables the whole nervous system to select relevant response in a few hundred milliseconds. (2) The competition delays the columns' response if incongruent stimuli(e.g., word "red" printed in blue ink) activate mutually inhibiting columns of the Broca area to name the ink color. (3) Assume the projection of the Wernicke area to the premotor area is not so strong as the fasciculus arcuatus. Then, in cases where other response is instructed than verbal one, the same stimuli do not delay the response. These results suggest the temporal competition should be the neural mechanism of cortical processing of the millisecond range.

678.8

DIVISION OF INPUTS BETWEEN THE HEMISPHERES REDUCES INTER-WORD INTERFERENCE.
Liederman, J.*, Thome, M., Sohn, Y.S., and Palomo, D., Psychology Dept., Boston University, Boston, MA 02215

We examined whether division of inputs between the hemispheres, as compared to single hemisphere presentation of all inputs, reduces the amount of inter-item interference. On the first display, a centrally-placed target word was presented. In the second display, 4 three-letter words were presented: 2 words to each hemisphere, or all four to a single hemisphere. In 50% of the trials, the target matched one of the 4 words; in 25% of the trials, the target did not match any of the words, and in 25% of the trials, the target word was a re-combination of two separate words in the second display. Half of these conjunction matches blended letters of words which were presented to a single hemisphere, and half were a blend of words presented to opposite hemispheres. Results indicated that performance was most accurate when inputs were divided between the hemispheres, and that this was especially the case when subjects needed to resist mistaking the conjunction for a match. This was not confounded by inter-item distance. We conclude that division of inputs between the hemispheres improves performance at least in part by reducing the number of inter-word intrusions as compared to intrusions among words presented to a single hemisphere.

678.9

NON-PHYSICAL MIND WOULD VIOLATE PHYSICAL LAWS.
David L. Wilson* Dept. of Biology, Univ. of Miami, Coral Gables, FL 33124

Beck and Eccles (PNAS, 1992) propose a mechanism by which voluntary movements occur through non-physical mental activity. Their model proposes that, as a consequence of volition, increased EPSPs occur because of quantum mechanical actions that increase the probability of exocytosis at hundreds of thousands of boutons on pyramidal cells. They claim that such events can occur "...without violating physical conservation laws" even though they view the causative mental events as not being part of the physical universe. I have earlier argued that a non-physical mind cannot produce changes in the physical universe without violating physical laws (Wilson, 1976, 1993). Have Beck and Eccles found a way to avoid such violation? Quantum mechanical effects such as those proposed by Beck and Eccles are random events, such as occur with radioactive decay. In the case of the Beck and Eccles model of mental events interacting with quantum probability amplitudes, the result would be distinctly non-random. Therefore, a set of neurons that fired at times linked directly to volition, but whose firings were not caused by physical events, would violate physical laws.

VISUAL PSYCHOPHYSICS AND BEHAVIOR III

679.1

Perceptual contour completion: a model based on local, anisotropic, fast-adapting interactions between oriented filters
Jochen Braun*, Ernst Niebur, Heinz G. Schuster†, and Christof Koch
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Long contours are perceptually salient despite missing segments and distracting contours in the background, evidently because the visual system is able to integrate information along the length of the contour. In principle, such integration of information can be accomplished by an iterative comparison of neighboring locations (Ullman and Shashua, *MIT AI Memo 1061*, 1988). However, it is unclear how this iterative scheme can be realized by a neuronal network in primary and secondary visual cortex. As a first step towards a neuronal realization, we simulated a network of oriented filters, with anisotropic interactions of a range matching psychophysical evidence (Polat and Sagi, *Vision Res*, 34: 73-78, 1994). In addition, we allowed interaction strengths to adapt in response to a given stimulus, implementing the iterative scheme of Shashua and Ullman.

The model was tested on gray level images composed of discrete 2-D Gabor elements (spatial period λ). Oriented filters were spaced by $\lambda/2$ in visual space and by $\pi/16$ in orientation. The initial strength of the interaction between filters (before adaptation took place) peaked at a center-to-center separation of 3λ and for roughly collinear configurations. Performance of the model was compared to that of simpler, non-adapting networks. The model exhibited contour integration in good qualitative agreement with human observer performance.

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679.2

Effects of Non-Local Interactions on the Perceived Direction of Motion of Fourier and Non-Fourier Line Segments
H.S.Orbach* and H.R.Wilson Univ. of Chicago, Chicago, IL.

A tilted (3.4' wide) bar moving (1, or 2, °/sec) in the vertical direction appears to move in the direction perpendicular to its orientation (63° to the horizontal) when seen through a small (0.375°) circular aperture. Vertical motion is perceived when two flanking apertures are added which show the ends of the bar. For increased aperture separation, the perceived direction of motion shifted back to the direction perpendicular to the orientation of the bar. An analogous experiment, with the center bar a non-Fourier contrast modulation, found similar interactions produced by non-Fourier and Fourier terminators. Changing aperture positions, we found a 2-dimensional interaction region with space constants of 1° and 0.4° (parallel and perpendicular to the bars' orientation). For a large separation of the flanking apertures, increasing the size of the center aperture biased the perceived motion back towards the vertical, indicating a strong effect of interaperture distance. This suggests a non-local interaction, after motion signals are combined by pattern units, that damps out when one unit is inactive but increases when both units are active. Models, based on simple, physiologically plausible, modifications of local models will be discussed.

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679.3

THE EFFECTIVE LUMINANCE OF COMPLEX STIMULI IN MOTION: FIRST-ORDER SIGNALS FROM SECOND-ORDER STIMULI. R. O. Brown*, UCSD, La Jolla, CA 92093-0109.

Models of visual motion distinguish first-order, or "Fourier" stimuli (e.g. a light bar moving on a dark background) from second-order, or "non-Fourier" stimuli (e.g. a B&W striped bar moving on a grey background equal to the mean luminance of the striped bar.). But the nonlinearity of luminance signals in the early visual system suggests that the effective luminance of a striped bar cannot be simply its space-averaged mean luminance.

In experiment 1, the effective luminances of moving striped bars were determined by a new technique, modified from the mimimum-motion method of Anstis & Cavanagh (1983). For 5/5 subjects, the effective luminances of striped bars (at 100%, 50% and 25% contrast) were significantly less than the means of their component luminances, as predicted by nonlinear compression.

In experiment 2, the same subjects made 2-alternative forced choice discriminations of the direction of motion of striped bars on grey backgrounds. All subjects performed well when the background equalled the mean luminance of the striped bars (a second-order stimulus), but motion discrimination was specifically impaired against the [darker] background equal to the effective luminance of the striped bars (a first-order stimulus in physical luminance).

Thus, even at low contrasts, second-order stimuli may generate first-order signals that support motion perception, while first-order stimuli may not. Studies of second-order motion stimuli that were not balanced for effective luminance should be interpreted with caution. *(Supported by PHS EY01711-18.)*

679.4

SIZE ILLUSIONS AFFECT PERCEPTION BUT NOT PREHENSION. M.A. Goodale*, S. Aglioti, and J.F.X. DeSouza. Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2.

There are many examples of visual illusions in which perception of the relative size of objects does not correspond to their real size . One such illusion is the so-called "Titchener circles" in which two target circles of equal size each surrounded by a circular array of either smaller or larger circles are presented side by side. Subjects typically report that the target circle surrounded by the array of smaller circles appears larger than the other. By adjusting the real size of the target circles, they can be made to appear equivalent in size. Although the perception of size is clearly affected by such manipulations, one might expect that the calibration of size-dependent motor outputs, such as grip aperture during prehension, might not be. To test this idea, we used a variation of the Titchener circles illusion in which two thin "poker-chip" disks were used as the target circles. Subjects were given the following instructions: if the disks appear equal in size, pick up the disk on the left; if they appear different in size, pick up the one on the right. Subjects used their right hand and grip aperture was tracked using standard opto-electronic recording (WATSMART). The relative size of the two disks was randomly varied so that on some trials the disks appeared perceptually equivalent but were physically different in size, and on other trials they were physically equivalent but perceptually different. We found that, while subjects' perception of the relative size of the two disks was affected by these manipulations, the scaling of their grip aperture (measured before contact with the disk) was not. Instead, it was entirely determined by the true size of the target disk. These results lend further support to recent suggestions that the visual mechanisms underlying perception and visuomotor control are largely independent. (Supported by MRCC Grant to MAG)

679.5

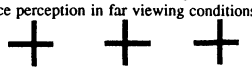
DISCRIMINATION OF VISUAL CATEGORIES IN RHESUS MONKEYS. R. Vogels*. Lab. Neuro- en Psychofysiologie, KUL, B-3000 Leuven, Belgium.

Several models have been formulated regarding the ability of humans to assign visual objects to categories (e.g. to identify an instance of a tree as a tree). Since behavioral methods alone were quite unsuccesful in deciding among these models, examining the representation of visual categories at the single cell level could be informative. As a prelude to this, I determined whether rhesus monkeys are able to categorize visual stimuli using a paradigm suitable for single unit recording. On each trial, a single stimulus was presented during fixation. Stimuli were color pictures of trees (the category to learn) or other objects which were similar to trees, but clearly belonging to another category for human observers. Care was taken that the tree instances, as well as non-trees, varied sufficiently in physical features. The monkey was required to make a rightward or leftward saccade if a tree or a non-tree, respectively, was presented. In order to avoid concurrent learning of the individual pictures, 20 to 40 instances of trees and of non-trees, of which some were new to the animal, were presented in a single session (400-600 trials). After the 3rd training session, the monkey responded correctly to the novel trees and non-trees, indicating categorical discrimination. Subsequent transfer tests in which the presence of simple features (e.g. color, texture, shape) was manipulated, ruled out the possibility that the monkey used a single visual feature as cue. More abstract features, such as leaves or branches, when isolated from others, kept some stimulus control, but was not sufficient to explain the discrimination. Thus, at least a combination of features, each not necessary nor sufficient, was controlling the behavior. These results show that the monkey responded to a class of physically dissimilar objects which form a natural category.
(Supported by G.S.K.E.)

679.6

VISUAL SURFACE PERCEPTION OF UNTEXTURED CROSS STEREOGRAM DETERMINED BY FIXATION POINT IN DEPTH IN NEAR VIEWING CONDITIONS Wu Zhou, W.M. King* **University of Mississippi Medical Center, Jackson, MS, USA 39216**

The cross stereogram shown below (Adapted from Nakayama&Shimojo, Sci., 257: 1357-1363, 1992) has no interior texture. The only source of horizontal disparity information is provided by the vertical limbs. What is the perceived depth of the interior portions of the horizontal limbs where disparity is not explicitly defined? Depth interpolation predicts a linear gradient of depth from the center of the cross to the ends of the horizontal limbs. The observer should see a vertical bar flanked by horizontal "wings" that are slanted toward or away depending on whether the vertical bar is seen behind or in front of the ends of the horizontal limbs respectively. Alternatively, the generic sampling principle (Nakayama and Shimojo,1992) states that the visual system acts as if it were viewing surfaces from a generic rather than an accidental vantage point, and predicts that the observer should see a horizontal bar in front of or behind a vertical bar. In near viewing conditions, we found that either configuration could be seen depending on where in depth the observer fixates. If the observer fixates the same depth as the vertical bar, the seen configuration is a vertical bar flanked by horizontal "wings" that are slanted toward or away the observer; however, if the observer fixates the same depth as the ends of the horizontal limbs of the cross, the seen configuration is a horizontal bar in front or behind a vertical bar. These observations can not be explained by either linear depth interpolation or the generic sampling principle. We suggest it is the fixation-dependent local disparity information that is important in interpreting this type of ambiguous untextured stereogram. The generic sampling principle might be important for surface perception in far viewing conditions, such as driving. (EY04045 to Dr. King)



679.7

MAINTAINING ATTENTION ON AN EXTRAFOVEAL STIMULUS ACTIVATES THE CONTRALATERAL SUPERIOR PARIETAL LOBULE IN A FEATURE DISCRIMINATION TASK: A PET ACTIVATION EXPERIMENT. R. Vandenberghe*[1,2], P. Dupont[1], A. Rosier[1,2], B. De Bruyn[2], L. Mortelmans[1] and G.A. Orban[2]. [1]PET Center, Dept. of Nuclear Medicine, UZ, GHB, [2]Lab. Neuro- en Psychofysiologie, Medical School, KULeuven, GHB, B-3000 Leuven, Belgium.

We studied the contribution of the superior parietal lobule in tonical engagement of peripheral attention and in ignoring a peripheral distractor. The 10 subjects, who gave their informed consent, performed an orientation discrimination task and a stimulus detection task in three different stimulus conditions: the stimulus was either a foveal grating, or an extrafoveal grating placed at 5 degrees to the left of the fixation point, or the foveal grating (the relevant stimulus) presented simultaneously with the extrafoveal grating (the distractor). Subtracting the extrafoveal target detection from the extrafoveal target identification condition revealed activation of the contralateral superior parietal lobule (at 62 mm posterior and 48 mm superior to the AC-PC line; $p<0.001$). This focus was also significant in the subtraction between the extrafoveal identification minus the foveal identification condition ($p<0.01$). In the presence of the extrafoveal distractor, the superior parietal lobule was activated ipsilaterally ($p<0.001$). These data indicate that, at least for feature discrimination, the superior parietal lobule is activated by tonical engagement of attention.
(Supported by HFSP & GSKE)

679.8

VISUAL ATTENTION MODULATES METACONTRAST: A NEW APPROACH TO THE "BINDING PROBLEM. S. Cobb, V. S. Ramachandran, D. Rogers-Ramachandran*. Psychology Department, UCSD, 0109, La Jolla, CA 92093-0109.

How does the visual system bind different fragments in the visual scene to create enduring object representations? When a central target square A is flashed briefly on the CRT (70 ms) and followed immediately two flanking squares for 60 ms in frame 2, the target is erased completely from consciousness ("metacontrast" or "backward masking"). We used this basic display to develop two striking new visual effects. 1) In experiment 1 we added two new squares (B & C) in frame 1 so that B appeared right next to the target (A) and C was off to one side on the corner of the CRT. When B and C were grouped perceptually, masking continued to occur but if B was grouped with A, masking was abolished completely, i.e., "binding" A and B perceptually into a single "object," rendered A immune from masking. 2) In experiment 2, we added two new squares (B & C) to frame 1 on either side of the target (A) to form horizontal row of three. And in frame 2 we added two new squares (one above and one below) to form a vertical column of four squares. When subjects paid attention to the vertical column, the central target square in frame 1 disappeared completely but when they switched attention to the horizontal row, the target reappeared with perfect clarity. We conclude that visual attention "binds" the target with the other squares on the basis of temporal synchrony and spatial proximity and renders it immune from metacontrast.

679.9

FILLING-IN MODEL PREDICTS AREA-SUPPRESSION FOLLOWS U-SHAPED FORWARD MASKING FUNCTION. K. F. Arrington*
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The filling-in theory[1] of surface brightness is tested under forward and backward area-suppression masking conditions. Under these conditions the brightness of a uniform area of a target disk is suppressed by a distant mask. The area-suppression of brightness follows a u-shaped function of stimulus onset asynchrony (SOA) for both forward and backward masking, unlike traditional metacontrast suppression which is usually assumed to be a contour suppression effect and which shows little forward masking[2]. Here the Boundary Contour System / Feature Contour System (BCS/FCS) neural network model[3] of brightness filling-in is evaluated under forward and backward masking stimulus conditions. The model accurately predicts that the area suppression is equal in strength for forward and backward masking and that the area suppression follows a u-shaped function of SOA for forward-masking. This u-shaped function is an emergent property of the filling-in dynamics; it occurs without incorporating delay, as has been done in sustained-transient theories that account for u-shaped functions of SOA in backward masking.

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679.10

IS HEADING BASED ON MOTION FIELDS OR MODELS OF A SCENE? J.Beusmans,* Nissan Cambridge Basic Research, 4 Cambridge Center, Cambridge, MA 02142, USA.

Neurophysiological research on egomotion is presently focused on cortical area MST, which is presumed to receive information about optic flow fields. We believe this focus is misguided on both theoretical and empirical grounds.

Our theoretical reservations derive from the fact that area MST is organized retinotopically, which implies that heading would be perceived in a retinotopic frame of reference. This seems a poor choice because observers move their eyes continually; MST's representation of a fixed direction of observer movement would thus change continually with eye movements.

Our empirical results question the assumption that heading is based directly on optic flow, the presumed MST input. First, we have found that the human perception of heading is more robust in a structured scene than in an unstructured one, even though the optic flow in both was roughly equivalent. The structured scene was a textured ground plane 60m deep; the unstructured scene was a cloud of 150 large dots whose depth varied randomly between 0 and 60m. A 3s image sequence simulated translation through these scenes at 6m/s; during the sequence, the subject fixated on a cross at eye height which required active eye movements. After about 1.5s, heading changed between 1 and 6 deg. Changes of 4 deg or more were always perceived with the ground plane, whereas such large changes generally went unnoticed with the flow generated solely by the cloud of dots. Second, subjects can perceive their heading in a scene consisting solely of spheres rotating about arbitrary axes through their centers.

These results challenge the assumption that heading is based directly on flow fields. As an alternative, we propose that the locomoting observer constructs a scene model and perceives heading based on its perceived motion within the scene. Posterior parietal cortex might be a more appropriate focus than MST for neurophysiological research on heading.

679.11

Short-Term Memory for Visual Motion. Tatiana Pasternak* and Tina M. Newman. Department of Neurobiology & Anatomy and Center for Visual Science. University of Rochester, Rochester, NY 14627

We examined short-term retention of visual motion signals with stimuli that require either local (gratings) or global (dynamic random dots) analysis of motion information. Previous studies have shown that these two types of stimuli may be processed at different levels in cortex.

Two macaque monkeys were tested in a matching-to-sample task in which they were required to match the direction of moving stimuli. On each trial, they viewed a sample stimulus moving in one of eight directions. After a temporal delay, the monkeys were presented with two choice stimuli moving in opposite directions, one of which matched the direction of the sample. The retention of local and global directional information was evaluated by varying the salience of the sample stimulus (e.g. contrast, motion coherence) and measuring motion thresholds over a range of temporal delays (0-4 sec) interposed between the sample and the choice stimuli. Performance declined gradually with increasing delays and the rate of decline was similar for both types of stimuli. This similarity suggests that short-term retention of directional information may depend on a single process even for very different types of motion information. The results also suggest that the representation of motion signals fades over a period of a few seconds.

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679.12

PERCEPTUAL FRAMING THROUGH NEURAL SYNCHRONIZATION A. Grunewald* and S. Grossberg. Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215.

The retinal image changes rapidly during self- or world-motion. Different processing rates of features within a single image mean that parts of subsequent images may get processed together. Such wrong conjunctions are typically experienced only under extreme conditions. The process that minimizes wrong conjunctions is called perceptual framing. A neural network model is proposed that implements this process. The model rapidly synchronizes activities across a neural network, thereby reducing the variability of neural response timing. This synchronization occurs due to cooperative feedback interacting with fast and slow coupled cells (Grossberg & Somers, 1991) and does not require central entraining.

The model accounts quantitatively for data indicating a reduction of variability of response onset latencies as one ascends in the visual hierarchy. Moreover, the model accounts quantitatively for temporal order judgment data indicating that 20ms stimulus onset asynchrony suffices for 75% accurate performance (Hirsh & Sherrick, 1961) and for spatial pooling data suggesting that pooling occurs over distances beyond the size of a single classical receptive field (Essock, 1990). The model predicts that bright stimuli that are presented too briefly to be detected may become visible if their size is increased.

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EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS IV

680.1

LONG TERM CHANGES IN HIPPOCAMPAL EXCITABILITY FOLLOWING OVEREXPRESSION OF GLUR6. A.E. Telfeian1*, M. During1, H.J. Federoff2, G. Mirchandani and A. Williamson1. Section of Neurosurgery, Yale Univ. School of Med., New Haven, CT (1) and Depts. of Neurosci. and Med., Albert Einstein Univ., New York (2).

We have previously shown that overexpression of the GluR6 kainate receptor subtype can be achieved by injecting the hippocampi of rats with an HSV-1 vector which expresses the GluR6 KA receptor (HSVGluR6; During et al, Soc Neurosci Abstr. 1993). These animals have limbic seizures following the injection. Seizures were not produced when HSVlac (expressing ß-galactosidase) was injected. These animals have served as controls.

When studied in hippocampal slices, the initial (12-48 hour) response obtained following the injection of HSVGluR6 was the presence of non-synaptic Na+-mediated spontaneous bursting. No bursting was seen in the CA1 cells of HSVlac-injected animals. The synaptic responses are normal in these animals. (Williamson et al, this meeting).

However, the physiological properties of CA1 cells in HSVGluR6-injected hippocampi changed dramatically over time. By two weeks post-injection, non-synaptic bursts were no longer seen in any of the cells studied. Moreover, at all times longer than 1 week following HSVGluR6 injection, synaptic inhibition was reduced and synaptically-evoked bursts in CA1 could be reliably produced. Both the fast and slow phases of the IPSP appear to have been affected. These changes persist for at least two months after the injection and were not seen in the HSVlac-injected animals at equivalent times. We have observed a similar pattern of pattern of hyperexcitability in CA1 cells epileptic patients. Therefore, overexpression of a single glutamate receptor subtype, may provide a new model for the human disease.

680.2

MULTI-COMPONENT EPSPs EVOKED FROM DENTATE GRANULE CELLS IN PILOCARPINE-TREATED RAT HIPPOCAMPUS. Masako Isokawa.* Brain Research Institute, CHS, UCLA, Los Angeles, CA 90024-1761.

Pilocarpine is a muscarinic acetylcholine receptor agonist and can produce a highly relevant model for human hippocampal epilepsy (Isokawa and Mello, 1991). We previously reported by intracellularly recording from dentate granule cells (DGCs) that, in this model, excitatory responses were increased in the perforant path neurotransmission. The present study was designed to investigate the mechanisms of this excitatory increase using the whole-cell patch clamp recording technique in slices. Male Sprague-Dawley rats (100-150g, N=23) were injected with pilocarpine (300mg/kg, i.p.). Fourteen rats showed spontaneously recurring seizures of 5-107 (average: 23.5 ± 8.4 SE) during the period of 1-3 months. In hippocampal slices prepared from these animals, single perforant path stimulation produced paired population spikes in DGCs. These spikes were often generated in an all-or-non fashion, although a weak current could occasionally separate them evoking only single spikes. When DGCs were patch clamped in a whole-cell configuration with CsMeSO3-containing electrodes, resting membrane potentials measured right after the whole-cell formation were in average -59.3 mV ± 1.39 SE, which was not significantly different from those in control rats (-60.7 ± 1.6, N=5) and in rats that were injected with pilocarpine but did not show spontaneous seizures (-57 ± 2.4, N=11). However, in DGCs of seizure-experienced rat hippocampus, single perforant path stimulation produced a complex, multi-component EPSPs with a significantly longer duration. The EPSPs were comprised of two or more peaks, and the induction of additional peaks after the primary EPSP was stimulus intensity dependent. This suggests that 1) additional peaks in the prolonged EPSPs are less likely to be spontaneously-generated miniature potentials superimposed on the evoked EPSPs, instead 2) perforant path stimulation may generate asynchronously arriving excitatory inputs that converge on DGC dendrites, summing up individually-evoked EPSPs in pilocarpine-treated rat DGCs. Supported by grants from NIH (NS31180 and NS 02808).

680.3

OVEREXPRESSION OF GLUR6 IN RAT HIPPOCAMPI PRODUCES SPONTANEOUS, NON-SYNAPTIC BURSTING IN VITRO. Anne Williamson*1, A. E. Telfeian1, P. Leone1, H.J. Federoff2 and M. During1, Section of Neurosurgery, Yale Univ.School of Med., New Haven, CT (1) and Depts. of Neurosci. and Med. Albert Einstein Univ., New York, NY (2)

Kainic acid injection has been extensively used as a model for temporal lobe epilepsy. We tested the hypothesis that an overexpression of kainate receptors alone would induce seizure activity. Overexpression of GluR6, a KA receptor subtype, was achieved by injecting the hippocampi of rats using HSV-1 as a vector (HSVGluR6; Federoff et al, 1990, PNAS, 89:1636). These animals experience limbic seizures within four hours following the injection. Animals injected with HSVlac, a vector which expresses ß galactosidase did not have limbic seizures and were used as a control.

Slices were made and whole-cell patch recordings performed in hippocampi 12 to 48 hours post-injection (n=78). The majority of CA1 and CA3 pyramidal cells, but not dentate granule cells, fired spontaneous bursts at rest in HSVGluR6-injected animals. This activity was not seen in the HSVlac-injected animals. However, the evoked synaptic responses in CA1 and CA3 in both HSVGluR6 and HSVlac-injected animals were normal.

Physiological and pharmacological studies demonstrate that these bursts were non-synaptic events mediated by intrinsic Na+ conductances. The spontaneous bursting was only seen within a narrow voltage range near rest (-55 to -65 mV) and was not blocked by APV and/or CNQX. Moreover, they were not blocked by local bath application of Cd2+. These bursts were mediated by Na+ conductances as TTX blocked the potentials and they were not seen when the local anesthetic derivative QX314 was included in the recording pipette. These data suggest, therefore, that the overexpression of KA receptors has rapid effects on the electrophysiological properties of hippocampal neurons. These changes may be important in the generation of synchronized activity which underlies temporal lobe seizures.

680.4

ENHANCED LTP IN HIPPOCAMPAL SLICES FOLLOWING *IN VIVO* PERINATAL HYPOXIA. FE Jensen*, CD Wang, and MC Stevens, Dept. of Neurology, Children's Hosp. Harvard Med School, Boston, MA 02115

We have previously shown that hypoxia is epileptogenic in immature rat pups *in vivo* (postnatal day (P) 10-12) but not in adults (*Ann. Neurol.* 29(6), 1991). Hippocampal and neocortical slices from P10 rats pups exposed to hypoxia showed evidence of hyperexcitability (*Soc. Nsci Abs.* 1992;18:159). In the present study, we evaluated the effect of prior *in vivo* hypoxia on long term potentiation (LTP) in hippocampal slices from P10 rats. Seizure activity was induced *in vivo* in P10 rats by exposure to hypoxia (4% O_2) and hippocampal slices were prepared following 10 mins recovery. Slices from hypoxic pups were coincubated with normal, nonhypoxic littermate controls. Extracellular synaptic potentials were recorded from s. radiatum of CA1 by stimulating CA3 Schaffer collaterals. LTP was induced by tetanic stimulation (100Hz for 1msec, repeated once after 20 sec) at an intensity evoking 1/2 of the maximal response. Slices from previously hypoxic rat pups showed enhanced LTP compared to controls. Over 1 hour, the mean % EPSP increase in hypoxic pup slices was significantly greater than slices from littermate controls ($p < 0.02$). The maximum % increase in EPSP in hypoxic slices was 107 $\pm$ 18% ($\pm$SE) (n = 10 slices, n = 10 pups) versus 55 $\pm$ 6% in slices from littermate controls (n = 6 slices, n = 6 pups) ($p < 0.05$). The latency to decay of LTP to 1/2 maximum increase was significantly longer in the slices from hypoxic pups (73 $\pm$ 21 min, $\pm$SD) than in controls (48 $\pm$ 26 min) ($p < 0.04$). These results show that in immature rats, prior *in vivo* hypoxia-induced seizure activity acutely increases the magnitude and duration of LTP. (Supported by NS31718, EFA, AHA, Milton Fund).

680.5

CORTICAL SYNAPTOSOMAL $^{45}Ca^{++}$ UPTAKE DIFFERENTIATES EPILEPTIC AND NON-EPILEPTIC MICE. A.F. Burroughs and M.J. Litzinger*Laboratory of Applied Neurobiology, Department of Pediatrics, University of Utah, Salt Lake City, UT 84132

Esplin et al. (Epilepsia,1994) have suggested that N-type presynaptic voltage sensitive calcium channels (VSCC) are different in epileptic (DBA/2J) and non-epileptic (C57/Bl) mice. Whole brain synaptosomal $^{45}Ca^{++}$ uptake studies were done to verify the physiological functions of presynaptic VSCCs in these mice. Additionally, cortical tissue synaptosomal $^{45}Ca^{++}$ uptake was done to determine the percentage of whole brain Ca^{++} flux due to cortical VSCC activity. $^{45}Ca^{++}$ uptake into synaptosomes was measured in the pre - eye mouse at postnatal day (PND) 8 and in the post - eye mouse at PND 16. Synaptosomes were depolarized with a 50 nM potassium solution to induce Ca^{++} flux across the membrane. Both the C57 and DBA mice showed $^{45}Ca^{++}$ synaptosomal uptakes that increased significantly (by >300%) between PNDs 8 & 16. At PND 8, cortical synaptosomes accounted for 77% of thewhole brain $^{45}Ca^{++}$ uptake in C57 mice and 74% of that in the DBA mice. At PND 16, cortical synaptosomes accounted for 79% of the whole brain $^{45}Ca^{++}$ uptake in the C57 mice and 82% of that in the DBA mice. Preliminary data shows the cortical synaptosomal $^{45}Ca^{++}$ uptake in DBA mice to be significantly greater ($p<0.001$) than the C57 at PND 16. This data suggests that presynaptic VSCCs are funtionally more susceptible to depolarization and, potentially, neurotransmitter realease. A loss of calcium homeostasis may play a role in generalized audiogenic seizures in the DBA/2J mouse.

680.6

GENETICALLY EPILEPSY-PRONE RATS DISPLAY GREATER AMOUNTS OF GluR 2/3 IMMUNO-REACTIVITY IN THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS THAN SPRAGUE-DAWLEY RATS. T.M. Goldenberg *, C.L. Morin, W.C. Gaza and C.E. Ribak, Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717.

Genetically epilepsy-prone rats (GEPR-9s) are used as a genetic model of epilepsy and display severe audiogenic seizures when exposed to acoustic stimuli. Previous research has shown that audiogenic seizures are initiated in the inferior colliculus where an apparent imbalance of excitatory and inhibitory amino acid activity exists. Immunocytochemistry with antibodies to the glutamate receptor subunit 2/3(GluR 2/3) in the inferior colliculus was used to compare the amount and distribution of receptors between the GEPR-9s and non-seizing Sprague Dawley (SD) rats. Light microscopy showed an increase of GluR 2/3 immunoreactivity in the ventral medial area of the central nucleus of the inferior colliculus of GEPR-9s. An analysis at the electron microscopic level revealed greater amounts of immunoreaction product in the postsynaptic structures of GEPR-9s. These data show significant differences in the amount of GluR 2/3 receptor in the inferior colliculus between the GEPR-9 and SD rats.
Supported by NIH grant NS 15669 to CER.

680.7

CORRELATING NEUROPHARMACOLOGICAL AND GENETIC CHANGES IN THE EL EPILEPTIC MOUSE. E. W. Johnson* and W. N. Frankel, The Jackson Laboratory, Bar Harbor, Maine 04609.

30% of all epilepsies occur without obvious pathology and may be genetic. Current therapeutic interventions have serious side effects and limitations. With genetic models for epilepsy, primary deficits can be more easily identified and examined as targets for new therapies.

EL mice exhibit spontaneous seizures at ^80 days of age but can be induced earlier by rhythmic vestibular stimulation. These tonic clonic seizures are accompanied by excessive salivation, head, limb and chewing automatisms and recur well into old age. Using EL mice, where known genetic determinates (*El1, 2* and *3*) can be identified unambiguously will greatly expedited the search for basic neuropharmacolgical mechanisms involved in endogenous epilepsy.

Modern neuropharmacological and molecular biological techniques make it possible to examine a variety of neuroactive compounds in the seizure focus. With *in vitro* quantitative autoradiography (QA), selective radioligands for specific receptor sites and second messengers are used to study changes in regional density and distribution. These approaches provide a fairly comprehensive picture of the anatomical distribution of neuromodulators in a seizure focus and may help to explain the etiology and cellular basis for epilepsy.

We are comparing brains from vestibularly stimulated EL mice and nonstimulated EL mice with two control strains, DDY and ABP as well as nonstimulated mice from all three groups at different ages.

Candidate genes that share the same relative position on the chromosome as the *El* genes and have a reasonable potential for affecting brain function are being studied using QA. Advances in the knowledge of the genetic and neuropharmacological basis of epilepsy should allow more precise and less destructive therapeutic interventions to be devised.

680.8

LOSS OF HIPPOCAMPAL INTERNEURONS IN A MODEL OF NEONATAL STATUS EPILEPTICUS. K.W. Thompson, A.M. Holm, C.G. Wasterlain* UCLA, Department of Neurology, Los Angeles, CA 90024.

The consequences of Status epilepticus (SE) in the neonate are not well understood. While childhood SE is associated with widespread neuronal necrosis, the lack of histological lesions in 15 d.o. rat brain after SE may indicate that the immature brain is selectively resistant to seizure-induced damage. We recently developed a model of perforant path stimulation in 15-16 d.o. rats which produces hippocampal lesions 24 hours post-stimulation. We now demonstrate permanent cell loss in the stimulated hilus 7 days after stimulation. The number of large pyramidal-shaped neurons within the tip of the dentate gyrus was 6.94$\pm$4.08 on the stimulated side compared with 25.69$\pm$11.59 on the unstimulated side ($p < 0.001$). Cell loss was also evident in the CA_3 pyramidal cell layer. Three of four animals showed loss of frequency-dependent paired pulse inhibition (27% inhibition before, 120% facilitation 1 week after, N.S.). These data suggest that focal status epilepticus can result in permanent neuropathological lesions in young animals, and that the limits of the immature brain's resistance or vulnerability to seizures have yet to be fully defined.

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680.9

MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF RAT BRAIN AFTER KAINIC ACID-INDUCED SEIZURES

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MRI diffusion weighted imaging (DWI), T2 weighted imaging (TWI) and MR Spectroscopy are non-invasive methods used for *in vivo* assessment of pathology and metabolic changes in human brain. In this report, ^{1}H-MRS was employed to study *in vivo* temporal metabolic changes and their correlation with DWI, TWI and histopathological stages in a rat model of TLE using kainic acid (KA). The KA injection (10 mg/Kg) induces a characteristic long lasting tonic clonic seizure followed by neuronal damage. Rat brains (n=5) were scanned at the level of the body of the hippocampus, spectra were recorded and the relative ratios of N-acetylaspartate (NAA), choline, and lactate (Lac) over creatine (Cr) were calculated. Our results show significant increase in Lac/Cr and NAA/Cr and a decrease in choline/Cr ratios during the ictal and early post-ictal phases correlated with increase signals in hippocampus and piriform cortex on DWI and TWI, as compared to control brains. Late post-ictally, NAA/Cr ratio decreased in correlation with the degree of neuronal loss. The significant ictal and early post-ictal increase in lactate and late post-ictal decrease in NAA reflect increased cellular activity and neuronal loss, respectively. Moreover, the early sharp decrease in choline, a membrane precursor, together with increase MR signal in specific areas of the limbic system prone to neuronal damage might represent an early index of neuronal injury during the early stages following the excitotoxic seizures.

680.10

HYPEREXCITABLE ASTROCYTES FROM HUMAN MEDIAL TEMPORAL LOBE STRUCTURES. N. Al-Rodhan*, M.G.Rioult, R. Villalba, W. T. Kim, D. Spencer and A. H. Cornell-Bell. Yale School of Medicine, New Haven, CT 06510.

To study astrocyte signaling in temporal lobe seizure pathology tissues were removed from 10 representative cases who underwent medial temporal lobectomies. Astrocyte cultures derived from areas of normal EEG and areas of seizure activity were compared using Ca^{2+} imaging (Fluo-3AM and confocal microscopy). Tissues including neocortex, hippocampus, parahippocampus and amygdala were cleaned of vessels and facia and treated with a single step digestion of papain and Trypsin/EDTA. Cultures were grown to confluence prior to Ca^{2+} imaging. Astrocytes from regions exhibiting hyperexcitable EEG records often exhibited spontaneous rapid, $Ca^{2+}{}_{in}$ transients prior to glutamate (Glu, 100 µM) application in the bath. Evoked responses often shut down the spontaneous $Ca^{2+}{}_{in}$ changes for minutes. In several cases Glu caused long-lasting reductions in $Ca^{2+}{}_{in}$ levels, a response never seen from cells cultured from normal EEG regions. Some astrocytes exhibited a rapid and prolonged increase in $Ca^{2+}{}_{in}$ that lasted for 75-100 sec followed by a silent period and then rapid $Ca^{2+}{}_{in}$ oscillations that persisted even in washout. In hyperexcitable cultures very few cells failed to respond to Glu. In the majority of cases the hippocampus and parahippocampus exhibited Ca^{2+} hyperexcitability in response to Glu, however there were examples where the neocortex or the amygdala was the only hyperexcitable tissue. These results imply astrocytes from any region of the medial temporal lobe may exhibit hyperexcitable $Ca^{2+}{}_{in}$ following Glu implying an active role of astrocytes in development of this hyperexcitability.

680.11

REPETITIVE ABNORMAL ELECTRICAL DISCHARGES IN BRAIN SLICES OF THE DENTATE GYRUS FROM EPILEPTIC PATIENTS. L.M. Masukawa*, K. Uruno, H. Wang, W.M. O'Connor, and M.J. O'Connor. Depts. of Neurology and Surgery, University of Pennsylvania Medical School and The Graduate Hospital, Philadelphia, PA 19146.

The population response of granule cells in the in vitro dentate gyrus brain slice from human epileptics during perforant path stimulation varies among patients. As we reported previously (Masukawa et al., Brain Res. 493: 1989), orthodromic responses range from single to multiple population spikes. In addition to these responses, specimens from a subpopulation of patients exhibit an unusual response which was characterized by repetitive negative field potentials. Each potential was approximately 100-200 msec in duration and the first potential had a latency of about 50 msec after perforant path stimulation. Such responses also could occur without stimulation in control ACSF and were sometimes revealed by exposure to 20 µM bicuculline. Antidromic stimulation could also drive this response. Several specimens generating this type of response also showed prominent molecular layer reorganization. Such waveforms are markedly similar to those observed in EEG records from epileptics during an epileptic discharge. Supported by NIH Grant #NS23077 to LMM.

680.12

CHRONIC FOCAL IRON-INDUCED EPILEPSY IN RAT; NEUROCHEMICAL, NEUROPHYSIOLOGICAL AND MORPHOLOGICAL OBSERVATIONS. E Ronne-Engström*, L Hillered, R Flink, L Kihlström, C Lindquist, Y Olsson, XJ Nie, H Carlson, Dpt of Neurosurg, Dpt of Neuropathology Uppsala University Hospital, Sweden.

Chronic focal iron-induced epilepsy in rats was studied with respect to extracellular levels of amino acids, electroencephalographic (EEG) changes including reaction to electrical cortical stimulation and histopathology. 93% of the animals developed spike or sharp wave activity on EEG. After 6 months the extracellular levels of amino acids aspartate (ASP), glutamate (GLU), aspargine (ASN), serine (SER), and glycine (GLY) were measured using intracerebral microdialysis both at the lesion side and in the contralateral hemisphere. The basal levels of ASP were significantly higher on both sides compared to a control group. There were unprovoked, spontaneous elevations of ASP and GLU up to 8x basal level. The levels of SER were significantly higher at the lesion side compared with the contralateral side. GABA levels were below the detection limit on the lesion side in all animals except for one, while it could be measured in five rats contralaterally. Electrical cortical stimulation was performed during the microdialysis and evoked seizure-like activity after stimulation in 5/8 animals. The amino acid levels displayed a pattern with elevations of mainly ASP and GLU in 4/8 animals. Histopathological changes were detected in all animals. Histological analyzis revealed a post-necrotic cyst, containing small vessels and macrophages, some loaded with iron pigment. The cyst was surrounded by astrocytes. This glial change extended into the white matter of the ipsilateral hemisphere and changes were also seen in hippocampus, in some animals bilaterally.

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID XII

681.1

ß-AMYLOID PEPTIDES STIMULATE GELATINASE ACTIVITY IN HIGH-DENSITY HIPPOCAMPAL CULTURES. S. Deb, C. Yu, and P. E. Gottschall*, University of South Florida, Department of Pharmacology, Tampa, FL 33612-4799

Little is known about the relationship between the Alzheimer plaque core ß-amyloid (Aß) peptide and the production of the so-called "acute phase" inflammatory mediators, many of which are also present in plaques. Tissue remodeling and repair enzymes that are critical in peripheral inflammatory responses are the matrix metalloproteinases (MMPs), and these enzymes are present in AD brain, they may exhibit "α-secretase" activity, and they appear to be involved in neurite extension. Thus, it was of interest to determine whether Aß peptides regulate the activity of MMPs in different brain tissues in culture. High density hippocampal cultures obtained from 18 day old rat embryos or enriched astrocyte cultures obtained from brains of 1 day old rat pups were incubated with Aß peptides (2.5 - 40 µM) *in vitro*. Media samples from these assays were measured for gelatinase (GLase) activity using zymography which was quantitated by densitometry. Both mixed hippocampal and astrocyte cultures showed high constitutive expression of a 58 kDa GLase. Treatment with Aß (1-40) for up to 72 h stimulated the activity of a 94 kDa GLase 5-10-fold and a 52 kDa GLase 20-fold in hippocampal cultures compared to untreated cultures. Aß (1-38) stimulated the activity of these two GLases to a lesser extent, whereas Aß (1-16) or Aß (25-35) exhibited little or no effect. Treatment of astrocytes with the four Aß peptides or fragments showed little or no effect on GLase activity after 24 h, and the 52 kDa activity was never observed under any condition in astrocytes. By immunoblot, the 94 kDa GLase was identified as gelatinase B (MMP-9), the 58 kDa GLase as gelatinase A (MMP-2) and the 52 kDa GLase as rat transin (stromelysin-1; MMP-3). These results indicate that Aß peptides stimulate the production of matrix degrading enzymes in hippocampal cultures and suggest they may play some role in development or growth of plaques, ie. abnormal neurite growth. In addition, rat transin, was apparently derived from neurons and not astrocytes. (supported in part by the Alzheimer's Association/Alice M. Melady Pilot Research Grant)

681.2

THE INVOLVEMENT OF SECRETED FORM OF AMYLOID β/A4 PROTEIN PRECURSOR IN THE NEURITOTROPHIC EFFECT OF NGF AND FGF. L-W. Jin* and T. Saitoh, Dept of Neurosciences, UC San Diego, La Jolla, CA 92093.

Previously we showed that a 17-mer peptide (APP319-335) within the secreted form of amyloid β/A4 protein precursor (sAPP) binds to the surface of B103 neuroblastoma cells and mediates both neurotrophic and neuritotropic effects of sAPP. An RMSQ peptide (APP330-333) within 17-mer blocked the binding and neuritotropic effect of 17-mer (J.Neurosci., in press). We used these peptides to study the involvement of sAPP in the effect of NGF and FGF, both of which modulate the level of sAPP in PC12 cultures (Neuron, 3:689, 1989). RMSQ peptide significantly decreased the number of neurites induced by NGF or FGF. This inhibition was overcome by either increasing the concentration of NGF or FGF, or by adding the 17-mer to the culture media. The RMSQ peptide, however, did not affect the survival-promoting effect of NGF or FGF. Thus, sAPP, in particular its 17-mer domain, is probably involved in the induction or the maintenance of neurites of PC12 cells treated with NGF or FGF.

681.3

A single amino acid substitution in the amyloid β/A4 protein precursor disrupts its neuritotropic activity.

Jean-Marc Roch*, Deborah Otero, and Tsunao Saitoh. UC San Diego.

We have previously shown that the secreted form (sAPP) of APP-695 is a growth-regulating molecule, a neuritotropic and neurotrophic factor, and that the domain responsible for all activities is from Ala319 to Met335 (Kang sequence), with a minimal essential sequence RERMS from Arg328 to Ser332 (Saitoh et al., *Cell* **58**:615, 1989; Roch et al., *J. Biol. Chem.* **267**:2214, 1992; Ninomiya et al., *J. Cell Biol* **121**:879, 1993; Jin et al., *J. Neurosci.*, in press, 1994; Yamamoto et al, *J. Neurobiol.*, in press, 1994;). In the present study, we synthesized 11-mer peptides containing this active domain, introduced mutations in the RERMS site, and compared the neurite extension activity of the mutant peptides to that of a peptide of wild-type sequence or KB75, a bacterially made sAPP-695. We found 3 mutant peptides with no activity. Using PCR and recombinant DNA methods, we engineered an APP cDNA encoding an APP variant with a single amino acid substitution, Glu→Ile in the RERMS site. The affinity for heparin of the mutant sAPP was identical to that of wild-type APP. However, the neuritotropic activity of the mutant sAPP was dramatically reduced, suggesting that the heparin binding and neuritotropic properties of APP can be separated. Thus, mutations in the APP molecule can alter its physiological function.

681.4

AMINO-TERMINAL REGION OF THE AMYLOID PRECURSOR PROTEIN STIMULATES MAP KINASE.

S.M. Greenberg*, W.Q. Qiu, D.J. Selkoe, A. Ben-Itzhak, K.S. Kosik. Brigham and Women's Hospital & Harvard Medical School, Boston, MA 02115.

We have previously demonstrated that the secreted form of the ß-amyloid precursor protein (ß-APP_s) stimulates mitogen-activated protein (MAP) kinases in PC12 cells. The amino-terminal half of ß-APP_s contains a region rich in cysteine residues followed by an acidic domain. In experiments to delineate the region of ß-APP_s responsible for activating MAP kinase, we found that reductive alkylation of disulfide linkages by dithiothreitol followed by iodoacetamide (but not iodoacetamide alone) entirely blocked the MAP kinase stimulation caused by medium enriched in ß-APP. We have confirmed the role of the cysteine-rich amino-terminal region by use of purified fragments of ß-APP_s expressed in *E. coli*. Fragments extending from the KpnI to the XhoI restriction sites (spanning the cysteine-rich and acidic domains) or from XhoI to BglII (most of the remainder of ß-APP_s) were cloned into the pGEX-2T vector. Purified ß-APP fragments were obtained by cleavage of fusion proteins with thrombin. We found that 10 min treatment with ß-APP(KpnI-XhoI) activated MAP kinase approximately 15-fold at a concentration of 50 nM, while XhoI-BglII had no effect. ß-APP(KpnI-XhoI), like intact secreted protein, was inactivated by reductive alkylation. Medium from CHO cells expressing APP with deletion of XhoI-BglIIstimulated MAP kinase as well. These results suggest that sites that activate MAP kinase reside in the amino terminal region of ß-APP_s and that, like other extracellular trophic factors such as the EGF family, they require structural loops created by disulfide linkages for activity.

681.5

ß-AMYLOID PRECURSOR PROTEIN EPITOPES BIND TO MICROTUBULES AND THEIR ASSOCIATED PROTEIN, TAU.

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Tubulin is one of several proteins associated to amyloid ß-protein (Aß) deposits, as well as neurofibrillary tangle in the brains of patients with Alzheimer's disease and related disorders. Although binding to tubulin may promote Aß fibril formation from the otherwise soluble peptide, Aß lesions may represent the proteolytic residual of a complex between the full length ß-amyloid precursor protein (ßAPP) or fragments thereof and tubulin. ßAPP C-terminal fragments expressed as GST fusion proteins, and immobilized on solid support, bound proteins in rat brain homogenates as well as human neuronal and glial cell lines. Both α and β tubulins bind to the 42 amino acids of Aß, as revealed by SDS-PAGE, followed by amino acid microsequencing and Western blotting. This binding was localized to the 1-28 N-terminal amino acids of Aß. Tubulin also binds to the predicted transmembranal sequence C-terminal to Aß. However this binding seems to be mediated by the microtubule-associated protein tau. Moreover, intact microtubules are required for these interactions since colchicine treatment abolished them. The C-terminal region of ßAPP has two microtubule binding sites, which may indicate that it is an integral part of the cytoskeleton and alteration of this complex may have a role in the amyloidogenic process characteristic of Aß deposition disorders.

681.6

BETA AMYLOID INDUCES TAU PHOSPHORYLATION AND DECREASED MICROTUBULE BINDING.

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Beta amyloid (βA) causes neuronal degeneration characterized by dystrophic axons and dendrites in primary rat and human neuronal cultures (Yankner et al., 1990; Busciglio et. al., 1992). The effects of aggregated forms of the βA (1-40) peptide were examined in rat E18 hippocampal and human fetal cortical cultures. Biochemical and immunocytochemical analysis was performed with the monoclonal antibody PHF-1, which recognizes a phosphorylated form of tau present in paired helical filaments. Treatment with βA resulted in marked induction of the PHF-1 antigen which preceded and accompanied neuronal degeneration. Pre-treatment with alkaline phosphatase completely abolished immunocytochemical staining of neurons with PHF-1 and PHF-1 immunoreactivity in Western blots, and increased immunoreactivity with the tau-1 monoclonal antibody, confirming the identity of this antigen as phosphorylated tau. Detergent extraction of βA-treated neurons under microtubule-stabilizing conditions demonstrated that most of the phosphorylated tau induced by βA is not bound to microtubules. In contrast, MAP-2 and non-phosphorylated tau were recovered in the detergent-resistant cytoskeletal fraction. These results suggest that βA induces tau phosphorylation which results in decreased binding of tau to microtubules. Abnormally phosphorylated tau in the AD brain has been reported to show decreased microtubule binding. These findings suggest a model in which βA causes cytoskeletal disruption and neuronal degeneration by inducing tau phosphorylation.

681.7

THE EFFECTS OF β-AMYLOID ON THE MOLECULAR MOTORS DYNEIN AND KINESIN

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Disruption of normal cellular transport is one plausible hypothesis for the pathogenesis of Alzheimer's disease (AD). β-amyloid is a 42 amino acid peptide which is thought to be processed from the amyloid precursor protein (APP) in endosomes or lysosomes, and whose abnormal accumulation in the neuropil is a hallmark of AD. Transport of APP to the cell surface has been reported to be a kinesin-dependent process, and transport of cell surface molecules from early to late endosomes has been reported to be a dynein-dependent process. The effects of β-amyloid on the motor proteins dynein and kinesin were examined using differentiated PC12 cells cultured in the presence of β-amyloid for nine days. Cells were harvested, washed in buffer, and lysed in SDS buffer for electrophoresis and immunoblot analysis. Immunoblots were visualized using antibodies against dynein intermediate chain (74 kd) and kinesin heavy chain (120 kd). Preliminary data indicates that both dynein and kinesin immunoreactivity are increased in PC12 cells cultured in the presence of 7 μg/ml β-amyloid, and that amounts of both motor proteins leveled out between 5 and 9 days in culture. As compared to levels present at the time β-amyloid was added to the culture medium, dynein intermediate chain showed a 40-fold increase and kinesin heavy chain showed a 9-fold increase respectively after nine days in culture. Effects of increasing concentrations of β-amyloid are currently under investigation. These data suggest that exogenous β-amyloid, whose production is a motor-dependent process, can increase intracellular levels of dynein and kinesin.

681.8

EXOGENOUS AMYLOID Aß STIMULATES THE ACCUMULATION OF AMYLOIDOGENIC FRAGMENTS OF APP IN TRANSFECTED CELLS

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Results of our previous investigations demonstrated that exogenous amyloid beta peptide, Aß1-42, is internalized and accumulates intracellularly in late endosomes or lysosomes in human cells and is resistant to degradation. We have analyzed the effect of internalized Aß on the metabolism of APP in stably transfected 293 cells by ^{35}S-methionine metabolic labeling, followed by immuno-precipitation. We found that the amounts of C-terminal, amyloidogenic fragments (particularly a 16 KDa fragment), are dramatically enhanced by treatment of the cells with Aß1-42, but not Aß1-28, which does not accumulate in cells. The amount of this fragment is greatly reduced in control, non-transfected cells incubated with Aß1-42. The stimulatory effect is not due to an increase in APP synthesis or an inhibition of the non-amyloidogenic processing of APP, since the amounts of soluble, N-terminal APP products secreted into the medium and non-amyloidogenic C-terminal fragments associated with the cell are the same in peptide-treated and control cells. The 16 KDa C-terminal fragment is highly enriched in the insoluble fraction of the cell lysate, and is colocalized with internalized ^{125}I-labeled Aß1-42. The continued internalization of exogenous Aß1-42 from the medium is not required for the stimulatory effect. Pretreatment of cells with Aß1-42, followed by removal and replacement with medium lacking peptide also results in accumulation of the amylodogenic fragments. Our results suggest that the ß-peptide stimulates its own accumulation by aggregating with newly synthesized amyloidogenic fragments, thereby protecting the Aß domain from degradation. Amyloid accumulation could be a "run away" process once amyloid aggregates have nucleated inside the cell. Supported the American Health Assistance Foundation and NIH NS31230

681.9

AMYLOID BETA-PROTEIN INTERFERES WITH THE UBIQUITIN-DEPENDENT PROTEIN DEGRADATION PATHWAY.

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Amyloid β-protein (Aβ) and ubiquitin are both components of amyloid plaques associated with Alzheimer's disease (AD) pathology. Abnormally high levels of ubiquitin and ubiquitin conjugates in AD brains are well documented. However, the significance of this observation is still unknown. One of the functions of ubiquitin is to target proteins for degradation. In our studies we found that $A\beta_{1-28}$ inhibits the ubiquitin-dependent degradative pathway. This effect was characterized using radiolabeled exogenous proteins such as lysozyme. The results showed that Aβ interferes with the step of ubiquitin conjugates degradation, catalyzed by the multisubunits 26 S proteosome. We also tested the ability of $A\beta_{1-28}$ fragments to inhibit degradation. We found that the amino-terminal-containing peptides were effective, although not to the same extent as $A\beta_{1-28}$. In addition, three new ubiquitin conjugates containing Aβ were detected on gel electrophoresis. We are currently investigating the function and significance of these ubiquitinated proteins. Our results suggest that impairment of ubiquitin-dependent protein degradation by Aβ may explain the accumulation of ubiquitin and ubiquitin conjugates in brain of AD patients.

681.10

AMYLOID ß-PROTEIN INDUCES THE EXPRESSION AND CELLULAR ACCUMULATION OF THE AMYLOID ß-PROTEIN PRECURSOR IN CULTURED CEREBROVASCULAR SMOOTH MUSCLE CELLS.

William E. Van Nostrand*, Judianne Davis-Salinas, Michael Wessel, and Susan M. Saporito-Irwin. Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine CA 92717-4025.

Deposition of the amyloid ß-protein (Aß) in senile plaques in the neuropil and in the media of intracortical and leptomeningeal blood vessels are hallmarks of Alzheimer's disease (AD) and related disorders including hereditary cerebral hemorrhage with amyloidosis-Dutch type. Cerebrovascular Aß deposits are also accompanied by degeneration of the smooth muscle cells in the affected vessels. Moreover, cerebrovascular smooth muscle cells have been implicated in the expression of AßPP and the production of Aß. In light of these suggestions, we investigated the effects of various length synthetic Aß peptides on the cellular degeneration and expression of AßPP in primary cultures of cerebrovascular smooth muscle cells. The cerebrovascular smooth muscle cell cultures were incubated alone or with 25 μM of various length Aß peptides for six days. Similar to untreated controls, cells that were incubated with 25 μM $A\beta_{1-39}$, $A\beta_{1-40}$, or a scrambled $A\beta_{1-42}$ peptide exhibited no signs of cellular degeneration nor any effect on the levels of AßPP. In contrast, cells incubated with 25 μM $A\beta_{1-42}$ showed signs of extensive cellular degeneration and a striking increase in the levels of cell-associated AßPP, however, no increase in the levels of secreted AßPP were observed. The levels of cell-associated, carboxyl terminal AßPP fragments were also markedly increased when the cerebrovascular smooth muscle cells were incubated with 25 μM $A\beta_{1-42}$ peptide. These studies suggest a cyclic mechanism whereby Aß can induce cellular degeneration and concomitant increased expression of cell-associated AßPP and potentially more Aß peptide leading to a spread of the cerebrovascular pathology of AD and certain related disorders.

CELL MIGRATION AND DIFFERENTIATION V

682.1

GnRH NEURONS MIGRATE ALONG THE MEDIAL TELENCEPHALON IN CLOSE ASSOCIATION WITH A SUBSET OF OLFACTORY NERVE AXONS.

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Several studies indicate that GnRH neurons of the septal-preoptic area and hypothalamus originate in the nasal area and migrate to the brain on the olfactory nerve. Previously, we found that GnRH neurons migrate dorsally and caudally along the medial edge of the telencephalon to the septal area in close association with a N-CAM and Ng-CAM enriched fiber bundle. In order to determine whether this fiber bundle was an extension of the olfactory nerve or another fiber bundle, we performed two experiments. First, we unilaterally ablated the olfactory placode on an E3 chick embryo, which prevented olfactory nerve outgrowth to the brain (and GnRH migration). We waited until E7 and then sacrificed the embryo and immunostained sections for N-CAM. N-CAM immunostaining was absent along the medial edge of the telencephalon on the operated side but was clearly visible on the unoperated side. Second, we applied filter strips impregnated with DiI to the olfactory nerve distal to the telencephalon to trace its full extent in an E7 chick embryo. Although most labeled axons appeared to terminate in the presumptive olfactory bulb, a small subset of labeled axons continued along the medial edge of the telencephalon in a location similar to that followed by GnRH neurons. These findings are consistent with the idea that GnRH neurons are delivered to the septal area on a transient extension of a subset of olfactory nerve axons. We are currently attempting to determine which cells in the olfactory epithelium give rise to these axons. This work was supported by NIH grant NS30047 (RBN).

682.2

CELL MIGRATION DURING DEVELOPMENT OF THE OLFACTORY SYSTEM IN THE NEWBORN OPOSSUM, *MONODELPHIS DOMESTICA*, *IN VIVO* AND *IN VITRO*.

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The olfactory system of the short-tailed opossum, *Monodelphis domestica*, develops almost entirely after birth. Here, we have studied the migration of cells which originate in the olfactory region during the first 10 postnatal days. Antisera to gonadotropin releasing-hormone (GnRH), to olfactory marker protein (OMP) and carnosine (both specific to olfactory cells) were used as markers. During the first posnatal days, migration of GnRH positive cells could be followed along the terminal and olfactory nerve and on the medio-lateral aspect of the forebrain. A second population of cells, which expressed both OMP and carnosine, was contiguous, but separated from GnRH cells. For further analysis, explants of whole brain attacched to their intact olfactory region from 3 day old opossum pups were cultured for up to 3 to 7 days. The explants showed a high preservation of the structures and of the immunoreactivities. Growth and maturation, although delayed, took place in culture and GnRH cells and OMP/carnosine positive cells followed the routes taken *in vivo*. The results demonstrate successful culture of whole-brain preparations with the intact olfactory system for studying cell differentiation and migration during development. (Financial support from Italian MURST and CNR, Swiss National Science Foundation).

682.3

SIMULTANEOUS MIGRATION AND PROLIFERATION OF NEUROBLASTS ORIGINATING IN THE ANTERIOR SUBVENTRICULAR ZONE OF THE NEONATAL RAT FOREBRAIN

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It has recently been shown using a retroviral lineage tracer encoding lacZ that the anterior part of the postnatal telencephalic subventricular zone (SVZa) is a discrete source of neurons destined for the olfactory bulb (OB), and that while en route to the OB the SVZa-derived cells traverse a highly restricted pathway (Luskin, Neuron, 11:173, 1993). In addition, the number of lacZ-positive cells along the full extent of the pathway, resulting from injections of retrovirus into the SVZa, increased as a function of the post-injection survival time, suggesting that the SVZa-derived cells divide as they migrate. In this study we examined the phenotype of the SVZa-derived cells and investigated whether they undergo cell division during their migration. Cells migrating to the OB were tagged by injections of retrovirus into the SVZa of neonatal rat pups and the lacZ(+) cells detected histo- or immunohistochemically in cryostat sections 3-4 days later. To determine if the SVZa-derived cells express a neuronal phenotype we used an antibody against neuron-specific tubulin (TuJ1), which is usually present in postmitotic neurons (Menezes & Luskin, J. Neurosci., Aug. '94). The cell proliferation marker, BrdU, administered 1-5 hr prior to sacrifice of the injected pups was used to identify S-phase cells. We found that the vast majority of lacZ(+), SVZa-derived cells were TuJ1(+), indicating their neuronal identity. Approximately 10% of the lacZ(+), SVZa-derived cells incorporated BrdU throughout the migratory pathway, indicating that some SVZa-derived cells proliferate while en route to the OB. Furthermore, some cells were triple-labeled by lacZ, TuJ1 and BrdU, directly demonstrating the presence of neuroblasts in the process of mitosis as they migrate. Thus, the SVZa-derived cells in the pathway are highly atypical of most other CNS neurons, which become postmitotic before migrating to their final position. Supported by Pew Charitable Trusts, NIH and March of Dimes (MBL), CNPq, Brasil (JRLM).

682.4

MOLECULAR CLONING OF ASTROTACTIN.

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Using affinity-purified anti-astrotactin antibodies, we have isolated a novel adhesion molecule from cDNA expression library of postnatal day 5 granule cells. The deduced amino acid sequence starts with a signal peptide, followed by an extracellular domain containing 3 EGF repeats and 2 fibronectin type III repeats in alternative orders, but no Ig C2 domains. Screening of genomic library yielded another novel gene with 50% homology, suggesting a new gene family.

By Northern analysis, the expression of this genes is restricted to developing brain, and appears to be neuron-specific. *In situ* hybridization demonstrates a specific temporal and spatial pattern of expression in immature neurons undergoing glial-guided migration and assembly into neural layers of cerebellum, hippocampus and cortex. Antibodies against a GST-fusion protein recognize a neuronal membrane protein with an apparent molecular weight of 100 kd on Western blot. *In vitro* functional assays with this antiserum demonstrate blocking of neuron-glia interactions and neuronal migration in microcultures of developing cerebellar cells. These results suggest that we have cloned the astrotactin molecule, which belongs to a new class of membrane signaling molecules, functioning in CNS neuron-glia interactions, including glial-guided neuronal migration.
Supported by NIH grant NS 15429 (MEH).

682.5

MULTIPOTENT IMMORTALIZED NEURAL PROGENITORS DIFFERENTIATE INTO GLIA & NEURONS FOLLOWING ENGRAFTMENT INTO THE SUBEPENDYMAL GERMINAL ZONE (SEZ) OF ADULT MICE. J.D.Flax, L.Villa-Komaroff* & E.Y.Snyder Depts. of Neurology & Neonatology. Harvard Medical Sch., Children's Hospital, Boston MA 02115.

It is becoming evident that CNS progenitors & germinal zones persist into adulthood. The SEZ in adult rodent brain contains progenitors capable of neuronal & glial differentiation in vitro. The function of adult SEZ cells in vivo remains controversial. Recent reports suggest they differentiate into "glioblasts" that migrate into parenchyma & replace lost astrocytes &/or oligodendrocytes, as well as olfactory bulb (OB) neurons &, perhaps, a small subset of other neurons. However, other reports suggest that SEZ cells are a self-renewing proliferative population that do not migrate out of the SEZ. Previously, we demonstrated that clonal immortalized CNS progenitor cell lines, following transplantation at various developmental time points, are capable of extensive cytoarchitectural integration, & of neuronal & glial differentiation along the neuroaxis, in a regional & temporal appropriate manner. The progenitors appear to differentiate appropriately in response to local microenvironmental developmental cues. The present study utilizes engraftment of these clonal neural progenitors into the SEZ or OB of adult animals as a tracer for the possible fate of endogenous SEZ progenitors & as an *in situ* biological assay for the signals that might regulate their fate. We found that engrafted progenitors differentiated into both glia & OB granule cell neurons when implanted into the SEZ. The results suggested that microenvironmental signals for inducing cell type specific differentiation (including neuronal) exist in some regions of the adult brain. The demonstration that immortalized CNS progenitors can differentiate & continue to express an exogenous gene (LacZ) following migration, suggests that this strategy may be useful for the repair (cell replacement) &/or delivery of exogenous gene products for gene therapy in the adult brain.

682.6

RE-EXPRESSION OF THE RADIAL GLIAL CELL MARKER, RC2, IN YOUNG ADULT MOUSE CORTEX FOLLOWING TARGETED NEURONAL DEATH. C.S. Hernit-Grant* and J.D. Macklis Dept. Neurology, Prog. Neuroscience, Harvard Med. Sch., Children's Hosp. Boston 02115

Embryonic neurons transplanted into regions of targeted photolytic neuronal cell death in neocortex of juvenile or adult mice selectively migrate into the neuron-deficient regions up to 800 μm over a period of 1-2 weeks (Macklis, J. Neurosci., 13(9), 1993). Here we study this migration directly in living slice preparations and investigate the potential role of radial glial cells as a structural substrate via re-emergence from postdevelopmental astrocytes.

Latex nanospheres conjugated to the cytotoxic chromophore chlorin e_6 were unilaterally injected into somatosensory cortex of 2-week old mice. Two weeks later, contralateral homotopic cortex received transcranial 674 nm laser illumination resulting in selective photolysis of retrogradely labeled callosal pyramidal neurons in layer II-III. After 2 weeks, mice received transplants of E17 neocortical cells pre-labeled with Di-I and PKH26. Three to seven days after transplantation fresh brains were cut at 150 μm, and the region containing the transplant was microdissected out, cultured, observed under fluorescence, and images collected with a digital confocal microscope. Transplanted cells displayed bipolar morphologies characteristic of migrating neurons and moved at speeds of upto 10 μm/hr. To study the potential role of radial glial cells, brains from another group were processed for fluorescence, RC2 and GFAP immunocytochemistry 3, 7 and 14 days after transplant. Glial cells outside the injection site reacted positively for RC2 or GFAP at all ages studied, and a small number reacted for both. RC2 reactive cells resembled the GFAP-positive reactive astrocytes, but had fewer processes and were fewer overall. GFAP positive reactive astrocytes in controls receiving transplants only or a sham injection of HBSS were found at all ages studied, but RC2 positive glia were present only within the injection site and only at 3 and 7 days and not at 14 days. These results suggest a role for RC2 positive glia during the selective migration of transplanted embryonic cells into neuron-deficient regions of young adult cortex. Further experiments are needed to determine if neurons associate closely with RC2 positive glia while migrating and whether these glia are of host or donor origin. Supported by HD28478, The Alzheimer's Assoc., MR Center Grant HD18655, The Rita Allen Foundation, and an NSF Postdoctoral Fellowship to CHG.

682.7

A NEUROGENIC SUBPOPULATION OF NEURAL CREST CELLS DEPENDS UPON TIMELY EXPOSURE TO ENVIRONMENTAL CUES. P. D. Henion[1], K. S. Vogel[2], S. L. Rogers[3*], and J. A Weston[1]. [1]Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403, [2]NCI-Frederick Cancer Research and Development Center, P. O. Box B, Frederick, MD 21702, [3]Department of Anatomy, Univ. of New Mexico, School of Medicine, Albuquerque, NM 87131.

We have previously proposed that distinct neurogenic and non-neurogenic neural crest subpopulations segregate very soon after crest cells arise from the neuroepithelium. We predicted that the putative neurogenic precursor subpopulation would be differentially responsive to and/or dependent upon specific environmental cues. We have tested the potential of the neural crest population to give rise to neurons both *in vivo* and *in vitro*. We have found that the lateral migratory pathway *in vivo* is unable to support neurogenesis among crest-derived populations with known neurogenic ability, and that neurogenesis among neural crest cell populations *in vitro* requires the timely availability of specific growth/instructional factor activities. These results support the notion that an apparently undifferentiated but developmentally distinct neurogenic subpopulation exists in the premigratory neural crest. Supported by NS29438.

682.8

CELL MIGRATION AND NOVEL DERIVATIVES OF CRANIAL NEURAL CREST AND PLACODES IN *XENOPUS LAEVIS*. A. Collazo*, J. Rubero, M. Bronner-Fraser, P. M. Mabee and S. E. Fraser. Beckman Institute 139-74, California Institute of Technology, Pasadena, CA 91125.

Our previous work has shown that neuromasts, the mechanoreceptors of the lateral line system of teleost fishes (zebrafish and *Betta*) and *Xenopus*, are derived from both neural crest and epidermal placodes (Collazo et al. *Science* 264:426, 1994). We expand our analyses for *Xenopus* by using DiI [(3) or (5)C18] to label small populations of neural crest, head mesoderm or placodal cells, and lysinated rhodamine dextran (LRD) to label individual neural crest cells. We used *in vivo* time lapse imaging of DiI labeled cells to study the migration of neural crest and placodes. Neural crest forming the first two branchial arches (mandible and hyoid) and last three arches originates, respectively, from midbrain and hindbrain. Crest fills the arches from ventral to dorsal with those cells contributing to the dorsal portion of the arch migrating later. We observe labeled neural crest cells in cartilage, neuronal, glial, connective tissue and neuromast cells. The first neural crest cells to migrate tend to form mostly cartilage not neural structures. The anterior-most neural tube did not form neural crest; instead, labeled cells migrated within the neural tube to positions in the hindbrain. Our study reveals some interesting differences from previous studies of neural crest in *Xenopus* (Sadaghiani and Thiébaud *Dev. Biol.* 124:91, 1987). We are unable to observe migration of neural crest before stage 18 and, at stage 15-16, the lateral population of "neural crest" is mostly head mesoderm. Development of the posterior and occipital lateral lines begins after stage 34. We observe labeled cells moving from one primordium (after the placode breaks up) to another along the lateral line nerve. We show primordia form hair cells by double labeling with the hair cell dye 4Di2Asp. By iontophoretically injecting single cells with LRD we are able to demonstrate that, unlike in zebrafish, cranial neural crest cells in *Xenopus* do appear to be multipotent. Single cell labeling of LRD also confirms our result that neural crest cells contribute to neuromasts. Support: MDA & NIMH.

682.9

ATP-MEDIATED EFFECTS ON NEURONS DURING ONTOGENY *IN VITRO*: FROM NEUROBLASTS TO PRIMARY NEURONAL CULTURES. D.C. Spray, R. Rozental*, M.F. Mehler, M. Morales, D. Vieira and J.A. Kessler. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Extracellular ATP plays an important role in cell proliferation and survival of hemopoietic stem cells even in the absence of cytokines. Since our previous studies suggested that cytokines that modulate hematopoiesis also govern neurogenesis (Mehler *et al.*, Nature **362**: 62, 1993) we evaluated the effects of extracellular ATP on neuroblasts as they underwent morphological and functional changes *in vitro* using dye-transfer (Lucifer yellow: L.Y.), Ca^{2+} imaging (Fura-2 AM) and double whole cell voltage clamp techniques. In naive neuroblasts maintained at 39°C (nonpermissive for T antigen expression), ATP (10-100 μM) transiently increased the steady-state $[Ca^{2+}]_i$ levels from ~ 100 nM to 10 μM without changing the internal pH. In parallel, ATP transiently uncoupled these cells as evidenced by a decrease in the extent of L.Y. spread and by a reduction in the strength of electrotonic coupling between naive neuroblasts. These responses were observed during a neuronal developmental stage in which chemosensitivity to glutamate, GABA and acetylcholine were not observed (outside out patches and Ca^{2+} imaging). In contrast to the latter neurotransmitters, the magnitude of ATP-mediated responses progressively decreased as these cells underwent morphological maturation and were dramatically lower in primary neurons. Since uncoupling has been correlated with cellular differentiation and ATP-mediated events seem to be developmentally regulated, we are currently investigating whether ATP elicited responses are implicated in the expression of membrane excitability during neuronal ontogeny.

682.10

GAP JUNCTIONS ARE PREVALENT IN THE DEVELOPING CEREBRAL CORTEX. J. G. Parnavelas* and B. Nadarajah. Department of Anatomy, University College London, Gower Street, London WC1E 6BT, U.K.

The neurons and glia that make up the mammalian cerebral cortex arise from neuroepithelial cells surrounding the lateral ventricles. These epithelial cells are linked with different types of specialized junctional complexes. The present study examines the distribution of gap junctions (patch-like membrane appositions with a gap of 2nm) during pre- and postnatal development of the rat cerebral cortex. Brains from rats of a number of embryonic stages and of postnatal animals up to 35 days were fixed with a mixture of aldehydes and processed for electron microscopy. Analysis of coronally-cut ultrathin sections showed that early in corticogenesis (E12-14), gap junctions were restricted in a monolayer of epithelial cells bordering the ventricle. At this stage, many gap junctions were seen between the opposing membranes of elongated cellular processes directed towards the ventricle. At later embryonic stages and in early postnatal life, this band of gap junctions appeared to move away from the ventricular zone and was observed in the regions between the intermediate zone and the deep part of the cortical plate including the subplate. Later in postnatal life, gap junctions were distributed throughout the cortical thickness with a conspicuous concentration in the deeper cortical layers at day 35. These junctions were formed predominantly between neuronal elements including the apical dendrites of pyramidal neurons. These observations suggest that gap junctions may provide a means of communication between proliferating, migrating and differentiating cells in the developing cerebral cortex.

682.11

D2 CYCLIN GENE FUNCTION IS IMPLICATED IN DIFFERENTIATION OF SPECIFIC NEURAL PRECURSORS IN BRAIN. ME Ross*, CJ MacNabb, and M Risken, Dept. of Neurology, Univ of Minnesota, Minneapolis, MN 55455

Cyclins are regulatory proteins which promote the progression of dividing cells through the cell cycle. D type cyclins mediate the transition from G1 into S phase and are thought to be widely expressed in mitotically active tissues. Their responsiveness to growth factors and their oncogenic capability supports the likely relationship of D cyclin regulation with cell growth and differentiation. We have isolated a cerebellar cDNA, MN20, which corresponds to a D2 cyclin message form, highly restricted to brain. DNA sequence data reveals that MN20 corresponds to a message containing 5 kb of 3' untranslated region (UTR) and 0.9 kb of open reading frame encoding D2 cyclin protein. Northern data reveal differential expression of MN20 using probes from either the extreme 3' UTR or a more 5' region. At postnatal day 6 the 3' probe hybridizes to a 7 kb message seen only in cerebellum while the 5' probe hybridizes to a 6 kb message found at varying levels in P6 cerebellum and 6 other tissues, raising the possibility that there are alternatively processed forms of D2 cyclin mRNA with distinct anatomic expression patterns. In situ hybridization using the 3' probe reveals that MN20 is not ubiquitous in brain, but is expressed in restricted neuronal precursor populations, e.g., in proliferating granule neuroblasts of P6 cerebellum but not hippocampus. Strikingly, the 3' MN20 probe labels presumed postmitotic neuronal precursors in embryonic cerebral cortex, but not dividing cortical neuroblasts. Data suggest that: 1) developing neuroblasts can select which components of the cell cycle machinery they employ, 2) post transcriptional regulation of D2 cyclin protein expression may be used to shape the development of particular brain regions, and 3) the D2 cyclin gene has additional functions beyond promoting the G1/S transition in the cell cycle. (Supported by NIH and the March of Dimes)

682.12

INTRACELLULAR RECORDINGS FROM PURKINJE CELLS IN ORGANOTYPIC CEREBELLAR CULTURES EXPOSED TO ARA C. R. Drake-Baumann[1,2] and F.J. Seil*[1,2,3], Neurology Research[1], VA Medical Center and Departments of Neurology[2] and Cell Biology & Anatomy[3], Oregon Health Sciences University, Portland, OR 97201.

Previous studies have shown that exposure of explant cultures of neonatal mouse cerebellum to the DNA synthesis inhibitor, cytosine arabinoside (Ara C), for the first 5 days *in vitro* (DIV) drastically reduced the granule cell population and compromised glial function (Seil, F.J. *et al.*, Brain Res. 186:393-408, 1980; Blank, N.K. *et al.*, Neuroscience 7:1509-1531, 1982). Myelination was absent and astrocytes failed to ensheath Purkinje cells (PC). Purkinje cells sprouted recurrent axon collaterals that hyperinnervated other PC somata and projected to PC dendritic spines.

Electrophysiological studies show that Purkinje cells of untreated (control) and Ara C treated explant cultures develop spontaneous electrical activity. Intracellular recordings from Purkinje cells of cerebellar explants between 13-21 DIV exhibit spike activity consisting of a mixture of complex and simple spikes. The complex spikes contain a fast rising action potential followed by a depolarizing potential on which a plateau and several spike-like components are superimposed. This type of activity has been observed in mature Purkinje cells *in vivo* and in slices *in vitro*. By contrast, Ara C treated Purkinje cells have mainly simple spike activity reminiscent of the type of activity seen in immature Purkinje cells. These observations suggest that the electrophysiological maturation of PC is arrested in Ara C treated explants. The lack of glial ensheathment of PC and/or the absence of parallel fiber input may play a role in the failure to develop complex spikes. (Supported by the U.S. Department of Veterans Affairs and NIH grant NS 17493).

DEGENERATIVE DISEASE: ALZHEIMER'S—MECHANISMS OF CELLULAR INJURY II

683.1

TEA AND Ca^{2+} -SENSITIVITY OF K^+ CHANNELS IN OLFACTORY NEUROBLASTS FROM ALZHEIMER'S AND NORMAL DONORS. M. Boakye, R. Etcheberrigaray*, V. Lubierman, B. Wolozin† and D. L. Alkon. *LAS*, NINDS and SGP, NIMH, National Institutes of Health, Bethesda, MD 20892.

Our laboratory has identified K^+ channel's regulation as one of the critical steps in associative memory storage. Because memory loss is the principal and earliest sign of Alzheimer's disease (AD), and because AD may be systemic, we studied K^+ channels in peripheral tissues. Using human fibroblasts, we have found that a 113 pS, tetraethylammonium(TEA)-sensitive channel is functionally absent in fibroblasts from AD patients. The present report extends our previous findings to human olfactory neuroblasts (HON), which make direct synaptic contacts with the central nervous system (CNS) and express neuronal markers. Patch-clamp techniques were used to study K^+ channels in HON from 8 AD patients and from 6 age-matched control individuals. In the cell attached mode, 57% of the control patches (n=70) and 56% of patches from AD cells (n=81) exhibited channel activity. Classified by conductance, we identified three types of K^+ channels of $\approx$ 25 pS(K-1), $\approx$ 115 pS(K-2) and $\approx$ 170 pS(K-3). Each channel type was found with about the same frequency in active patches from AD and control individuals (%AD/%control): K-1 = 52/62; K-2= 35/25; K-3= 41/35. Clear TEA sensitivity differences were observed, however, between AD and normal cells for the K-2 channel. The K-2 channel was blocked by 10 mM TEA in all 9 control cells explored, while the activity of the same channel from AD cells remained unchanged even after 100 mM TEA addition (n= 6), $p < 0.001$, Fisher's test. Initial results indicate that in AD cells, the K-2 channel requires significantly elevated $[Ca^{2+}]$ (2 mM) facing the inner side of the membrane, since its activity is dramatically reduced by EGTA addition. No activity was observed at $[Ca^{2+}]$ below 2 nM. EGTA removal (or Ca^{2+} elevation) restores K-2 activity. In contrast, the K-2 channel from control cells was unaffected by Ca^{2+} removal, remaining active in high as well as in low $[Ca^{2+}]$. These results indicate important differences in K^+ channel regulation in AD HON as compared to HON from control individuals. Worth noting is the lack of TEA sensitivity in AD cells. These results, together with our previous observations in fibroblasts, further indicate alterations of K^+ channel function in Alzheimer's, that could also occur in the CNS and thus contribute to memory loss early in the course of the disease.

683.2

SSCP ANALYSIS OF TRANSFORMING GROWTH FACTOR BETA 3 IN THE CHROMOSOME 14 LINKED FAD PEDIGREES

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Chromosome 14-linked Familial Alzheimer's Disease represents a genetically well defined form of AD, with early onset of symptoms and autosomal dominant transmission. However, this FAD is identical to the more common "sporadic" AD in its pathology and clinical course. Because the Chr 14 mutation primarily affects age of onset rather than significantly reshaping the pathology, transcripts from the minimal candidate region known to regulate cell status are particularly good candidate genes. One such gene, TGFB3, is located just within the distal boundary of the minimal candidate region, between D14S76 and D14S61. SSCP analysis was used to scan the seven TGFB3 exons, 1101 bp of non-nontranslated 5' transcript and 711 bp of sequence 5' to transcriptional start site, followed by sequencing of conformational polymorphisms. Rare non-pathogenic sequence polymorphisms were detected in both affected and control cases, each of these leading to silent substitutions. However, in three of the major Chr 14-linked FAD kindreds (FAD 1, 2, and 4), we found no evidence for potentially pathogenic mutations.

683.3

β-AMYLOID NEUROTOXICITY IN HYBRID NEUROBLASTOMA CELLS: ROLE OF NITRIC OXIDE. W.D.Le*, W.J.Xie, L.Colom, R.G.Smith and S.H.Appel, Dep.of Neurology, Baylor College of Med., Houston, TX 77030.

Despite many recent studies have focused on the molecular events associated with β-amyloid-mediated neuronal cell death *in vitro*, the involved mechanisms remain unknown. Data have been reported emphasizing alterations in calcium homeostasis, activation of EAA receptors, substance P receptors and serine proteases in β-amyloid-mediated neurotoxicity. In the present paper we document that nitric oxide (NO) plays an important role in synthetic β-amyloid 1-40 mediated neurotoxicity in our hybrid neuroblastoma cell line MES 23.5. Cytotoxicity was evaluated by FDA/PI vital staining and morphological assessment. Confocal microscope and the calcium sensitive dye Fluo-3 were employed to visualize changes in intracellular calcium concentration. Clear calcium increases were observed by dye Fluo-3 imagining in some cells four hours after the addition of β-amyloid to the culture. Inhibitors of VGCC had no effect on β-amyloid cytotoxicity. Removal of calcium from the medium partially reversed the toxic effects of β-amyloid as did antagonists of the NMDA receptor MK-801 and AP4. Interestingly, β-amyloid (20-40 μM) increased NO production in the cells by 3-4 fold as measured by cGMP formation. The effects of β-amyloid on NO production were calcium dependent and partially attenuated by NMDA receptor antagonist MK-801. Furthermore, NO scavengers (hemin and reduced hemoglobin) and NO synthase inhibitors (L-NMMA and L-NIO) significantly blocked β-amyloid induced NO production as well as neurotoxicity. Inhibitors of the neutral cysteine proteinase calpain, however, had no effect on β-amyloid neurotoxicity. These results suggest a critical role for a calcium-activated, possibly mediated by NMDA receptors, increased synthesis of NO as a significant mechanism in the β-amyloid induced apoptotic cell death *in vitro*.

683.4

INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) EXPRESSION IN ALZHEIMER'S MICROVESSELS. M.A. Dorheim*, W.R. Tracey, and P. Grammas. Department of Pathology, Univ. of Oklahoma HSC, Oklahoma City, OK 73104, Pharmaceutical Products Division, Abbott Labs, Abbott Park, IL 60064.

The vascular production of NO, which is potentially neurotoxic, may have important pathophysiological consequences in the central nervous system. We have previously shown increased NO production and elevated expression of endothelial cell NOS in isolated microvessels (MVs) in Alzheimer's disease (AD). Since iNOS provides quantitatively more NO over a longer period, its expression could be important in AD. The objective of this study was to determine if iNOS can be induced in the cerebral microcirculation and whether its expression is altered in AD. MVs were isolated from rat brains and human cortices from AD patients and age matched non-demented controls. MVs were homogenized and the soluble protein fraction isolated for slot blot analysis with iNOS antibody. iNOS recognizes the 130 kD and does not cross react with the constitutive isotype. Immunodetection was completed using chemiluminescence and relative iNOS expression was quantitated by scanning densitometry. Isolated rat MVs treated with IL1ß, interferon-γ, and lipopolysaccharide expressed a 3 fold increase in iNOS. AD MVs expressed twice the amount of iNOS compared to control. These data suggest that iNOS expression in brain endothelium could be a significant source of NO in AD brains. (Supported by NS grant 30457, OCAST and the Glenn Foundation).

683.5

RELATIONSHIP OF PROTEASE-RESISTANT PROTEIN, AMYLOID AND TUBULOFILAMENTOUS PARTICLES TO THE AGENT OF SPONGIFORM ENCEPHALOPATHY. H. Narang. Dept Psy. SUNY Stony Brook NY 11794.

Senile plaques, hallmarks of Alzheimer's disease (AD), are also seen in Down's syndrome, and some transmissible spongiform encephalopathies (TSEs). Histochemical studies have revealed: i) amyloid ß-protein (APP) plaques, both in AD and TSEs; ii), Protease-resistant protein (PrP) plaques observed only in TSEs. The APP and PrP are coded by two different normal genes. Unique tubulofilamentous particles (TP) seen in all TSEs have a novel three layer structure; a ssDNA lying sandwiched between two layers of protein, the inner being the PrP. Narang (Res Virol 143, 381, 1992) suggested that in the infected cell the ssDNA may code an "accessory" protein which has an ability to cleave and bind precursor PrP 33-35 into a chain of PrP 27-30. As the modified PrP molecules are added into the chain, assembly of scrapie-associated fibrils (SAF) takes place while ssDNA wraps around SAF/PrP. After an outer protein coat has been added it forms the TP. The SAF which do not acquire ssDNA and the outer protein coat accumulate to form PrP plaques.

Interlinking of ssDNA or part of a viral DNA with the host PrP or the amyloid gene can lead to the post-translation modification of the PrP or APP. An unusual palindromic six base (TACGTA)n repeat sequence was obtained from ssDNA from scrapie and bovine spongiform encephalopathy. During sequencing of the ssDNA, about 280bp of the APP751 mRNA gene was also sequenced from two of the clones. The over expression of the APP gene appears to be a critical requirement that actually contributes to AD pathology. Activation of the interlinked viral DNA with the amyloid or PrP gene would initiate excess production of APP and PrP. This would occur in transformed cells and gradually lead to plaque formation. The expression of the APP gene might be influenced by point mutations, environmental stimulus, stress and injury.

683.6

PROPENTOFYLLINE INTERFERES WITH ALZHEIMER'S DISEASE-AMYLOID- AND OX ANTIBODIES' RECOGNITION OF ACTIVATED MICROGLIA A. McRae[1]*, K. Rudolphi[2], Y. Nakamura[3], P. Schubert[3] [1]University of Goteborg, Dept Anat & Cell Biol Göteborg, Sweden 41390, [2]Hoechst AG, Frankfurt a.M., Germany, [3]Max Planck Institute of Psychiatry, Martinsried, Germany

Propentofylline (PPF) has been shown by our groups to exert neuroprotection and to depress the ischemia-induced immunostaining of microglial antigens in the gerbil brain upon in vivo treatment. In view of the presumed relevance of a pathological microglial activation for neuronal damage, we attempted to investigate possibilities of pharmacological interference and the underlying mechanisms under defined in vitro conditions. Microglial cell cultures collected from 2-3 weeks old mother cultures of newborn rats were replated and further cultivated in FCS-DMEM for 7 days in the presence or absence of 50 M PPF. Immunostainings were performed with antibodies against OX 18, OX 42 and amyloid precursor protein (APP) and with CSF from AD patients (AD CSF). Non treated microglia were intensely stained with OX 18, OX 42 and AD CSF. PPF treated microglia displayed a loss of OX 18 and AD CSF immunoreactivity. In contrast, APP immunoreactivity was slight in the non treated microglia cultures but was enhanced in PPF treated cultures. The findings indicate that prolonged exposure of activated microglial cells in culture with PPF influences their antigen properties, possibly by favoring A2 adenosine actions. Thus PPF treatment could provide a neuroprotective therapy for AD in which an ongoing activation of microglial cells is considered to be relevant for nerve cell death.

683.7

DEGENERATIVE CHANGES IN BRAIN MICROVASCULATURE AND AMYLOID ß PROTEIN IN ALZHEIMER'S DISEASE (AD) R.N. Kalaria*, D.R.D. Premkumar and P. Hedera. Departments of Neurology and Pathology, Case Western Reserve University and UH Alzheimer Center, Cleveland, Ohio 44106, USA

Considerable evidence suggests cellular abnormalities in the brain vasculature in AD. While it is unclear whether these abnormalities are a consequence of amyloid deposition, it is likely they affect perfusion or permeability functions. We used immunocytochemical markers to examine changes in the cerebral endothelium and basement membrane (BM) in relation to the presence of amyloid ß protein (Aß) and apolipoprotein E (APOE) in AD and non-AD aging control subjects. Double immunostaining with antibodies to vascular markers, CD34 and CD31, revealed absence of endothelial staining in many capillary profiles that appeared to retain their BMs stained by antibodies to collagen IV. Such differential labelling suggested capillaries with collapsed or degenerated endothelium. In addition, BMs were often seen to contain circular bodies that did not appear to be pericytes. These peculiar features were largely restricted to the neocortex and lacking in brain regions free of Aß deposits. The abnormalities, also apparent in Down's syndrome, were largely absent in other non-AD disorders. We suggest this profound vascular phenomenon is relatively specific to brain amyloidosis that implies anomalies in integrity of brain microvasculature in AD. Supported by grants from NIA and ADRDA.

683.8

VITAMIN E DELAYS ONSET OF NEUROLOGIC DISORDER IN TRANSGENIC (TG) MICE EXPRESSING HUMAN AMYLOID PRECURSOR PROTEIN (APP) VARIANTS. K. K. Hsiao, R. Johannsdottir, W. Yunis, and H. B. Clark*. Departments of Neurology and Pathology, University of Minnesota, Minneapolis, MN 55455

We created Tg mice expressing human APP variants that exhibited a progressive neurologic disorder manifesting as an increase in behaviors characteristic for fear and apathy, accompanied by hypertrophic astrocytic gliosis with diminished glucose metabolism in the hippocampal formation, parahippocampal area, amygdala, and cerebral cortex. No extracellular amyloid deposition, neurofibrillary tangle formation, or neuronal loss was observed. Although the morphological substrate for neural dysfunction in these mice has not yet been defined, their behavioral and pathologic abnormalities resembled the age-dependent changes that we observed in some aged non-Tg mice within the same FVB strain, suggesting that aberrant expression of APP in Tg mice may accelerate age-dependent neural dysfunction in a manner characteristic for the specific strain of mouse under study. Vitamin E was administered intraperitoneally to animals in two affected Tg lines. Animals receiving vitamin E remained healthy significantly longer than control mice ($p<0.001$). Since vitamin E is a free-radical scavenger, these results indicate that free-radical formation may be involved in the pathogenesis of then neurologic disorder in these Tg mice. Whether the protective effect of vitamin E is due to its ability to ameliorate a harmful activity of APP or cause a general deceleration of the aging process is unknown.

683.9

DISTRIBUTION OF APOLIPOPROTEIN E PHENOTYPES IN ALZHEIMER'S DISEASE. Dale M. VanderPutten*[1,2], Ginger S. Johnson[3], Carl R. Merril[1]. [1]Laboratory of Biochemical Genetics, NIMH, Washington, DC 20032, [2]Monoclonetics Int. Inc., Houston, TX 77027, [3]Molecular Geriatrics Inc., Lake Bluff, IL 60044

We have confirmed a risk of developing Alzheimer's disease (AD) associated with the apolipoprotein E4 (apoE4) allele by phenotypic analysis of apoE4 protein in plasma, cerebrospinal fluid (CSF) and brain ($z = 4.6$). We have found significantly ($p < 0.001$) lower levels of apoE in AD patients' CSF but not in plasma, frontal cortex or hippocampus. ApoE4 levels in apoE 4/4 homozygotic AD CSF were not different from normal control levels. Analysis of apoE 3/4 heterozygotic individuals showed differences between apoE 3 and 4 levels in AD CSF but not AD plasma or normal CSF. Quantitative RT-PCR analysis suggested the variation in relative amounts of apoE 3 and 4 in CSF results from differential metabolism apoE 3 and 4 proteins in the AD CNS. We hypothesize this difference in apoE metabolism may be an important factor in the development of AD.

683.10

NEURONAL APOLIPOPROTEIN E IMMUNOREACTIVITY IS INCREASED IN PICK'S DISEASE. N.W. Kowall*[1,2], C. Abraham[2] and A.C. McKee.[1,2]. [1]Geriatric Research Education Clinical Center, Bedford VAMC, Bedford MA 01730; [2]Depts. of Pathology, Medicine, & Neurology, Boston U. Sch. of Med.

In the normal central nervous system, apolipoprotein E (ApoE) is synthesized and secreted by astrocytes, taken up by neurons and transported down axons. One of the characteristic histological features of Pick's disease is the presence of ballooned Pick Cells that may develop as a result of impaired axonal transport. We defined the distribution of ApoE in samples of affected cerebral cortex from 13 patients with Pick's disease using specific anti-human ApoE antibodies. In some cases prominent ApoE immunoreactivity was present in neurons – including ballooned Pick cells and Pick bodies which contain phosphorylated tau and neurofilament. Staining was confined to astrocytes in cases with profound neuronal loss. ApoE immunoreactivity was also associated with parenchymal and vascular amyloid deposits when present. The accumulation of ApoE within damaged neurons, its association with phosphorylated cytoskeletal proteins, and the over-represention of the E4 allele in these patients, strongly supports a role for apoE in the pathogenesis of Pick's disease.

683.11

APOLIPOPROTEIN E EXPRESSION AND EFFECTS OF DENERVATION AT NEUROMUSCULAR JUNCTIONS IN MOUSE SKELETAL MUSCLE. M. Akaaboune, M. Villanova, B.W. Festoff, M. Verdière-Sahuqué and D. Hantaï*. INSERM U.153 and Université Paris VI, 75005 Paris, France; KCVAMC and KUMC, Kansas City, MO 64128 and KS 66160.

Apolipoprotein E (ApoE) allele E4 has been associated with late onset familial Alzheimer's disease (AD). In AD brain, ApoE is localized to the cerebrovascular amyloid plaque and neurofibrillary tangles. In a previous study we demonstrated that β-amyloid protein precursor and α_1-antichymotrypsin, found accumulated in senile plaques in AD brain, were localized at neuromuscular junctions (NMJ). In the current study we asked whether ApoE was also localized at NMJ and was affected by denervation, using immunocytochemistry and Western blotting. The results indicate that ApoE was present in normal muscle at the NMJ, within intramuscular nerves, and on endothelial cell surfaces. Following axotomy, ApoE disappeared rapidly from intramuscular nerves. However, at the NMJ, ApoE lingered several days before disappearing. Such experiments in muscle after denervation may shed light on the mechanism of synapse loss as well as plaque deposition in AD.

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683.12

OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IS INCREASED IN ALZHEIMER'S DISEASE. M.F. Beal,* U. MacGarvey, and P. Mecocci. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Oxidative damage to DNA may play a role in both normal aging and in neurodegenerative diseases. The mitochondria are the major source of free radicals in the cell, and recent evidence has shown decreases in cytochrome oxidase activity in Alzheimer's disease (AD), both in platelets and in cerebral cortex. Cytochrome oxidase inhibition leads to increased free radical generation in isolated mitochondria. Prior studies showed evidence for increased lipid peroxidation in AD postmortem brain tissues. We examined whether AD is associated with increased oxidative damage to both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) in postmortem brain tissue. We measured the oxidized nucleoside, 8-hydroxy-2'-deoxyguanosine (OH^8dG), in DNA isolated from 3 regions of cerebral cortex and cerebellum in 13 AD and 13 age-matched controls. There was a significant 3-fold increase in the amount of OH^8dG in mtDNA in parietal cortex of AD patients as compared with controls. In the entire group of samples there was a small significant increase in oxidative damage to nDNA and a highly significant 3-fold increase in oxidative damage to mtDNA in AD as compared with age-matched controls. These results confirm that mitochondrial DNA is particularly sensitive to oxidative damage, and they show that there is increased oxidative damage to DNA in AD, which may contribute to the neurodegenerative process.

683.13

OVERREPRESENTATION OF CYP2D6B MUTANT ALLELE IN LEWY BODY VARIANT OF ALZHEIMER'S DISEASE. Tsunao Saitoh*, Yu Xia, Xiaohua Chen, Eliezer Masliah, Richard DeTeresa, Michael Alford, Douglas Galasko, Clifford Shults, Leon J. Thal, Lawrence Hansen, and Robert Katzman. Department of Neurosciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0624

Approximately one-quarter of neuropathologically confirmed cases of Alzheimer's disease (AD) also have brainstem and neocortical Lewy bodies, constituting a Lewy Body Variant (LBV) of AD. Since Lewy bodies are a pathologic hallmark of idiopathic Parkinson's disease (PD), this sub-population of AD patients may have the risk factors shared by PD patients, in addition to the risk factors for AD. Analyses of cytochrome P450 CYP2D6-debrisoquine 4-hydroxylase mutant B allele (CYP2D6B), susceptibility gene for PD, revealed an association of this allele with the LBV of AD. Further, it was found that patients with the CYP2D6B allele have less developed AD neuropathology than those with the wild type CYP2D6 allele. On the other hand, analyses of apoliprotein E allele 4 (ApoE ε4), susceptibility gene for AD, revealed an association of this allele with the LBV of AD. Thus, genetically, LBV is like AD and PD at the same time. The association of CYP2D6B with the LBV of AD and its effect on neuropathology implies that this subpopulation of AD patients may require therapeutic strategies different from those applied to pure AD.

DRUGS OF ABUSE: ADDICTION/TOLERANCE

684.1

THE RELATIONSHIP BETWEEN OPIOID AGONIST INTRINSIC EFFICACY AND TOLERANCE. Alokesh Duttaroy, Ka Wa Chan, Sukrut Shah and Byron C. Yoburn*. College of Pharmacy, St. John's University, Queens, NY 11439.

Tolerance is a reliable consequence of repeated exposure to opioid agonists. Furthermore, the magnitude of tolerance has been shown to be dependent upon agonist intrinsic efficacy. Continuous infusions of high intrinsic efficacy compounds (*e.g.*, fentanyl) produce less tolerance than infusions of lower intrinsic efficacy drugs (*e.g.*, morphine). However, it has been reported that when opioid analgesics are administered intermittently the degree of tolerance is not significantly affected by intrinsic efficacy (Duttaroy *et al.*, *FASEB J.*, 7: A704, 1993). In the present study the role of intrinsic efficacy was investigated by comparing intermittent and continuous administration dosing protocols using 3 opioid agonists (morphine, etorphine and fentanyl). In the continuous administration study, mice were infused s.c. (osmotic minipumps) with 5 - 40 times the ED_{50} of each drug for 168hrs; the pumps were removed and 24hrs later mice were tested for analgesia (tailflick) in cumulative dose-response studies. Controls were implanted s.c. with inert placebo pellets. With increasing infusion doses, the magnitude of tolerance increased for all three analgesics. However, maximal tolerance of $\approx$ 3 fold was observed for fentanyl and etorphine at 40 times the ED_{50}; whereas $\approx$ 4 fold tolerance was produced by an infusion 10 times the ED_{50} for morphine. In another study, the effect of intermittent administration of morphine and etorphine on analgesic tolerance was examined. Mice were injected s.c. once per day for 7 days with saline or 10 times the ED_{50} for morphine or etorphine; 24hrs following the last injection mice were tested for analgesia. Although both morphine and etorphine produced tolerance, the degree of tolerance was similar for both drugs. Intermittent morphine produced a 1.7-fold shift in the ED_{50}, while intermittent etorphine produced a 2.1-fold shift in the ED_{50}. These results indicate that under conditions of continuous administration intrinsic efficacy is a determinant of the magnitude of tolerance to opioid analgesics. However, when opioid agonists are administered intermittently, intrinsic efficacy appears to have little impact on tolerance. (Supported by NIDA DA 04185)

684.2

COMPARISON OF THE ATTENUATION OF THE OPIOID WITHDRAWAL SYNDROME BY 7-NITROINDAZOLE, A BRAIN-SPECIFIC NITRIC OXIDE SYNTHASE INHIBITOR, WITH THAT OF OTHER NITRIC OXIDE SYNTHASE INHIBITORS. A.S. Kimes*[1], D.B. Vaupel[1], and E.D. London[1,2,3]. NIDA IRP, NIH, Balto., MD, 21224; [2]Dept. Radiology, Johns Hopkins Sch. Med., Balto., MD 21204; and [3]Dept. Pharmacol. Exp. Ther., Univ. MD Sch. Med., Balto., MD 21201.

Inhibitors of nitric oxide synthase (NOS) attenuate some signs of opioid withdrawal (Kimes et al., Psychopharmacology 112:521, 1993). The purpose of the present study was to compare the potency of a brain-specific NOS inhibitor, 7-nitroindazole (7-NI) with that of less selective NOS inhibitors, L-N^G-nitroarginine (L-NNA), L-N^G-nitroarginine methyl ester (L-NAME), and N(5)-(1-iminoethyl)-L-ornithine (L-NIO). We also compared the attenuation of the withdrawal by NOS inhibitors to that produced by clonidine. Morphine dependence was produced by the subcutaneous implantation of 1 morphine pellet (75 mg) on Day 1. On Day 4, the following drugs (doses in mg/kg) were administered 1 h before naloxone challenge (0.5 mg/kg): L-NNA (1, 3, 10, 18 & 30); L-NAME (10, 18, 30, & 56); 7-NI (1.8, 5.6, 18, 30, 56, & 100); L-NIO (1, 10, 56, 100 & 300); clonidine (0.01, 0.03, & 0.1). All of the NOS inhibitors decrease wet dog shakes, weight loss, diarrhea, and grooming. The selective NOS inhibitor, 7-NI also attenuates the expression of other signs (mastication, salivation, and penis licks/ejaculations), the expression of which are either unchanged or increased by the less selective NOS inhibitors. The expression of many signs are affected similarly by clonidine and 7-NI (weight loss, diarrhea, wet dog shakes, grooming and mastication), but clonidine uniquely attenuates the expression of abnormal posturing. The potencies of the NOS inhibitors to reduce the frequency of wet dog shakes during opioid withdrawal were similar for 7-NI and L-NAME. L-NNA was almost 4-fold more potent, and L-NIO was less than half as potent as either 7-NI and L-NAME. In conclusion, our results show that NOS inhibitors attenuate withdrawal at least as effectively, but not as potently, as clonidine, and suggest that 7-NI, which does not increase blood pressure, may be a good candidate for human use.

684.3

NALOXONAZINE PRETREATMENT IMPAIRS MORPHINE-INDUCED ACQUISITION OF PLACE-PREFERENCE BUT NOT STIMULATION OF DOPAMINE RELEASE Tanda G., R.Longoni, L.Spina, E.Carboni* and G. Di Chiara Dpt. of Toxicology Univ.of Cagliari (Italy)

Although opiates stimulate dopamine (DA) release and the firing activity of DA units, the role of DA in opiate reinforcement is debated. We now report that naloxonazine (15 mg/Kg i.p. 20 h before) fails to modify the stimulation of DA release in the n. accumbens elicited by morphine (1.0 and 5.0 mg/kg s.c.) but prevents the acquisition of place-preference induced by a single pairing with morphine. The results suggest that DA does not mediate the primary reinforcing properties of opiates. However, since naloxonazine fails to extinguish heroin self-administration (Negus et al. JPET, 265, 1249, 1993) DA might act as a secondary reinforcer to maintain opiate self-administration.

684.4

ANABOLIC ANDROGENIC STEROIDS: AN ASSESSMENT OF THEIR ADDICTIVE POTENTIAL. A.R. Lumia, S.T. Diekman and M.Y. McGinnis* Biopsychology Program, Skidmore College, Saratoga Springs, NY 12866 and Dept. of Cell Biology and Anatomy, Mount. Sinai School of Medicine, New York, NY 10029.

We employed a three-chambered place preference test apparatus to examine the reinforcing and addictive potential of anabolic androgenic steroids (AAS) in adult Long-Evans male rats. Testing consisted of three phases. First, all rats received a pretest which allowed each animal 20 min to explore all three chambers and establish a place preference. For the second (conditioning) phase, the animals were divided into two groups: an experimental group receiving twelve daily injections of 1mg testosterone propionate (TP) alternating with propylene glycol (PG), and a control group receiving only PG. Injections were given 15 min prior to placement in a chamber associated with either TP or PG for 45 min. In the third phase the rats received two place preference tests. In the first test all rats received PG and time spent in each chamber was recorded. In the second test they were given either TP or PG and tested again for place preference. Results showed that rats receiving TP paired with a particular chamber spent significantly more time in that chamber. PG-treated rats did not show a place preference. The potential for AAS addiction is suggested by the findings that 1) rats can discriminate the presence of AAS from a control substance, and 2) that AAS are positively reinforcing in a place preference test. (supported by a grant from the Harry Frank Guggenheim Foundation)

684.5

EVIDENCE THAT IBOGAINE INHIBITS DOPAMINE RELEASE VIA A KAPPA RECEPTOR MECHANISM. Malcolm S. Reid*, Kang Hsu, Patricia Broderick+, S. Paul Berger. UCSF/VAMC, Substance Abuse Treatment Research 116W, 4150 Clement St., San Francisco, CA 94121; +Pharmacology Dept., CUNY Medical School, 138th St and Convent Ave., New York, NY 10031.

Ibogaine has been proposed as a clinical treatment for stimulant and opiate addiction. In the present study *in vivo* microdialysis was used to determine the effects of ibogaine on the extracellular levels of dopamine in the nucleus accumbens and striatum of freely moving Sprague-Dawley rats. In addition, the effects of the non-selective opiate antagonist naloxone and the kappa receptor opiate antagonist norbinaltorphimine (NorBNI), alone and in combination with ibogaine, were also studied. All drugs were tested locally by perfusing them through the microdialysis probe. Ibogaine was tested at several doses (10-6M to 10-3M) and it was found that it produced a biphasic dose-response curve, whereby lower doses (10-6M to 10-4M) produced a dose-dependent decrease in dopamine levels while higher doses (5x10-4M and 10-3M) produced an increase in dopamine levels, seen in both the nucleus accumbens and striatum. Naloxone and NorBNI (10-6M to 10-4M) had no effects on dopamine, except the highest dose of naloxone (10-4M) which produced an increase in dopamine levels. Co-administration of naloxone or NorBNI (10-6M or 10-4M) with ibogaine (10-4M) blocked the decrease in dopamine levels produced by ibogaine alone, suggesting that the ability of ibogaine to inhibit dopamine release is mediated by kappa opiate receptors. Further studies on the dopamine stimulatory properties of ibogaine are underway.

684.6

Δ^9-TETRAHYDROCANNABINOL AND THE SYNTHETIC CANNABINOID CP-55,940 INDUCE EXPRESSION OF *c-FOS* mRNA IN STRESS-RESPONSIVE NUCLEI OF RAT BRAIN. M. Herkenham* and L. S. Brady. Section on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Little is known about the functional consequences of acute cannabinoid (marijuana) intoxication. Rats were injected acutely i.p. with Δ^9-tetrahydrocannabinol (10 mg/kg) or the synthetic cannabinoid CP-55,940 (1, 3, or 10 mg/kg) and sacrificed 40 min later. Brains were processed for *in situ* hybridization of the riboprobe for the immediate-early gene *c-fos*. Induction of *c-fos* mRNA expression occurred in a set of structures that comprise the central effectors of the stress response—paraventricular nucleus of the hypothalamus, anterior lobe of the pituitary, locus coeruleus, ventral lateral septum, central nucleus of the amygdala, and paraventricular nucleus of the thalamus. The two drugs produced similar patterns of responses, and responses to CP-55,940 were somewhat dose-dependent. Motor structures, notably those containing dense cannabinoid receptors, did not show elevated *c-fos* mRNA levels. Thus the activated structures reflect the animals' psychological (cannabinoids elevate plasma corticosterone and appear to be stressors in rats) and motoric states (cataleptic) and not the primary sites of action of the drug.

684.7

DELETION OF ß-ENDORPHIN IN THE MOUSE BY TARGETED GENE MUTAGENESIS. M. Rubinstein, M. Japón, E. C. Chan, R. Allen*, and M. J. Low, The Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201, U.S.A.

The endogenous opioid peptides are proposed to modulate neuroendocrine circuits, autonomic reflexes, analgesia, memory, and learning through binding to a family of G-protein coupled receptors. Our laboratory has initiated a program to test the function of the individual components of the endogenous opioid system by gene targeting in embryonic stem cells. ß-endorphin is produced in three discrete areas; the arcuate nucleus, the nucleus tractus solitarius, and the pituitary by the posttranslational processing of proopiomelanocortin (POMC). Because POMC is also important as a precursor for ACTH and melanocyte stimulating hormones (MSH), we designed a gene targeting vector that encodes a truncated POMC prohormone selectively deficient in the ß-endorphin peptide sequences (Nucleic Acids Research 21:2613-2617, 1993). Mice homozygous for the mutated POMC allele are developmentally normal, grow to maturity and reproduce. HPLC chromatography and RIA of hypothalamic and pituitary extracts show that the truncated POMC prohormone is authentically processed to ACTH and MSH in the total absence of ß-endorphin-like immunoreactivity. As a result, the mice have normal hypothalamic-pituitary-adrenal function under basal and restraint-stress induced conditions. The neurons that normally produce ß-endorphin are intact and the wide-spread fiber distribution from these neurons is also normal and detectable with an antiserum to ACTH on brain sections. Preliminary data suggest that swim-stress induced analgesia is unaltered in the ß-endorphin deficient mice. Additional behavioral tests are ongoing. We conclude from these studies that ß-endorphin is not an essential neuropeptide for development of the nervous system or organization of the neuroendocrine circuits controlling the hypothalamic-pituitary-gonadal and adrenal axes. The lack of an obvious nociceptive phenotype suggests that enkephalins, dynorphins, and possibly nonopioid mechanisms are either sufficient for analgesia or can compensate for the loss of ß-endorphin in the 129SV/EV X C57Bl/6N genetic background.

685.1

EXCITOTOXIC-TYPE NECROSIS IN THALAMUS PRECEDES MOTOR SEIZURES IN A RAT MODEL OF WERNICKE'S ENCEPHALOPATHY. P.J. Langlais*, S.X. Zhang, G.S. Weilersbacher, T. Corso, and J.W. Olney. Psychology Dept., San Diego State University, San Diego, CA, 92182 and Psychiatry Dept., Washington University. Sch. Med., St. Louis, MO, 63110.

In the pyrithiamine-induced thiamine deficient (PTD) rat model of Wernicke's encephalopathy, thalamic necrosis has been linked to elevated extracellular glutamate (Langlais & Zhang, 1993) and activation of the NMDA glutamate receptor (Langlais & Mair, 1990). Motor seizures are frequently observed in late stages of PTD treatment. We undertook the current study to determine the role of seizures in thalamic necrosis. In PTD rats perfused prior to onset of motor seizures, electron microscopic evaluation revealed cytomorphologic changes of neurons in the anteroventral (AV) and gelatinosus (Ge) thalamic nuclei that are consistent with a glutamate excitotoxic process. In PTD rats administered thiamine prior to seizures and recovered for 7 days there was significant neuronal loss in the ventroposterolateral (VPL), AV, and Ge nuclei. In animals given thiamine after onset of seizures, necrosis was more severe in VPL and was evident in midline and intralaminar regions. These observations demonstrate that seizures may aggravate but are not prerequisite to excitotoxic necrosis and early administration of thiamine can prevent more widespread damage. We propose that the critical events leading to excitotoxic necrosis are thiamine deficiency-induced reductions of energy production, elevated glutamate levels, and histamine-enhanced NMDA receptor activity. Supported by AA 07466, AG05681, MH14677, NS29481, and RSA MH38894.

685.2

HYPERACTIVATION OF GLUTAMATE PATHWAYS INDUCES THALAMIC CYTOPATHOLOGY RESEMBLING WERNICKE-KORSAKOFF ENCEPHALOPATHY. JW Olney*, NB Farber, T. Corso, J Labruyere, SX Zhang, PJ Langlais. Washington Univ., St. Louis MO 63110 and San Diego State University, San Diego, CA 92182.

Wernicke-Korsakoff encephalopathy (WKE) is a brain damage syndrome associated with alcoholism and thiamine deficiency (TD). Feeding a thiamine deficient diet to rats and treating them simultaneously with pyrithiamine induces a TD brain damage syndrome that closely resembles both the pattern and type of cytopathology seen in WKE. Acute neuron-necrotizing lesions in various thalamic nuclei is a conspicuous feature of WKE in humans and of the TD syndrome in rats. Langlais et al (1990,1993) have shown in the TD rat that there is a striking elevation of extracellular glutamate (Glu) in vulnerable thalamic regions as the lesions are developing, and that these lesions can be prevented by pretreatment with MK-801, a powerful antagonist of NMDA Glu receptors. Collins & Olney (1982) have shown that acute lesions can be induced in the ventral posterolateral (VPL) nucleus of the thalamus by hyperactivation of cerebrocortical neurons that project to VPL and use Glu as transmitter; these lesions can be prevented by MK-801 pretreatment (Clifford et al., 1989). In the present study, we have performed an electronmicroscopic analysis of the acute cytopathology induced in VPL of the TD rat and compared it with acute cytopathology induced in VPL by hyperactivation of Glu-ergic corticothalamic afferents to VPL. In both of these syndromes the cytopathological reaction was found to have the characteristic features of Glu-induced excitotoxicity. Acute edematous swelling of presynaptic dendrites and neuronal cell bodies, degenerative changes in mitochondria and endoplasmic reticulum and clumping of nuclear chromatin typifies both syndromes, as does sparing of presynaptic axon terminals. These findings are consistent with the hypothesis that brain damage in human alcoholics may be mediated by an unleashing of the excitotoxic potential of endogenous Glu. Supported by AA 07466, AG 05681, MH 14677, NS 29481 and RSA MH 38894.

685.3

ABNORMAL MITOCHONDRIAL MEMBRANE POTENTIAL IN HUMAN NEUROLOGICAL DISORDERS. S.D. Handran*, A.M. Moudy, M.P. Goldberg, D.C. DeVivo[a], S.M. Rothman. Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110 and [a]The Neurological Institute of New York, NY 10032.

Several neurodegenerative diseases are characterized by selective neuronal losses which may result from a defect in energy metabolism. A working hypothesis states that an underlying impairment of mitochondrial function may result in decreased ATP production, increased glutamatergic receptor activation, and loss of intracellular calcium homeostasis (Beal et al, *TINS* 16:125, 1993). We investigated human fibroblasts obtained from patients with mt-DNA mutations that directly result in impaired energy metabolism. We have previously shown (*Soc. Neurosci. Abstr.* 19:1502, 1993) abnormal calcium homeostasis in fibroblasts from patients with MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes). We have extended this investigation by measuring mitochondrial membrane potential with the dual emission fluorescence dye JC-1 and confocal microscopy. Ratio images were analyzed by dividing the 590 nm emission (potential sensitive) by the 527 nm emission (potential insensitive). MELAS fibroblasts had significantly decreased mitochondrial membrane potential relative to control patients ($p=0.002$, Mann-Whitney) as determined by measuring the area of polarized membrane (having ratio values ≥ 1) to total mitochondrial area. This decreased membrane potential may compromise the ability of the mitochondrion to contribute to cytosolic calcium homeostasis, possibly through diminished calcium uptake. Chronic impairment in the neurons of patients with these and other neurodegenerative disorders may result in the disease phenotype. Supported by NS19988 and NIH5-T32NS07057-15.

685.4

A LYSO GM1 DERIVATIVE (LIGA20) GIVEN ORALLY AFTER CORTICAL THROMBOSIS REDUCES INFARCT SIZE AND ASSOCIATED COGNITION DEFICIT. Guidotti, A*; Kharlamov, A., Zivkovic, I. and Costa, E.; FGIN, Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, D.C. 20007

A bilateral, photochemically induced thrombotic lesion of rat sensorimotor cortex (approximately 3 mm in diameter and 25 mm^3 in volume) is associated with a persistent cognition (learning and memory) deficit which was evaluated with water maze tasks. The semisynthetic N-dichloroacetyl sphingosine derivative of lyso GM1 (LIGA20) administered after the lesion either i.v. or p.o. reduced the infarct size by 30 to 40% and attenuated the associated cognition deficit by limiting the extent of damage of neurons at risk located in the area surrounding the infarcted core (i.e., area penumbra). The LIGA20 protection was dose and time dependent. Maximal protection was afforded by a single injection of 34 μmol/kg of LIGA20, i.v., 1 hr after lesion or by 270 μmol/kg, p.o. administered 1 and 24 hr after the lesion. The protective effect of LIGA20 was observed when the drug was administered i.v. up to 6 hr after the lesion. The protective efficacy of the oral administration of LIGA20 maybe related to its physicochemical properties (molecular area, surface potential, collapse pressure, dipole moment) which, unlike those of GM1, allow absorption from the gastrointestinal tract. LIGA20 given orally reaches the brain promptly and is rapidly inserted into the neuronal membranes. In the brain LIGA20 does not reduce Ca^{2+} influx but selectively inhibits the pathological amplification of Ca^{2+} signalling elicited by persistent stimulation of ionotropic glutamate receptors in the area penumbra by a yet unknown molecular mechanism.

685.5

STRIATAL TOXICITY OF 3-NITROPROPIONIC ACID PREVENTED BY THE AMPA ANTAGONIST NBQX. L.Turski* and H.Ikonomidou . Research Labs of Schering AG, Berlin, Germany and St. Louis Children's Hospital, St. Louis, USA.

3-Nitropropionic acid (3-NP), which irreversibly blocks succinate dehydrogenase (complex II), is reported to be a selective striatal neurotoxin in rodents and primates. Chronic systemic administration of 3-NP to rats leads to low grade metabolic disturbances in the striatum and to striatal neuron damage (sparing NADPH-diaphorase containing neurons). These observations support the hypothesis that disturbances in mitochondrial energy metabolism can lead to axon-sparing glutamate-like toxicity in the brain and that such processes may be involved in the pathogenesis of chronic neurodegenerative disorders in humans.

Aged (18-24 months old) Wistar rats were subjected to a chronic (over 28 d) continuous treatment with 3-NP (24 mg/kg/d) or with 3-NP and the competitive AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepro-pionate) antagonist NBQX (6-nitro-7-sulphamoyl-benzo[f]quinoxaline-2,3-dione; 24 mg/kg/d) by means of osmotic minipumps implanted i.p. Seven days after discontinuation of the treatment with 3-NP or 3-NP and NBQX, the rats were sacrificed by transaortic perfusion-fixation and the brains were serially sectioned and prepared for light microscopy. Two out of eight 3-NP and vehicle treated rats died in the course of chronic treatment. Morphological analysis of the brains from six remaining rats treated with 3-NP and vehicle showed consistent damage in the striatum and less damage in the hippocampus and cortex of three animals. Treatment of seven additional rats with 3-NP and concurrently with NBQX did not produce lethality and brain damage. These observations suggest that AMPA antagonists may be of benefit for preventing chronic degeneration triggered by 3-NP.

685.6

TRAUMA CAUSES GLUTAMATE-TYPE CYTOPATHOLOGY IN INFANT RAT BRAIN. C. Ikonomidou*, Y. Q. Qin, J. Labruyere and J. W. Olney: Depts. of Psychiatry and Pediatric Neurology, Washington University, St. Louis, MO 63110.

Glutamate (Glu) is an excitatory neurotransmitter in the mammalian CNS, but it also has neurotoxic properties when administered systemically to infant animals. In rats we have shown that infants are much more susceptible than adults to the excitotoxic actions of exogenous Glu, NMDA or to hypoxia-ischemia, and that peak sensitivity to these conditions occurs between postnatal days 6 and 10. In this study we explored the neuropathologic changes that follow contusion injury to the infant rat brain at the age of peak sensitivity to Glu excitotoxicity. The contusing device consisted of a perforated stainless-steel tube 40 cm long that guided a falling weight onto a footplate resting upon the infant skull (at 3mm anterior and 2 mm lateral to lambda). A 1 mm footplate diameter, a 2.5 mm depression of the skull surface and a force of 165 g/cm were the selected conditions. Animals were anesthetized and percardiac perfusion fixed at 0, 2 and 6hrs after trauma. Histologic analysis revealed focal damage at the point of impact and this was surrounded by a zone of neuronal necrosis that progressively expanded over a period of 6 hrs. The cytopathologic changes consisted of swollen dendrites, degenerating neurons with pyknotic nuclei and markedly swollen cytoplasm (bulls eye profiles), and dark cells with vacuolated cytoplasm. Electronmicroscopic analysis revealed that these lesions were of the axon-sparing type and resembled in all aspects lesions caused by exogenous Glu. The NMDA antagonist MK-801, when administered 15 min before or 15 min after the trauma, significantly inhibited development of Glu-type pathology around the trauma site. We conclude that traumatic injury in the infant rat brain triggers excessive release of endogenous Glu followed by a cascade of events resulting in excitotoxic neuronal degeneration. Glu antagonists may prove beneficial in preventing brain damage associated with head trauma in pediatric patients. Supported by AA 07466, RSA MH 38894 (JWO) and a grant from Schering AG.

685.7

EXCITATORY AMINO ACID ANTAGONISTS DECREASE GANGLION CELL DEATH FOLLOWING OPTIC NERVE TRANSECTION IN ADULT RATS.

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Damage to the adult mammalian optic nerve produces retrograde degeneration of >95% of all retinal ganglion cells (RGCs) within four weeks. Work from our laboratory suggests that axotomy induced degenerative processes may include an excitotoxic component. This study tested the excitotoxic hypothesis of RGC death by examining the neuroprotective efficacy of the NMDA antagonist MK-801 and the AMPA/Kainate antagonist CNQX on RGCs following optic nerve transection. RGCs were retrogradely labeled by injection of the carbocyanine dye DiI in their lateral geniculate nuclei. Four days later both optic nerves were transected in two groups, one that received 5µl intravitreal injections of MK-801 and CNQX, and a 2nd group received saline injections. RGC density 28 and 90 days after transection was determined from wholemounts at 400X for n=6 retina per group. Florescent double labeling by SMI-31 phosphorylated neurofilament immunohistochemistry was used to examine the reaction of the ganglion cell fiber layer in retina from each group.

MK-801/CNQX treatment did not induce abnormal intra-retinal RGC axon sprouting. Treatment with MK-801/CNQX did provide significant protection of RGCs at 28 days (9% to 33.5% of all RGCs), but by 90 days the RGC density in treated cases was identical to that in non-treated optic nerve transection (ONX) cases.

28 days	RGCs / mm^2)	% Lost	% Spared
Normal	1209 ± 312	-	-
ONX	61 ± 25*	95%	5.0%
MK-801 + CNQX	239 ± 120[1,2]	80.2%	19.8%

*ONX < Normal, p<.0001; Normal > MK801/CNQX >ONX, p<.0001

These results are consistent with the presence of an excitotoxic component in the process of axotomy induced retinal ganglion cell death which may involve both NMDA and AMPA type EAA receptors. (Supported by NS 28852 and NS 08803).

685.8

GLUTAMATE RECEPTOR MEDIATED DOPAMINERGIC TOXICITY DUE TO SDH INHIBITION

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Metabolic deficiencies have been detected in patients with Huntington's, Parkinson's and Alzheimer's Diseases suggesting a metabolic defect as a common etiological feature. Several laboratories have shown that intrastriatal administration of an irreversible succinate dehydrogenase (SDH) inhibitor, 3-nitropropionic acid (3-NPA), or reversible SDH inhibitor, malonate, produced lesions that were "excitotoxic", further suggesting that compromised metabolism could trigger an excitotoxicity (J. Neurochem. 61:1147 & 1151, 1993; J. Neurosci.13:4181, 1993).In this study, we examined the possible involvement of glutamate receptors in metabolic stress-induced loss of dopaminergic (DA) neurons from rat mesencephalic (MN) culture. MN neurons were plated in DMEM plus 10%FBS/10%horse serum. Previous studies showed that DA neurons were sensitive to medium change but could be maintained for up to 3 weeks if fed with conditioned medium [CM; obtained from MN cultures at 6 days in vitro (div)]. Prior to metabolic stress studies, glutamate receptor mediated toxicity was assessed. At 12div, 10-500µM NMDA or kainate (KA) was added to the cultures. After 24 hr, the medium was replaced with CM, allowed to recover for 48 hr and then examined for ^{3}H-DA uptake. NMDA and KA caused a dose dependent loss of DA uptake (EC_{50} 16 and 26µM, respectively). Staining for tyrosine hydroxylase positive cells confirmed cell loss. In metabolic stress studies, cultures were treated with 0.1-0.5mM 3-NPA. Inhibition of SDH produced a dose dependent loss of ^{3}H-DA uptake (EC_{50} 0.18mM). Coincubation with the NMDA antagonist MK-801 (1µM) provided nearly complete protection vs. 0.25mM 3-NPA and significant, but less, protection vs. 0.5mM 3-NPA. The nonNMDA antagonist, CNQX (15µM), was not protective. These studies demonstrate the sensitivity of cultured neurons to NMDA and KA toxicity and further show that metabolically stressing DA neurons results in neurotoxicity that is mediated in part by the NMDA receptor.

685.9

GLUTAMATE-ELICITED $[Na^+]_i$ INCREASE IMPAIRS NEURONAL CALCIUM BUFFERING BY MITOCHONDRIA.

L. Kiedrowski* and E. Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington, DC 20007.

An exposure of primary cultures of cerebellar granule cells to glutamate (50 µM) applied in a Mg^{2+}-free medium for 4 min increases somatic $[Na^+]_i$ up to 100 mM and after glutamate removal $[Na^+]_i$ fails to decrease promptly. In contrast, $[Ca^{2+}]_i$, after an initial increase to about 5 µM, decreases to 1.5 µM during glutamate exposure. Moreover, a sudden drop in $[Ca^{2+}]_i$ to 350 nM is observed immediately after glutamate removal when $[Na^+]_i$ is still elevated. These data indicate that mechanisms other than the plasmalemmal Na^+/Ca^{2+} exchanger are responsible for these decreases in somatic $[Ca^{2+}]_i$. Thapsigargin (1 µM) fails to affect the $[Ca^{2+}]_i$ decrease during and after glutamate exposure indicating that intracellular Ca^{2+} stores controlled by endoplasmic Ca^{2+} ATPase do not contribute significantly to Ca^{2+} buffering. In contrast to thapsigargin, mitochondrial inhibitors CCCP (1 µM) and antimycin A1 (1 µM) evoke large increases in $[Ca^{2+}]_i$, up to several µM, when applied during or immediately after glutamate exposure. Both CCCP and antimycin A1 fail to affect $[Ca^{2+}]_i$ when applied without glutamate or 15 min after glutamate removal when the basal $[Ca^{2+}]_i$ is restored. When after glutamate removal $[Na^+]_i$ is artificially decreased to 5 mM, by application of external Na^+ (5 mM) with gramicidin (5 µM), $[Ca^{2+}]_i$ drops to 150 nM and then increases to 350 nM when 100 mM Na^+ is applied. These $[Na^+]_i$-dependent changes in $[Ca^{2+}]_i$ are observed also in a Ca^{2+}-free medium. Our data suggest that the glutamate-evoked $[Na^+]_i$ increase, which seems to be associated with a decrease in $[K^+]_i$, reduces the efficacy of Ca^{2+} buffering by mitochondria.

685.10

INHIBITION OF GLUTAMATE UPTAKE IN VIVO IS NOT SUFFICIENT FOR INDUCING NEURONAL DAMAGE.

L.Massieu, A. Morales-Villagrán and R. Tapia*. Instituto de Fisiología Celular. UNAM. México D.F. México.

Exposure of neurons to high concentrations of glutamate cause their degeneration and death.The clearence of this amino acid from the synaptic cleft depends on their transport by high affinity uptake mechanisms. We have studied by microdyalisis the effect of the administration of two inhibitors of the glutamate transporter, L-trans-pyrrolidine-2,4-dicarboxylate (PDC) and dihydrokainate (DHK), on the extracellular concentration of endogenous amino acids in the rat striatum in vivo. We have correlated the changes in the concentration of glutamate and aspartate with injury to striatal cells, evaluated 5-7 days after drug administration by determination of glutamate decarboxylase (GAD) and choline acetyltransferase (ChAT) activities as well as by histological examination. DHK (50 mM) and PDC (25 mM) produced similar remarkable increases in the extracellular concentration of glutamate (12-fold) and asparte. However, the former induced also elevations in the concentration of other amino acids. When glutamate transport inhibitors were directly injected in the rat striatum, clear neuronal damage was observed only after DHK (400 nmol/µl) administration, which produced a significant reduction of both ChAT (46%) and GAD (44.5%) activities, relative to the contralateral striatum. This decrease was partially prevented by administration of the NMDA antagonist, MK-801 (1 mg/Kg ip. 30 min before), and the non-NMDA antagonist, NBQX (50 nmol/µl coinjected with DHK). Neither PDC nor two other inhibitors of glutamate uptake, DL-threo-ß-hydroxyaspartate and L-aspartate-ß-hydroxamate, induced striatal cell damage. The neurotoxic effects of DHK could be explained by direct activation of glutamate postsynaptic receptors rather than by the increased glutamate concentration.

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INGESTIVE BEHAVIORS VII

686.1

GASTRIC BRANCH VAGAL AFFERENT AND EFFERENT CONTRIBUTIONS TO GASTRIC MACRONUTRIENT EMPTYING.

A. Baldessarini, T.H. Moran, C.F. Salorio, P.R. McHugh*, and G.J. Schwartz. Dept. of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The gastric branch of the vagus nerve plays a critical role in the control of gastric macronutrient emptying, because gastric branch vagotomy results in significantly accelerated emptying of all liquid macronutrients. This effect of gastric branch vagotomy could reflect the loss of vagal afferent input, vagal efferent output, or both. The gastric emptying of a range of caloric concentrations (0.2 - 0.8 kcal/ml) of 5 ml macronutrient loads was evaluated at 5, 10, 20 and 40 minutes in rats with: 1) selective afferent gastric branch vagotomy, 2) selective efferent gastric branch vagotomy, and 3) in sham controls. In control rats, increasing caloric concentration slowed gastric emptying of carbohydrate (glucose), protein (peptone) and fat (oleic acid) loads. In addition, equicaloric loads of peptone and oleic acid (0.2 kcal/ml) emptied more slowly than glucose, demonstrating some macronutrient-specific control of emptying. Gastric emptying in selective gastric branch de-afferentated rats, although accelerated, still revealed both caloric and macronutrient-specific controls. Selective gastric branch de-efferentation, however, resulted in much more significant acceleration of gastric emptying, similar to that seen in total gastric branch vagotomized rats. These results suggest that while gastric branch vagal afferent fibers play some role in the control of nutrient emptying, other non-gastric vagal and spinal afferents, and gastric efferents may be more critical for the control of gastric emptying and food intake. Supported by DK19302.

686.2

SUBDIAPHRAGMATIC VAGAL AFFERENT CONTRIBUTIONS TO THE MICROSTRUCTURE OF LIQUID MEAL INTAKE.

G.J. Schwartz*, C.F. Salorio, and T.H. Moran. Dept. of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The vagus nerve provides the major neuroanatomical pathway linking gastrointestinal and central nervous system sites involved in food intake control. Surgical transection of this link during total subdiaphragmatic vagotomy has profound effects on meal size and body weight. Because these vagotomies involve transection of both sensory and motor vagal fibers, they do not reveal the specific contributions of afferent and efferent vagal components to feeding. Electrophysiological studies have shown that vagal afferent fibers with gastrointestinal receptive fields are responsive to mechanical and chemical gut stimulation with short latency, suggesting that vagal afferents signals may have the ability to modulate ingestive patterns within a meal. Using automated lick analysis, we evaluated the pattern of licking of milk diet over the first 2.5 hours of the dark cycle in male Sprague Dawley rats with and without selective subdiaphragmatic vagal rootlet deafferentation, before and after surgery. Although both sham and afferent vagotomized rats showed equivalent total numbers of licks, vagotomized rats showed longer bursts of licking, fewer bursts, and faster initial cumulative rates of licking than sham controls. These results demonstrate that removal of vagal afferent input from the gut alters the pattern of food intake in ways that suggest the removal of potential negative feedback signals,.and underscore a role for vagal afferent neurophysiological signals in determining the moment-to-moment control of feeding behavior. Supported by DK19302 and the Whitehall Foundation.

686.3

BOMBESIN INHIBITION OF SODIUM APPETITE BUT NOT WATER INTAKE IN RATS WITH LESIONS CENTERED ON THE AREA POSTREMA (AP). G.L. Edwards*, T. Wang and J.D. Power. Dept. of Physiol. & Pharmacol., Coll. of Vet. Med., Univ. of Georgia, Athens, GA 30602.

Recent studies indicate that bombesin acts to inhibit sodium appetite caused by depletion of sodium (Soc. Neurosci. Abstr. 19: 1263, 1993; Soc. Neurosci. Abstr. 19: 1822, 1993). Rats with lesions centered on the AP (AP-lesions) are reported to consume increased amounts of saline (Am. J. Physiol. 264: R1242, 1993). Since the AP is a circumventricular organ and lacks a blood-brain barrier it is possible that bombesin acts in the AP to inhibit sodium appetite. When treated with 4 μg/kg bombesin I.P., rats with AP-lesions decreased their 2h "need-free" intake of 2% saline compared to 2% saline intake when injected with sterile physiological saline I.P. ($P < 0.02$). In addition, bombesin (4 μg/kg I.P.) reduced sodium depletion-induced 2% saline intake in both AP-lesioned and unlesioned rats ($P < 0.05$). Conversely, water intake induced by isoproterenol (25 μg/kg) was not reduced by bombesin in either rats with lesions of the AP or intact control rats ($P > 0.05$). These data indicate that bombesin is effective at reducing sodium appetite in rats with AP-lesions. Thus, sites other than the AP are capable of mediating the effects of peripherally administered bombesin on sodium appetite. (Supported by NIH DK 42533)

686.4

DISTRIBUTION OF FOS-LIKE IMMUNOREACTIVITY IN RAT BRAIN FOLLOWING ISOPROTERENOL TREATMENT AND THE EFFECTS OF CONVERTING ENZYME INHIBITION OR ANGIOTENSIN II BLOCKADE. M.J. McKinley, B.J. Oldfield*, Howard Florey Institute of Experimental Physiology and Medicine, Univ.of Melbourne, Parkville, 3052, Vic., Australia.

Subcutaneous (s.c.) administration of the β-adrenergic agonist isoproterenol causes copious water drinking in rats which is blocked by angiotensin inhibition. The aim of the present experiment was to map the distribution of neurons in the brain of the rat which increased Fos production in response to isoproterenol and to test the effect of the angiotensin II antagonist losartan or the converting enzyme inhibitor captopril (at 2 different doses) on this response. Rats were injected with isoproterenol (Winthrop, 50μg/kg s.c.) and killed 2h later. Immunohistochemistry was used to detect Fos-like immunoreactivity (Fos-LI) in paraformaldehyde-fixed sections of brain. Fos-LI was increased in neurons in the lamina terminalis, in particular those in the subfornical ogan (SFO), median preoptic nucleus (MnPO), and organum vasculosum of the lamina terminalis (OVLT). It increased also in the hypothalamic supraoptic and paraventicular nuclei, bed nucleus of the stria terminalis, central nucleus of the amygdala, locus ceruleus, parabrachial nucleus, nucleus tractus solitarii, ventrolateral medulla and area postrema, but not in control rats injected with s.c. isotonic saline. Losartan (Dupont-Merck, 100mg/kg, intraperitoneal) or captopril (Bristol-Myers Squibb, 50mg/kg s.c.) which block isoproterenol induced drinking, also blocked Fos-production in the lamina terminalis, but not in the other regions, whereas a lower dose of captopril (0.5mg/kg s.c.) which potentiates isoprotetrenol-induced drinking, potentiated the increase in Fos-IR in the lamina terminalis. Thus, isoproterenol results in the activation of neurons in the SFO, OVLT and MnPO secondary to the action of Ang II. Activation of these neurons in the lamina terminalis appears to be crucial for the induction of thirst.

686.5

UNCONDITIONED AND CONDITIONED EXPRESSION OF cFOS TO AMPHETAMINE AND LITHIUM CHLORIDE IN RAT BRAIN DURING TASTE AVERSION LEARNING. M.W. Swank, G.E. Schafe, and J.L. Bernstein*. Department of Psychology, University of Washington, Seattle, WA 98195.

Amphetamine and lithium chloride (LiCl) are both effective unconditioned stimuli (USs) in the formation of learned taste aversions. However, the mechanism of action of these drugs is quite different with the area postrema and related emetic circuitry critical to the response to LiCl but not amphetamine. Induction of Fos-like immunoreactivity (FLI) in rat brain was examined two hours following *ip* injection of either d-amphetamine sulfate (3 mg/kg) or LiCl (6 mEq/kg). Amphetamine strongly induced FLI in striatum while LiCl did not. In most other regions where FLI was detected (e.g. nucleus tractus solitarius (NTS); lateral septum; paraventricular nuclei of the thalamus and hypothalamus) substantially more FLI was noted in animals treated with LiCl than in those treated with amphetamine. In the lateral pontine parabrachial nucleus (PBN), however, amphetamine and LiCl induced comparable, significant elevations of FLI. After a single prior pairing with either LiCl or amphetamine, exposure to a saccharin solution induced significant taste aversions and significant FLI in intermediate NTS relative to unpaired controls. Overall, these results indicate that PBN is a site of common activation by these two very different drugs during acquisition. The response to taste re-exposure points to a population of cells in intermediate NTS which have a common role in taste aversion expression despite different US activation patterns.

686.6

IS THE NMDA RECEPTOR INVOLVED IN THE RESPONSES TO AMINO ACID IMBALANCED DIETS? **D.W. Gietzen*, B.G.Truong AND B. Dang. Dept. VM: Anat., Physiol., Cell Biol. and Food Intake Lab., Univ. California-Davis, Davis CA 95616.**

The anorectic responses to imbalanced amino acid diets (IMB) have been associated with neural activity in the prepiriform cortex (PPC), an area essential for the initial depressed feeding response to IMB. Because the principal neurons of the PPC contain glutamate, we measured the intake of IMB after acute injection of the NMDA receptor antagonist, D(-)-2-Amino-5-phosphonopentanoic acid (AP5), into the PPC. After stereotaxic placement of bilateral guide cannulae aimed at the PPC and prefeeding a low-protein basal diet (BAS) for 10 days, rats were given 0.5 μl of either AP5 (4 nM in artificial CSF, N=11) or vehicle (aCSF, N=8) just before feeding IMB at the onset of darkness. BAS intake on the day prior to injections was taken as control for each rat. Coordinates for the PPC were: A 11.4, L 3.7, D 6.5 (Paxinos and Watson, 1982). Intake of IMB was increased to 88% of control in the AP5 group ($p = 0.024$ vs aCSF group, that ate 55% of control) at 6-9 hr after introduction of IMB, a crucial time period in the feeding responses. Intake values for 6-9 hr were: AP5 group = 3.59 ± 0.41g, aCSF group = 2.29 ± 0.38g, $p = 0.038$). Although feeding at other time points was not affected, the 6-9 hr increase was sufficient to increase intake of IMB nearly to a significant level at 24 hr ($p = 0.058$). We conclude that the NMDA receptor in the PPC may have a role in mediating the anorectic responses to IMB diets. **Supported by USDA CSRS 90-37200-5440, NIH DK35747 & DK42274.**

686.7

Gastric emptying of corn oil emulsions during oral, gastric, or concurrent oral/gastric infusions. **J.M. Kaplan*, W. Siemers and H.J. Grill. Dept. Psychology, U. Pennsylvania, 19104.**

We showed previously that substantially less corn oil emulsion empties from the stomach when ingested orally than when infused gastrically. In the first experiment, we sought to determine whether the full inhibitory effect can be achieved when only a portion of the total load is orally delivered. Gastric contents were recovered after 6-min single or concurrent infusions across which the intraoral:intragastric infusion ratio [100:0, 0:100, 50:50, 25:75; net infusion rate = 1.0 ml/min] was varied. Half as much oil emptied during exclusively oral infusions as during exclusively gastric infusions. The concurrent infusions were equally effective at slowing oil emptying as the exclusively oral infusion. Preliminary results of a second experiment indicate that oil emptying is equally rapid following gastric infusions (@ 1.0 ml/min) of either 50% corn oil or pre-ingested 50% corn oil (recovered from donor rats). These two experiments show that oral inhibition of oil emptying does not depend on the amount ingested, the number of swallowing actions, or on enzymatic alteration of the corn oil. **Supported by DK-42284 and DK-21397.**

686.8

FEEDING RELATED ELEVATION IN PLASMA CORTICOSTERONE LEVELS IN INTACT AND CHRONIC DECEREBRATE RATS. R.J. Seeley,*[***] R.R. Sakai,[*] J.C.K. Donahey,[*] B.S. McEwen,[**] and H.J. Grill[*] [*]Depts of Animal Biology and Psychology University of Pennsylvania, [**]Neuroendocrinology Lab, Rockefeller University, [***]Dept of Psychology, University of Washington, Seattle WA, 98195.

The neural basis for the feeding-related increase in corticosterone (CORT) was explored using the chronic decerebrate (CD) rat in which the caudal portion of the brainstem is neurally isolated from the forebrain by a complete transection of the neuraxis. Tail blood samples were taken before and after a gastric intubation of either 8 ml milk-diet or water following overnight deprivation. Pre-intubation baseline CORT levels were comparable in CD and intact controls. In intact rats, CORT levels increased significantly after the water intubation (110% above baseline). By contrast, no water response was observed in the CD rat. Both groups, however, showed a dramatic elevation in CORT following the nutritive load (intacts: 250% above baseline; CD: 440% above baseline). Additionally, tail blood samples were taken 1 hr after each of 5 intubations of milk diet spread evenly throughout the 12 hr light cycle. CD rats showed CORT levels that were slightly elevated from those of the intact controls, but the rise and fall of CORT levels through the light cycle in CD rats exactly paralleled those of the intact controls. The CD rat's normal-like CORT profile may reflect the competence of the HPA axis in the transected brain to mediate neuroendocrine responses. If so, the current results indicate that the function of the HPA axis can be modulated by humoral signals associated with food deprivation and caloric loads. Alternatively, the CD rat's CORT profile may have been mediated by the autonomic nervous system without a significant forebrain contribution.

686.9

STIMULATION OF FEEDING BEHAVIOR IN THE RAT BY INTRA-HYPOTHALAMIC INJECTION OF 8-BR-cAMP. E.R. Gillard*, A.M. Khan, A.U. Haq, R.S. Grewal, and B.G. Stanley. Departments of Neuroscience & Psychology, University of California, Riverside, CA 92521, USA.

Although several neurotransmitters acting in and around the lateral hypothalamus (LH) have been implicated in the control of eating, it is unknown which, if any, second messengers mediate their effects. To begin to address this, food intake was measured 1, 2, and 4 hrs after injection of 8-bromoadenosine 3',5'-cyclic monophosphate (8-BR-cAMP, a membrane-permeant cAMP analogue) into the perifornical hypothalamus (PFH) of freely feeding adult male Sprague Dawley rats. Each dose of 8-br-cAMP (0, 1, 10, 50 and 100 nmol, dissolved in artificial cerebrospinal fluid vehicle) was administered in a final injection volume of 0.3 µl. Dose-dependent stimulation of eating was observed, with doses of 50 nmol and 100 nmol eliciting average 2 hr intakes of 5.9 and 15.7 g, respectively. To determine the locus of this effect, we compared the effects of 8-br-cAMP injected into the PFH and six other areas bracketing the PFH (LH, anterior and posterior LH, paraventricular nucleus of the hypothalamus, amygdala, and thalamus). Consistent eating-stimulatory effects were observed in the LH and PFH at doses ≥ 50 nmol (2 hr intakes ≥ 3.4 g). Thalamic injections resulted in a more variable feeding response which achieved significance only at 100 nmol. In contrast, injections into all other sites failed to stimulate eating. These results suggest anatomical specificity of 8-br-cAMP's effects, with likely sites of action being the LH/PFH and/or thalamus. It is possible that neurotransmitters known to influence eating in the hypothalamus may do so via the cAMP second-messenger system.

686.10

HYPOTHALAMIC GALANIN IN RELATION TO CIRCULATING INSULIN AND FAT INGESTION. S.F. Leibowitz*, J.I. Koenig and A. Akabayashi. The Rockefeller University, N.Y., N. Y. 10021 and The Immunobiology Research Institute, Annandale, N.J. 08801

Galanin (GAL) is densely concentrated in neurons of the hypothalamic paraventricular nucleus (PVN). When injected into the hypothalamus, GAL reduces insulin secretion and stimulates feeding behavior, causing a preferential increase in fat ingestion. The present studies in albino rats reveal an inverse relationship between endogenous GAL peptide (measured via RIA), specifically in the PVN, and circulating levels of insulin, while GAL and fat ingestion are positively related. Three experiments were conducted: 1) With measurements taken at several intervals across the light/dark cycle (8 time points, n=7/time), GAL levels in the PVN, as opposed to other hypothalamic nuclei, increased significantly during the middle phase of the feeding cycle, while insulin levels were declining and natural fat ingestion increased. PVN GAL and circulating insulin levels were inversely correlated ($r=-0.32$, $p<0.05$) across the feeding cycle. 2) In rats (n=28) on macronutrient diets, GAL levels in the PVN, but not other hypothalamic areas, were inversely correlated with circulating insulin levels ($r=-0.41$, $p<0.05$) but positively related to daily fat intake ($r=+0.64$, $p<0.01$). 3) In rats (n=11) given 5 daily injections of GAL antisense oligonucleotides into the PVN, GAL levels and daily fat intake decreased by 35% ($p<0.05$ compared to sense controls), while circulating insulin levels tended to rise (+46%, $p<0.10$). This inverse relation between endogenous GAL and insulin under natural feeding conditions is consistent with another report at this meeting (Tang et al.) showing insulin in diabetic rats to reduce GAL gene expression and peptide levels, specifically in the area of the PVN.

686.11

THE RELATIONSHIP OF AMOUNT OF SOUP IN THE STOMACH TO THE CONTROL OF TEST MEAL INTAKE IN MEN AND WOMEN. H. R. Kissileff*, T. Wentzlaff, R. N. Pierson, and F. X. Pi-Sunyer. New York Obesity Research Center, St. Luke's-Roosevelt Hospital and Columbia Univ., 1111 Amsterdam Ave. New York, NY 10025.

If a critical level of gastric tension, induced by volume, triggers the cessation of eating, graded volumes of preloads should lead to proportional reductions in intake of a subsequent test meal, thereby preserving constancy of total (preload + meal) volume, as preload size increases. This hypothesis holds only if meals and preloads empty at the same rates. To test this hypothesis, five women and six men in good health, nonobese and without medical problems, ate a yogurt shake test meal 30 min after tomato soup preloads. Emptying of the preload from the stomach was measured by radionuclide scintigraphy (see table for the volume of soup remaining at the start of the test meal.) The preloads and test meals were given with at least two days between. Preload sizes ranged from 160 to 800 ml in 160 ml steps. A no preload condition was included.

Test meal intake was reduced as a function of preload size in a linear fashion only between 320 g and 640 g in women and only at the 160 g compared to 0 and 800 g compared to 640 g preloads volume in men (see Table below for details).

Preload (g) ⟶	0	160	320	480	640	800	LSD
WOMEN intake (g)	896	809	771	627	482	431	137
preload in stomach (g)	0	59	89	245	235	445	83
MEN intake (g)	1032	837	825	822	792	482	192
preload in stomach (g)	0	23	65	194	265	285	110

Unless the preloads of soup differentially affect test meal emptying, gastric tension, induced by volume, by itself, is not a critical signal for normal meal termination in humans. (Support: NIH - DK36507 & DK26687)

686.12

METABOLIC REGULATION OF FOOD INTAKE IN THE WHITE-CROWNED SPARROW. R. D. Richardson, T. Boswell, R. J. Seeley, M. Ramenofsky, J. C. Wingfield, and S. C Woods*. Depts of Psychology and Zoology, University of Washington, Seattle, WA. 98195.

Migratory birds rely on increased fat storage and fatty acid utilization to meet seasonal changes of energy expenditure and as a result increase food intake and fat stores before migration. To determine if their feeding behavior is sensitive to carbohydrate utilization, white-crowned sparrows (*Zonotrichia leucophrys gambelii*) maintained on short daylength (9L15D) were injected IP with 2-deoxy-D-glucose (2-DG), 2,5-anhydro-D-mannitol (2,5-AM), or insulin, drugs that elicit feeding in mammals. Low doses of 2-DG (25 or 50 mg/kg) had no effect on food intake and higher doses (100 or 300) mg/kg) significantly suppressed feeding after 1 and 2 hours. No dose of 2-DG increased meal size. Analogously, low doses of 2,5-AM (25, 50 or 100 mg/kg) had no effect on food intake, and higher doses (300 and 600 mg/kg) significantly suppressed intake. Low doses of insulin (0.5 or 1.0 U/kg) also had no effect on food intake and higher doses (2.0 or 4.0 U/kg) significantly suppressed feeding and induced hypoglycemia after 1 and 2 hours. These data suggest that decreased carbohydrate metabolism does not elicit feeding in this species. Importantly, all of these drugs may increase plasma fatty acids in birds. The fatty acid tributyrin (100, 300, 600 or 2000 mg/kg IP) significantly suppressed 60-min and 120-min intake dose-dependently in these birds, and equicaloric glucose (1200 mg/kg) had no effect. We conclude that white-crowned sparrows are unresponsive to manipulations of carbohydrate metabolism, but are responsive to increases of plasma lipids.

686.13

EATING SUPPRESSION AND BODY WEIGHT LOSS BY LATERAL HYPOTHALAMIC (LH) NMDA RECEPTOR BLOCKADE WITH D-AP5. B.G. Stanley*, V.L. Willett III, H.W. Donias and M.G. Dee II. Departments of Neuroscience & Psychology, University of California, Riverside, CA 92521.

We have recently shown that intense eating is elicited by LH injections of glutamate, N-methyl-D-aspartic acid (NMDA), or non-NMDA receptor agonists (Brain Res. 1993, ***613***, 88-95 & ***630***, 41-49). To examine whether LH glutamate and NMDA receptors might mediate natural eating, LH injection of the competitive NMDA receptor antagonist D-AP5 (10 nmol/0.3 µl of artificial CSF) was tested for anorectic effects in adult male Sprague-Dawley rats. First, we examined whether pretreatment with LH injection of D-AP5 would suppress eating elicited by LH NMDA (10 nmol) and found a dose-dependent effect, with 10 nmol of D-AP5 suppressing the 9.8 gm NMDA-elicited eating response by 71%. In separate groups, the effects of bilateral LH injection of this dose of D-AP5 were tested on eating elicited by 24 hrs of food deprivation or by the onset of the nocturnal eating period. With food deprivation, the 11.9 gm eating response 30 min after vehicle injection was reduced 61% by D-AP5. For nocturnal eating, the 4.1 gm response 30 min after vehicle was reduced 34% by D-AP5. Some other behavioral effects were also noted. Finally, to determine the effects of long-term treatment, D-AP5 was bilaterally injected into the LH at the onset and midpoint of the dark-phase for 8 consecutive days. Compared to vehicle injection, D-AP5 produced marked and prolonged reductions of daily food intake and loss of body weight. Food intake normalized when the injections were stopped. These findings suggest that endogenous LH glutamate may act in part via NMDA receptors to influence eating behavior and body weight.

687.1

CNTF ENHANCES SPROUTING IN PARTIALLY DENERVATED MUSCLE. S.G. Siegel*, A.W. English, Dept. of Anatomy & Cell Biology, Emory University, Atlanta, GA 30322.

Following partial denervation of skeletal muscles, the remaining intact motoneurons develop sprouts which innervate a limited portion of the denervated fibers. In adult rats, each neuromuscular compartment of the lateral gastrocnemius (LG) muscle receives innervation from precisely one branch of the muscle nerve. After partial denervation by transection of the branch to the medial compartment (LGm) of the LG muscle, newly formed sprouts do not cross the compartmental border. To investigate whether exogenous ciliary neurotrophic factor (CNTF) can augment the natural sprouting response, rat LGm fibers were denervated. CNTF was administered by intramuscular injection (2-20μg/kg) into the LG muscle once daily for 7 days. Animals were sacrificed one day after the last injection, and the tissue was analyzed for sprouts. The motor end plates and nerve terminals were visualized immunohistochemically by the presence of clustered fluorescent alpha-bungarotoxin and FITC-conjugated antibody to the 200kd neurofilament protein, respectively. Sprouts from intact motoneurons were identified as thin offshoots from the nerve terminals or preterminal axon. CNTF administration resulted in an increase in the proportion of end plates with sprouts, suggesting that the natural sprouting response to partial denervation can be enhanced. However, sprouting from motoneurons in the intact compartment did not lead to reinnervation of fibers in the denervated compartment.

CNTF was provided by Regeneron Pharmaceutical, Inc.

687.2

ANTEROGRADE INFLUENCES ON COLLATERAL NEURONAL SPROUTING IN THE SYMPATHETIC NERVOUS SYSTEM. G. A. Kuchel*, C. Richard and M.C. Bastien Montreal General Hospital Res. Inst.,1650 Cedar,Montreal,Quebec,Canada H3G 1A4

In contrast to retrograde signals for collateral sprouting (ie. target-derived NGF), anterograde influences on this form of plasticity are less well delineated. Using a NE-uptake assay, Dornay et al. (*J.Nsc.* 5:1522) concluded that an intact preganglionic innervation was essential for the development of collateral sprouting in the rat pineal gland after a unilateral denervation. This target receives bilateral innervation from the two superior cervical ganglia (SCG). We have quantified fluorescent immunohistochemistry for tyrosine hydroxylase (TH) as an index of collateral sprouting (*Exp.Neurol.*124:381).

As shown earlier, 1 day after a unilateral denervation (R-ICN cut) the rat pineal gland exhibited a 50% decrease in the area fraction representing TH immunoreactive profiles (TH-IR). Collateral sprouting then took place with an increase to 78% and 76% of control values at 3 and 10 days, respectively. When this lesion was immediately preceded by a decentralization of the "intact" SCG (L-CSTx), TH-IR was 71% and 72% of control values at 3 and 10 days, suggesting that much of the collateral sprouting response was still intact. Further studies will be required to evaluate the possibility that degenerating preganglionic neurons "releasing" neurotransmitters during critical periods of signaling supplant usual anterograde signals for lesion-induced postganglionic collateral sprouting.

687.3

EFFECT OF SCIATIC NERVE SECTION ON SPROUTING BY SPARED ROOTS IN DORSAL HORN OF ADULT RATS. B. Zhang,* I. Fischer and M. Murray, Dept. Anatomy and Neurobiology, Medical College of Pennsylvania, Phila., PA 19129.

Lumbosacral dorsal rhizotomy, sparing one root, induces sprouting by that root in adults (Polistina, et al., '90). Sciatic nerve section enhances regeneration of the central process of crushed dorsal root in adults (Richardson, et al, '87). Sciatic nerve lesions are also accompanied by decreased synthesis of CGRP and SP by DRG neurons and depletion of these peptides from the dorsal horn (Nothias et al., '93; Himes and Tessler, '89). In neonates sciatic lesion induces sprouting by central processes into the dorsal horn unaccompanied by depletion of peptides (Reynolds and Fitzgerald, '92). We combined unilateral sciatic nerve section with bilateral spared root preparation to determine whether the peripheral lesion would enhance sprouting by the spared root in adult rats. The immunocytochemical staining was compared on the two sides using antibodies against neuromodulators and cytoskeletal proteins. CGRP and SP levels increased on the sciatic nerve lesioned side at 3 days and the CGRP projection was expanded, SP returned to normal (or less) at 10 days and CGRP returned to normal (or less) at 20 days, indicating a transient effect of sciatic lesion in this paradigm. The sprouting of central projections however blocks or delays the downregulation of peptide synthesis normally evoked by peripheral nerve lesion. Immunocytochemical staining of cytoskeletal proteins high molecular weight (HMW) tau, MAP 1B and phosphorylated MAP 1B showed no significant differences, suggesting that central sprouting in this paradigm is not associated with axonal elongation.

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687.4

SPROUTING OF INTACT AFFERENTS INTO THE DENERVATED CUNEATE NUCLEUS OF ADULT RATS AFTER DORSAL RHIZOTOMY. P.E. Garraghty[1,2*], D.R. Sengelaub[1,2], A.C. Mills[3], and N. Muja[1]. [1]Dept. Psych. and [2]Prog. Neural Sci., Indiana Univ., Bloomington, IN 47405; [3]Dept. Biol., Middle Tenn. St. Univ., Murfreesboro, TN 37132.

In 1991, Pons et al. (Science, **252**, 1857) reported a remarkably extensive topographic reorganization in SI (area 3b) of macaque monkeys that had undergone dorsal rhizotomies extending from the second cervical (C2) to the fifth thoracic (T5) level some years earlier. Here, we evaluate the hypothesis that this reorganization was due to the formation of new connections between intact afferents and neurons that had been denervated by the rhizotomies. Thus, we have performed comparably extensive, dorsal rhizotomies in adult rats followed by anatomical tracing and electrophysiological mapping experiments. Using peripheral skin injections of a conjugate of cholera toxin subunit B and horseradish peroxidase, we have observed aberrant expansions of gracile projections into the cuneate nucleus within 3 months of the deafferentation. Moreover, this new growth apparently forms functional connections because cortical recordings in forelimb regions of SI in these same animals reveal neurons that are responsive to stimulation of hindlimb skin surfaces innervated by afferents that normally project to the gracile nucleus. These results suggest that substantial new growth is possible in the mature central nervous system, and that such new growth may well account for the substantial functional recovery detected in earlier experiments in the so-called "Silver Springs monkeys." (Supported by SO7 RR07031)

687.5

PROGRESSIVE ENTORHINAL LESIONS ACCELERATE HIPPOCAMPAL SPROUTING IN RATS. T.S.Carrigan, M.L.McQuilkin, and J.J.Ramirez*. Neuroscience Program and Department of Psychology, Davidson College, Davidson, NC 28036.

Entorhinal cortex (EC) lesions are known to induce sprouting by the crossed temporodentate pathway (CTD) in rats. Previous evidence from our laboratory indicates that two-stage (progressive) lesions of the entorhinal area accelerate recovery from spatial alternation deficits associated with unilateral EC lesions and increase the synaptic efficacy of the CTD by 250% in as little as 6 days postlesion.

The purpose of this study was to determine anatomically whether progressive lesions accelerate CTD sprouting. Animals were assigned to one of four groups: progressive lesion, control, priming lesion, or one-stage lesion. The postlesion proliferation of the CTD was analyzed using quantitative autoradiography. ^{3}H-proline was injected into the intact EC at the time of the one-stage or secondary lesion of the contralateral homologue. Six days later the rats were killed and the brains were prepared for autoradiographic analysis. The CTD sprouting response was quantified by creating a ratio of the silver grain density evident in the outer molecular layer of the dentate gyrus contralateral and ipsilateral to the ^{3}H-proline injection (C/I ratio). Neither the priming lesion nor the one-stage lesion resulted in a significant sprouting response at 6 days postlesion. Progressive lesions increased the C\I ratio by approximately 300% in both the dorsal and ventral leaves of the dentate gyrus. The lesion sizes of the one-stage and progressive treatment conditions were similar.

We conclude that progressive entorhinal lesions enhance the rate of sprouting by the crossed temporodentate pathway. Taken together, the anatomical, electrophysiological, and behavioral results indicate that CTD sprouting is functionally significant and behaviorally meaningful.

Supported by NSF grant IBS-9020151 and NIMH grant MH47895-02.

687.6

THE ENTORHINAL CORTEX REDUCES GRANULE CELL COLLATERAL SPROUTING IN ORGANOTYPIC SLICE CULTURES OF MOUSE HIPPOCAMPUS. B.W. Coltman*,[1], F.E. Dudek[2], and C.F. Ide[1]. [1]Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118 and [2]Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523

Organotypic slice cultures of murine hippocampal tissue provide an *in vitro* model system for the controlled study of mossy fiber collateral sprouting into the dentate molecular layer(DML). We hypothesized that the presence of afferent innervation would decrease the degree of mossy fiber collateral sprouting in the DML. Alternate slices derived from the mid-hippocampal region were cultured either with or without attached entorhinal cortical tissues. Bodian silver staining confirmed the presence of fiber tracts between the dentate gyrus and entorhinal cortical tissues. Timm's heavy metal staining and densitometry were utilized to assay the degree of mossy fiber sprouting. Attached entorhinal cortex significantly reduced the degree of mossy fiber sprouting in the DML after ten days *in vitro* ($p < 0.001$; n=30 pairs). To confirm that damage to mossy fiber axons was not responsible for mossy fiber sprouting in the DML, alternate pairs of hippocampal slices were subjected to either complete transection of their mossy fiber projection (at the point where the fibers leave the hilus) or left undisturbed. Slices receiving mossy fiber lesions displayed no significant increase or decrease in mossy fiber sprouting into the DML as compared to adjoining control slices receiving no mossy fiber lesions (n=10 pairs). Therefore, mossy fiber collateral sprouting into the dentate molecular layer is not a consequence of damage to mossy fibers, but rather, may be regulated by the presence of afferents from the entorhinal cortex *in vitro*. Supported by DOD grant #2-89/116/88-150.

687.7

DELAYED DEGENERATION DIFFERENTIALLY AFFECTS THE TIME COURSE OF SPROUTING BY AFFERENT SYSTEMS REMAINING IN THE MOLECULAR LAYER OF THE DENTATE GYRUS FOLLOWING LESIONS OF THE PERFORANT PATH. B. Shi* and B. B. Stanfield. Lab. of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

Axons in peripheral nerves and in certain central pathways have been shown to undergo very slow Wallerian degeneration in WLD mutant mice. It has recently been shown that in WLD mutant mice there is a delay in the intensification of acetylcholinesterase histochemical staining in the molecular layer of the dentate gyrus following lesions of the entorhinal cortex. The intensification of the acetylcholinesterase staining is indicative of sprouting of septohippocampal fibers in the dentate molecular layer. Thus, it appears that delayed post-lesion reactive sprouting is associated with the delayed degeneration of cut central axons in this mutant. We have studied the time course of changes in the septohippocampal and the hippocampal commissural projections following interruption of the perforant path in WLD and normal (C57BL/6J) mice by injecting wheat germ agglutinin conjugated horseradish peroxidase into the septum or contralateral hippocampus and processing the tissue sections for TMB histochemistry. In normal mice changes in the distribution of labeled septal and commissural axons indicative of sprouting in the dentate molecular layer are seen as early as three days post-lesion. The earliest similar changes are found in WLD mutant mice is seven days post-lesion, when an increase in the density of labeled septal axons begins in the outer molecular layer. The delay in the sprouting of commissural axons in the mutant is even longer. Changes in the distribution of labeled commissural axons in the dentate gyrus of WLD mice are first seen twelve days post-lesion. These results confirm that post-lesion reactive axonal sprouting can be delayed in the CNS of WLD mutant mice. In addition, our results indicate that the extent of this delay may differ among axonal fiber systems. These findings are consistent with the notion that various CNS axonal systems may respond differentially to sprouting cues and are reminiscent of differences found in the regenerating response exhibited by sensory and motor axons in the WLD mutant after peripheral nerve cuts.

687.8

AXONAL GROWTH AND TERMINAL SPECIALIZATION OF MOSSY FIBERS IN DEVELOPING RAT PUPS: AN NCAM-H AND NEO-TIMM'S STUDY. J.P. Leite*, T.L. Babb, G.W. Mathern, J.K. Pretorius and T. Seki. UCLA School of Medicine, Los Angeles, CA 90024-1769.

The postnatal axogenesis and synaptogenesis of mossy fibers (MF) were studied in normal Sprague Dawley rats between 2 and 120 days of age. Axonal growth was demonstrated by the transient expression of the embryonic form of neural cell adhesion molecule (NCAM-H). NCAM-H was broadly expressed throughout the hippocampal formation at post natal (PN) day 2. Overall staining was decreased at PN 7, with preferential labelling of the MF pathway. Maximum expression was observed at PN 16, and a decrease of staining was observed at PN 30 and 120.

The Neo-Timm method was used to identify the progressive incorporation of the histochemically active zinc in maturing MF synapses. At PN 2 there was almost no zinc in granule cells or MFs; however, a very slight staining was observed in the highly cellular area of the hilus. At PN 7 some fibers and puncta were observed in the subgranular zone and in stratum lucidum and CA3 pyramidale. By PN 16, the distribution of MF puncta was qualitatively similar to adult hippocampus (PN 120) although Timm staining was less intense and puncta appeared smaller. Maximum accretion of MF terminals in the CA3 stratum lucidum occurred between PN 14 and PN 24. This study demonstrates that during development NCAM-H expression precedes the period of maximum incorporation of MF terminals. Increasing amounts of zinc in MF boutons might regulate the final maturation of MF synapses. Supported by NS 02808, KO8-NS 01603, NIH-FIC and CNPq (Brazil).

687.9

DEVELOPMENTALLY REGULATED LOCALIZATION OF A PURKINJE CELL-SPECIFIC TRANSCRIPT IN DENDRITES. F. Bian[1,3], K. Schilling[4], G. Cole*[2,3], and J. Oberdick[2,3]. [1]Ohio State Biochemistry Program, [2]Department of Cell Biology, Neurobiology and Anatomy, and [3]The Biotechnology Center, The Ohio State University, Columbus, OH 43210. [4]Abteilung Anatomie und Zellbiologie der Universitat, 89069 Ulm, Germany.

Several mRNAs thought to be important for neuronal growth and plasticity have been found to be localized in dendrites. Cerebellar Purkinje cells (PCs) are ideal for studying the role such transcripts play due to both the size and monopolar orientation of their dendrites. *In situ* hybridization has been performed for three PC-specific transcripts known to encode proteins highly concentrated in PC dendrites. Of the mRNAs coding for these three markers, L7, PEP-19 and CaBP28K, only that for L7 is abundantly detectable in PC dendrites. In addition, it appears that the amount of L7 mRNA in dendrites is much greater during the early postnatal period than in adulthood, suggesting that direct translation of the L7 protein in dendrites may be particularly important during dendritic outgrowth. This is consistent with several observations made of Purkinje cells in primary dissociated cultures of embryonic cerebellum. Under conditions of high K+, PC dendrites consistently take on a more elongated morphology relative to their stunted appearance in normal medium. Under the same high K+ conditions, expression of both the L7 protein and β-galactosidase driven from an L7-*lacZ* fusion gene, are qualitatively higher based on histological assay. In particular, in immature cultures, PC dendritic processes are L7 positive only in the high K+ condition. Thus, the L7 protein may be important for the growth and continuing plasticity of PC dendrites.

687.10

RHAMM-LIKE IMMUNOREACTIVITY IN RAT BRAIN AND RHAMM-DEPENDENT INHIBITION OF TYROSINE HYDROXYLASE-POSITIVE FIBER OUTGROWTH FROM INTRAOCULAR BRAINSTEM TRANSPLANTS. A-Ch Granholm*, M.L. Price, W.A. Staines[1], E.A. Turley[2], and J.I. Nagy[2]. Dept. Basic Science, Univ. Colorado HSC, Denver, Colorado, [1]Dept. Anatomy, Univ. Ottawa, Ontario, [2]Dept. Physiology, Univ. Manitoba, Winnipeg, Manitoba, Canada. Studies *in vitro* have suggested that RHAMM (Receptor for Hyaluronic Acid Mediated Motility) plays a role in neurite extension and motility. Anti-RHAMM antibodies (A-R) were found to label axons with a distribution similar to the efferents of the locus coeruleus (LC). We investigated if RHAMM plays a role in the outgrowth of LC fibers from fetal brainstem grafts. Adult rats received intraocular transplants of fetal brainstem tissue containing LC. The fetal tissue was treated with A-R, control antiserum, RHAMM mimicry peptide (RMP) corresponding to one of the hyaluronic acid binding domains, or control peptide with a scrambled amino acid sequence - at grafting and weekly for 5 weeks postgrafting. Transplants treated with A-R or RMP grew significantly less than the controls, and contained fewer tyrosine hydroxylase-immunoreactive neurons. The graft innervation of the host iris was also significantly reduced in both the A-R and RMP treated groups. Nerve fibers showing strong staining with A-R in the adult brain were also immunoreactive for dopamine beta hydroxylase (DBH). Three weeks following treatment with moderate doses of DSP-4 there was depletion of these double stained fibers but other populations of single-labelled, central DBH-positive fibers remained. Collateral sprouts induced in DBH-positive fibers following DSP-4 treatment were intensely labelled with anti-RHAMM antibodies. Confirmation that the immunoreactivity seen represents a true isotype of RHAMM is under investigation. The results suggest the participation of RHAMM in nerve fiber growth *in vivo*. Supported by USPHS grant MH49661.

687.11

ROLE OF THE HYALURONAN RECEPTOR RHAMM IN NEURITE EXTENSION AND MOTILITY IN PRIMARY NEURONS AND NEURONAL AND GLIAL CELL LINES. U. N. Frankenstein*, J. Hacking, M. Z. Hossain, E. A. Turley and J. I. Nagy. Department of Physiology and Manitoba Institute of Cell Biology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba CANADA R3E 0W3,

RHAMM (Receptor for Hyaluronan Mediated Motility) has been identified as a receptor for the extracellular matrix component hyaluronan (HA) and was recently shown to be essential for the locomotion of normal and transformed peripheral cells. In view of similarities in molecular processes that govern cell locomotion and growth cone migration, we investigated whether RHAMM also contributes to cell migration of CNS-derived cells *in vitro*. Immunohistochemical studies of cultured primary neurons, astrocytes and microglia as well as of several neuronal and astrocytic cell lines revealed a punctate form of RHAMM labelling in cell bodies, along processes and at growth cones. By Western blot analyses, neuronal cell lines such as PC12, NG108-15 and NSC-34 cells were found to express major RHAMM forms with apparent MW of 75 and 116 kDa, while glial cells contained 50 and 72 kDa forms with HA binding capacity. Treatment of NG108-15 cells with dibutyryl-cAMP led to enhanced expression of RHAMM with a clear increase in RHAMM immunolabelling in these cells. Monoclonal and polyclonal anti-RHAMM antibodies as well as peptides corresponding to the HA binding domains of RHAMM significantly inhibited neurite migration, glial cell process elongation and motility. An antibody directed against a repeat sequence present in RHAMM significantly stimulated neurite migration of NSC-34 cells and rat primary neurons. Collectively these results demonstrate the presence of RHAMM in neural cells and suggest a critical role for HA/RHAMM interactions in the mediation of neurite migration and glial cell locomotion.

688.1

The Time Course of Axon Elimination in the Splenium of the Rat Corpus Callosum. J.H.Y. Kim*& J.M. Juraska. Neuroscience Program, Univ. of Illinois, Champaign, IL 61820

We have previously reported preliminary data indicating that axon number changes in the rat corpus callosum after 15 days of age (Kim & Juraska, 1990). In adult rats, we found significant dorso-ventral and rostro-caudal variations in axonal density within the splenium, which could potentially influence axon number calculations if the splenium is not sampled extensively. Therefore, we re-examined axon number in the rat splenium at 15, 25 and 60 days of age. The posterior fifth of the corpus callosum, which contains the axons from the visual cortex at all of these ages (Kim et al., 1992), was extensively sampled with electron microscopy. Axon density was multiplied by the area of the posterior fifth from adjacent 1μ sections to determine axon number.

There was a significant decrease in axon number between day 15 and 25, and also between day 25 and 60. Thus, axon withdrawal in the rat splenium continues after the adult-like distribution of callosal projections within the visual cortex is established (Olavarria & Van Sluyters 1985). Preliminary results indicate that there is a sex difference in the timing of axon withdrawal in the rat splenium which will be further investigated. Supported by NSF IBN 9310945.

688.2

EARLY DEVELOPMENT OF THALAMO-CORTICAL AND GANGLIONIC EMINENCE PROJECTIONS IN THE HAMSTER. C. Métin and P. Godement*. Institut A. Fessard, CNRS, 1 av. de la terrasse, 91190 Gif/Yvette, FRANCE

The formation of long-range projections in the brain requires that axons navigate along precise pathes and recognize their final targets. In mammals, the thalamus and the neocortex are connected via reciprocal connections and are separated by the ganglionic eminence. We have studied the development of these projections, from embryonic day E11 to E14 in hamster embryos, corrresponding to stages when these fibers begin their growth. Small injections of carbocyanine dyes (DiI, DiA) were done in fixed hamster embryo brains to label anterogradely and retrogradely the projections from small territories of the thalamus, cortex and ganglionic eminence.

Anterograde labeling shows that at E11.5, thalamic fibers reach the medial ganglionic eminence (GE), and cortical fibers reach the lateral GE. Surprisingly, retrogradely-labeled neurons are found in close proximity to the growth cones of thalamic and cortical fibers in the medial and lateral GE, respectively. Thus, at this age, there are reciprocal connections between the GE and the thalamus and neopallium. Injections into the ganglionic eminence confirm the existence of this early projection. At E12.5, thalamic fibers accumulate at the frontier between the lateral GE and the neopallium. They cross into the intermediate zone of the neopallium one day later, at E13.5. The most advanced reach the dorso-medial region of the parietal subplate and few or no fibers are found in more rostral or caudal levels. From E11.5 to E12.5, corticofugal fibers grow to and within the GE. At the frontier between the neopallium and GE, they make a right-angle turn. The first cortical fibers reach the dorsal thalamus at E13.5. At this age, retrogradely labeled cells are observed in the lateral part of the parietal cortical plate, and in the ventro-basal region of the dorsal thalamus, suggesting that these regions are the first that establish reciprocal connexions.

These observations show that thalamic and cortical fibers project to the ganglionic eminence before reaching their targets. At early stages, the GE interconnects the diencephalic and neopallial compartments of the brain. Therefore, interactions of thalamic and cortical fibers with the GE and/or its projections could be involved in the establishment of thalamo-cortical connectivity during early development.

688.3

THE POSTNATAL DEVELOPMENT OF THE ABERRANT LONGITUDINAL BUNDLE AND THE CORPUS CAL LOSUM IN BALB/cWah1 MICE. S.L.Schmidt ;D.Wahlsten* and N.Parra. Department of Psychology, University of Alberta, T6G 2E9, Canada .

The BALB/cWah1 mouse strain has approximately 50% callosal defective animals. In the brains of these acallosal mice, an aberrant longitudinal bundle (LB) is consistently found. This study was designed to investigate the distribution of cells and terminals of the LB and the corpus callosum (CC) during postnatal development. At ages ranging from 1-20 postnatal days (PND), 36 BALB/cWah1 mice were anaesthetized and intracardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde (PFA). Carbocyanine dyes, DiI or DiA, were inserted into the CC of normal animals or the LB of callosal defective mice. The brains were stored in 4% PFA, in darkness, for 3- 9 months. After the appropriate time, each brain was embedded in gelatin and sectioned. The sections were stained with bis-benzimide and examined in an epifluorescence microscope. Analysis was focused on the parietal cortex. The distribution of cells and terminals of normal and callosal defective animals (totally or partially absent CC) did not differ. At PND 5 and PN10 axons were seen traveling through the neocortex and terminating in layer I. At PND 10 the granular layer was clearly identified and it was found devoid of callosal or LB projections. From 15-20 PND the gaps in layer IV were confirmed and the callosal and LB cells were mainly found in layers III and V. From 10-20 PND the pattern of callosal and LB projections corresponded to those portions of the parietal cortex which did not contain dense aggregates of granule cells. It was concluded that the distribution of cells and terminals of the LB resembled a normal ipsilateral CC. This indicates that the appropriate targets are being located by the LB axons.

688.4

PEP-19 IMMUNOHISTOCHEMISTRY REVEALS THE DEVELOPING DIRECT PURKINJE -VESTIBULAR COMPLEX PROJECTION IN THE RAT J. J. Velier[1], A. B. Scheibel[2,3*], R. S. Fisher[2,3], and H. V. Vinters[1,3] Depts. of [1]Pathology (Division of Neuropathology), [2]Anatomy and Cell Biology, and the[3]Brain Research Inst., UCLA, Los Angeles, CA 90024

We performed an immunohistochemical examination of the developing direct purkinje-vestibular complex projection in the embryonic rat. The antibody utilized to discriminate Purkinje cell axons from others within the developing metencephalon was a rabbit polyclonal antibody produced against the Purkinje cell enriched antigen, PEP-19, identified as a 7.6 kDa peptide containing regions of similarity to a number of proteins thought to interact with calcium, including S100b, calpain, and calmodulin. A previous immunohistochemical examination of PEP-19 in the adult rat showed immunolocalization to purkinje cells and a minor proportion of neurons within the dorsal cochlear nucleus. The earliest age we have so far examined, embryonic 17 (E17), shows no immunopositive axonal profiles within the vestibular complex (superior and lateral vestibular nuclei). At E20, well organized fascicles are seen passing through the deep cerebellar nuclei and the pontocerebellar isthmus. These axons are observed to enter into the vestibular complex and apparent synaptic contacts upon cells of all sizes within the lateral vestibular nucleus are seen. From these intial findings we conclude that the direct purkinje-vestibular complex projection is established between E17 and E20.

688.5

CALLOSAL AGENESIS IS SECONDARY TO DEFECTS IN HIPPOCAMPAL COMMISSURE FORMATION IN MOUSE EMBRYOS. D.J. Livy*. Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Pioneer axons of the corpus callosum (CC) use the hippocampal commissure (HC) as a guiding substrate to cross the telencephalic midplane in normal mice. In mice with callosal agenesis, CC axons arrive at their proper location on time to cross midplane, but HC development is delayed, preventing its use by the CC axons as a guiding substrate. The routing and timing of hippocampal axon growth through the HC was studied in Balb/cWah1 (Wah1-50% CC defect), 129/J (129-70% CC defect), and a sixth generation cross of Wah1x129 ($C129F_6$-100% total CC absence) mouse embryos using perfusion fixation and the insertion of crystals of the carbocyanine dyes DiI and DiA into the hippocampal fimbria. Specimens ranged from 0.300g to 0.600g body weight or 15 to 17 days chronological age. Selected sections were also stained using bis-benzamide to show background morphology.

In Wah1 embryos, the first crossing of HC axons was seen at about 0.450g-0.480g which is about 1 day later than that seen in normal $B6D2F_2/J$ hybrid embryos. In 129 embryos, first crossing was not seen until about 0.520g - 0.550g, about 2 days later than in normal hybrids. No HC axons were seen to cross midplane in the $C129F_6$ embryos prior to 0.600g. The arrival and movement of axons in all three strains appeared normal, but their traverse of midplane was blocked by the presence of a cleft which extended deep in the septal region.

Supported by NSERC grants to S.K. Malhotra and D. Wahlsten.

688.6

MULTIPLE GUIDANCE CUES ARE INVOLVED IN THE SEGREGATION OF PIONEERING PATHWAYS IN THE CORTEX AND INTERNAL CAPSULE: EVIDENCE FROM REELER A.R. Bicknese*, W. Wang, A. Sharma Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794

We compared pathway formation during cortical development in normal and reeler mutant mice using the carbocyanine dyes DiI and DiA to trace axons in fixed tissue, and immunolabeling with antibodies to chondroitin sulfate proteoglycans (CSPG) to identify the extracellular matrix (ECM). By embryonic day (E)11, neurons begin extending axons from the preplate zone (PPZ) which has intense CSPG labeling. By E12, cortical plate (CP) neurons begin splitting the PPZ into the marginal zone (MZ) and subplate (SP), which retain dense CSPG labeling. CP axons transverse the SP, turn and then obliquely cross the intermediate zone (IZ), exiting the cortex close to the VZ. Reelers retain the PPZ, and their IZ and CP are mixed in one layer. Axons of "CP" neurons extend obliquely across this layer and exit near the VZ. In both normal and reeler, pioneer efferents hesitate in a zone above the forming striatum. If later arriving (E12-13) thalamic axons encounter the waiting efferents, they fasciculate. The time period of co-extension is brief. In normal neocortex, thalamic axons selectively extend within the subplate and its CSPG As the striatum matures, thalamic axons extend laterally around expanding striatum, to enter the subplate and its CSPG. CP axons continue to exit the cortex near the VZ. Thus the two systems are physically separated and axons do not encounter each other after E14. Reeler E14 "CP" develops streaks of CSPG intermingled with efferent neurons and axons allowing reeler thalamic afferents to encounter cortical axons. Thalamic axons selectively extend within the streaks of CSPG, rarely fasciculating with cortical axons. Our evidence indicates that if thalamic and cortical axons guide each other in development, it occurs in a limited number of axons. Multiple guidance cues produce pathway segregation in the forming cortex and internal capsule.

688.7

POTENTIAL ROLES OF THE CEK5 AND CEK8 RECEPTOR TYROSINE KINASES IN THE DEVELOPMENT OF NERVOUS SYSTEM ARCHITECTURE. J. A. Holash* and E. B. Pasquale, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Many receptor-tyrosine kinases have been shown to play important roles in nervous system development as they transduce the effects of extracellular "growth factors" with neural activity. The Eph subclass represents a major branch of receptor tyrosine kinases that are highly expressed in the developing nervous system. We have examined the spatial distribution of two Eph-related kinases, Cek5 and Cek8, in the embryonic peripheral nervous system and visual system. At Hamburger and Hamilton stage 24, both Cek5 and Cek8 appear to be expressed in outgrowing motor axons. High levels of phosphotyrosine immunoreactivity correspond to the sites of Cek5 and Cek8 immunoreactivity. At 6 days *in ovo*, Cek8 expression becomes restricted to a subset of spinal neurons, while Cek5 is expressed throughout spinal nerves. By 8 days *in ovo*, Cek5 expression in peripheral nerves decreases, but appears high perineurally, whereas Cek8 expression remains high within the nerves themselves.

Cek5 and Cek8 are also expressed in the developing visual system. The pattern of Cek8 expression suggests that it plays a role in the formation or maintenance of the ganglion cell pathway from retina to optic tectum. Cek8 is expressed first in the undifferentiated retinal neuroepithelium, and subsequently, as the retina differentiates, becomes restricted to ganglion cell axons. By 8 days *in ovo*, Cek8 expression is apparent throughout the optic nerve, and by 10 days *in ovo*, Cek8 immunoreactivity outlines the optic tract. Cek5 appears to play a role in the development of retinotectal specificity. It is highly expressed in the ventral portions of the developing retina and optic nerve, but only weakly expressed in the dorsal portions of these structures. Immunostaining of retinal explants demonstrates that Cek5, Cek8 and phosphotyrosine immunoreactivities can be localized to neuronal processes. This work was supported by NIH Grant HD26351.

688.8

CORTICOSPINAL TRACT IN THE DEVELOPING RAT SPINAL CORD: THE PARENT AXONS AND PROJECTION COLLATERALS. M. Nagashima* and Y. Inoue.
Department of Anatomy, Hokkaido University School of Medicine, Sapporo 060, Japan.

A detailed morphogenesis of the corticospinal tract of the rat neonate ranging from postnatal day 1 (P1) to P11 was investigated using anterograde labeling with fluorescent dye, DiI, optimized by confocal laser scanning microscopy (MRC-500, Bio-Rad). The parent axons constituted a compact bundle which was constantly enclosed in the ventral part of the dorsal funiculus. The trajectories of individual axons were not parallel to each other within the bundle. In addition, there was an aberrant fascicle which ran ventrally along the main bundle in the sacral segments. The parent axons could be divided into two categories; pioneer axons and follower axons. In contrast with the pioneer axons which progressed by regular time course, the growth cones of follower axons were distributed over a wide range. The majority of the projection collaterals toward the target area emanated from the rectangular branching, however, a number of them ran in various directions. Multiple collaterals from the single parent axon were often observed. The projection fibers repeated branching and their arbors terminated onto the preferential phenotype of the target neurons within the dorsal horn, the intermediate substance or the ventral horn in the spinal gray matter.

688.9

AXONAL ARBORIZATIONS OF THE PRIMARY AFFERENTS SERVING DIFFERENT VESTIBULAR END-ORGANS IN ZEBRAFISH EMBRYOS. S.E. Fraser* and B. Chapman, Division of Biology, 139-74, Caltech, Pasadena, CA 91125.

In adult vertebrates, primary vestibular afferents serving the different semicircular canals and otolithic organs form distinct though overlapping regions of axonal arborization in the vestibular nuclei. While there is an obvious role for this specific connectivity in sensory processing, little is known about the developmental mechanisms which are responsible for establishing the connections. In particular the relative contributions of inherent positional cues, trophic factors, and patterns of neuronal activity to the development of specificity have not previously been examined.

As a first step in answering these questions we have studied the axonal arborization patterns of primary vestibular afferents in normal zebrafish embryos. In order to label the afferents we used iontophoretic injections of lipophilic dyes into the cristae and maculae in the inner ears of fixed zebrafish specimens. After allowing time for the dyes to diffuse along the axons, we examined the patterns of arborization in area octavolateralis in the hindbrain using confocal microscopy of whole mounts of the labeled embryos.

We find that the primary afferents serving the different vestibular end-organs have recognizably different projection patterns. The pattern of axonal arborization of afferents from a given end-organ, however, is highly stereotyped and reproducible between animals at a given age. Double labeling experiments using DiI (C3) and DiI (C5) injected into different canal cristae in the same animal have shown that there is good separation of the arbors of the populations of axons serving different end-organs.

Further experiments are currently underway to test whether the development of normal patterns of axonal arborization in the vestibular system depend on the presence of normal neuronal activity, as has previously been shown to be true in the visual system.

688.10

AXONOGENESIS AND THE EARLY APPEARANCE OF GABA-IMMUNOREACTIVE NEURONS DURING DEVELOPMENT OF THE CNS IN A TELEOST. L Ohlin and P Ekström*, Dept. of Zoology, Univ. of Lund, Sweden.

During early CNS development, a small number of neurons give rise to pioneer axons that form an axonal scaffold. It is not known at which stage these neurons aquire neurotransmitter phenotype, or which transmitter(s) they utilize. In an attempt to approach this question we have studied the early development of the axonal scaffold in embryos of a teleost fish, the three-spined stickleback, with immunocytochemical techniques. We used monoclonal antibodies against acetylated α-tubulin (MAb 6-11B-1) that labels the axons of the early axonal scaffold, and polyclonal antibodies against γ-aminobutyric acid (GABA). GABA is known to exert various neurotrophic actions in the developing nervous system. The antibodies were applied to semithin Araldite sections and to wholemount embryos. Both single and double labeling experiments were performed. Hatching occurs between 144-168 h postfertilization (PF). At 36h PF, two 6-11B-1 immunoreactive (6-11B-1ir) ventral tracts are located in the mesencephalon. At 48h PF, two 6-11B-1ir ventral tracts extend from the mesencephalon throughout the spinal cord. At 51h PF, the first GABA-immunoreactive (GABAir) neurons appear in the ventral prosencephalon, ventral mesencephalon and in the spinal cord. At 60h PF, the 6-11B-1ir ventral tracts were connected by numerous commissures all along between the mesencephalon and the spinal cord. Also, an additional lateral ventral tract appeared. At 66 h PF, the first GABAir cell groups have grown by addition of GABAir neurons, and GABAir cell bodies and fibers are closely associated with the 6-11B-1ir tracts that constitute the axonal scaffold. Thus, GABAergic neurons appear only when the axonal scaffold is established. We suggest that the first pioneer neurons are not GABAergic but that GABAergic neurons make an important contribution to the formation of the early neuronal network.

688.11

COMMISSURE FORMATION IN THE EMBRYONIC BRAIN OF THE GRASSHOPPER. G.S. Boyan* and H. Reichert, Zoology Institute, University of Basel, Rheinsprung 9, CH-4051 Basel, Switzerland

A fundamental event in the normal development of the insect brain is the establishment of correct bilateral connections between the brain hemispheres. We have studied the formation of the very first commissural fiber pathways in the embryonic grasshopper brain using immunocytochemical and intracellular dye injection techniques. During early embryogenesis a set of approximately 130 neuroblasts differentiates from the neuroepithelium of the head to form the bilaterally organized proto-, deuto- and tritocerebral brain segments. In the midline of the brain itself, however, an additional set of precursor cells differentiates from the neuroepithelium anterior to the stomodium, and ventrally between the pars intercerebral lobes of the protocerebral segment. We have identified one large central cell flanked symmetrically on each side by a pair of smaller neuroblasts. At 29% of embryonic development the processes of cells expressing the glial antigen Annulin are seen surrounding all these midline cells as well as many of their progeny. By 30% the most lateral neuroblast on each side has produced a lineage of progeny from which a clearly recognizable pair of cells can be shown to direct the first axonal processes medially across the midline of the brain. These pioneer cells have been morphologically identified via intracellular dye injecton and anti-HRP immunostaining, and express the antigen to fasciclin II as they fasciculate with one another across the midline. The pathway established by these midline pioneers provides a scaffold first for fibers originating from lineage-identified cells at the posteric-medial border of the pars intercerebralis, and then for fibers from the optic lobes. Each subsequent group of fibers crossing the brain reiterates a pattern of antigen expression and fasciculation similar to that described for the midline pioneers. Supported by the Swiss NSF.

688.12

MOLECULAR MECHANISMS OF EMBRYONIC BRAIN DEVELOPMENT IN *DROSOPHILA MELANOGASTER*. S. Therianos, S. Leuzinger, F. Hirth, C. S. Goodman and H. Reichert*. Dept. of Zoology, University of Basel, CH-4051 Basel, Switzerland, and HHMI, Dept. of Molecular and Cell Biology, University of California, Berkeley, California 94720

We are using the powerful genetic and molecular genetic techniques that are available for the study of embryonic development in *Drosophila* to investigate the molecular mechanisms involved in building a complex brain. Embryonic development of the *Drosophila* brain is comparable to that of other insects. During the period of embryogenesis in *Drosophila*, complex cerebral hemispheres are formed. These cerebral hemispheres are interconnected by the brain commissure, which is pioneered by a pair of axons that navigate along a midline intercerebral bridge. The differentiation of this brain commissure depends on the *com* gene. In *com* mutants the brain commissure is severely reduced, and only a small subset of commissural fascicles are formed. The cerebral hemispheres are linked to the ventral nerve cord by paired brain connectives, which interconnect the initially separated procephalic and ventral neurogenic regions. The formation of these paired brain connectives depends on the *sim* gene. In *sim* mutants paired connectives do not form, and aberrant ectopic midline interconnections between brain and ventral nerve cord are established by axons which navigate along the midline stomatogastric nervous system structures. Our future goal is to carry out a comprehensive molecular genetic analysis of the set of genes involved in brain development. (Supported by SNSF and HHMI).

688.13

ESTABLISHING FUNCTIONAL RELATIONSHIPS BETWEEN GENES IN ABL-REGULATED PROCESS ES BY ANALYSIS OF *DROSOPHILA* MUTANT PHENOTYPES. J.-L. Juang*, F. M. Hoffmann. McArdle Laboratory 77for Cancer Research, University of Wisconsin-Madison., Madison, WI 53706

The function of the *Abl* protein tyrosine kinase in *Drosophila* is of interest because it relates to the transforming oncogene in Abelson murine leukemia virus. *Drosophila* provides a well characterized system in which to analyze the developmental consequences of specific mutations by examining mutant phenotypes. Five genes, *disabled (dab), enabled (ena), failed axon connections (fax), fasciclin I (fasI)* and *fasciclinII (fasII)* have been examined to analyze their functional interaction with the *Abl*. We have observed defects in axon outgrowth and/or pathfinding in embryonic CNS of these mutants. We also used enhancer trap lines to visualize a small subset of axons. Our preliminary data suggests the axon tract defects observed in mutant embryonic CNS are due to defects in cell adhesion mechanisms critical to growth cone pathfinding. Antibody staining of CNS-derived cell lines have indicated that the *Abl, dab,* and *ena* proteins are present in the neuronal cytoplasm, neurites, and the growth cone regions .

688.14

NEUROTROPHIN-3 HAS A CHEMOTROPIC EFFECT ON CORTICAL NEURONS. D.D.M. O'Leary* and M.M. Daston
Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA.

NT-3 promotes the regrowth of transected corticospinal axons in adult rat spinal cord (Schnell et al Nature 367:170, 1994) and has been speculated to have a tropic effect on developing corticospinal axons. To address this potential role, we have utilized a chemotaxis chamber which is normally used to study cell migration. The chamber has 48 wells that are separated into upper and lower compartments by a membrane containing small pores. Dissociated cells are placed in the upper compartment and normally remain on the membrane's upper surface. When a soluble chemotropic agent is added to the lower chamber cells pass through the pores onto the membrane's bottom surface. By reducing the pore size, we have been able to study directed neurite growth, rather than cell chemotaxis, since neurites, but not cells can pass through the membrane.

The tropic effect of NT-3 containing medium or medium conditioned with explants of basilar pons, cerebellum or olfactory bulb on cortical neurites was tested. Cortical cells dissociated from P1 rats were plated on the top surface of the membrane. 24 hrs later, neurites on the bottom surface of the membrane were visualized immunohistochemically and counted. The number of neurites on the bottom surface of the membrane was increased 2 to 5 fold by the explant conditioned medium compared to normal unconditioned medium. NT-3 containing medium resulted in a 10 fold increase in the number of neurites on the bottom surface of the membrane. The NT-3 effect is completely abolished when the gradient is eliminated by adding NT-3 containing medium to both the upper and lower chambers, suggesting that the effect is due to chemotropism and not simply enhanced neurite outgrowth. Experiments are in progress to determine if corticospinal neurons are among the NT-3 responsive population and whether these neurons express *trkC* in situ.

688.15

DEVELOPING RAT CORTICOTHALAMIC FIBRES DEFASCICULATE WHEN THEY REACH THE PERIRETICULAR NUCLEUS. N.C. Adams and R.W. Guillery*. Department of Human Anatomy, University of Oxford, South Parks Road, OX1 3QX, England.

Corticothalamic fibres undergo extensive reorganisation between the cortex and the thalamus. To examine this reorganisation as connections are being formed, we placed small crystals of the lipophylic dyes DiI and DiA in the cortex of aldehyde fixed brains of rats between embryonic day (E)15 and postnatal day (P)1. After 4 to 8 weeks the brains were sectioned, counterstained with DAPI and viewed with epiflourescence and confocal microscopy.

At E15 the first axons from the cortex can be seen entering what will later be the internal capsule. At this age, there is no discernable order in the direction that the fibres take, with many growing fibres crossing each others' paths. At E16, there is a denser fibre projection, with fibres aggregating into bundles as they leave the cortex. Fibres at E17 leave the parietal cortex and form distinct fascicles which pass in a relatively straight path towards the thalamus. On reaching the area of the internal capsule occupied by the perireticular nucleus these bundles break down. Defasciculation is accompanied by a dramatic change in the course taken by the corticofugal fibres, which form a characteristic latticework of fibres. The lattice continues to the inner border of the reticular nucleus, where the fibres adopt an almost direct approach towards their thalamic targets.

Perireticular cells, with their cortically projecting axons (N.C. Adams *et al* (93) *Soc. Neurosci. Abs.* **19**, p45) and their position in the path of growing fibres to and from the neocortex are uniquely placed to have a role in the reorganisation of axons between the cortex and the thalamus. Perireticular cells are thus likely to be involved in guiding the corticothalamic axons to their correct destination, and in so doing, create the lattice.

This work was supported by the Wellcome Trust.

NEUROTRANSMITTER SYSTEMS AND CHANNELS: CHANNELS

689.1

MUSCLE ACETYLCHOLINE RECEPTOR CHANNEL FUNCTION DURING NERVE REGENERATION. R.A. Maselli*, E.J. Lane, T.L. Ashton, M. Lee, and M. Chao. Lab. of Neurology, University of California, Davis, CA 95616.

Two distinct types of acetylcholine receptor (AChR) channels are present at the synaptic membrane of vertebrate skeletal muscle. One type is characterized by high conductive (HC) fast kinetics, and the other type is characterized by low conductance (LC) slow kinetics. In an effort to define the role of innervation in the expression of these two classes of AChR channels we performed crush injuries to the sciatic nerves of adult mice. This was followed by sequential single channel recordings performed in freshly dissociated fibers from the flexor digitorum muscle. Up until two days after crush the percentage of LC channels were less than 1% of the total observed events and similar to the controls. At three days post crush the percentage of LC channels started to rise, and by day five more than 80% of the openings were the LC type. This was followed by a progressive decrease of the percentage of LC type, and by eight days post crush less than 40% of the opening were LC type. We conclude that neural factors are important to regulate the expression of either type of channels and that single channel recordings are useful to monitor the course of reinnervation.

689.2

DECREASED ACTIVITY OF Na CHANNELS IN PITUITARY MELANOTROPES FOLLOWING THE ONSET OF DOPAMINERGIC INNERVATION. J.C. Gomora*, A. Navarrete, A. Marin and G. Cota. Dept. of Neurosciences, Cinvestav, Mexico, DF 07000.

High-threshold Ca^{2+} currents in rat melanotropes undergo a drastic inhibition during the early postnatal period, concomitant with the onset of dopaminergic innervation (Gomora et al., *Soc. Neurosci. Abstr.* 19:1128, 1993). We have now investigated whether the long-term effects of innervation include regulation of other voltage-gated ion channels. Melanotropes were dissociated from non- and fully-innervated rat intermediate pituitary lobes (postnatal days, PN, 3 and 11, respectively), kept in culture for 5-24 h, and then subjected to whole-cell patch clamp. Standard recording solutions and activating pulses to +20 mV (HP -80 mV) were used. Peak Na^+ inward current decreased from -543±54 pA (mean±SE, n=14) in PN3 cells to -283±46 pA (n=14) in PN11 cells. In contrast, the average values for cell capacitance and K^+ outward current amplitude did not significantly differ between these two sets of cells. Thus, the dopaminergic innervation of melanotropes reduces Na^+ current density, but does not seem to affect the long-term activity of K channels.

689.3

FUNCTIONAL EXPRESSION OF Ca^{2+}-ACTIVATED K^+ CURRENTS IN EMBRYONIC CHICK SYMPATHETIC NEURONS DEVELOPING *IN SITU* AND *IN VITRO*. M. E. Wisgirda* , S. Raucher & S. E. Dryer, Program in Neuroscience, Florida State University, Tallahassee, FL 32306

We have examined the expression of whole-cell $I_{K(Ca)}$ in embryonic chick sympathetic neurons developing *in situ* and *in vitro*. $I_{K(Ca)}$ first appears relatively late in embryonic development (E13) and reaches its mature density at E18. Voltage-activated Ca^{2+} currents (I_{Ca}) are present throughout these stages. When sympathetic neurons are isolated at E13 and maintained in cell culture for an additional five days, they do not express $I_{K(Ca)}$ although I_{Ca} is expressed at normal density. Culturing sympathetic neurons in the presence of 5 ng/ml NGF caused a significant ($p < 0.05$) increase in $I_{K(Ca)}$ expression, although this effect appeared to be restricted to a subpopulation of cells (17 out of 34 neurons). Similarly, co-culture of sympathetic neurons with ventricular myocytes stimulated $I_{K(Ca)}$ expression ($p < 0.05$ compared to controls). By contrast, co-culture of sympathetic neurons with spinal cord explants, which make functional contacts with sympathetic cells, did not promote $I_{K(Ca)}$ expression. The developmental expression of $I_{K(Ca)}$ is dependent upon extrinsic factors, possibly including target-derived trophic factors. Suported by NIH Grant NS-27013.

689.4

TRANSIENT EXPRESSION OF N-TYPE CALCIUM CHANNELS IN THE FOREBRAIN OF HUMAN INFANTS IDENTIFIED WITH [^{125}I] ω-CONOTOXIN GVIA. K. E. Ward*, J. M. McIntosh, B. M. Olivera, and F. Filloux. Depts. of Neurology, Pediatrics, Psychiatry, and Biology, University of Utah, Salt Lake City, UT 84132.

Presynaptic voltage-gated Ca^{2+} channels (N-type) are thought to play an important role in the mature brain by regulating Ca^{2+}-dependent neurotransmitter release. However, little is known of their role in the developing brain. Recently, members of our group have described transient expression of N-type Ca^{2+} channels in discrete regions of postnatal rat brain (Filloux et al., Dev. Brain Res., 78:131-136, 1994). We now describe the transient expression of N-type Ca^{2+} channels during human brain development. Postmortem human brain samples from the cerebellum and dorsolateral prefrontal cortex were obtained from 11 postnatal subjects, ages 1-6 months, and 8 adults, ages 26-66 years old. Slide-mounted, 10μm-thick tissue sections from each area were radiolabeled with [^{125}I]ω-conotoxin GVIA. Autoradiograms were generated and intensities of binding were quantitated using computerized image analysis.

Results showed no qualitative differences in the intensity of [^{125}I]ω-conotoxin GVIA binding in cortical or cerebellar gray matter between infants and adults. However, there was a striking difference when comparing forebrain white matter in these ages. Specifically, infants had significantly higher binding intensity in cortical white matter when compared to adults (infants=1.60 fmol/mg, adults=0.25 fmol/mg; $p<.0005$). Hence, it would appear that transient expression of N-type Ca^{2+} channels also occurs in at least some discrete regions during human brain development. The increased expression of N-type Ca^{2+} channels in early development of forebrain white matter may be a useful parameter in identification of certain forebrain neurodevelopmental pathologies.

689.5

A NOVEL CALCIUM-DEPENDENT OSCILLATION IN POST-NATAL MOUSE LATERAL GENICULATE NEURONES. Nikki MacLeod, Alex Johnston & Gavin Swanson*. *Physiology Dept., University of Edinburgh & TINS, Cambridge*, UK.*

Underlying the bursting behaviour of adult thalmic neurones is a 5 Hz oscillation which is generated by alternating activation and deactivation of two steeply voltage-dependent currents I_T, a transient calcium current and I_H, a slowly inactivating current carried by both sodium and potassium ions. Both of these currents and the consequent membrane oscillation are activated by *membrane hyperpolarisation*, induced by synaptic inhibition or intracellular current injection.

In a recent series of experiments investigating the properties thalamic neurones in brain-slices prepared from mice between P7 and P17 we have discovered a voltage and calcium-dependent membrane oscillation which is activated by *membrane depolarisation*. The oscillation is usually present at the cells' resting potential (-35 to -50mV) and induces spontaneous firing of complex action potentials, which often have an initial fast, TTX-sensitive component and a later slow component which is blocked by bathing the slice in low Ca medium containing cadmium ions. Neither the wide part of the action potential or the underlying membrane oscillation are affected by adding TTX, but are enhanced by low concentrations of 4AP which is known to facilitate calcium entry into neurones. The oscillatory behaviour is stopped by small membrane hyperpolarisations, while small depolarisations increase the frequency and ultimately lead to inactivation of the associated action potentials. Large hyperpolarisations induced by inward current injections do not induce the classical 5Hz oscillation described above, even though the cells showing the HT oscillation have a well developed LTS and I_H. However, in some of the younger neurones (P7-P8) showing the depolarisation-induced oscillation, membrane hyperpolariation reveals TTX & bicuculline-sensitive giant depolarising potentials of up to 60 mV which can lead to fast spike generation if threshold is reached.

689.6

POSTNATAL CHANGES IN FIRING PROPERTIES OF NEURONS IN THE NUCLEUS TRACTUS SOLITARII. A. Vincent, F. Tell* and A. Jean. Dept. Physiologie, URA CNRS 1832, Neurobiologie et Neurophysiologie Fonctionnelles, Fac. St Jérôme 13397 Marseille cedex 20, France.

Whole cell recordings of NTS neurons were carried out on rat brainstem slices at different postnatal ages (P0-3, P5-10, P15-21 and P>30 days). Resting potential remained unchanged while input resistance decreased from P0-3 to adulthood. Duration of action potentials (APs) shortened with age. APs amplitude increased from birth to P>30 with a peak at P5-10. The percentage of cells that possessed an A-like potassium current augmented with age. Although IA amplitude did not increase significantly, IA decayed more rapidly at P0-3 than in the adulthood. Thus, unlike previously reported, IA is present at birth but is significantly altered postnatally. Therefore, firing properties of NTS neurons are not fully mature at birth. Depending on the age, processing of synaptic inputs by NTS neurons would be different and therefore generation of integrative processes such as the control of autonomic functions might be altered.

OTHER FACTORS AND TROPHIC AGENTS I

690.1

Localization of Rse, a Novel Tyrosine Kinase Receptor, in the Mammalian CNS; Comparison with Other Tyrosine Kinase Receptors. Mark Armanini*, Lanway Ling, Klaus Beck, Franz Hefti, Melanie Mark, Paul Godowski, Siao Ping Tsai, Arnon Rosenthal, Paul Moran, Ingrid Caras, and Heidi Phillips. Depts. of Neuroscience and Cell Genetics, Genentech Inc., 460 Point San Bruno Blvd., SSF, CA.

Rse is a novel tyrosine kinase receptor isolated from rat and human brain RNA. Rat and human **Rse** share 90% amino acid identity, containing extracellular, transmembrane, and intracellular domains. Northern analysis reveals **Rse** expression to be most abundant in brain. Here, in-situ hybridization was performed to identify specific neuronal structures expressing this gene.

In-situ hybridization revealed **Rse** to be widely expressed throughout the adult rat brain, but restricted to specific regions. Expression in the hippocampus was observed primarily in CA1, to a much lesser extent in CA3 and CA4, and undetectable in the dentate gyrus. High levels of expression were observed in the cortex, caudate, olfactory bulbs, and cerebellar granule cells, but undetectable in the midbrain, brainstem, and medial septum. Sections from the adult human cortex and hippocampus also revealed high levels of **Rse** expression in these structures.

In the E15.5 mouse and 8 week human embryo, expression of **Rse** was undetectable in the CNS. Thus, expression appears to be turned on later in development, with highest levels being maintained in the adult. The regulation of **Rse** expression in response to a combination fimbria-fornix/enterohinal lesion was also investigated.

The localization, developmental patterns, and response of **Rse** expression to injury were compared with other recently described tyrosine kinase receptors of the mammalian CNS.

690.2

K-252a INDUCES TYROSINE PHOSPHORYLATION OF THE FOCAL ADHESION KINASE AND NEURITE OUTGROWTH IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS Anna Coco Maroney*, Lorraine Lipfert, M. Elizabeth Forbes, Marcie A. Glicksman, Nicola T. Neff, Robert Siman and Craig A. Dionne Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380

The protein kinase inhibitor K-252a has been shown to promote cholinergic activity in cultures of rat spinal cord (Glicksman et al., J. Neurochem., 61: 210-221, 1993) and neuronal survival in chick dorsal root ganglion cultures (Borasio, Neurosci. Lett. 108: 207-212, 1990). To determine the mechanism by which K-252a acts as a neurotrophic factor, we examined the effects of this molecule on a human neuroblastoma cell line, SH-SY5Y. K-252a induced neurite outgrowth in a dose-dependent manner. Coincident with neurite outgrowth was the early induction of 125 and 140 kDa tyrosine phosphorylated proteins. Inhibition of protein kinase C with GF-109203X did not prevent K-252a induced tyrosine phosphorylation or K-252a dependent neurite outgrowth suggesting that the neurotrophic effects mediated by K-252a are independent of protein kinase C inhibition. We have identified one of the phosphosubstrates as the pp125 focal adhesion protein tyrosine kinase (Fak). Induction of phosphorylation coincided with increased Fak activity and appeared to be independent of ligand/integrin interaction. The induction of Fak phosphorylation, by K-252a, was also observed in LA-N-5 cells and primary cultures of rat embryonic striatal cells, but not in PC12 cells. The protein kinase-C independent induction of tyrosine phosphorylation and the identification of Fak as a substrate of K-252a-induced tyrosine kinase activity suggests that this compound mediates neurotrophic effects through a novel signaling pathway.

690.3

LPA, A NEURITE OUTGROWTH INHIBITOR, ACTIVATES MAP KINASE IN N1E-115 NEUROBLASTOMA CELLS. G.J.A. Ramakers* and W.H. Moolenaar. Division of Cellular Biochemistry, Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam, The Netherlands.

The phospholipid lysophosphatidic acid (LPA) has recently been identified as an albumin-bound serum factor, that induces DNA synthesis and stress fiber formation in fibroblasts by activating both classic and novel signaling pathways through a putative 40 kD receptor coupled to heterotrimeric G-proteins (Moolenaar, Trends Cell Biol. 4: 213; 1994). The 40 kD LPA receptor is highly expressed in brain and in the neuroblastoma cell line N1E-115, which differentiates to a sympathetic phenotype upon serum witdrawal. In differentiated N1E-115 cells LPA induces rapid neurite retraction through a novel signaling pathway independent of classic second messengers (Jalink et al., Cell Growth Differ. 4: 247; 1993). As protein tyrosine kinase and phosphatase inhibitors interfere with LPA-induced neurite retraction in N1E-115 cells, we investigated protein tyrosine phosphorylation in N1E-115 cells by LPA after serum-starvation, using anti-phosphotyrosine immunoblotting.

LPA (at 100 nM and higher), thrombin and insulin, but not FGF, EGF or glutamate transiently stimulated the phosphorylation of p42 MAP kinase with a peak at 2 min. LPA-induced MAPK phosphorylation was inhibited by pertussis toxin and various suppressors of protein kinase C (PKC), indicating an involvement of the heterotrimeric G_i protein as well as PKC. A model will be presented in which LPA-induced MAP kinase parallels, but is independent of neurite retraction, and serves as a trigger to drive N1E-115 cells back into the cell cycle.

690.4

CONDITIONED MEDIA FROM A TRANSFORMED GLIAL-LIKE CELL LINE ENHANCES NEURITE OUTGROWTH AND GAP-43 EXPRESSION. J.R. Madsen,* O. Weisfeiler, A. Greenspan and L.I. Benowitz. Dept. Neurosurgery, Children's Hospital; Program in Neuroscience, Harvard Medical School, Boston, MA, 02115.

The glial environment exerts a profound effect upon neuronal development and regeneration. To identify molecules that enhance or suppress axonal outgrowth, we have been coculturing primary neurons from postnatal day 3 rat cerebellum with two closely related transformed glial-like cell lines (C27 and C17). The neuronal response was quantitatively assayed by measuring the expression of the neuronal growth-associated protein, GAP-43, using Western blotting. As reported previously, neurons grown on the C27 line expressed considerably higher levels of GAP-43 than when grown on C17 cells. To determine whether secreted factors contribute to this difference (rather than cell-to-cell contact), we examined neurite outgrowth in the presence of conditioned medium (CM) from C27 and C17 cells. Again, neurite outgrowth was significantly higher in cultures containing C27 CM as compared with C17 CM. The neurite-promoting activity of the C27 cells is detectable in serum-free as well as serum-containing CM, and can be concentrated by ultrafiltration with a size cutoff > 10kD . The C27 and C17 CMs do not differ in immunoreactive laminin, but do show other differences in protein composition by HPLC. Several of the monoclonal antibodies developed in this laboratory to identify epitopic differences between surface molecules of C17 and C27 also identify specific differences between the CM proteins of the two cell lines by Western analysis. Further work is directed towards identifying the CM antigens which these antibodies recognize and to determine which molecules in CM or on the cell surface contribute to the control of neurite growth.
Support: NIH NS01471 (JRM) and the Boston Neurosurgical Foundation.

690.5

GAP-43 IN THE SUPERIOR CERVICAL GANGLIA AND IRIDES OF THE RAT AFTER DECENTALISATION. X-E. Hou and A. Dahlström*. Dept. of Anatomy and Cell Biology, Göteborg University, Göteborg, S-413 90 , Sweden.

The distribution of GAP-43-like immunoreactivity (LI) in the superior cervical ganglia (SCG) and irides of adult SD rats was studied using confocal laser scanning microscopy (CLSM). In the SCG of control rats, GAP-43-LI was mainly located in the nerve terminals around, but not inside, the principal neurons and very little in nerve bundles. After 24 hours decentralization the GAP-43 positive nerve terminals disappeared, while the principal neurons became weakly GAP-43-LI positive. Three days after decentralisation, GAP-43-LI was again observed in nerve terminals, and also appeared in axon bundles, while principal neurons appeared negative. SIF cells did not show clear changes after decentralisation. In the irides of control rats, GAP-43-LI was present and distributed in a patchy pattern in axon bundles, around blood vessels and in the terminal network. Double immunolabeling showed that GAP-43-LI colocalized with TH-LI, but appeared to distribute differently from SP-like (SP-LI) in the subcellular structures. A semi-quantification of GAP-43-LI in the irides was performed using CLSM. The intensity and density of the nerve terminal network in the dilator plate were registered. After 3 days of decentralisation, the intensity and density of the GAP-43-positive network terminal was significantly increased.

The results indicate that GAP-43-LI is normally present in nerve fibers and terminals of the post-ganglionic adrenergic SCG neurons in adult rats. The modulations in GAP-43-LI observed in cell bodies and nerve terminals after decentralisation suggests that these autonomic neurons have a capacity for remodelling and plasticity which may partly be regulated via the pre-ganglionic neurons.

690.6

IDENTIFICATION OF INTERLEUKIN-6-EXPRESSING NEURONS IN DIFFERENT REGIONS OF RAT BRAIN. R.A.Gadient* and U.H.Otten, Dept.Physiology, Univ.Basel, CH-4051 Basel, Switzerland

Interleukin-6 (IL-6) belongs to a family of neurotrophic cytokines which includes ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M and IL-11. This family of multifunctional proteins shares a common signal-transducing receptor subunit (gp130). We focused on IL-6, which promotes neuronal survival, differentiation and ameliorates the effects of excitotoxins. In a recent study using reverse transcription-polymerase chain reaction (RT-PCR) we showed that both IL-6 and IL-6 receptor (gp80) transcriptes are found in normal rat brain in a region-specific manner and are developmentally regulated. To identify the cellular origin of IL-6 and its receptor, we established a sensitive non-radioactive in situ hybridization technique. In line with our RT-PCR data the most pronounced IL-6 mRNA signals were observed in hippocampus and hypothalamus. Low expression was seen in the striatum. The IL-6 mRNA signal was predominantly located in neuronal cells, such as the pyramidal and granular cells of the hippocampus and the Purkinje cells of the cerebellum. These findings implicate IL-6 in the regulation of so far unidentified neuronal functions.
This study was supported by the Swiss National Foundation for Scientific Research (Grant 31-39121.93) and by the BMFT (Grant 01KL9303).

690.7

INTERLEUKIN-1ß EXPRESSION DURING DEVELOPMENT OF THE MOUSE BRAIN. J.L. Scripter* and C.F. Ide. Neuroscience Training Program and Department of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118

Interleukin-1ß (IL-1ß) is a cytokine which modulates inflammation and immune responses in the periphery. Expression of IL-1ß has been demonstrated in the adult brain after trauma and may be regulated during cell death. Since normal development of the brain involves programmed cell death, we investigated the developmental profile of IL-1ß immunoreactivity (ir) in the perinatal mouse brain using antiserum to human recombinant IL-1ß for immunocytochemistry on E18, P0, P3, P6 and >P30 animals. We also synthesized a 447-bp cDNA probe (to murine IL-1ß mRNA) for use in *in situ* hybridization studies on E18, P0, P6, P10 and >P30 mouse brains. Grains were counted and immunoreactivity was analyzed using quantitative image analysis. At E18, moderate to high expression of mRNA, but no detectable IL-1ßir, was present. At P0, peak mRNA levels were observed and IL-1ßir was found in cells of all brain areas, especially in the cerebral cortex. At P6, mRNA levels declined and IL-1ßir was equivalent to levels and areas found at P0 and P3. Importantly, at P10, IL-1ß mRNA declined in all brain regions except the olfactory bulb and the CA1 region of the hippocampus. In adults there was no detectable IL-1ß mRNA except in the olfactory bulb; IL-1ßir was present in scattered cells throughout the brain at low levels, prominently in the temporal cortex and the cerebellar granule cell layer. Thus, IL-1ß is regulated during the early perinatal period and may be involved in the neural immune response associated with natural cell death in the developing CNS. Supported by DOD grant #2-89/116/88-150 to the Tulane/Xavier Center for Bioenvironmental Research.

690.8

EFFECTS OF INTERLEUKIN-1 AND INTERLEUKIN-2 ON RAT HIPPOCAMPAL NEURONS IN CULTURE. F.Z. Wang*, H. Yao and A.S. Ding. Dept. of Neurobiology, Institute of Basic Medical Science, Beijing 100850, China

As it was reported that of various brain regious the hippocampus has the greatest abundance of interleukin-1(IL-1) and interleukin-2 (IL-2), as well as their receptor, we first examined the neurotrophic effect of recombinant human interleukin-1β (rhIL-1β) and recombinant human interleukin-2 (rhIL-2) on cultured hippocampal neurons in vitro. The results showed that in the presence of rhIL-1β (100U/mL) or rhIL-2 (100U/mL), the numbers and length of neurite outgrowth were markedly incresed as compared with that seen in the control cultures. From long-term cultures, the cell size and the number of surviving neurons were high than those observed in the parallel series grown with non-rhIL-1β and non-rhIL-2 medium. Then the effects of rhIL-1β nd rhIL-2 on the cultrued rat hippocampal neurons were studied using intracellular recording. Experimental results showed that all of the neurons tested with rhIL-1β (100U/mL) exhibited a 4.20±1.86 mV membrane hyperpolarization. 50% of the neurons perfused with rhIL-2 (100U/mL) showed a depolarization of 11.12±2.71 mV in amplitude accompanied by bursting activity. However, a 3.25±0.63 mV hyperpolarization was observed in the neurons perfused with rhIL-2 in higher concentration (1000U/ml). These results indicate that the cytokins IL-1β and IL-2 can affect not only on the neuronal survival but also on the excitability of hippocampal neurons, by which the immune system may influence the function of neurons in CNS.

690.9

EFFECTS OF ENDOTHELIN-1 ON THE EARLY DEVELOPMENT OF CHICK EMBRYOS. L.Hsu* and A.Y.Jeng. Department of Biology,Seton Hall University,South Orange,NJ 07079 and Research Department,Ciba Pharmaceuticals,Summit,NJ 07901.

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide originally isolated from conditioned medium of porcine aortic endothelial cells. We have previously found that ET-1 enhances neurite outgrowth from phorbol ester-treated dorsal root ganglion explants. The purpose of the present study was to examine the effects of ET-1 treatment on the development of the nervous system in chick embryos. Eggs were incubated at 37°C and treated with ET-1 (50 pmol) through a window in the shell. A single dose of ET-1 on day 1 or 3 did not affect brain development. However, about 12% of the treated embryos had eye malformations (smaller or absent). Repeated treatment with 50 pmol ET-1 from days 2-4 resulted in significantly larger heads and trunks when observed on day 7, while the size of the eyes and limbs were comparable to the controls. The overall lengths of the diencephalon and the telencephalic and optic lobes in the treated groups were increased. Similar effects on brain development were also noted when embryos were treated with a single dose of 50 pmol ET-1 on day 5. These results suggest that ET-1 has growth-factor-like effects on brain development and that embryos are most responsive to treatments around day 5.

690.10

RELATIONSHIP OF *c-neu* ONCOPROTEINS AND THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFr) IN DEVELOPING RAT CEREBRAL CORTEX. P.E. Kuhn* and M.W. Miller. Cell. and Devel. Biol., Rutgers Univ., Piscataway NJ 08854, Res. Serv., V.A.M.C., Iowa City IA 52242, and Depts. of Psychiatry and Pharmacology, Univ. of Iowa Coll. of Med., Iowa City IA 52242.

Similarities between *c-neu* oncoproteins and EGFr were examined with antibodies directed against the carboxyl ends of the oncoprotein and the EGFr. During the first two postnatal weeks, both antibodies identified neuronal cell bodies distributed throughout cortex. Hence, both proteins may be interrelated. The transient pattern of oncoprotein immunostaining correlated with the transient expression of a 35 and a 300 kDa protein. The anti-EGFr identified a 50 kDa protein which was expressed transiently during the first two postnatal weeks. After immunoprecipitating cortical tissue with the anti-oncoprotein antibody, evidence of the EGFr+ 50 kDa protein in the supernatant was eliminated; the precipitate retained all of the immunolabeling. In the reverse experiment, tissue was immunoprecipitated with the anti-EGFr; the 35 and 300 kDa proteins remained unprecipitated in the supernatant. Immunoprecipitation with either antibody did not affect detection of the oncoprotein+ or EGFr+ proteins with different profiles of temporal expression. The EGFr+ 50 kDa protein shares an epitope with a *c-neu* oncoprotein(s). Thus, the 50 kDa protein (a) forms a heterodimer with a *c-neu* oncoprotein or (b) is a component of a larger *c-neu* oncoprotein (e.g., the 300 kDa protein). Supported by the V.A. and N.I.H. (DE07734, AA06916, and AA07568).

690.11

LOCALIZATION OF RETINOID-BINDING PROTEINS AND RETINOID RECEPTORS SUGGESTS A FUNCTION FOR RETINOIC ACID IN DEVELOPING AND ADULT DOPAMINE-INNERVATED BRAIN AREAS. RH Zetterström,[1] A Tomac,[1] M Eriksdotter-Nilsson,*[1] I Nennesmo,[2] U Eriksson,[3] L Olson,[1] Depts. of [1]Neuroscience and [2]Pathology, Huddinge Hospital, Karolinska Institute, and [3]Ludwig Institute for Cancer Research, Stockholm Branch, Stockholm, Sweden.

Retinoic acid, the active metabolite of retinoids (vitamin A compounds), is thought to act as a gene regulator via ligand-activated transcription factors. These are retinoic acid receptors (RAR's) and retinoid X receptors (RXR's), both existing in three forms α, β and γ. In the intracellular regulation of retinoids there are also four binding proteins involved: cellular retinol binding protein (CRBP) type I and II and cellular retinoic acid binding protein (CRABP) type I and II. We have used in situ hybridization to localize the possible presence of the mRNA for CRBP I, CRABP I, RAR β and RXR γ in the developing and adult rat brain and imunohistochemistry to also investigate CRBP I- and CRABP I-immunoreactivity (IR). RAR β and RXR γ are extremely highly expressed in the dopamine-innervated areas caudatus putamen, nucleus accumbens and tuberculum olfactorium in the newborn This expression seems to decline during postnatal development. The striatum of the newborn rat is strongly CRBP I-IR in a patch+marginal zone manner, sparing the intervening "matrix," while at the same time containing many evenly distributed CRABP I-IR neurons. In the adult striatum there is much less CRBP I-IR while a population of evenly distributed strongly CRABP I-IR neurons remain. These CRBP I- and CRABP I-patterns are also seen at the mRNA level. Preliminary studies of human postmortem tissue suggest that a similar population of CRABP I mRNA positive- and CRABP I-IR neurons are present in the human caudate and putamen. Our data suggest that retinoids are important in the development and adult dopamine-innervated areas of the brain.

690.12

DEVELOPMENTAL INTERACTIONS BETWEEN CHOROID NEURONS AND THEIR SMOOTH MUSCLE TARGET CELLS D.C. Darland, G.G. Leblanc*† and R. Nishi, Department of Cell Biology and Anatomy and †Department of Biological Structure and Function, Oregon Health Sciences University, Portland, OR 97201.

The choroid neurons of the avian ciliary ganglion innervate the smooth muscle cells surrounding the arterial vasculature of the choroid layer of the eye. These neurons are cholinergic and express somatostatin as a neuropeptide. When grown in culture, the smooth muscle cells secrete Activin A (AA), which has been shown to increase the expression of somatostatin in the neurons of the ciliary ganglion. We have characterized the target cells to determine expression of smooth muscle markers over time in culture. The cells lose expression of smooth muscle specific actin (SMSA) when placed in culture, but redifferentiate over time. Treatment with bFGF increases proliferation of the cells while suppressing the expression of muscle markers. In contrast, TGFβ rapidly increases expression of SMSA while suppressing proliferation. We have used RT/PCR to isolate and clone a fragment of AA from E12 choroid, showing that AA is present in vivo, as well as being expressed in vitro. We have initiated studies to examine the time course of AA expression during choroid smooth muscle cell differentiation in culture in addition to parallel studies of AA expression in the choroid layer in vivo.

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690.13

CHARACTERIZATION OF SOLUBLE FACTOR ACTIVITIES IN DEVELOPING CHICK CILIARY GANGLION - IRIS SYSTEM. B.A. Link, W.R. Woodward*, and R. Nishi. Dept. of Cell Biology and Anatomy, Oregon Health Sciences Univ., Portland OR 97201.

The differentiation of the developing avian iris and innervation by ciliary ganglion neurons is as an excellent system to study neuron - target influences. The developing chick iris is composed of two muscle groups, the myoepithelial or smooth-type muscle constrictor and the striated-type muscle dilator. Myoepithelial cells are believed to further differentiate to the striated-type muscle and give rise to a contractile dilator at E13. Previous studies (Landmesser and Pilar, 1974 J. Physiol. 241:737) have shown that the iris is initially innervated by the ciliary ganglion at embryo day 8 (E8). Coincident with innervation of the iris is a period of cell death in the ciliary ganglion which results in a 50% loss of the neurons. To investigate potential reciprocal influences, we have utilized mitogenic and neurotrophic assays to identify soluble activities from the ciliary ganglion and the target iris. Soluble extracts from E8 to E19 irises were prepared and applied to a heparin-agarose column. Heparin binding and non-binding fractions were tested for 3[H]-thymidine incorporation in murine AKR-2B cells in the presence or absence of exogenous heparin. Neurotrophic activities (+/- heparin) were determined by the ability to support survival of cultured E8 ciliary ganglion (CG) neurons. Soluble extracts from E8 to E14 CGs were assayed for mitogenicity as described. From iris extracts we observed developmental increases in mitogenic activity with the greatest activity in the heparin binding - heparin dependent fraction, We observed iris neurotrophic activity to increase with development in both heparin binding - heparin dependent and non-heparin binding fractions. As in the developing iris, heparin binding - heparin dependent mitogenic activity was found in the ciliary ganglion, but with a less dramatic developmental increase. In vitro differentiation of iris explants is currently being used to assess the roles of these soluble extracts on iris ontogeny. Supported by NS25767.

690.14

Transforming Growth Factor alpha immunoreactive nerve fibers in the rat and mouse intestinal tract P. Hoffmann, J. Lakshmanan, J.M. Zeeh, L. Liu, M. Sottili, L. Barajas, M.G. Ervin*, and V.E. Eysselein Harbor UCLA Medical Center, Torrance, CA 90509

Epidermal Growth Factor (EGF) and Transforming Growth Factor α (TGFα) have been shown to modulate mucosal blood flow, mucus production and secretion, and motility in gastrointestinal tissues in addition to their mitogenic actions. These effects are important since they may mediate EGF/TGFα's capability to protect intestinal mucosa against acute inury. The aim of the present study was to show morphological evidence of the involvment of TGFα in the regulation of the enteric nervous system by demonstrating TGFα immunoreactivity in nerve fibers of stomach and colon in rats and mice. Tissue specimens were fixed in Zamboni's solution, frozen in OCT compound and cut with a cryostat. A polyclonal antibody, raised against conjugated rat TGFα, was used to perform immunohistochemistry. Binding was visualized using an avidin biotin peroxidase technique as described previously. Beaded nerve fibers were detected in the muscle and submucosal layers of stomach and colon in rats and mice. A weak immunoreactivity was also detected in neurons of the myenteric plexus of both species. Detection of TGFα immunoreactivity in neural structures of intestinal tissues gives morphological evidence that TGFα released from these nerve fibers may be the endogenous source to induce EGF/TGFα's nonmitogenic actions in vivo.

690.15

THE EFFECTS OF TRANSFORMING GROWTH FACTOR-α (TGFα) ON NEURONAL AND GLIAL CELLS ON THE DEVELOPING MEDIAL SEPTAL AREA.
I.E. Mazzoni* and R.L. Kenigsberg, Centre de Recherche Pediatrique, Hôpital Ste-Justine, Montreal, Quebec, CANADA, H3T 1C5.

We have examined the effects of TGFα on different neuronal and glial cell populations in primary cultures of dissociated fetal rat medial septal cells. TGFα, like epidermal growth factor (EGF), was found to induce a dose-dependent decrease in choline acetyltransferase (ChAT) activity which was accompanied by a parallel decrease in the number of acetylcholinesterase positive neurons. The effects of TGFα on ChAT enzymatic activity were first evident after 4 days of continuous exposure to this growth factor. However, although the dose-response profiles of TGFα and EGF on ChAT enzymatic activity were similar (i.e. maximal inhibition evident around 10 ng/ml), TGFα consistently produced a more pronounced inhibition of ChAT activity when compared to EGF in sister cultures. When we combined maximal effective doses of TGFα with those for EGF, the decrease in ChAT activity was not additive, thus suggesting a common mechanism of action for both growth factors. In addition, like EGF, TGFα induced a marked proliferation of astrocytes and microglial cells in the fetal septal cultures without affecting the number of oligodendrocytes. The inclusion of the antimitotic 5-fluorodeoxyuridine completely abolished both the glial cell proliferation and the decrease in ChAT enzymatic activity induced by TGFα. Consequently, TGFα is indirectly affecting cholinergic cell differentiation via responsive glial cells. Competitive binding studies on different cell populations from the medial septum showed that TGFα is more effective in displacing EGF binding to its high affinity receptor, while the opposite is seen for the low affinity EGF receptor. Finally, although TGFα when applied in the absence or presence of an antimitotic did not appear to affect the number or glutamic acid decarboxylase (GAD) immunoreactivite neurons (GABAergic) as assessed by GAD immunocytochemistry, the intensity of the immunoreaction appeared to be somewhat higher in TGFα-treated cultures when compared to controls. Quantitative effects of TGFα on GABAergic neurons will be examined by measuring GAD activity radiometrically. These results suggest that TGFα can differentially affect neuronal and glial cell populations in the developing rat medial septal area both directly and indirectly.

690.16

TRANSFORMING GROWTH FACTOR (TGF)-ß1 REGULATES RAT BRAIN TGF-ß TYPE II RECEPTOR mRNA LEVELS. T.E. Morgan*, D.K.Sarkar†, W.Vale△, and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191; †Department of VCAPP, Washington State University, Pullman, WA 99164-6520; △Salk Institute, San Diego, CA 92186-5800.

TGF-ß1 is a multifunctional peptide that plays an important role in peripheral wound healing. Numerous laboratories have found TGF-ß1 in the damaged and diseased brain and its potential activities are being explored. Three distinct TGF-ß receptors are detected in the mammalian central nervous system. Previously, we showed that TGF-ß1 mRNA levels increase in hippocampal microglia after partial deafferentation by unilateral perforant path transection of adult rats. We now report a several fold increase of radioimmunoassay detectable TGF-ß1 peptide in the ipsilateral hippocampus after perforant path transection; moreover, a cRNA probe specific for TGF-ß type II receptor detected increased type II receptor mRNA levels in the ipsilateral cortex (area including wound site) and hippocampus after transection. The predominant cell type expressing TGF-ß type II receptor mRNA is OX-42 immunopositive (macrophage/microglia). Cultured microglia contain TGF-ß type II receptor mRNA. Treatment of cultured microglia with human recombinant TGF-ß1 caused TGF-ß type II receptor mRNA levels to increase in a dose-dependent fashion. These results suggest autocrine features in the activities of TGF-ß1 in the rodent brain and continue to implicate microglia/macrophage as a major mediator of TGF-ß1 function in the brain. Supported by Alzheimer's Association Los Angeles Chapter (Turken Award - TEM) and AG-07909 (CEF).

690.17

DOES TGF-ß REGULATE CHROMAFFIN CELL PROLIFERATION? N. Wolf, S. Bieger, K. Unsicker* and K. Krieglstein. Dept. of Anat.&Cell Biol., Univ. Heidelberg, D-69120 Heidelberg, Germany.

Transforming growth factor-ßs (TGF-ßs) are pleiotropic cytokines with prominent roles in the regulation of cell proliferation, migration, differentiation, and extracellular matrix. We have previously found (Flanders et al., Development 113: 1991; Bieger et al., SN Abstr. 19:1993) that sympathoadrenal progenitor cells in the embryonic mouse adrenal gland as well as adult rat and bovine chromaffin cells synthesize and release TGF-ßs. Western blotting using an antibody to the TGF-ß receptor type II indicates that preparations of bovine adrenal medullary cells also have a TGF-ß receptor component. Chromaffin cells of the rat adrenal medulla are capable to divide, in a decreasing fashion, from birth to adulthood (Tischler et al., Int. J. Devl. Neurosci. 7: 1989), and NGF, FGF, and IGFs are known to stimulate synergistically chromaffin cell proliferation in vitro (Frödin and Gammeltoft, PNAS 91:1994). Since a physiological role for the chromaffin cell-derived TGF-ßs has not been established as yet, we were interested whether TGF-ß would interfere with the capacity of chromaffin cells to divide and respond to various mitogens. Chromaffin cells were dissociated from P6 rat adrenal medullae as previously described and cultured for 7 days under serum-free conditions. Cultures received FGF-2, IGF-II, retinoic acid (RA), and TGF-ß1 in various concentrations and combinations. BrdU was added at 10 µM during the final two days. Cultures were then processed for the co-visualization of BrdU- and tyrosine hydroxylase immunoreactivities. FGF-2, IGF-II and RA, applied single or in combinations, caused up to 30-fold increases in chromaffin cell proliferation. TGF-ß1, applied at 1, 2.5, or 10 ng/ml, significantly reduced the factor-mediated increases in proliferation. This suggests that TGF-ß, released by chromaffin cells, together with glucocorticoids and PACAP (Frödin and Gammeltoft, 1994), which is released from intramedullary nerve terminals, must be considered as negative regulators of chromaffin cell division. DFG Un34/16-1

690.18

ANALYSIS OF ASTROCYTE PCR PRODUCTS OBTAINED WITH DEGENERATIVE PRIMERS TO TGFß/GDNF. D.O. Dean*, D.G. Schaar, C.F. Dreyfus & I.B. Black. Dept. of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854.

One of the characteristic features of Parkinson's Disease is degeneration of midbrain dopaminergic neurons. Recently, a new trophic factor, Glial cell line-derived neurotrophic factor (GDNF), which rescues these neurons, has been described. It shares homology with members of the Transforming Growth Factor-ß (TGF-ß) superfamily through the number and spacing of cysteine residues. Previously, our laboratory has shown that GDNF is expressed by cultured type 1 astrocytes from a number of brain regions. We now report the use of degenerative PCR to isolate TGF-ß/GDNF-related cDNAs from two of these regions.

Degenerate PCR primers directed towards amino acid sequences conserved among members of the TGF-ß superfamily (and in particular GDNF) were synthesized. RT-PCR was performed, utilizing these highly degenerate primers with total RNA isolated from cultured substantia nigra and cortex type 1 astrocytes as a template. The PCR products were subcloned, classified by insert size and representatives of each sequenced.

We obtained seven different inserts (4 from substantia nigra and 3 from cortex), of which five showed no significant homology to any nucleotide sequence in either the Genebank, or EMBL databases. Four of these sequences also had potential open reading frames. The remaining two sequences were highly homologous (92-96%) to either mouse TFE3 (a transcription factor), or mouse heat shock protein 86 (a member of the hsp90 family). These clones apparently represent the rat homologues of the aforementioned genes.

In sum, we have isolated two previously described and five novel cDNAs. We are presently characterizing the trophic and growth activities of these novel clones, using different brain neuronal populations.
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690.19

RECOMBINANT HUMAN IGF-1 AND IGF ANALOGUES PROMOTE SURVIVAL OF CEREBRAL CORTICAL AND CEREBELLAR NEURONES IN HYPOGLYCAEMIC CONDITIONS. S.J. Harper, T. Priestley, A. Macaulay, M.A. Cascieri, G.G. Chicchi & R.G. Hill.* Merck Sharp & Dohme Ltd, Terlings Park, Harlow Essex. UK. & Rahway, New Jersey.

Insulin and insulin-like growth factors (IGF-I, IGF-II) are closely related polypeptides which are found in the CNS and which promote neuronal survival and neurite outgrowth. They are each associated with specific cell surface receptors and several soluble binding proteins (IGF-BPs) which are involved in regulating function and availability. The two analogues of IGF-1 were produced by site directed mutagenesis: [Gln3, Ala4, Tyr15, Leu16]IGF-1 and a B chain analogue in which the first 16 amino acids of IGF-1 were replaced by the first 17 amino acids of insulin. These analogues have significantly reduced binding affinity for IGF-BPs.

Neuronal cultures were established using embryonic (E15 - E17) rat cortex and PnD4 rat cerebellum in order to determine whether these IGF analogues were as effective as native recombinant human IGF-1 in protecting against cell death induced by hypoglycaemia. Cultures were maintained in DMEM +10% FCS in the presence of mitotic inhibitors for 5 days (cerebellum) and 6-8 days (cortex). DMEM was then replaced with Lockes solution, a minimal salt solution containing no glucose, or Lockes containing IGF-1 or analogues. After 48 hours, Lockes solution was removed and this and the remaining cells were assayed for LDH, an established method for assaying cell survival. Results are expressed as percentage released LDH of total. In the presence of either IGF-1 or either of the IGF-1 analogues the percentage LDH released from cortical and cerebellar neurones was significantly reduced (approx. 50%) indicating that these growth factors promote survival. More importantly, these data show that the IGF analogues with reduced affinity for IGF-BPs are as effective as IGF-1 in promoting cell survival and their reduced affinity for IGF-BPs has no deleterious effect on their neuroprotective function.

690.20

AURINTRICARBOXYLIC ACID (ATA), A NEUROPROTECTIVE COMPOUND, INDUCED THE TYROSINE-PHOSPHORYLATION CASCADE IN PC12 CELLS.
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ATA is known to protect neurons from apoptotic cell death by inhibiting of calcium-activated endonucleases. With PC12 cells, a neuronal model cell line, ATA prevents cell death under serum free condition. However, direct inhibition of endonucleases by ATA was not observed in isolated nuclei of PC12 cells.

The western blot analysis of PC12 cell lysate with anti-phosphotyrosine antibodies revealed that pre-treatment with 100 µM ATA for 2 minutes induced phosphorylation of the tyrosine residues of some proteins. The major tyrosine-phosphorylations induced by ATA were not observed in PC12 cells treated by NGF or EGF. However, ATA, as well as NGF or EGF, induced the tyrosine-phosphorylations of PI3 kinase and SHC in PC12 cells. The induction of tyrosine-phosphorylation by ATA was not observed in NIH3T3 cells. Moreover, ATA did not protect NIH3T3 cells from cell death under serum free condition.

These results suggest that the neuroprotective action of ATA is mediated by the activation of a membrane receptor and the subsequent stimulation of the tyrosine-phosphorylation cascade.

690.21

CO-ADMINISTRATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND EPIDERMAL GROWTH FACTOR (EGF) ELICITS NEURONAL MORPHOLOGIC DIFFERENTIATION OF PC12 CELLS. D.W. Fink, Jr*. Div. Cytokine Biol., Lab Cell Biol., Center for Biologics Evalua tion and Research, FDA, Bethesda, MD 20892.

Cultured PC12 cells possess receptors for and respond to an array of peptide growth factors including nerve growth factor (NGF) and EGF. Addition of either NGF or EGF to PC12 cells elicits activation of cognate tyrosine kinase (TK) receptors resulting in rapid, enhanced tyrosine phosphorylation of a series of substrates that is similar for both NGF and EGF. While NGF promotes neuronal differentiation of PC12 cells, EGF invokes a mitogenic response. The neuroactive peptide, VIP has been reported to promote a partial neuronal differentiation in neuroblastoma cells, an effect attributed to VIP-mediated increases in cAMP levels. The possibility that a combination of EGF-stimulated TK activity and the cAMP-stimulating effect of VIP could support an NGF-like neuronal differentiation response in PC12 cells was investigated.

Co-administration of EGF and VIP to PC12 cell cultures for up to 6 days results in robust neuritic outgrowth. The neurites approximate, in length, those obtained by NGF treatment for comparable periods of time, but are less complex in their arborization. In the presence of 500 nM VIP, the neurite outgrowth response can be obtained with concentrations of EGF as low as 1 ng/ml. The neuronal morphologic differentiation induced by EGF + VIP is not inhibited by the NGF-selective inhibitor, K-252a. These results suggest that peptides such as VIP and EGF which are partial, or ineffective neurotrophic agents may interact cooperatively eliciting a neuronal morphologic differentiation similar to that observed for the identified neurotrophin, NGF. One possible inference suggested by these data is that together, cAMP-dependent protein kinase and tyrosine kinase activities are sufficient to support neurite outgrowth, a characteristic feature of neuronal differentiation.

690.22

INHIBITION OF HUMAN NEUROBLASTOMA GROWTH BY A SPECIFIC VIP ANTAGONIST

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Vasoactive intestinal peptide (VIP), was shown to be a mitogen for sympathetic neuroblasts (Pincus et al., Nature 343,564,1990), and for human neuroblastoma (cell line-NMB), a tumor of the sympathetic nervous system (Wollman et al., Brain Res. 624,339,1993). Northern blot analysis revealed VIP mRNA transcripts in the neuroblastoma (cell line-NMB) suggesting an autocrine growth factor role for VIP in neurogenesis and tumor propagation. Measurements of thymidine incorporation and cell numbers now showed that a potent VIP hybrid antagonist(neurotensin$_{1-6}$-VIP$_{7-28}$; Gozes et al., Endocrinology 125,2945,1989) inhibited cell division in this neuroblastoma cell line. The hybrid VIP antagonist also inhibited lung cancer growth (Moody et al., Proc. Natl. Acad. Sci. USA 90, 4345, 1993), indicating a new therapeutic strategy against prevalent cancers including neuroblastoma, the most common solid malignancy of children less than 5 years of age.

690.23

CSF-1 AND ITS RECEPTOR ARE EXPRESSED IN DEVELOPING BRAIN AND MUTATION OF CSF-1 IMPAIRS BRAIN DEVELOPMENT. M.D. Michaelson*, P. Bieri, J.W. Pollard, M.F. Mehler, H. Xu, J.C. Arezzo, and J.A. Kessler. Depts. of Neuroscience and Dev. & Mol. Biology, Albert Einstein College of Medicine, Bronx, NY 10461.

Colony stimulating factor-1 (CSF-1) was originally identified as a specific growth factor for cells in the mononuclear phagocyte lineage, and subsequently was also found to be an important factor in maternal-fetal regulation. We find that CSF-1 is a growth factor in the nervous system as well. Nuclease protection studies demonstrate that CSF-1 mRNA is expressed *in vivo* in developing and adult mouse brain in various regions, including hippocampus, striatum, cerebellum and cortex. CSF-1 mRNA is found in whole brain samples as early as embryonic day 13, and is detectable through adulthood with some temporal variation. RT-PCR analysis shows splice variants of CSF-1 mRNA are expressed which encode soluble, and not membrane-bound, growth factor. Additionally, mRNA for c-fms, which encodes the CSF-1 receptor, is readily detectable in the developing and adult CNS. Regional studies correlate c-fms expression with CSF-1 mRNA expression. Treatment of primary cultured neurons from five brain regions with CSF-1 promotes survival and process outgrowth.

To define the role of CSF-1 *in vivo*, electrophysiologic techniques were employed on *op/op* mice, which are homozygous for an inactivating CSF-1 mutation. These animals display aberrant visual and auditory evoked potentials, using both extracranial and intracranial recordings, indicative of abnormal cortical function. The expression of CSF-1 and its receptor *in vivo*, the effects of CSF-1 on neurons *in vitro*, and the neurological deficits in *op/op* mice suggest that CSF-1 is an important factor in CNS development.

690.24

PERIOSTEUM ALTERS THE TRANSMITTER PHENOTYPE OF SYMPATHETIC NEURONS. S.E. Asmus*, R.J. Schotzinger and S.C. Landis. Dept. of Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106.

Factors released by sweat glands induce a phenotypic switch in the sympathetic neurons that innervate the glands. To determine if other sympathetic neurons undergo a target-regulated transmitter switch, the innervation of periosteum, the connective tissue covering of bone, was examined. Adult rat periosteum contained sympathetic fibers characterized by choline acetyltransferase (ChAT) activity and staining for acetylcholinesterase (AChE) and vasoactive intestinal peptide (VIP). Prenatally, however, periosteal innervation displayed noradrenergic properties - catecholamine (CA) fluorescence and tyrosine hydroxylase (TH). During the early postnatal period, periosteal fibers exhibited both TH and VIP. Between postnatal days (P) 14 and 21, the phenotypic transition was complete; CA and TH decreased, while the number of fibers stained for AChE and immunolabeled only for VIP increased. Neonatal treatment with the adrenergic neurotoxin, 6-hydroxydopamine, reduced both the developmental expression of noradrenergic traits and VIP and AChE in the adult, confirming that a transmitter switch had occurred. To determine if periosteum induces the neurochemical switch, periosteal flaps from P2 rats were transplanted under P1 rat hairy skin, which receives only noradrenergic sympathetic innervation. One and two weeks after surgery, TH- and CA-containing fibers were present in the transplants. Four to six weeks after transplantation, no CA were detected, but VIP-containing fibers were observed. To examine possible factors governing the switch, periosteum of transgenic mice with a disruption in the gene for leukemia inhibitory factor (LIF), a cholinergic differentiation factor, were assayed. Identical distributions of VIP and AChE fibers were observed in transgenic and control mice, indicating that LIF is not required for the switch to occur. These results suggest that periosteum, in addition to sweat glands, regulates the neurochemical differentiation of sympathetic neurons and supports the hypothesis that target tissues influence the transmitter expression of their innervating neurons.

690.25

EFFECT OF DIFFERENTIATION AGENTS ON CHOLINERGIC CHARACTERISTICS OF A HUMAN NEUROBLASTOMA CELL LINE. R.D. Crosland*, Toxinology Div., U.S. Army Med. Res. Inst. Infec. Diseases, Frederick, MD 21702.

LA-N-2 is a cell line derived from a human peripheral neuroblastoma. It has a partially cholinergic phenotype and is a potential *in vitro* model of peripheral cholinergic neurons. It does not demonstrate, however, depolarization-stimulated, Ca^{2+}-dependent release of acetylcholine (ACh). The object of this study was to induce Ca^{2+}-dependent release of ACh in LA-N-2 cells with various differentiation agents.

Cells were treated with 1 mM dibutyrl cAMP (dibucAMP), 0.25 nM leukemia inhibitory factor (LIF) and/or 3.8 nM nerve growth factor, or 10 µM retinoic acid (RA) for 9-14 days in F12/EMEM with FBS. Treated cells were loaded with [^{3}H]choline for 30 min at 37° and washed. The amounts of cellular and released (5 min, RT), labeled and unlabeled ACh and choline were determined by HPLC. None of the differentiation agents induced Ca^{2+}-dependent release of [^{3}H]ACh. Some of them, however, had marked effects on other aspects of cholinergic function. LIF increased [^{3}H]choline uptake, [^{3}H]ACh synthesis, and ACh content 250% and Ca^{2+}-independent release of [^{3}H]ACh 70% compared to untreated cells. RA increased [^{3}H]choline uptake, [^{3}H]ACh synthesis, and ACh content 30% and basal release of [^{3}H]ACh 130%. DibucAMP increased [^{3}H]choline uptake 20% and decreased ACh content 60%. LA-N-2 cells may be a good model for studying aspects of peripheral cholinergic function.

690.26

EFFECTS OF 2-CHLOROADENOSINE AND FGF ON MYENTERIC NEURONS IN DISSOCIATED CELL CULTURE. M.J. Saffrey*, K.H. Schäfer[1] and G. Burnstock. Dept. Anat. Dev. Biol.,Univ. Coll. London, UK and [1]Dept. Anat., Univ. Saarland, Homburg, Germany.

Purines have been reported to have trophic actions in the nervous system, and may act synergistically with growth factors. We have investigated the effects of basic FGF and the stable adenosine analogue, 2-chloroadenosine (2-CA) on neurite outgrowth and survival of enteric neurons in dissociated cell culture. Myenteric ganglia were obtained from postnatal day 7 rat small intestine by a novel method using a combination of enzymatic digestion and mechanical agitation and then dispersed by further enzyme treatment. The resulting cells (neurons and glia) were grown in serum-free medium. After 48 hr, cultures were immunolabelled for the neuronal marker, protein gene product 9.5 (PGP 9.5). The numbers of PGP 9.5 positive neurons were counted and neurite lengths measured using an image analysis system. FGF (10ng/ml) caused a 45% increase in neuronal survival, and an increase in neurite length of 94.8%. 2-CA (10^{-5}-10^{-7}M) had no effect on neuronal survival, but increased neurite outgrowth in a dose-dependent manner. This increase was maximal (36.9%) at 10^{-5}M and was blocked by the P_1 purinoceptor antagonist, 8-sulphonyl-phenyltheophylline. When 2-CA was added together with bFGF, a greater increase in neurite outgrowth was seen at 2-CA concentrations of both 10^{-5} (158%) and 10^{-6}M (157%). FGF-induced neuronal survival was unchanged upon addition of 2-CA. The proportion of process-bearing neurons increased in the presence of both agents. The ratio of neurons to glial cells was similar in all conditions. These observations indicate that bFGF promotes both survival and neurite outgrowth of enteric neurons and that purines may not only directly influence neurite outgrowth, but also possibly act synergistically with FGF on neurite outgrowth in this system. Thus interactions between purines and trophic factors may be of importance in the development and regeneration of enteric neurons.

691.1

EFFECTS ON DEVELOMENTAL AND ADULT NEUROCHEMISTRY OF CHRONIC NEONATAL TREATMENT WITH AN INHIBITOR OF ORNITHINE DECARBOXYLASE IN THE RAT. A. Contestabile,M. Sparapani,M. Virgili, F. Facchinetti and E. Ciani (Spon: European Neuroscience Association),Dept. of Biology,Univ. of Bologna,Italy.

Neonatal rats were treated from postnatal day 0 to 24 with daily s.c. injections of the suicide ornithine decarboxylase inhibitor,DFMO. In addition to some somatic effects,the treatment resulted in permanent decrease of brain weight which was mainly due to a 40% decrease of the cerebellum. While ornithine decarboxylase inhibition was only effective during the first phase of the treatment,the levels of putrescine and spermidine were decreased in the cerebellum and forebrain up to 3 days after the end of the treatment. Significant alterations of neuronal and glial chemical markers were measured in the cerebellum of treated rats once adults. Developmental treatment with DFMO activates some mechanism able to keep low levels of putrescine and spermidine even in the presence of a progressive lack of ornithine decarboxylase inhibition. Furthermore,low developmental levels of the two polyamines mainly affect regions where substantial neurogenesis does occur during the treatment, such as the cerebellum.

691.2

NEUROTROPHIC EFFECTS OF PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) ON CEREBELLAR GRANULE CELLS IN CULTURE. T. Taniwaki*, S.P. Becerra †, G.J. Chader †, J.P. Schwartz. Clinical Neuroscience Branch, NINDS, and †Laboratory of Retinal Cell and Molecular Biology, NEI, NIH, Bethesda, MD 20892

Pigment epithelium-derived factor (PEDF) is a neurotrophic agent identified and purified from conditioned medium of retinal pigment epithelium cells. PEDF has been shown to enhance neurite outgrowth and neuronal differentiation in human Y-79 retinoblastoma cells. It became of great interest to determine whether PEDF has neurotrophic effects on neurons in primary culture from central nervous system regions other than the eye. Cultures of cerebellar granule cells, prepared from 8-day postnatal rats, were treated with 5-500 ng/ml recombinant human PEDF. The PEDF-treated group contained more cells compared to the control group, when assayed either by the MTS assay (aqueous soluble tetrazolium formazan assay) or by immunocytochemistry for neuron-specific enolase. The difference became larger with longer culture times (up to 7 days) and was dependent on the dose of PEDF. Furthermore, the effect was 10-20 fold larger in the absence of serum than in its presence. PEDF had no effect on incorporation of BrdU (mitosis). Use of an enzyme-linked immunoadsorbent assay to measure neurite outgrowth showed no effect of PEDF on neurite extension. Fragments of rPEDF lacking the reactive site for serine protease inhibitor (serpin) still possess neurotrophic activity. These results demonstrate that PEDF has a neurotrophic survival effect on cerebellar granule cells in culture which is not related to serpin activity such as is seen with the glia-derived nexins.

691.3

MOTOR ABNORMALITIES INDUCED BY X-IRRADIATION AT BIRTH ARE DELAYED BY PERINATAL GM1 TREATMENT.

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Since GM1 was reported to enhance neurite formation after damage of central neurons by different agents, we study its effects on motor abnormalities and neurochemical changes in cerebellum (CE) induced by a 5 Gy single dose of X-rays (X) applied to rats up to 72 h after birth. GM1 30 μg/g were s.c. injected using different protocols: 1) 4 daily doses after X; 2) 1 dose 1 h before and other immediately after X; 3) 3 daily doses before and 3 daily doses after X. Noradrenaline (NA) was assayed fluorometrically and motor function was quantified using an "ad-hoc" test.

At postnatal day (PN) 30, indicators of motor function in X-treated rats that received GM1, no matter the protocol used, returned to nonirradiated values. However, at PN 90 no differences in motor function were found between GM1-treated and uninjected exposed rats, except for protocol 1. None of the protocols modified the changes in NA levels induced by X.

Then, GM1 (in particular, when repeatedly administered after X) may produce a long-term delay in the appearance of X-induced motor abnormalities, probably due to an action(s) on central pathways involved in motor output.

691.4

RECOMBINANT HUMAN GLIAL GROWTH FACTOR SUPPORTS THE PROLIFERATION OF HUMAN SCHWANN CELLS *IN VITRO*. J. L. Rutkowski[1], C. J. Kirk[2], M. Lerner[1], R. Toms[2], A. G. Knapp*[2], and G. I. Tennekoon[1]. [1]Dept. of Pediatrics, Univ. of Michigan, Ann Arbor, MI 48109, and [2]Cambridge NeuroScience, One Kendall Square, Bldg. 700, Cambridge, MA 02139.

Glial growth factors (GGFs) belong to a family of growth factors including the heregulins and neu differentiation factor that arise from the same gene by alternative splicing. Products of this gene initiate diverse biological functions in different cell types. Human and bovine cDNA clones encoding several mRNA isoforms have been identified, and a recombinanat human protein (rhGGF2) was shown to stimulate the proliferation of rat Schwann cells *in vitro*. We examined the mitogenic activity of rhGGF2 on human Schwann cells by bromodeoxyuridine (BrdU) incorporation using an ELISA. Whereas rat Schwann cells respond similarly to either human or bovine GGF, an increase in BrdU uptake could only be observed in human Schwnn cells when treated with rhGGF2. The EC50 for rhGGF2 was approximately 170 pM in both species. Due to the possibility of fibroblast contamination, stimulation of DNA synthesis was confirmed in human Schwann cells by double-labeling with antibodies to s-100 and BrdU. Moreover, rhGGF2 was effective in expanding human Schwann cell cultures in the presence of cAMP-elevating drugs which both synergize with rhGGF2 and suppress fibroblast growth. The population doubled every 5-7 days, and the cells have been carried at least 10 passages. The abillity to generate large numbers of human Schwann cells *in vitro* will faciliatate studies on nervous system transplantation and mechanisms of human peripheral nerve disease. (Supported by NS21700 and the Johns Hopkins Center for Alternatives to Animal Testing.)

691.5

RECOMBINANT HUMAN GLIAL GROWTH FACTOR: TEMPORAL KINETICS AND SIGNAL TRANSDUCTION IN SCHWANN CELLS. C.J. Hoban, J. Sudhalter, N. Mahanthappa, S. M. Goldin*, D. Gwynne and C. Kirk. Cambridge NeuroScience Inc. One Kendall Square, Building 700, Cambridge, MA 02139.

Glial growth factors (GGFs) are products of the neuregulin gene family that are expressed in the nervous system (Marchionni *et al.*, Nature 362:312-318,1993). Recombinant human GGF2 (rhGGF2) is a potent mitogen for Schwann cells. We examined two aspects of rhGGF2 action on Schwann cells *in vitro*: the temporal kinetics of DNA synthesis and activation of signal transduction pathways. Purified rhGGF2 stimulates DNA synthesis in both a dose and time dependent manner. Exposure to rhGGF2 for various time periods before the addition of ^{3}H-thymidine labeling media stimulates DNA synthesis maximally when added at 22 hr, 50% when added at 7 hr and 20% when added at times less than 2 hr (to 5 min).

In addition, we have used compounds that inhibit either tyrosine kinases or protein kinase C (PKC) to determine if the signal for DNA synthesis is acting through these signal pathways. We show that induction of DNA synthesis by rhGGF2 is inhibited by either staurosporine, a PKC inhibitor, or genistein, a tyrosine kinase inhibitor. Our data suggest that rhGGF2 can stimulate DNA synthesis in Schwann cells through two different signal transduction pathways, a PKC-mediated as well as a receptor-tyrosine kinase pathway. We also examined the early events stimulated by rhGGF2 on second messenger pathways that signal directly to the nucleus. Within 1 hr of treatment, rhGGF2 activates several transcription factors to bind to DNA. These data describe some of the earliest events that are initiated through the binding of rhGGF2 to high affinity cell surface receptors on Schwann cells that lead to the generation of a mitotic signal.

Because rhGGF2 is an important growth regulator for several distinct cell types, our goal is to determine the biochemical basis for these pleiotropic responses induced by rhGGF2 effects in different cell types and tissues.

691.6

A ROLE FOR SCHWANN CELL-ASSOCIATED HEPARAN SULFATE IN MODULATING THE ACTIVITY OF THE NEUREGULIN, rhGGF2. J. Sudhalter, N. K. Mahanthappa, J. Lucas, E. Gamzu*, and M. A. Marchionni. Cambridge NeuroScience, 1 Kendall Sq., Bldg. 700, Cambridge, MA 02139.

Glial growth factor 2 (GGF2) is a potent Schwann cell mitogen, which is expressed in neurons and is one of several products of the neuregulin gene (Marchionni et al. 1993, Nature 262: 312-318). Previously Ratner and co-workers (PNAS 85: 6992-6996, 1988) described a neuronally-associated Schwann cell mitogen bound to cell surface heparan sulfate proteoglycan (HSPG) whose mitogenic activity can be inhibited by heparin. Several products of the neuregulin gene have been purified using heparin affinity chromatography, and Schwann cells have HSPG associated with both their plasma membrane and their basement membrane (Carey et al. 1987, JCB 105: 1013-1021). We have thus investigated the effects of heparin on the biological activity of rhGGF2. We show that heparin inhibits the mitogenic activity of rhGGF2 and blocks p185 receptor tyrosine kinase activation on Schwann cells. In contrast, D-glucuronic acid and chondroitin sulfate A had no effect. Heparin added to other known Schwann cell mitogens (bFGF, PDGF-BB) produced little or no inhibition. When Schwann cells were treated with 4-methylumbelliferyl-B-D-xyloside to inhibit proteoglycan assembly, we observed a nearly total loss of mitogenic response to rhGGF2. We hypothesize that rhGGF2 may contain a heparin binding domain and acts through cell-surface HSPGs to mediate its effects. This co-receptor model of biological activation, which consists of HSPG and a high affinity tyrosine kinase receptor for maximal activity is well-supported for other heparin-binding growth factors (Rapraeger et al. 1991, Science 252:1705-1707; Higashiyama et al. 1993, JCB 122: 933-940).

691.7

PROMOTION OF PERIPHERAL NERVE REGENERATION BY A SOLUBLE NEUREGULIN, rhGGF2: STUDIES OF AN ENTUBULATION MODEL *IN VITRO*. N. K. Mahanthappa*. Cambridge NeuroScience, One Kendall Sq., Bldg. 700, Cambridge, MA 02139.

Glial growth factors (GGFs) comprise a group of proteins, first identified in bovine pituitary extract, that promote the proliferation of Schwann cells in vitro (Marchionni et al. 1993, Nature 262:312-318). Recombinant human GGF2 (rhGGF2) is one of several differentially-spliced products of the neuregulin gene. We have previously shown that rhGGF2 promotes Schwann cell proliferation without significantly affecting the expression of a number of Schwann cell associated proteins (Bermingham-McDonogh et al. 1993, Soc. Neurosci. Abstr. 19:1098). The proliferative activity may be of therapeutic utility in peripheral nerve damage and disease. Since Schwann cells are known to provide trophic support for a variety of neural populations, simple expansion of the resident Schwann cell population in a site of disease or damage may promote regeneration and survival.

The goal of the current study is to examine the therapeutic utility of GGF2 in the promotion of nerve regeneration by analyzing an *in vitro* model of the entubulation surgery commonly used to treat peripheral nerve gap injuries. In the current model, a fragment of neonatal rat superior cervical ganglion is placed in one end of a segment of polyethylene tubing (1.9 mm x 10mm) that has been filled with culture medium containing bovine dermal collagen ± rhGGF2. The collagen is allowed to gel and the tubes are maintained in culture for up to 10 days. Preliminary results suggest that rhGGF2 promotes Schwann cell proliferation, and Schwann cell invasion of the tube in a dose-dependent manner.

691.8

CONTROLLED RELEASE OF rhGGF2 FROM A MICROPOROUS COLLAGEN/COMPOSITE MEMBRANE. S. M. Goldin, C. Kirk, L. I. Mortin*, R. Toms, E. M. Taylor, F. R. Wason, and J. Sudhalter. Cambridge NeuroScience, 1 Kendall Square, Bldg. 700, Cambridge MA 02139.

To optimize the therapeutic potential of growth factors in nerve regeneration, it is desirable to control their delivery to the site of nerve repair. Recombinant human glial growth factor 2 (rhGGF2), a product of the neuregulin gene, stimulates rat Schwann cell proliferation and DNA synthesis in cell culture [Marchionni et al. 1993, Nature 362:312]. The halflife of biological activity is less than 24 hours when soluble rhGGF2 is added to a polyethylene nerve guide tube reconnecting the two cut ends of rat sciatic nerve. The initial goal of this study was to determine whether rhGGF2 can be immobilized and released in quantities sufficient to enhance Schwann cell proliferation within a nerve guide tube. This technology will then be employed in a rat sciatic nerve model of nerve repair. A composite microporous structure incorporating collagen has been developed for this purpose. RhGGF2 was exposed to this microporous membrane (thickness 150 μm) under mildly acidic conditions. Under these conditions secondary bonds within the collagen matrix are broken, and reform when the pH is raised in a manner that incorporates rhGGF2 within the membrane.

Employing stimulation of Schwann cell [^{125}I]-uridine incorporation *in vitro* as a marker for release of rhGGF2, the rate of release was determined to exceed 0.5 x 10^{-6} pmole/hr per mm^2 of membrane surface over a period of at least two weeks. A mathematical model of a 1.4 mm diameter nerve guide tube lined with this membrane predicts that at the aforementioned release rate, [rhGGF2] would increase at a rate of >10 pMolar per hour. Based on preliminary estimates of the halflife of soluble rhGGF2 within a nerve guide tube *in vivo* in rats, and an EC50 of ~100 pM for stimulation of Schwann cell proliferation ([^{125}I]-uridine incorporation) *in vitro*, the model predicts that by this means of controlled release, [rhGGF2] will exceed that required for stimulation of Schwann cell mitogenesis for a two week period.

691.9

GLIAL GROWTH FACTOR 2, A NOVEL MUSCLE TROPHIC FACTOR. R. M. Sklar, G. Gao, C. Kirk, I. Isaacs, D. Gwynne*, R. McBurney, M. Marchionni, Cambridge NeuroScience, One Kendall Square, Bldg.700, Cambridge, MA 02139

Recently, the expression of one or more products of the neuregulin gene in peripheral and motor neurons has been described by *in situ* hybridization (Marchionni, et al., Nature 1993;**362**:312-318). One of these neuregulins, acetylcholine receptor-inducing activity (ARIA), has been shown to stimulate the expression of several genes encoding components of the developing neuromuscular junction in chick muscle culture (Falls, et al., Cell 1993;**72**:801-815; Corfas & Fischbach, J. Neuroscience 1993;**13**:2118-2125). A secreted form of neuregulin, rhGGF2, has been cloned, based on its mitogenic activity on Schwann cells, and expressed in CHO cells. The possibility that rhGGF2 may have trophic effects on muscle cells in culture was investigated. rhGGF2 was mitogenic for subconfluent quiescent human myoblasts, but did not inhibit myoblast fusion. Differentiation of clonal human myoblasts in the continuous presence of rhGGF2 resulted in greater numbers of myotubes after six days of differentiation (Sklar, et al., J. Cell. Biochem. 1994;**18D**:540). The increase in myotube numbers was the result of an inhibition of myotube death as measured by propidium iodide uptake. rhGGF2 effects on muscle culture suggest that this neuronal-derived factor may regulate muscle growth *in vivo*.

691.10

NEUREGULIN, rhGGF2, STIMULATES TRANSCRIPTION OF THE AChR DELTA SUBUNIT GENE. S. A. Jo[1], M. A. Marchionni[2], I. J. Isaacs[2], and S.J. Burden[1]*. [1]Biology Department, Massachusetts Institute of Technology, Cambridge, MA 02139 and [2]Cambridge NeuroScience Inc., One Kendall Square, Building 700, Cambridge, MA 02139.

Motor neurons induce the aggregation of acetylcholine receptors (AChR) at neuromuscular synapses. We have shown that a signal in the synaptic basal lamina at the neuromuscular synapse stimulates transcription of the AChR δ subunit gene in the synaptic nuclei of skeletal myofibers (Jo and Burden, Development 115: 673-680, 1992). Because the neuregulin gene is known to encode an AChR-inducing activity, ARIA (Fall et al., Cell 72: 801-815, 1993), we were interested in determining whether products of the neuregulin gene might be the basal lamina signal. We treated C2 myotubes, which were stably transfected with a gene fusion between 1.8 kb of 5' flanking DNA from the AChR δ subunit gene and the human growth hormone (hGH) gene, with neuregulin (rhGGF2, Marchionni et al., Nature 362: 312-318, 1993), and we found that neuregulin causes a 2.4 fold increase in hGH expression. In contrast, neuregulin did not increase hGH expression from C2 myotubes stably transfected with a metallothionein promoter-hGH gene fusion. Thus, neuregulin activates transcription of the AChR δ subunit gene. Moreover, RNase protection assays show that neuregulin increases the level of endogenous δ and ε subunit mRNAs by 2-fold and 4-fold, respectively. Neuregulin induces the rapid tyrosine phosphorylation of a p185 protein in C2 myotubes, indicating that neuregulin regulates transcription of AChR gene through tyrosine phosphorylation. In addition, we show that the *cis*-acting elements for neuregulin-mediated stimulation of δ subunit gene expression are contained in the same 181 bp of 5' flanking DNA from the AChR δ subunit gene that confers synapse-specific expression *in vivo*.

691.11

β-A ACTIVIN IS REGULATED BY SYNAPTIC ACTIVITY AND IS EXPRESSED IN THE DEVELOPING RAT BRAIN. K. I. Andreasson*, C. A. Barnes and P. F. Worley. Depts of Neurology and Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205, and Depts. of Neurology, Psychology and Div. of Neural Systems, Univ. of Arizona, Tuscon, AZ 84724

β-A activin is a member of the transforming growth factor ß superfamily of secreted proteins. By differential screening, we have identified it to be rapidly regulated by neuronal activity. β-A activin mRNA is induced in the dentate gyrus with a single maximal electroconvulsive shock within one hour and peaks at two hours. β-A activin mRNA is induced in NMDA dependent synaptic stimulation. In chronically implanted animals, β-A activin mRNA is strongly induced in hippocampal granule cells ipsilateral to high frequency LTP stimulus one hour after treatment. This induction is eliminated with pretreatment of the animals with MK-801. In adult brain, β-A activin is expressed at basal levels in layers II/III and V/VI of the cortex and in the dorsal striatum. Treatment with MK-801 obliterates basal expression in layers II/III and significantly reduces expression in layers V/VI, indicating that expression of ß-A activin mRNA is dependent on excitatory glutamatergic synaptic activity. Deafferentation of the visual cortex with the intraocular injection of tetrodotoxin reduces basal expression of β-A activin in monocular visual cortex in layers II/III and V/VI. In developing brain, β-A activin mRNA is expressed in cortical plate of fetal and post-natal brain, as well as in the differentiating neurons of the developing striatum. Expression is maximal in cortical plate around the time of birth and steadily decreases until baseline levels are reached between post-natal days 10-15. The expression of ß-A activin is an example of differential regulation of an immediate-early gene in developing and synaptically active adult brain. Supported by HD00992, AG09219, and EY09347.

691.12

TRANSMISSION ELECTRON MICROSCOPY REVEALS A REDISTRIBUTION OF MAP2 IN NEURO-2A NEUROBLASTOMA CELLS AFTER GANGLIOSIDE TREATMENT. L.-J Wang, G. Yorke*, R. Colella and F.J. Roisen. Dept. of Anat. Sci. & Neurobiol., Univ. of Louisville Sch. of Med., Louisville, KY 40292.

Our previous immunofluorescent studies of Neuro-2a cells exposed to ganglioside GM1 demonstrated enhanced microtubule (MT)-dependent neuritogenesis, increased microtubular network density, and a redistribution of MAP2 from the perikarya to the distal neuritic processes. In the current study, immunogold-labeled specimens were analyzed with computer assisted morphometry. Neuro-2a neuroblastoma were grown *in vitro* with or without GM1 (150 μg/ml) for 24 hr and embedded in Lowicryl. Thin sections were incubated with anti-tubulin and anti-MAP2 and colloidal gold labeled second antibody, 10 and 20 nm respectively. The number of gold particles per μm^2 was determined in the microspines, neuritic processes and perikarya of a minimum of 50 cells per treatment group. The results demonstrate that: (a) GM1 enhances the area of spine-like projections ($p<0.05$); (b) more MAP2 per unit area was found in spine-like projections after GM1 treatment ($p<0.001$); (c) more MAP2 was found in neurites after GM1 exposure ($p<0.05$); and (d) MAP2 seems more closely associated with actin-rich subcortical cytoplasm than with MT. In contrast, the location of tau protein, another MAP, was not changed after GM1 treatment. These results are consistent with previous qualitative studies demonstrating that GM1 increases the spine-like projections. Since MAP2 is associated with actin in dendritic spines, perhaps GM1 shifts MAP2 from interactions with MT to actin-filaments which form the basis of growth cone development. This suggests that gangliosides may be involved in the determination of axonal or dendritic fate by selectively changing the distribution of MAP2 and thus regulating microtubule stability and actin-mediated growth cone activity. Studies are in progress to determine the effect of GM1 on the synthesis of MAP2 and tau protein. Supported by a grant from Alliant Community Trust Fund, Louisville, KY.

691.13

ANTIBODY TREATMENT IN VIVO AS A MEANS OF BLOCKING PROTEIN ACTIVITY: EFFECTS ON INTRAOCULAR GRAFTS OF ANTI-INSULIN LIKE GROWTH FACTOR-1 AND ANTI-CELLULAR-CELL ADHESION MOLECULE ANTIBODIES. MB Giacobini*[1], VR Sara[2], B Öbrink[3], L Olson[1]. [1]Dept of Neuroscience, and [3]Cell & Molecular Biology, Karolinska Institutet, Stockholm, Sweden, [2]School of Life Science, QUR Gardens Point Campus, Brisbane, Australia.

Blocking antibodies can be used in order to study the normal roles of proteins such as trophic factors and cell adhesion molecules in the developing brain by reducing or eliminating the effects of these factors. The role of IGF-1 in olfactory bulb maturation was studied using the technique of intraocular transplantation. Olfactory bulbs from E15–E17 fetuses were transplanted into the anterior chamber of the eye of adult host rats. Transplants were treated with either 300 ng truncated IGF-1, two different IGF-1 polyclonal antisera, two different non-immune sera, 15 µg IGF-1 binding protein, or vehicle alone. Treatments were administered by preincubation just prior to grafting and by 5 µl injections into the anterior chamber on days 5, 10 and 15 postgrafting. Olfactory bulb grafts grew significantly larger after treatment with IGF-1 antisera treatment than after any of the other treatments. Similar anti-IGF-1 antibody treatment of grafts of cerebral cortex had no such effect. Immunohistochemical studies of the olfactory bulb grafts revealed no detectable differences between treatments with regard to several markers tested. These studies have established a system for studying the endogenous effects of trophic factors on developing brain tissue by antibody treatments. Studies of the role of the cell adhesion molecule C-CAM during brain development is currently under progress using a similar experimental paradigm.

691.14

EFFECT OF THROMBIN AND 14-AMINO ACID PEPTIDE AGONIST OF THROMBIN RECEPTOR ON PURE OR MIXED GLIAL CO-CULTURES OF SEPTAL NEURONS. Th. Debeir, J. Benavides and X. Vigé*. Synthélabo Recherche, Preclinical Research Department, BP 110, 92225 Bagneux Cedex, France.

A role of thrombin in neuropathological processes is suggested by the fact that this protease is secreted by glial cells. An imbalance between thrombin and PN-1 (its natural inhibitor) seems to be involved in the pathogenesis of several neurological diseases including Alzheimer's disease. In vitro thrombin alters neurite outgrowth and, at low concentrations, increases choline acetyl transferase (ChAT) activity in a mixed neurons/glia culture of septal cells. To characterize further the effect of thrombin on cholinergic neurons we have compared the effect of thrombin and of the 14-amino acid peptide agonist of the thrombin receptor on a pure culture of septal neurons and on co-cultures of septal neurons/glia. Septal cells were grown in defined medium, onto a monolayer of glial cells for co-cultures. Cells were treated one day after plating. ChAT activity and MTT reduction (index of cell viability) were assayed at the 5th day in vitro.

In pure septal neurons, low concentrations of thrombin (up to 30 nM) did not affect ChAT activity or MTT reduction. However, 100 nM thrombin decreases ChAT activity and MTT reduction by 44% and 17%, respectively. In co-cultures, thrombin displayed a biphasic effect. Low concentrations (1 nM) increased ChAT activity by two fold whereas high concentrations (100 nM) decreased it (-83%). At the high concentration, thrombin was neurotoxic, as indicated by a large decrease in MTT reduction (-90%). Thrombin effects on ChAT activity were partially mimicked by the 14-amino acid peptide both in septal cells (no effect at 0.1 µM and -63% at 100 µM) and in co-cultures (+17% at 0.1 µM and -28% at 100 µM). This peptide did not affect MTT reduction. Thus, thrombin's effects on cholinergic neurons seem to be mediated, at least in part, by thrombin receptors and glial cells seem to play a major role in thrombin action.

691.15

EXPRESSION AND REGULATION OF ESTROGEN RECEPTOR (ER) AND ITS MRNA IN A SUBPOPULATION OF RAT DORSAL ROOT GANGLION NEURONS M.M. Oblinger*, J. Pickett, N. Taleghany, L. DonCarlos[1] Dept. Cell Biol. and Anat., Chicago Med. School, North Chicago, IL and [1]Dept. Anat. and Neurobiol., Loyola Univ., Chicago, IL

While considerable information is available on estrogen receptors (ER) in brain, very little is known about the expression and functional roles of ER in peripheral neurons. A recent study using in situ hybridization histochemistry with oligonucleotide probes reported that virtually all dorsal root ganglion (DRG) neurons in rat express ER mRNA (Sohrabji et al., 1994, J. Neurosci. 14:459). In the present study, we re-examined this issue using immunocytochemistry to study ER protein localization in the DRG, in situ hybridization with ^{33}P-labeled cRNA probes to examine ER mRNA in various lumbar (L3-6) DRG, and PCR to amplify ER mRNA sequences from the DRG. DRG were harvested from adult female Sprague Dawley rats 21 days after ovariectomy with either continuous hormone replacement via implants of 17β-estradiol-filled capsules (+E), or with no hormone replacement (ovx). Immunocytochemistry revealed robust nuclear staining for ER in many of the small-sized DRG neurons, but only infrequent staining of large and medium-sized cells. In situ hybridization also showed that ER mRNA expression was significant in the small, nociceptive DRG cells but less apparent in large neurons. ER labeling in the DRG was higher in the ovx than in the +E group, and more ER-positive neurons were apparent in L6 DRG compared to other lumbar ganglia. Preliminary findings on ER function in the DRG indicate that the expression of several proteins localized to small cells, such as peripherin, varies with the estrogen status of the animal.

691.16

STEROID AND NEURONAL REGULATION OF THE INDEPENDENT FATES OF NUCLEI OF MUSCLE DEO1 DURING METAMORPHOSIS IN *MANDUCA SEXTA*. C. D. Wright* and J. W. Truman. Department of Zoology, University of Washington, Seattle WA 98195

Metamorphosis in the moth *Manduca sexta* involves a dramatic reorganization of the skeleton and neuromuscular system. To understand the regulation of this reorganization, we have focused on the fate of a larval, abdominal body-wall muscle, DEO1. This muscle degenerates after pupal ecdysis and then regrows to become the adult muscle DE5. Examination of the muscle at the time of pupal ecdysis revealed 2 classes of nuclei. Using the terminal deoxynucleotidyl transferase method to detect degraded DNA, we found that one class of nuclei undergo degeneration starting on day 2 after ecdysis. At the same time, the other set of nuclei show incorporation of bromodeoxyuridine indicating the initiation of mitogenesis and the start of growth of muscle DE5. Both nuclear degeneration and nuclear proliferation require the rise in the titer of steroid hormones, the ecdysteroids, which occurs after pupal ecdysis. In addition, axotomy experiments show that intact innervation is required for the mitogenesis but not for the nuclear degeneration. Hence, the muscle regrowth requires a local cue from the motoneuron as well as the systemic cue provided by the steroid.

691.17

EXPRESSION OF THE MONOCYTE CHEMOATTRACTANT JE/MCP-1 FOLLOWING CEREBRAL CORTICAL LESIONS. R.M. Klein*, W.-L. Liu, E. Hausmann, and N.E.J. Berman. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160-7400

Monocyte chemoattractant protein-1 (MCP-1), the product of the mouse early response gene JE, is a chemokine which is stimulated by the cytokine, tumor necrosis factor alpha (TNF-α) and by IgG. In previous studies, we have shown that injured neurons express TNF-α and Fc receptors, suggesting that they may provide signals which direct CNS repair processes. In the present studies, we examined expression of JE/MCP-1 following lesions of the visual cortex. Aspiration lesions of posterior cortex were made in mice. Following a 2 day survival, RNA was extracted from the lesioned and nonlesioned hemisphere. RNA from intact mouse brain was used as a negative control, and RNA from LPS-stimulated J774 cells was used as a positive control. Northern analysis was performed to detect JE/MCP-1 transcripts. Samples from injured cortex showed expression of JE/MCP-1, which was absent from intact brain and from the uninjured hemisphere. Taken together, our results suggest that injured neurons produce signals such as TNF-α which stimulate expression of chemoattractant factors such as JE/MCP-1. This chemokine attracts cells of the monocyte-macrophage lineage to the site of injury where they function in phagocytosis and direction of tissue remodeling. Supported by MH38399, HD02528 and NS32202.

691.18

PURIFICATION OF CRANIN, A MUCIN-LIKE LAMININ BINDING MEMBRANE PROTEIN, FROM ADULT SHEEP BRAIN. N. R. Smalheiser* and E. Kim. Dept. of Pediatrics, MC 5058, Univ. of Chicago, Chicago, IL 60637.

Cranin is a 120 kDa integral membrane glycoprotein which binds the long arm of laminin in a calcium-dependent manner, and which expresses carbohydrate chains susceptible both to N-glycanase and O-glycanase (J Neurosci Res 36: 528 '93). Because of its extremely low abundance in brain membranes, we have undertaken to purify cranin from adult sheep grey matter. Tissue (2 kg) was homogenized, crude pellets rinsed with high salt/urea buffer, and solubilized in NP40 buffer. After sequential elutions from DEAE, con A and Jacalin lectin columns, material was passed over laminin affinity beads (at physiologic ionic strength) and eluted with 10 mM EDTA/EGTA. Repeating the last step purified cranin nearly to homogeneity, with a yield of ~100 µg as assessed by amino acid analyses. Aliquots of cranin which are detected strongly by laminin-binding and lectin blotting assays (con A, Jacalin or PNA) do not stain well by Coomassie Blue or silver stains. Cranin isolated from either E14 chick or adult sheep brain exhibited charge heterogeneity in 2-D blots, with pI centered at 5.7. It was readily digested by O-sialoglycoprotease, which selectively attacks proteins with a high density of sialylated O-linked saccharides. Though cranin appears to be distinct from dystroglycan (pI = 3.7), it closely resembles a set of low abundance selectin-binding proteins described in the immune system, both in their mucin-like modifications as well as similarities in binding their respective ligands (calcium dependent, inhibited by periodate oxidation, inhibited by fucoidin and sulfatides). Supported by NIH NS 26055, HD 09402.

691.19

CHARACTERIZATION OF HCNP PROCESSING ENZYME.
Y.Otsuka, K.Ojika*, Y.Tsugu and N.Matsukawa. 2nd Dept. of Int. Med., Nagoya City Univ. Med. Sch., Nagoya 467, Japan.

HCNP is a peptide which stimulates acetylcholine synthesis in medial septal nuclei explant culture. The peptide consists of eleven amino acids located at N-terminal lesion of its pro-protein, and suggested to be processed by the cleaving enzyme. We characterized the HCNP processing enzyme in Wistar rat hyppocampus. Hippocampus from 10-11 day old rats were sonicated in 50mM MES-NaOH buffer (pH6.0) containing 150mM NaCl, 3mM KCl, 1mM EDTA and 1mM DTT, and centrifuged at 15000rpm for 20min. The supernatant was incubated at 37°C with purified HCNP pro-protein or synthetic peptide which consisted of 26 amino acids from N-terminal of the HCNP pro-protein. The reaction was terminated by adding TFA and subjected to HPLC equipped with C18 column to measure the enzyme activity. The activity was determined by the height of the peak with retention time identical to that of de-acetylated HCNP (free-HCNP) in the HPLC. The peak was also analyzed by amino acid sequencer and mass spectrum, and confirmed it as free-HCNP. The enzyme was eluted between 58-100kDa in the molecular sieving column, and retained on DEAE column at pH7.6 and G-Butyl column at pH6.0. Optimal pH of the enzyme activity was 5-6, and the activity was inhibited by Chymostatin and E-64.

691.20

CLONING AND CHARACTERIZATION OF A NEW FACTOR RELATED TO ARIA/NDF/GGF.
H. Chang and W. Gilbert*. Dept. of Cell. and Mol. Biol., Harvard Univ., Cambridge, MA 02138.

Alternative spliced products of the ARIA/Neu differentiation factor (NDF)/Glial growth factor (GGF) gene have a wide range of activities, including stimulation of glial cell proliferation and regulation of acetylcholine receptor synthesis in muscle. Early experiments suggested that NDF is the ligand for the Her2 receptor, a member of the EGF receptor family. But more recent results indicated that NDF directly interacts with the Her4 receptor, another member of the EGF receptor family, raising the possibility that additional factors similar to ARIA/NDF/GGF may be the ligand for Her2. We used a PCR-based strategy to identify new factors related to ARIA/NDF/GGF, and cloned a new gene from an adult rat brain cDNA library. The new factor contains both the Ig-like domain and the EGF-like domain, and has a domain structure very similar to the ARIA/NDF/GGF proteins. At least two more alternative spliced forms of the new factor are also cloned. We expressed one form of the new factor in COS-7 cells, and our preliminary results show that the recombinant protein induces tyrosine phosphorylation of a 185 kD protein in the MDA-MB 453 cells. The phosphorylated protein could be the Her2 receptor or another member of the EGF receptor family. We are currently characterizing the new ARIA/NDF/GGF related gene and trying to determine the identity of the 185 kD phosphorylated protein.

NUTRITIONAL AND PRENATAL FACTORS

692.1

POST-TERM PATTERNS OF CARDIAC RATE AND VARIABILITY IN PREMATURELY-BORN INFANTS SUFFERING FROM APNEA OF PREMATURITY.
V.L. Schechtman*, J.A. Henslee, M.Y. Lee & R.M. Harper, *Brain Research Inst., UCLA Sch. of Med., Los Angeles, CA 90024; and Southwest SIDS Research Inst., Lake Jackson, TX 77566.*

Prematurely-born infants have higher heart rates and reduced heart rate variability at term relative to fullterm neonates (Eiselt et al., 1993), suggesting that prematurity may have long-lasting effects on cardiovascular control. In the present study, we assess post-term development of heart rate and its variability in prematurely-born infants who continue to suffer from apnea of prematurity (AOP).

Six-hour recordings of EKG and respiration were obtained from infants with AOP born at gestational ages of 24 to 35 weeks. Heart rate and its variability were assessed during three periods of regular respiration in each recording of the premature infants and similar recordings of fullterm infants at comparable post-conceptional ages.

At term, infants suffering from AOP showed higher heart rates and reduced heart rate variability relative to fullterm neonates. Over the first month of postnatal life, however, the fullterm infants showed an increase in heart rate and a reduction in variability, such that by 1 mo after term, there were no significant heart rate differences between fullterm infants and prematurely-born infants suffering from AOP. The two groups of infants continued to show comparable heart rates and variabilities at all ages up to 6 mo after term. Thus, neither prematurity nor AOP appears to have a long-lasting effect on cardiac rate or overall cardiac variability.
Supported by HD-22695.

692.2

EFFECTS OF CORN-BASED DIET ON THE DEVELOPMENT OF THE GABAERGIC INNERVATION OF THE SOMATOSENSORY CORTEX IN THE RAT. IMMUNOCYTOCHEMICAL STUDIES.
S. Orozco-Suárez , A. Feria-Velasco* and A. Del Angel. Unidad Invest. Med. Patol. Exptl., Hosp. de Oncología, CMN, S-XXI, IMSS, México, D. F. and Div. de Biol. del Desarrollo, Unidad de Invest. Bioméd. de Occidente, IMSS, Guadalajara, Jal. MEXICO.

Restriction of specific nutrients interferes with growth and development of central nervous system producing alterations in physiology, biochemistry and morphology of brain and cerebellum. To determine the effect of corn-based diet on the development of GABAergic innervation of cerebral cortex, female Wistar rats, 60 days old were fed for 6 weeks with 1) corn-based diet (with 8% protein), and 2)normal diet (commercial diet for rodents, 23% protein). The morphometric studies were performed in the pups at 1,7,14,21,30 and 60 days after birth, and postnatal development of GABAergic elements was analyzed by GABA and GAD-immunocytochemistry. Radial distribution of cells and laminar numerical densities were calculated at each stage of development by substantial qualitative observations. Results of morphometric studies showed that body and brain stem weight, and thickness of cerebral cortex of the corn-fed group, were significantly reduced compared to their controls. At postnatal stages the maturation of GABA-immunoreactive elements showed a reduction in cell number, cell size and different cell development at each postnatal stage in the corn-fed rats. Results suggest that protein restriction plays an important influence on development and differentiation of the GABAergic system in cerebral cortex. Moreover, as corn is restricted in tryptophan and lysine, it is important to further investigate to which extent this restriction also participates in the results obtained in the present work.

692.3

LONG-LASTING CHANGES IN DOPAMINE RECEPTOR DENSITIES IN THE NUCLEUS ACCUMBENS OF PRENATALLY STRESSED RATS.
C. Henry, J. Arsaut, A. Sarrieau*, M. Le Moal, J. Demotes-Mainard. INSERM U-259 and U-394, 33077 Bordeaux, France.

Restraint stress applied to pregnant rats during late pregnancy results in offspring exhibiting some behavioral and neurobiological alterations. Particularly, this procedure of prenatal stress can cause long-lasting changes in the hypothalamo-pituitary-adrenal axis of the offspring, that persist in adult animals. Moreover, prenatally stressed rats show an increased propensity to develop amphetamine self-administration. For this reason, we studied by quantitative autoradiography the densities of D1, D2 and D3 dopamine receptor subtypes in the striatum and nucleus accumbens at postnatal day 90 in either prenatally stressed or control rats, using [3H]-SCH23390, [3H]-sulpride and [3H]-7-OH-DPAT as ligands, respectively. Our results show that in adult offspring, the prenatal stress significantly induce i) a mild increase in D1 receptor binding in both striatum and nucleus accumbens; ii) an increase of D2 receptor binding in the nucleus accumbens; iii) a decrease of D3 receptor binding in the nucleus accumbens. These data indicate that prenatal stress induces long-lasting changes in the dopamine sensitivity in nucleus accumbens, which possibly could participate in the increased propensity to amphetamine self-administration. The mechanism responsible for this alteration remains to be elucidated, but the impaired control of corticosterone could possibly be involved in long-term changes in the mesolimbic dopamine system.

692.4

EFFECTS OF PRENATAL PROTEIN MALNUTRITION ON LTP AT THREE AGES OF DEVELOPMENT.
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The effects of gestational protein malnutrition on the establishment, maintenance, and decay of LTP across the perforant path/dentate granule cell synapse were examined in freely-moving rats at 15, 30, and 90 days of age. Measures of population spike amplitude (PSA) and EPSP slope were used to assess the magnitude and duration of LTP. Significant enhancement of both measures was obtained from all malnourished (6/25) and control (25/25) animals at 15 days of age. The magnitude of enhancement obtained 6/25 rats was significantly below that of controls over the first 24 hrs. post-tetanization. Beyond 24 hrs., both groups showed continuous rise in these meaures. To determine what percentage of this rise was the result of tetanization and what percentage resulted from normal development, input/output (I/O) curves were obtained daily from PND 16-30 from non-tetanized pups of both diet groups. Subtraction of developmental increases revealed that tetanization effects decayed to baseline between days 4 and 5 post-tetanization. At 30 days of age, approximately 50% of 6/25 animals showed no significant change in PSA measures, while EPSP slope enhancement was delayed 1-3 hrs. The remaining 50% of 6/25 animals did not differ from controls, i.e., initial enhancement of both measures was maintained for 5-18 hrs., decaying to baseline by 24 hrs. Approximately 50% of 90 day old 6/25 animals showed a decrease in both measures $\geq$1 hr. after tetanization. At 3 hrs., enhancement was comparable to initial levels obtained from the remaining 6/25 animals and controls. Results indicate an inverse relationship between the impact of the dietary insult and age, and suggest that altered GABAergic interneuronal activity may underlie the LTP deficits obtained from 6/25 rats.
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692.5

OXIDATIVE STRESS RISKS WITH HIGH POLYUNSATURATED FATTY ACID DIETS IN BRAIN/HEART/KIDNEY/MAMMARY/LIVER OF FEMALE SHR RATS

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Aging can be increased by accelerating the rate of initiation of random free radical reactions or by increasing ingestion of easily oxidized dietary components. Dietary polyunsaturated fatty acids (PUFA) of omega-3 and omega-6 type, known to have some beneficial cardiovascular effects, are susceptible to peroxidation under multiple conditions that encourage oxidative free radical formation. We studied the effects of long-term feeding with dietary omega-3 and omega-6 PUFAs on 1) blood pressures and 2) in vitro tissue lipid peroxidation in female spontaneously hypertensive rats. After 16 weeks of feeding with either control (5% corn oil, AIN) or high fat diet (19% Menhaden oil; MO and 20% Corn oil; CO), no differences in blood pressure were observed among groups. In whole brain homogenates, autooxidation averaged 282% and iron-ascorbate catalyzed peroxidation 1225% compared to baseline oxidation in all diet groups. Marked differences occurred between the omega-3 and omega-6 (MO and CO respectively) high PUFA diets on baseline oxidation, autooxidation and iron-ascorbate catalyzed oxidation in various tissues. MO caused a 675% increase in basal oxidation, 2624% increase in autooxidation and 4244% increase in iron-ascorbate catalyzed oxidation in mammary gland compared to the CO diet. Similarly, a 400% increase in iron-ascorbate catalyzed oxidation occurred in liver, 225% in heart, 120% in kidney and 50% in aortae in MO compared to CO, respectively. The results indicate that 1) brain is extremely susceptible to iron-catalyzed peroxidation; 2) neither high omega-3 nor high omega-6 PUFA diets afford protection from hypertension in female SHRs and 3) high omega-3 fatty acid diets markedly increase the oxidative aging risk of multiple organs.

692.6

ELEVATED BLOOD COPPER:ZINC RATIOS IN ASSAULTIVE YOUNG MALES

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There has recently been increased research in studying the association between chemical imbalances and violent behavior. In the treatment of over 3000 assaultive young males, The Carl Pfeiffer Treatment Center has observed elevated blood copper:zinc ratios. The objective of this study was to determine if violent and non-violent patients had significantly different copper and zinc levels and copper:zinc ratios. The sample consisted of all male subjects of the Center and for an 8 week period that were 3 to 18 years old. The test subjects had a history of frequent assaultive incidents, while the control subjects had an absence of such incidents. There were 135 violent and 18 non-violent patients. The mean copper:zinc ratios of 1.40 for violent and 1.02 for non-violent were shown to be significantly different ($p < 0.05$). The mean serum copper levels were 98.5 and 86.6 mcg/dl and plasma zinc levels were 75.4 and 87.4 mcg/dl for violent and non-violent subjects, respectively. These relationships suggest a strong correlation between assaultive behavior and abnormal metal metabolism. Clinical studies are underway to determine if correcting these chemical imbalances will result in improved behavior.

692.7

EFFECTS OF PROTEIN DEFICIENT DIETARY REGIMENS, PRE-TERM BIRTH AND HYPERTENSION ON RAT NEONATAL BEHAVIOR.

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The effect of various metabolic factors on the development of neonatal reflexes and on learning and memory capacity in adulthood was studied in the rat. Female rats of the Wistar strain were subjected to protein deficient dietary regimens (commercial animal food at a reduced weight regimen of 80% or a diet containing casein 6% as the only protein component). Another group of animals was subjected to a caesarean cut at day 19 of pregnancy in order to induce a pre-term birth of pups. Spontaneous hypertensive rats (SHR) were used to examine the possible influence of gestational hypertension on rat brain maturation. We found that protein deficient dietary regimens were accompanied by a retardation in the development of neonatal reflexes and a reduction in learning and memory capacity in adulthood. Differences were also found between control animals and pups born by caesarian cut or from SHR mothers, above all for eye opening time and body weight.

692.8

BIOMAGNETIC ASSESSMENT OF THE INTEGRITY OF THE FETAL CENTRAL NERVOUS SYSTEM.

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Perinatal identification of infants at risk for neurological dysfunction is a challenging but important clinical problem. At present, ultrasound evaluation of CNS-mediated cardiac reactivity to external stimuli or spontaneous movement is the dominant technique for assessing the fetal nervous system. Beat-to-beat variability is also believed to be a good index of fetal well-being, but this is not easily assessed by ultrasound. Biomagnetic techniques offer an attractive alternative to ultrasound examination. The fetal magnetocardiogram (MCG) is readily recorded as early as 14 weeks, and in some cases it is possible to directly record fetal brain activity via magneto-encephalography. Using a 37-channel biomagnetometer we have demonstrated centrally-mediated, stimulus-evoked fetal heart-rate accelerations in a 34-week old fetus. Heart-rate increased to presentation of external sounds and spontaneous movements. Interestingly, the heart-rate returned to baseline when the auditory stimuli were left on for an extended period of time. Examination of "habituation-time" may provide novel insights into fetal CNS function. MCG also provided data on inter-beat variability and morphology of the fetal PQRST waveform. Auditory-evoked brain activity was also recorded successfully from a 34-week old fetus. The data demonstrated clear brain reactivity with an onset latency of 130 milliseconds.

692.9

REPRODUCTIVE SUCCESS IS COMPROMISED IN RATS OVERFED AS NEONATES.

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On postnatal day 4, Long-Evans female rat pups were randomly assigned to one of three groups: 1) mother reared in litters of 9 (MR), 2) gastrostomy-fed for ten days to match the growth of the MR group (WM), and 3) gastrostomy-fed with excess formula to accelerate growth (OF). On day 14 the gastrostomy-fed animals were returned to lactating dams in litters of 8 pups each. All animals were weaned on day 22 and maintained thereafter on laboratory chow. On day 170, plasma glucose readings were taken using tail blood. The animals were bred the following day. On day 190 glucose readings were again recorded.

6 of 9 MR females (67%), 5 of 11 WM females (45%), and 4 of 11 OF females (36%) produced litters. Pregnant glucose levels of the successful females were significantly lower than glucose levels of females who failed to produce pups ($p<.001$). Pre-pregnant glucose levels were significantly higher in OF females and an analysis of variance also indicated that pre-pregnant glucose levels were different among the three groups ($p<.01$, ANOVA; OF x WM, $p<.05$, t test; OF x MR, $p<.01$, t test).

These findings support the hypothesis of several researchers that high fuels experienced in early development can permanently alter adult metabolism. The issue of whether or not central regulatory control systems were altered in addition to peripheral metabolic systems by these nutritional manipulations in early development remains to be determined.

692.10

PRENATAL MALNUTRITION ON THE DEVELOPMENT AND MATURATION OF CA3 HIPPOCAMPAL PYRAMIDAL CELLS IN THE RAT.

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The effects of postnatal malnutrition (6% casein diet) in the somatic size, length of the apical dendrite, number of dendritic branching and the thorny excrescence area of CA3 hippocampal pyramidal cells were studied in rats of two ages (30 and 90 days old). A total of 144 cells impregnated with rapid-Golgi were selected for morphometric analysis. Results showed that postnatal malnutrition produced significant decreases ($p<0.05$) in the all parameters studied. These findings indicate that postnatal malnutrition produces severe alterations in the maturational and development patterns which in these cells ocurrs during posnatal life. We have found similar results in pre and postnatal malnutrition studies (García-Ruiz M et. al., Brain Res. 625: 203, '93). Thus, only postnatal malnutrition can affect these maturation patterns in CA3 pyramidal cells, as well as, chronic malnutrition. These anatomical deficits could be affect the functional integrity of the intrahippocampal activity of the CA3 field. **Supported by DGAPA IN-204892, IN-204093 and CONACYT fellowship 83601.**

692.11

PRENATAL MALNUTRITION ON THE BASKET (GABAergic) DENTATE GYRUS CELLS IN THE RAT.

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The effect of prenatal malnutrition (6% casein diet) on the somatic size, in basket cells was measured in the fascia dentata in rats of 30 days of age, using the Golgi technique. The density of GAD-like immunoreactive cell was studied in the fascia dentata and hippocampal formation, divided into three levels (rostral, medial and caudal). Results showed a significant reduction in the major axis (-15%), perimeter (-16%) and area (-20%) in fusiform cells. In all three levels, numbers of GAD-immunoreactive cells were significantly increased in the hilius of fascia dentata in relation with the hippocampus. The bigger amounts of those cells were localized in the middle part of the fascia dentate. In addition, preliminary data showed increased number of these cells in an older age (i.e. 220 days). The morphological alterations observed in these cells may affect at least in part, the functional integrity of the hippocampal formation, since they have inhibitory activity on the granule cells. **Supported by DGAPA IN-204892 and IN-204093.**

692.12

POSTNATAL MALNUTRITION ON THE DEVELOPMENT AND MATURATION OF GRANULE DENTATE GYRUS CELLS IN THE RAT.

I. Granados*, A. Díaz, L. Cintra, A. Aguilar, M. García-Ruíz, and S. Díaz-Cintra. UISI-IMP, SSA. 14410 and Centro de Neurobiología, UNAM, México, D.F. 04510.

The effects of postnatal malnutrition (6% casein diet) in the somatic size, number of dendritic branching and the spine density of granule cells were studied in rats of 30 days old. A total of 115 cells impregnated with rapid-Golgi were selected for morphometric analysis. Results showed that postnatal malnutrition produced significant decreases ($p<0.05$) in the number of spines in proximal and middle dendritic segments as well as dendritic density in the 7 of the 8 intersected rings, studied. These findings are similar to those found in the study of CA3 pyramidal cells and indicate that postnatal malnutrition produces severe alterations in the maturation and development patterns which in these cells occurs during postnatal life. Thus, these anatomical deficits may be affect the functional integrity of the intrahippocampal activity initiated in these granule cells. **Supported by DGAPA IN-204892, and IN-204093,** IN-202891.

692.13

THE SEVERITY OF NEONATAL ALCOHOL-INDUCED CEREBELLAR PURKINJE CELL LOSS IN RATS DEPENDS ON THE TIMING OF ALCOHOL EXPOSURE: A STEREOLOGICAL STUDY. J. D. Thomas[1]*, C. R. Goodlett[2], E. A. Wasserman[1], & J. R. West[3]. [1]University of Iowa, [2]IUPUI, [3] Texas A&M.

Short term binge-like alcohol exposure during the brain growth spurt in rats depletes Purkinje cells in the cerebellum, but the severity of loss depends on the timing of exposure. Two consecutive days of binge-like alcohol exposure beginning before, but not after, postnatal day (PD) 7 significantly reduces the density of Purkinje cell profiles in the cerebellar vermis (Hamre & West, Alc. Clin. Exp. Res., 1993). Those findings, however, were based on biased counting methods from which estimates of total Purkinje cell number could not be obtained. The present study investigated the effects of two-day alcohol exposure on Purkinje cell number using a stereological method (the optical fractionator) which provides an unbiased estimate of total cell number. Sprague-Dawley rat pups were randomly assigned within litter to five treatment groups. Three alcohol-exposed and one control group were gastrostomized and artificially reared from PD 4 through 9. All alcohol-exposed groups received 6.6 g/kg/day of alcohol, producing peak blood alcohol concentrations of 390 mg/dl. One group was exposed to alcohol on PD 4 & 5 (4/5), a second group on PD 8 & 9 (8/9), whereas a third group was exposed during both periods (Comb). Control groups included the gastrostomy control (GC) and a normally reared suckle control (SC). On PD 55, subjects were perfused, the cerebellum was sectioned, and Purkinje cell number was determined with an Olympus/BICO computerized stereological system. Purkinje cell numbers were reduced in all alcohol-exposed groups [Comb: 53% of GC; PD 4/5: 57% of GC; PD 8/9: 83% of GC]. The PD 4/5 group did not significantly differ from the Comb group and both had significantly more severe cell loss than the PD 8/9 group. These results confirm the greater susceptibility to alcohol-induced Purkinje cell loss early in the neonatal brain growth spurt. Moreover, these unbiased methods now show that the severity of cell loss is greater than previously estimated and that even exposure late in the brain growth spurt can produce significant reductions in Purkinje cell number. Supported by Grants AA05523 (JRW) & AA09596 (CRG).

692.14

OFFSPRING OF A SECOND MATING SHOW AN ENHANCED ANALGESIC RESPONSE TO MORPHINE. M.L. Pilati*, S.H. Tritt, D.L. Tio and A.N. Taylor. Department of Psychology, University of California, Los Angeles, CA 90024.

It has been demonstrated that sensitivity to opiates declines as a function of multiparity in the rat. The impact of this maternal change on offspring sensitivity has not been studied. In order to determine if parity has an impact on offspring responsiveness to opiates, female Sprague-Dawley offspring from a first and second breeding were tested for their analgesic responsiveness to morphine. At 30-36 days of age subjects were tested for their hotplate latency 30 and 60 min after administration of 5mg/kg morphine. Prior to drug administration baseline latency was established. There was a significant effect ($p<.01$) of parity on baseline and 30 min latencies, due to offspring from the second mating showing higher latencies. The significance at 30 min was still present after correcting for baseline differences. It is not clear whether the observed difference is a result of maternal age (dams were 3 months old at first breeding and seven at the second) or parity. The data do, however, indicate that maternal history may significantly alter offspring sensitivity to drugs and possibly other aspects of behavior. (Supported by NIH-HD07228, UCLA Psychoneuroimmunology Program, and VA Medical Research Service.)

692.15

INCREASED LOOK DURATION IN PAIRED COMPARISONS BY RHESUS MONKEY INFANTS WITH N-3 FATTY ACID DEFICIENCY (N-3 FAD).

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N-3 fatty acids are integral components of neural and retinal membranes. Perinatally deficient primate and human infants show low levels of n-3 fatty acids in cerebral cortex and retina, delayed development of visual acuity and abnormal electroretinograms.

Nine infants deficient both pre- and postnatally and 8 standard nursery infants received paired comparison tests at 2, 5, 9 and 13 weeks with both 6 pattern-pairs and 6 face-pairs, each set presented on a separate day. Familiarization consisted of 30 sec of fixation. Tests (familiarized vs novel card) were 10 sec on each side, semi-randomized. For each stimulus set, tests were conducted both immediately and after 24-hours.

Fixations by deficient infants were consistently longer in both the immediate and 24-hr tests for patterns ($p<.05$) and approached significance for faces ($p<.08$).

Table 1	Pattern Sets (6 pairs)		Face Sets (6 pairs)	
	Immediate*	24-Hour*	Immediate	24-Hour
Standard	1.18 + .05	1.18 + .06	1.20 + .05	1.1 + .05
Deficient	1.52 + .05	1.43 + .06	1.48 + .05	1.4 + .05

Look duration has been inversely associated with speed of visual processing. N-3 FAD may slow processing.

692.16

EEG POWER SPECTRA ANALYSIS OF MALNOURISHED LACTATING RATS. L. Cintra*, S. Ramírez, F. Mena, P. Durán and C. Escobar. Centro de Neurobiología, UNAM. A.P.70228, México, D.F., 04510.

Cerebral activity of normal (N) and malnourished (M) lactating female Sprague-Dawley rats was registered during a 24h period, during and after suckling and without their pups. Rats were malnourished 5 weeks before mating, throghout gestation and lactation. N rats showed a synchronized pattern during milk ejection. Using power spectra analysis in N rats, we found a significant increase of theta activity in the first third of the lactation period, an increase of delta waves in the second third and a significant increase of theta activity in the last third of this period. M rats showed a significant increase of theta activity during the last lactating days, however during the first third of the period they showed increase in delta waves. In both groups N and M delta waves increased when pups were retired from the nest. We also found ultradian rhythms in delta, theta and high frequency activity; N rats showed 4 main peaks and M rats presented 8 peaks, with phase advance in respect to N rats. These results suggest that protein malnutrition installed 5 weeks prior to conception, can produce alterations in the electrical activity of lactating rats (Supported by DGAPA IN-202891).

692.17

SLEEP-WAKE PATTERNS IN MALNOURISHED, REHABILITATED AND CONTROL RATS OF 60 DAYS OF AGE.
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This study was designed to evaluate the sleep-waking cycle of 6% protein malnourished, postnatally rehabilitated and control male Sprague-Dawley rats at 60 days of age. Malnutrition was established before mating and was continued throughout gestation and postnatal life. Rats were rehabilitated by the "cross fostering" method switching at birth malnourished pups to well nourished dams. Occipital EEG and neck muscles EMG activity were obtained with bipolar stainless steel electrodes. Polygraphic vigilance states were obtained for a 24h period and scored visually in 12 second epochs. Postnatally rehabilitated rats showed a similar distribution of their vigilance states as their controls during the light and dark phase, as well as in the 24h period. In contrast, malnourished rats showed an increased proportion of SWS during the dark phase and in the total 24h period and a phase shift in the distribution of REM-sleep. These data reveal that malnutrition affects the mechanisms that regulate sleep-wake patterns and suggest that postnatal nutritional rehabilitation can prevent those alterations. (Supported by DGAPA IN-202891 and PADEP 30383).

692.18

EARLY OLFACTORY LEARNING IN UNDERNOURISHED RATS.
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Rat pups are attracted to odors experienced in the nest, emanating from the dam or litter mates. The acquisition of this preference to odors is associated to maternal care and is considered an early associative learning process. When different odors are presented to the pups in a two-choice test they exhibit enhanced orientation to those experimented in the nest.

Morphological studies have reported that undernutrition in the rat retards cellular migration and functional maturation in the olfactory bulb. The present study examined the olfactory behavioral response of prenatal- (U-R), postnatal (U), pre- and postatally undernourished- (U-U) and control (C) Wistar rat pups (n=30) in a two-choice olfactory test. Undernutrition during gestation was induced by restricting the mother's food intake to 50% of the diet, two weeks before mating and during gestation. At birth U-U and U pups were postnatally undernourished by daily removing the pups for 12 h and placing them in an incubator. Pups were tested on postnatal days 1 to 12 using maternal shavings and fresh shavings' odor. The latency to show a response was lower in the U-U and U ($p<0.01$) than U-R and C . Also, the proportion of pups that exhibited a preference to maternal odor was higher in U and U-U ($p<0.01$), although all groups showed a significant preference to maternal odor in contrast to fresh odor. Our data suggest that undernutrition does not restrict the pups ability to establish early olfactory associations, nor to express an olfactory preference, however, postnatal undernutrition excerted an important influence by motivating the pups to respond faster to the maternal odor source. (Supported by PADEP DCCH9151).

692.19

EFFECTS OF CHRONIC UNDERNUTRITION ON THE AMPLITUDE AND LATENCY OF THE N13 AND N20 COMPONENTS OF THE SEP IN CHILDREN. H.Hesse, M.F.Rivera, M.A.Zavala, I.Díaz, W.Mejía*, G.J.Quirk, Dept.Physiology, National Autonomous University of Honduras and Odontopediatric Center,Ministry of Health, Tegucigalpa, Honduras and Center for Neural Science, New York University,NY,NY 10003.

An estimated 50% of Honduran children suffer from chronic undernutrition. To assess the consequences of such deprivation on CNS physiology, we measured the central conduction time (CT), using the somatosensory evoked potential (SEP), of 14 children ages 7-10 with heights below the 3rd percentile for their age and 17 age-matched controls. The two groups also differed significantly in nutritional intake, socioeconomic variables, achievement in Bender's neurointegrative test and hematocrit (control mean=39; undern.=36; $p<.01$, Scheffe F-test), but not birth weight. The children were stimulated over the median nerve with 0.3ms shocks at a rate of 2-3Hz and a voltage sufficient to produce a just visible abduction of the thumb (motor threshold, MT). The cervical N13 and cortical N20 components were recorded over positions C5S-EPc and C3-A1, respectively. In addition to motor threshold, recordings were also taken at .75MT and 1.25MT to assess the response to varying stimulation intensities. Mean CT (N20 latency-N13 latency) for the undernourished group (6.64ms) did not differ significantly from the controls (6.38ms, $p=.3$), nor was there a difference in the amplitudes of the N13 and N20 waves with intensity. However, in 5/14 undernourished subjects whose hematocrit was extremely low the CT was 1.8 standard deviations greater than controls ($p<.001$, t-test). Thus, CT may be affected in a proportion of subjects by a postnatal dietary lack, while the CNS of others may be "protected".

PATTERN FORMATION, COMPARTMENTS, AND BOUNDARIES III

693.1

THALAMOCORTICAL AXON MORPHOLOGY IN NEONATAL RAT SOMATOSENSORY CORTEX IS RAPIDLY ALTERED BY PERIPHERAL DAMAGE S. Catalano*, R.T. Robertson^ and H.P. Killackey^', Depts of 'Psychobiology and of ^Anatomy and Neurobiology, University of California, Irvine, CA 92717; *Dept. Molecular and Cell Biology, University of California, Berkeley, CA 94720

Jensen and Killackey ('87, J.Neurosci. 7:3544) reported that infraorbital nerve section on the day of birth alters both the somatotopic pattern within S1 and the morphology of thalamocortical terminal arbors in the adult. Given the close relationship between individual thalamic terminations and the cortical pattern in the adult rat, we sought to examine whether neonatal infraorbital nerve section would alter the initial development of individual thalamocortical terminal arbors. We therefore examined the morphology of individual axons on P 3 in rats subject to infraorbital nerve section on the day of birth and compared them to axons of the same age from normal rats. Axons were labeled with DiI and photoconverted axons were later reconstructed from coronal vibratome sections. In normal animals at P 3, terminal arbors within layer IV are relatively restricted, spanning an average of 144.0 um. Following infraorbital nerve section on the day of birth, P 3 arbors are significantly wider, spanning 249.2 um ($t[5]=3.66$, $p=0.015$). The number of branches per terminal arbor does not differ from normal animals. Our observations suggest that the role of the periphery in guiding terminal arbor formation is exerted both very rapidly and at the level of the single thalamic axon. Further, these results imply that there is a tight coupling between individual axon terminal arbor morphology and pattern formation in rat somatosensory cortex. Supported by NSF BNS90-22168, NIH NS07351 and NIH NS30109.

693.2

PRENATAL DELINEATION OF THE STRIOSOMAL SYSTEM BY SELECTIVE TRANSCRIPTION FACTOR EXPRESSION. E. Fusco*, J.D.Milbrandt[1], M.J. Iadarola[2], and A.M. Graybiel. Dept. Brain & Cognitive Sciences, MIT, Cambridge, MA 02139; [1] Dept. Pathology, Washington Univ. Sch. of Medicine, St. Louis MO 63110; [2]Neurobiol & Anesthesia Br, NIDR, NIH Bethesda MD 20892.

Transcription factors (TFs) are proteins that can bind to DNA and regulate the transcription and therefore the expression of other genes. Selective TF expression is thus key for the differential gene expression that controls developmental fates. We have analyzed the prenatal expression of several TFs by immunocytochemical techniques to determine their expression in the mouse forebrain and, in particular, in the striatum. We report that the TFs: NGFI-A (zif268, egr1, krox24), Fos/FRA (OGS lot no. 392051), and FRA have a remarkably restricted expression in embryonic forebrain, and that, over a prolonged period of development, they are selective markers for the striosomal system of the striatum. The general spatial and temporal patterns of expression of these TFs are similar, especially in the striosomal system, but they are not identical. NGFI-A & Fos/FRA expression is first detectable at E16 and largely restricted to the striatum and olfactoamygdaloid regions. FRA immunoreactivity, which should represent several members of the Fos family, has more intense and broader expression. At E15, FRA is already clearly detectable in the striatum and also in other well circumscribed forebrain regions (as major examples: olfactory, limbic and hippocampal regions). Within the striatum, these TFs are all first expressed by scattered cells. Patches of TF-positive neurons are present by E18 and persist until after birth. To determine whether these patches were protostriosomes, we marked cells destined for striosome fate by injecting BrdU at E11 and by double-labelling for BrdU and FRA at E19 (by which time BrdU-positive protostriosomes are clearly detectable). Almost all the striosomes marked at E11 by BrdU injection, were, at E19, double-labelled with FRA. The expression pattern showed by these TFs suggest that they may function in relation to the development of the striosomal system.
Supported by NIH 5R01 HD28341.

693.3

RECIPROCAL INFLUENCES OF NIGRAL (DOPAMINE) NEURONS AND STRIATAL PATCH NEURONS IN DISSOCIATED CO-CULTURES. E. Aronica*, L. Costantini, and A. Snyder-Keller. Wadsworth Center for Laboratories and Research, N.Y. State Dept. Health, and S.U.N.Y. School Public Health, Albany, NY 12201.

In our previous studies of transplants of nigral tissue into dopamine (DA)-depleted rats, we found that the inclusion of embryonic striatal tissue increased the efficacy of these transplants, and this effect was greatest with the youngest striatal tissue containing primarily patch neurons (Costantini et al., Exp. Neurol. 1994). We have now employed dissociated cultures to examine whether the survival of DA neurons, as well as striatal patch neurons, is increased in co-cultures of nigral and striatal cells taken from different age embryos. Striatal patch neurons were labelled by *in vivo* BrdU injection on E13 + E14. The number of BrdU-labelled neurons was much higher in E14 striatal cultures (58%) as compared to E18 (10%) or E20 (4%) striatal cultures at 1 DIV, and the percentage in all cultures decreased over the next two weeks. The inclusion of E14 nigral cells attenuated this decline. Similarly, the number of DA (TH-immunoreactive) neurons in E14 nigral cultures decreased with time *in vitro* (10% at 1 DIV to 3% at 15 DIV), and this decline was attenuated by the inclusion of E14, but not E18 or E20, striatal cells. Thus, the survival of nigral DA neurons and striatal patch neurons in culture appears to be enhanced in the presence of the other. These reciprocal influences may be relevant to the *in vivo* development of the nigrostriatal system, as well as the possible potentiation of transplanted cells. (Supported by MH46577.)

693.4

AGGREGATION OF STRIATAL PATCH AND MATRIX NEURONS IN DISSOCIATED CULTURES: INFLUENCE OF CO-CULTURED NIGRAL CELLS. A. Snyder-Keller*, L. Costantini, and E. Aronica. Wadsworth Center for Laboratories and Research, N.Y. State Dept. Health, and S.U.N.Y. School Public Health, Albany, NY 12201.

The relative importance of intrinsic and extrinsic factors in the formation of striatal patch/matrix organization is still a matter of debate. Krushel et al (1989) reported that striatal patch neurons selectively reassociate *in vitro*. We pursued this observation using striatal tissue dissected from E14, E16, or E20 rat embryos, dissociated with trypsin, plated on coverslips placed in 24-well culture dishes, and grown under different culture conditions. Striatal patch neurons were labelled by *in vivo* BrdU injection on E14 or E13 + E14. Aggregation was influenced by a variety of culture conditions: substrate, medium, whether grown face-up in the wells or on inverted coverslips, etc. On polylysine-coated inverted coverslips, striatal neurons from all ages were fairly evenly distributed when grown in defined medium. Under these conditions, aggregation was most notably increased when striatal neurons were grown in the presence of nigral cells. However, within these aggregates BrdU-labelled patch neurons were not obviously associated with one another, and were just as frequently seen outside the aggregates. These findings indicate that the aggregation of striatal cells in culture is greatly influenced by "extrinsic" conditions, including the presence of nigral cells. Although these influences were not specific to striatal patch neurons, they may nonetheless relate to the clustering of patch neurons *in vivo*. (Supported by MH46577.)

693.5

TRANSIENT c-*fos* EXPRESSION IN RESPONSE TO DOPAMINE D1 AGONISTS IN THE DEVELOPING STRIOSOMES. E. Arnauld, J. Arsaut, R.M. Bluthé*, J. Demotes-Mainard. INSERM U-394, 33077 Bordeaux, France.

In intact adult mice, dopamine D1 receptor activation only induces c-*fos* in the most caudal region of the striatum. In contrast, when dopaminergic receptors are previously sensitized by lesion of the nigro-striatal pathway, the expression of c-*fos* in response to D1 agonists is present throughout the striatum. Therefore, c-*fos* induction by D1 agonists requires a state of altered responsiveness of dopamine receptors. Since the D1 receptors develop in the striatum prior to the D2 subtype, we further examined in intact animals the postnatal ontogeny of the c-*fos* response to D1 agonists. *In situ* hybridization of c-*fos* messenger ribonucleic acid (mRNA) in the developing mouse striatum showed that during a transient developmental period extending from postnatal days 3 to 12, injection of the D1 agonist SKF 38393 results in a c-*fos* expression scattered throughout the striatum and observing a patchy distribution. After postnatal day 15 the D1 receptor-mediated c-*fos* hybridization signal had almost disappeared and lost its patchy distribution, except in the most caudal division of the striatum where a strong expression appeared at postnatal day 3 and persisted in adults. Since this transient response of c-*fos* gene to D1 dopamine agonist parallels the developmental pattern of striatal dopamine innervation, of D1 dopamine receptor distribution and the delineation of the striosomal compartment, this study raises the questions of : i) the possible role of D1 receptor-mediated gene expression in the ontogeny of striatal compartments, and ii) the significance of a persisting D1-induced c-*fos* expression in the most caudal part of the adult caudate-putamen.

693.6

DEVELOPMENTAL AND GENETIC ANALYSIS OF *hyh*, A MUTANT WITH ABNORMAL CEREBRAL CORTICAL MORPHOGENESIS. K. M. Allen*[1], H. Sweet[2], M. Davisson[2], R. Bronson[2], and C. Walsh[1]. [1]Dept. of Neurology, Beth Israel Hospital, Harvard Medical School, Boston, MA. [2]The Jackson Laboratory, Bar Harbor, ME.

Whereas the most striking features of the mouse mutant hydrocephalus with hop gait (*hyh*) are massive lethal dilation of the lateral ventricles, a large third ventricular cyst, and an abnormal "hopping" gait (Bronson and Lane, Dev. Brain Res. 54:131, 1990), our preliminary examination of adult and neonatal *hyh* brains has shown several disorders of cerebral cortical development that were not previously noted. Firstly, there are morphogenetic abnormalities and disruptions of laminar patterning of the medial cortical regions including dentate gyrus, Ammon's horn, subiculum, and cingulate cortex. Secondly, the migration of some cerebral cortical neurons is disrupted forming islands of heterotopic cells along the lateral ventricles. The presence of large islands of ectopic cerebral cortical neurons has not been observed in other inherited forms of hydrocephalus. These heterotopias may reflect a specific effect of *hyh* on cortical neuronal migration or may be a secondary effect of hydrocephalus.

In order to more precisely map *hyh* on Chromosome 7, the coinheritance of several simple sequence length polymorphism (SSLP) markers and the mutant phenotype was analyzed in over 300 mice from a backcross and two independent F_2 populations. Localization of the recombination breakpoints allowed the definition of an approximate 2 cM candidate region between *D7Mit75* and *D7Mit56*. This corrected location of *hyh* is about 10 cM proximal to the proposed location based on a smaller cross. High resolution mapping places *hyh* near several potential candidate genes on proximal Chromosome 7 which are being analyzed for involvement with *hyh*. Furthermore, closely linked genetic markers will allow prenatal diagnosis of *hyh* mutant animals before they display gross abnormalities. A comparison of brain development in mutants and normal littermates will facilitate an understanding of the specific abnormalities of forebrain morphogenesis present in the *hyh* mice.

693.7

REGULATION OF PHENOTYPE EXPRESSION IN THE STRIATUM. William F. Silverman*, Yaakov Pollack[1] and Yoram Solberg. Center for Brain Research and [1]Unit of Microbiology & Immunology, Ben-Gurion University, Beer Sheva, 84105 ISRAEL

We have previously reported an increase in the expression of the gene coding for tyrosine hydroxylase (TH) in subsets of dopamine (DA) neurons, related to the establishment of synaptic connections with their target cells in the striatum. We have also observed that certain gene products in the striatal neurons are expressed at the same time that TH mRNA is up-regulated, suggesting that DA projections from the SN are simultaneously conducting regulatory messages in both anterograde and retrograde directions. In order to test this, we injected 6-hydroxydopamine unilaterally into the SN of newborn rats. Forty-eight hours later this treatment had effectively eliminated the DA innervation to the striatum on the injected side, but failed to reduce the expression of neurotensin or calbindin, leading us to formulate an alternate hypothesis: Induction of these striatal phenotypes is a function of afferent innervation from non-DA areas. Postmortem injections of the carbocyanine dye DiO into the striatum has subsequently demonstrated that at birth and through the first day, virtually all striatal afferents originate from nigral DA neurons. At P2, when activation and peptide expression are first observed in the striatum, a robust afferent innervation was observed to arrive from both cerebral cortex and ventral thalamus, in addition to the midbrain DA group, raising the possibility that input from these areas might influence expression of specific striatal phenotypes in the perinatal rat. Supported by the Israel Institute for Psychobiology (WFS) and the Clore Foundation (YS).

693.8

STAINING FOR ACETYLCHOLINESTERASE REVEALS DIFFERENT PATTERNS IN THE THALAMUS AND CORTEX OF PERINATAL RATS, MICE, AND HAMSTERS. R.W. Rhoades*, N.L.Chiaia, and C.A. Bennett-Clarke. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Acetylcholinesterase (AChE) is a marker for thalamocortical axon terminals in perinatal rats and has been used extensively to study the development of these fibers. Studies in which we attempted to used this marker in developing mice and hamsters demonstrated striking differences in the expression of this enzyme in the three species. In developing rats, AChE staining heavily labels neurons in the ventral posterolateral (VPL) and ventral posteromedial (VPM) thalamic nuclei and reveals a somatotopically organized pattern of clusters in the developing cortex. In both hamster and mouse, VPM neurons were relatively lightly labelled and heavy staining in VPL appeared to be contained in afferent fibers. This was confirmed by its disappearance after medial lemniscus lesions. AChE staining in the cortices of both of these species resulted in a negative image of the distribution of thalamocortical afferents. This image appeared to result from reduced AChE cortical neuronal staining and was first apparent as a crude representation of the body in lamina V and VI and then as a more precise image in lamina IV. Staining for both AChE and Nissl substance in developing mice indicated that the dense AChE staining was located in the septae. These results demonstrate a dramatic species difference in AChE expression by thalamic and cortical neurons and prompt caution in the general use of this enzyme is as a marker for developing thalamocortical axons.

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693.9

INFRAORBITAL NERVE TRANSECTION AND AXOPLASMIC TRANSPORT BLOCKADE HAVE SIMILAR EFFECTS UPON PRIMARY AFFERENT AND SECOND ORDER NEURONAL MARKERS IN THE RAT'S TRIGEMINAL BRAINSTEM COMPLEX C.A. Bennett-Clarke*, N.L. Chiaia, R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Transection of the infraorbital nerve (ION, the trigeminal [V] branch that supplies the mystacial vibrissae follicles) has specific effects on the chemistry of both primary afferent and second-order neurons in the V brainstem complex. Such lesions cause a marked upregulation of galanin in the central arbors of damaged primary afferent axons and reductions in the staining of second-order cells for both cytochrome oxidase (CO) and the calcium binding protein, parvalbumin (PA). Blockade of axoplasmic transport has less severe effects on the population of fibers in the developing ION than transection and it does not disrupt peripheral activation of V brainstem cells. Nevertheless, such blockade produces changes in V primary afferent and second-order neuronal markers that closely mimic those observed after nerve transection. Transport blockade causes a marked up-regulation in galanin in V primary afferents and, in V nucleus principalis, V nucleus interpolaris, and the magnocellular part of V nucleus caudalis, these galanin-positive axons are arrayed in a clustered and somatotopic fashion which matches that of the mystacial vibrissae follicles. Transport blockade also causes reductions in the density of both CO and PA staining in the brainstem and a loss of the normally observed patterning that corresponds to that of the vibrissae follicles.

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693.10

EFFECTS OF AXOPLASMIC TRANSPORT BLOCKADE ON MYELINATED AXON NUMBER IN THE INFRAORBITAL NERVE AND CENTRAL VIBRISSAE-RELATED PATTERNS. S. Zhang*, N.L. Chiaia, R.S. Crissman, C.A. Bennett-Clarke, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Axonal transport was blocked in the developing infraorbital nerve (ION the trigeminal branch that supplies the mystacial vibrissae) between P-0 and P-6 by application of implants impregnated with varying amounts of colchicine or vinblastine. Effects upon numbers of myelinated fibers in this nerve were assessed by light microscopic examination of toluidine blue-stained sections and effects upon central vibrissae-related patterns were evaluated by histochemical and immunocytochemical staining. The average number of myelinated axons in the normal ION (N=20) on P-6 was 9,931 ± 1,241. The average percentage of myelinated axons that remained in the ION of animals subjected to transport blockade (N=18) ranged from 14.1 ± 3.7% for the animals that received implants with high colchicine concentrations to 51.7 ± 17.7% for the rats that received low doses of vinblastine. Regardless of the number of myelinated axons that were preserved in the ION, the effects on central vibrissae-related patterns were the same. The cytochrome oxidase (CO)-dense patches in the brainstem corresponding to the vibrissae follicles were absent and the density of staining was markedly reduced. The vibrissae-related CO pattern in the thalamus was also absent, but the density of staining was not markedly decreased. In cortex, staining for serotonin or labelling axons with Di-I revealed a banded pattern similar to that observed after nerve transection.

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693.11

TRANSECTION OF, AND AXOPLASMIC TRANSPORT BLOCKADE IN, THE INFRAORBITAL NERVE IN NEWBORN RATS REDUCE EXPRESSION OF ACETYLCHOLINESTERASE BY SOMATOSENSORY THALAMOCORTICAL AXONS. N.L. Chiaia*, C.A. Bennett-Clarke, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Acetylcholinesterase (AChE) is transiently expressed by thalamocortical axons in perinatal rats and it has been used extensively to study the development and plasticity of these axons. The use of AChE as a marker for thalamocortical fibers in plasticity studies requires that its expression not be significantly altered by manipulations employed in such experiments. The present results demonstrate that transection of the infraorbital nerve (ION) or blockade of axoplasmic transport in this nerve with colchicine or vinblastine markedly reduces the expression of AChE in developing thalamocortical fibers. Newborn rats sustained either transection of the ION or application of a vinblastine- or colchicine-impregnated implant to this nerve and were killed on postnatal day 7. The cortex was either stained for AChE or Di-I was used to label thalamocortical afferents. Staining with AChE in experimentally manipulated animals revealed very light bands of labelling in the portion of the cortex representing the vibrissae follicles contralateral to the treated nerve. In some animals no periphery-related AChE staining was visible in the experimental hemisphere. ACHE staining demonstrated dense somatotopically organized patches in the cortex that retained its normal input. Tracing with Di-I revealed a banded pattern of thalamocortical afferents in the experimental cortices of all animals evaluated.

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693.12

NMDA RECEPTOR MEDIATED MOTOR NEURON DENDRITE GROWTH Robert G. Kalb*, Yale University, New Haven CT. 06510

Adult mammalian motor neurons receive 20,000-50,000 synaptic inputs and this innervation is a function of the size and geometry of the neuronal dendritic arbor. We performed a quantitative examination of the postnatal growth of rat motor neuron dendrites until the adult architecture is achieved. Motor neuron cell body cross sectional area and dendritic growth in the radial and rostrocaudal axes increase monotonically while the growth of dendritic arbor/motor neuron and the number of dendritic branches is biphasic. These later features undergo overabundant growth in the first three weeks of life then regress to the adult pattern.

The timing and organization of afferent ingrowth into the spinal cord have been proposed to regulate dendrite growth during development. We have examined the participation of synaptic activity in dendritogenesis. We find that blockade of the NMDA subtype of glutamate receptor in neonates with either MK-801 or aminophosphonovaleric acid inhibits the growth of dendritic arbor/motor neuron by predominantly inhibiting dendritic branching. Blockade of the NMDA receptor in adult animals has no effect on dendritic geometry. Thus NMDA receptor activation promotes the development of a more complex motor neuron dendritic tree during the naturally occurring remodeling period in early postnatal life. The activity-dependent maturation of the dendritic tree is likely to influence the quantitative and qualitative aspects of convergent innervation to motor neurons and have lifelong effects on their computational capabilities.

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693.13

DIFFERENTIAL AFFINITY OF HOST SEROTONIN AXONS FOR INTRASTRIATAL GRAFTS OF DIFFERENT ANATOMICAL ORIGIN. P. Pierret*, H. Moukhles, G. Campistron and G. Doucet. Département de pathologie et Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

Grafts of fetal striatal or cortical tissue have been shown to be innervated by host serotonin (5-HT) neurons following implantation into ibotenic acid-lesioned striatum of adult rat. In contrast, very few 5-HT axons were visualized in grafts of fetal substantia nigra after implantation into the unlesioned striatum of adult rat, suggesting a differential affinity of 5-HT axons for CNS grafts of different anatomical origin. Nevertheless, we have recently demonstrated that implantation into newborn rat results in strong 5-HT innervation of grafted mesencephalic tissue, indicating a developmental regulation of this affinity. To test this hypothesis further, we compared the 5-HT innervation of fetal striatal and mesencephalic grafts after implantation into the unlesioned striatum of rats aged 1 to 25 d. Immunocytochemistry for 5-HT, tyrosine hydroxylase and DARPP-32 was performed 2 months after transplantation, to visualize 5-HT axons and assess mesencephalic and striatal grafts, respectively. Numerous 5-HT-immunoreactive axons were present inside ventral mesencephalic transplants (devoid of 5-HT cell bodies) only after implantation into recipients less than 14 d old. In contrast, striatal grafts were richly 5-HT-innervated in older as well as newborn recipients. Interestingly, 5-HT fibers invaded only DARPP-32-positive areas, i.e. true striatal portions of the "striatal" grafts, even in newborn recipients. Therefore, the affinity of 5-HT axons for "striatal" grafts was not affected by the age of the recipient. Furthermore, 5-HT axons showed a differential affinity for striatal and non-striatal portions of the "striatal" grafts. We conclude that a postnatal developmental regulation in the affinity of host 5-HT axons is a special feature of their relationship with ventral mesencephalic tissue.

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693.14

ENGINEERING OF ARTIFICIAL HIPPOCAMPAL NETWORKS. J.J. Hickman, K.E. Foster, D.A. Stenger[1], A.E. Schaffner[2]*, Yong-Xin Li[2] and J.L. Barker[2]. Science Applications International Corporation, Mclean, VA 22102; [1]Center for Biomolecular Science and Engineering, Naval Research Laboratory, Washington, D.C. 20375 and [2]Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

The creation of chemically defined, patterned surfaces has allowed us to study the development of neuronal networks *in vitro*. We are using patterned, self assembled monolayers (SAMs) to control the adhesion and direct the neurite outgrowth of hippocampal neurons. Controllable variables in the preparation of *in vitro* networks such as surface composition, growth media and cell preparation all play important roles in determining neuronal viability and pattern fidelity. The patterned surfaces have been characterized by XPS, imaging XPS and contact angle measurements both before and after cell culture. We have begun electrophysiological recordings from cells grown on SAMs. We will apply what we learn from this study to an understanding of embryonic neuronal development and circuit formation as well as to the repair of damaged neuronal systems *in vivo*.

694.1

EFFECTS OF INFRAORBITAL NERVE TRANSECTION ON THE ORGANIZATION IN INTRACORTICAL CONNECTIONS WITHIN THE RAT'S PRIMARY SOMATOSENSORY CORTEX T.D. King*, C.A. Bennett-Clarke, N.L. Chiaia, H.P. Killackey, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Intracortical projections within lamina IV of the vibrissae representation of the rat's primary somatosensory cortex (the barrelfield) are generally restricted to the septal regions and avoid the dense aggregates of lamina IV neurons referred to as the barrels. In the present study, anterograde and retrograde tracing with biotinylated dextran amine (BDA) was used to determine whether transection of the infraorbital nerve (ION, the trigeminal branch that supplies the vibrissae follicles) on either the day of birth (P-0) or P-7 altered the organization of intracortical connections in layer IV of the barrelfield. In rats that sustained ION transection on P-0 and survived to adulthood, there was a marked reorganization of intracortical connections in lamina IV of the barrelfield. Both retrogradely labelled cells and anterogradely labelled axons were present within the dense aggregates of granule neurons that remained in this lamina. In contrast, the pattern of intracortical connections in layer IV of rats with ION transections on P-7 appeared normal. Labelled cells and anterograde labelling was largely restricted to the septae and formed a negative image of the clusters of granule cells. These results suggest that altered input to the barrel cortex must be achieved very early in postnatal development to alter intracortical connections in this lamina.

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694.2

CENTRAL PROJECTIONS OF INTER-WHISKER PRIMARY AFFERENTS SHORTLY AFTER BIRTH AND IN ADULTHOOD: GALANIN AND SINGLE AXON STAINING PATTERNS. P.H. Young*, N.L. Chiaia, C.A. Bennett-Clarke, R.W. Rhoades, P.J. Shortland & M.F. Jacquin. Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis MO 63104; Anatomy, Medical College of Ohio, Toledo OH 43699; Neurology, Washington Univ. Sch. Med., St. Louis MO 63110.

Mechanisms subserving pattern formation in the rat whisker-barrel system are unknown, although most believe peripheral connections are necessary. In part because NGF can preserve excess ganglion cells and alter pattern formation, Henderson et al. (*J. Neurosci.* 14, '94) suggest that patterns form as a result of a neurotrophically specified, disproportionate death of ganglion cells that project to inter-whisker surfaces. If such cells project centrally to regions intervening between surviving and neighboring whisker projections, their death would form "septa", i.e. somatotopic patches. For this hypothesis to be tenable, inter-whisker and whisker ganglion cells should have central projection patterns that are mirror images of each other; one should resemble a "honeycomb", the other should resemble barrels. To test this, inter-whisker surfaces were selectively denervated at birth (by electrocautery of the skin between rows of whiskers) and their central projections stained by galanin histochemistry (White et al., *Dev. Brain Res.* 72, '93) on postnatal days 3 or 6. Alternate sections were stained for cytochrome oxidase to reveal whisker patches. At both ages, galanin immunoreactive terminals were sparse in brainstem whisker regions, although they occurred most often between whisker patches, sometimes to the extent that the stained fibers produced a honeycomb pattern. However, in normal adults, primary afferents with inter-whisker receptive fields had collaterals that usually ended in whisker patches; on rare occasion they terminated between whisker patches. Thus, staining patterns in neonates, but not adults, support the above-stated hypothesis.

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694.3

ANATOMICAL AND FUNCTIONAL REORGANIZATION OF THE CUNEATE NUCLEUS AFTER NEONATAL FORELIMB REMOVAL IN THE RAT. R.D. Lane*, N.L. Chiaia, H.P. Killackey, and R.W. Rhoades Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699 and Dept. of Psychobiology, Univ. of California, Irvine CA 92717

Most previous anatomical studies have shown that large caliber, low-threshold primary afferents exhibit only limited central sprouting after peripheral nerve damage in either neonatal or adult animals. In this study, forelimb removals were carried out in newborn rats that were then allowed to survive until adulthood and used in terminal anatomical or physiological experiments. Sciatic nerve afferents to the brainstem were labelled with a combination of WGA-HRP and CT-HRP in 6 normal rats and 8 animals that sustained neonatal forelimb removals. All of the experimental rats and none of the controls had dense HRP labelling that extended out of the gracile nucleus into the cuneate nucleus. Multi-unit recordings demonstrated the presence of hindlimb-related activity in the cuneate nuclei of all manipulated rats. Single unit recordings demonstrated the existence of cuneate neurons (49% of 49 recorded) that responded to hindlimb stimulation. Most of these (67%) had split receptive fields that also included the stump of the forelimb. Electrical stimulation of the sciatic nerve and brachial plexus demonstrated that 18 (37%)of 49 cells tested could be activated from both sites. These results are consistent with the conclusion that low-threshold, hindlimb-related primary afferents invade the cuneate nucleus after neonatal forelimb removal and make functional contacts with neurons in this nucleus.

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694.4

EFFECTS OF FORELIMB DAMAGE AT DIFFERENT AGES ON PEPTIDE DISTRIBUTION AND LECTIN BINDING IN THE CERVICAL DORSAL HORN. W.H. Bauer*, B. Hoeflinger, N.L. Chiaia, C.A. Bennett-Clarke and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Rats that sustained forelimb removal on either embryonic day (E-) 16 or the day of birth (P-0), or transection of the brachial plexus as adults had sections through the cervical dorsal horn stained for galanin, calcitonin gene-related peptide (CGRP), or the lectin *Bandieria simplicifolia*-I (BS-I) ≥30 days after these lesions. There were age-related differences in the effects of peripheral nerve damage upon each of these markers. Damage to the brachial plexus in adulthood caused a significant increase in the density of galanin immunoreactivity in the medial portion of layers I and II and the appearance of galanin immunoreactivity in layers III and IV of the cervical dorsal horn. Such lesions resulted in significant reductions in the density of CGRP immunoreactivity in layers I and II and decreased BS-I binding in lamina II. Forelimb removal at birth resulted in no significant change in the density of galanin immunoreactivity in layers I and II, but in the appearance of galanin-immunoreactive fibers in layers III and IV. Neonatal forelimb removal resulted in no significant change in the density of CGRP immunoreactivity in layers I and II, but in a significant reduction in BS-I binding in the medial portion of lamina II. Forelimb removal on E-16 increased galanin immunoreactivity in layers III and IV, but had no effect on either CGRP immunoreactivity or BS-I binding in the cervical dorsal horn.

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694.5

CHRONOTOPIC ORGANIZATION OF PRIMARY AFFERENTS IN THE RAT'S TRIGEMINAL SPINAL TRACT. R.S. Crissman*, F.A White, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Trigeminal (V) ganglion cells with different phenotypes have different birthdates. Larger neurons positive for neurofilament protein (NF) and which give rise to large myelinated axons are born early in V ganglion neurogenesis with 27.3% born on E-10.5, 30.3% born on E-11.5, and 37.0% born on E-12.5. In contrast, CGRP-positive ganglion cells are born later, 51.5% on E-13.5 and 42.1% on E-14.5. The difference in birthdates for these two classes of V ganglion cells is reflected by the position of their axons in the V spinal tract. NF-positive axons are located throughout the depth of the V spinal tract, but are more dense in its deeper portion. In contrast, CGRP-positive fibers are largely restricted to the superficial portion of the tract. Ganglion cells that bind the lectin *Bandierea simplicifolia*-I (BS-I) are also born late with 70.9% being generated on E-13.5. BS-I-positive axons are also restricted to the superficial V spinal tract. Immunocytochemical results were supported by electron microscopy. The larger myelinated axons are located in the deep portion of the V spinal tract and there are relatively few unmyelinated fibers in this region. The percentage of myelinated axons is reduced superficially. These results are consistent with the conclusion that axons are added just below the pial surface in the developing V spinal tract with the earliest arriving axons eventually forming its deepest part and later arriving fibers forming its superficial portion.

Supported by DE 07734 and NS 28888

694.6

IMMATURE THALAMOCORTICAL SYNAPTIC RESPONSES IN NEONATAL MOUSE BARREL CORTEX. A. Agmon*[1] and Diane K. O'Dowd[1,2], Depts. of [1]Anatomy and Neurobiology and [2]Developmental and Cell Biology, University of California, Irvine, CA 92717.

We have extended our previous study of the development of thalamocortical synaptic responses in mouse barrel cortex (J. Neurophys. 68, 345, 1992) to include the first 3 postnatal days (P0-P2) and the deep cortical layers. Using the thalamocortical slice preparation, whole-cell recordings were obtained from neurons in all cortical layers, and synaptic currents evoked by electrical stimulation of the thalamocortical pathway. To study the morphological basis of these responses, selected cells were stained with Lucifer Yellow, and in the same slices (after fixation) thalamocortical axons were labeled with DiI(5). Synaptic responses were evoked in deep (layers V/VI) neurons as early as P0, and in the incipient layer IV by P2. Responses were exclusively excitatory; they were highly labile and were often irreversibly depressed after a small number of stimuli. Current-voltage relationships indicated that the dominant component in these responses was mediated by non-NMDA glutamate receptors. In P2 animals, a single electrical stimulus to the thalamus evoked a prolonged sequence of small synaptic events lasting up to 400 ms, apparently due to temporally-dispersed transmitter release. Supported by NS30109.

694.7

PRENATAL DEVELOPMENT OF THE RAT CORTICOTRIGEMINAL TRACT. D.A. Randall* and W.E. Renehan. Division of Gastroenterology, Henry Ford Health Sciences Center, Detroit, MI 48202

The rodent trigeminal system has served as a model of somatosensory development and plasticity for over two decades. It is interesting to note, however, that most investigations have focussed entirely on the ascending trigeminal pathways, with little attention paid to the maturation of the descending inputs to the trigeminal neuraxis or the developmental interactions between the ascending and descending projections. We have addressed this issue by using the carbocyanine dye DiI to study the prenatal development of the corticotrigeminal (CT) projection in the rat. Crystals of DiI were placed in the region of the barrelfield cortex of fixed brain tissue from rats that had been sacrificed on embryonic (E) days 18 and 20 and postnatal (P) day 0 (the day of birth). Numerous labeled CT axons were clearly visible in the ipsilateral medullary pyramid at E18. At this age, CT fibers could be seen approaching the medial border of the rostral ipsilateral trigeminal brainstem nuclear complex (TBNC), but no terminals were identified within the confines of the trigeminal subnuclei. At E20, however, numerous CT axon terminals were visible in the rostral ipsilateral TBNC. A lesser number of axons were also identified in the caudal aspect of the ipsilateral TBNC. In addition, labeled axons were seen in the rostral portion of the contralateral TBNC. The disposition of labeled CT axons in P0 animals was similar to that noted in adult animals, with the density of the terminals in the contralateral TBNC exceeding that seen in the ipsilateral brainstem. These results indicate that the CT pathway in the rat develops in an ipsilateral-to-contralateral, rostral-to-caudal sequence in the late prenatal period. Supported in part by DE07734.

694.8

EFFECTS OF WHISKER DEPRIVATION OR CORTICAL IMPULSE BLOCKADE ON THE DEVELOPING CORTICOTRIGEMINAL PROJECTION. W.E. Renehan* & M.F. Jacquin. Lab. of Gastro., Gust. & Somatic Sensation, Henry Ford Hospital, Detroit, MI 48202; Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis MO 63104; Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110.

We know that daily whisker trimming from birth alters the receptive field size and character of many trigeminal (V) brainstem cells without affecting primary afferent responses or projection patterns, brainstem cytochrome oxidase staining patterns, or brainstem somatotopy (Jacquin et al., *J. Comp. Neurol.* '94). Deprivation-induced response alterations may then reflect disrupted inhibitory mechanisms, the preservation of immature circuitry, altered V brainstem circuitry due to altered activity-based competitive interactions, use-dependent plasticity of existing circuits, and/or an indirect effect of direct changes in cortical circuits (Simons & Land, *Nature*, '87) that are imposed on V brainstem neurons by more effective cortico-V projections. The latter is particulary compelling because the patterning and extent of the cortico-V projection is altered by V nerve injury at birth, and cortical inputs contribute to V brainstem receptive fields in normal rats. To further evaluate the cortical substrate hypothesis, 2 experiments were done. In 1-2 month old rats whose mystacial whiskers were trimmed daily from birth, WGA-HRP injections into the barrel cortex labeled cortical fibers whose extent, density and pattern were normal in ipsilateral and contralateral V brainstem nuclei. However, the incidence of cells in V nucleus interpolaris that were discharged by electric shocks in the contralateral internal capsule was significantly higher. This suggests that whisker deprivation impacts on "subtle" aspects of cortico-V circuits. To determine whether these changes are prompted by the brainstem or cortex, slow-release polymers containing TTX are being applied to the newborn cortex. Effects on the cortico-V projection will be reported. Supported by NIH DE07734.

694.9

DO DENDRITES RESPECT WHISKER PATCH BORDERS IN THE DEVELOPING NUCLEUS PRINCIPALIS? CONFOCAL ANALYSIS. P. Robinson, J.A. DeMaro, J.J.A. Arends, J. Christensen, G.J. Bailey*, T.A. Woolsey & M.F. Jacquin. Dept. Neurology and Neurological Surgery, Washington Univ. Sch. of Med., St. Louis, MO 63110; Dept. Anatomy and Neurobiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

In the developing somatosensory cortex, granule cell dendrites come to be largely confined to individual barrels by selective pruning and reorientation relative to thalamocortical patch boundaries. In the developing VPM thalamus, the opposite appears to occur (Brown et al., *Soc. Neurosci. Abstr. 19*:47, '93). To study this issue in trigeminal nucleus principalis (PrV), the brainstem link in the whisker-barrel neuraxis, rats were sacrificed on postnatal days 0-6. Single PrV cells were stained with Lucifer yellow in aldehyde-fixed transverse slices and the resultant labeling and whisker patch-related autofluorescence were reconstructed manually and by confocal microscopy. The extent to which a single cell's dendritic tree was confined to its patch of origin was determined by computer-assisted merging of the dendrite and patch images. We find that at birth, almost all PrV cell dendrites are restricted to their "home" patch, although there appear to be a greater number of proximal dendrites per cell, less polarity in the dendritic tree as a whole, and a greater number of dendritic appendages per cell than at later ages. By postnatal day 6, a greater number of cells have dendrites that span multiple patches, and other features also more closely approximate the adult-like morphology. However, in the adult PrV, most dendrites do not span beyond their "home" patch. These data suggest that PrV dendrites develop according to a different set of rules than those operating in the VPM thalamus and layer IV of barrel cortex. This may reflect the fact that primary afferent projections are quite mature in the newborn PrV when large numbers of PrV cells have yet to die of natural causes, and that PrV dendrites serve a different function than VPM dendrites in adults. NIH DE07734, NS17763.

694.10

PLASTICITY AT THALAMOCORTICAL SYNAPSES REVEALS A SYNAPTIC BASIS FOR DEVELOPMENTAL CRITICAL PERIODS. M. C. Crair* and R. C. Malenka. Departments of Psychiatry and Physiology, University of California, San Francisco, CA 94143.

It has long been suspected that there could be a synaptic basis for the developmental critical period seen in the cortical representation of the sensory periphery. We present evidence that LTP and LTD-type phenomena might be responsible for one such developmental critical period in the somatosensory cortex of the rat. Using an *in vitro* thalamocortical slice preparation we tested the lability of monosynaptic response in the barrel cortex of rats to stimulus of the somatosensory thalamus and found that under a pairing protocol (depolarization to -10 ± 5 mV with 100 stimuli at 1 Hz using a blind whole-cell patch), animals beyond the critical period did not display significant plasticity (1 out of 10 cells from animals P8-P14 potentiated more than 10%), whereas thalamocortical synapses in younger animals potentiated easily (150 ± 20% in 9 out of 10 cells from animals age P3-P7). Elucidation of the mechanisms responsible for this developmental switch would aid not only our understanding of critical periods *per se*, but also add to the growing body of evidence suggesting that synaptic plasticity commonly seen *in vitro* may have important *in vivo* ramifications as well.

694.11

THE TIME-COURSE OF PLASTIC CHANGES IN THE BARREL CORTEX OF ADOLESCENT RATS. S. Glazewski* and K. Fox. *Dept. Physiology, University of Minnesota, MN 55455*

Long-term deprivation (60 days) of all but the D1 vibrissae in adolescent rats (P28), results in expansion of the D1 representation in layers II/III of the barrel cortex. To test how rapidly this expansion occurs, we assayed plasticity in rats deprived for 5-7 days and compared the results with those obtained from rats deprived for 60 days. Two kinds of deprivation were used: one group had their vibrissae trimmed every day (VT), the other group had their vibrissae carefully pulled out every two days (VP). The receptive fields of 298 layer II/III cells located above barrels immediately surrounding the D1 barrel were measured using post-stimulus time histogram analysis. We found that functional plasticity occurred in both long- and short-term deprived animals but that the underlying processes were different. Short-term deprivation resulted in suppression of deprived vibrissae input (0.55±0.13 spikes per stimulus in VT and 0.43±0.28 in VP vs. 1.22±0.25 for age matched controls), without a change in the spared D1 vibrissa input. In contrast, long-term deprivation did cause potentiation of the spared vibrissa input (0.96±0.17 spikes per stimulus versus 0.45±0.15 at P28 and 0.41±0.15 in adults). These results suggest that attenuation of deprived vibrissae input might be a prerequisite for expansion of the spared vibrissae representation. Supported by NS27759.

694.12

AUGMENTATION OF NEURAL ACTIVITY AND CORTICAL GROWTH IN ENUCLEATED RATS D. Zheng* and D. Purves Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have examined the metabolic activity and size of the adult rat primary somatosensory cortex (S1) after neonatal eye removal to ask whether altered neural activity affects the growth of the neocortex. Activity in S1 was analyzed by comparing the cortical vascularization of enucleated rats with littermate controls; postnatal growth was determined by direct measurement of S1 and its component parts. The overall density of blood vessels in the enucleated animals was 11% greater in the primary somatosensory cortex, and 16% greater within the cortical barrels that represent the whisker pad. In parallel with this change, measurements of S1 and its component parts in the enucleated animals showed that cortical regions with increased levels of ongoing activity were larger than their counterparts in the littermate controls. Thus there was an increase in the average size of both S1 (11%) and of the barrels in the whisker pad representation (20%) in the experimental rats. This correlation of increased metabolic activity and cortical growth provides evidence that neural activity stimulates the elaboration of cortical circuitry during postnatal development. Such activity-modulated cortical growth during maturation may be the mechanism by which early experience encodes information in the developing central nervous system.

694.13

THE EXPRESSION OF DIFFERENT CYTOCHEMICAL MARKERS IN NORMAL AND AXOTOMIZED NUCLEUS GRACILIS PROJECTING DORSAL ROOT GANGLION CELLS IN THE ADULT RAT.

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The aim of this study was to analyze the expression of a number of cytochemical markers in normal and axotomized nucleus gracilis projecting dorsal root ganglion cells. L4 and L5 lumbar dorsal root ganglion neurons projecting to the nucleus gracilis were retrogradely labeled with Fluoro-Gold (FG) and analyzed immunohistochemically for their content of substance P (SP), calcitonin gene-related peptide (CGRP), nitric oxide synthase (NOS), galanin, galanin message-associated peptide (GMAP), neuropeptide Y (NPY) and carbonic anhydrase as well as affinity to RT97, choleragenoid and to griffonia simplicifolia lectin I - isolectin B_4 (GSL-B_4).The analysis was made in uninjured rats and in rats which had been subjected to unilateral sciatic nerve transection and partial resection three weeks earlier.

Six and nineteen percent, respectively, of the FG-labeled cells showed SP- and CGRP-like immunoreactivity. Following nerve injury none of the cells showed immunoreactivity for either SP nor CGRP. NOS-like immunoreactivity was found in two percent of the cells normally and in ten percent after injury. Galanin- and GMAP-like immunoreactivity were found in two and three percent, respectively, of the cells normally and in 22 and fourteen percent, respectively, after injury. Thirty-four percent of the cells were carbonic anhydrase positive normally, and 42 percent after injury. Seventy-five percent of the cells showed RT97-immunoreactivity normally and twelve percent after injury. Choleragenoid-like immunoreactivity was found in 99 percent of the cells normally and in 81 percent after injury. Two percent of the cells were labeled by GSL-B_4 in the normal cases. After injury, however, no double-labeling was found. No NPY-like immunoreactivity was found in these cells normally, but after nerve injury 96 percent of the cells showed NPY-like immunoreactivity.

These results show that peripherally axotomized nucleus gracilis projecting neurons undergo marked alterations in their cytochemical characteristics, which may be significant for the structural and functional plasticity of somatosensory systems after injury.

694.14

POSTNATAL DEVELOPMENT AND PLASTICITY OF TERMINALS AND SYNAPSES IN SUPERFICIAL MEDULLARY DORSAL HORN: STEREOLOGICAL ANALYSIS.

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Mature receptive fields in the superficial dorsal horn develop later than in other "second-order" somatosensory nuclei (Fitzgerald, *J. Physiol. 364,* '85), and associated critical periods for deafferentation-induced plasticity differ. To further our understanding of the bases for these observations, EM stereology was applied to developing connections and morphology in laminae I and II of nucleus caudalis in normal rats. At birth, postnatal (P) days 1, 4, 17 and 90 there were 6.4 ± 4.6, 13.2 ± 6, 16.3 ± 2.7, 22.6 ± 1.3 and 35.2 ± 6.6 synapses per 100 μm^3, respectively. At corresponding ages there were 12.5 ± 2.9, 18 ± 8.5, 18.9 ± 2.3, 31.8 ± 13.3 and 29.6 ± 2.5 terminals; 4.1 ± 4.6, 10.5 ± 4.0, 4.4 ± 2.0, 2.3 ± 0 and 0.2 ± 0.2 degenerating profiles; 9.2 ± 1.9, 5.1 ± 2.1, 5.5 ± 3.4, 0.3 ± 0.1, and 0 growth cone-like profiles per 100 μm^3. Thus, there is a gradual and protracted accretion of connections and terminals through P17, and a corresponding attrition of degenerating and growth cone-like profiles. After infraorbital nerve section at birth, at P1, 4, 17 and 90 there were 10.5 ± 6.9, 9.8 ± 3.7, 21.8 ± 2.6 and 38.7 ± 5.2 synapses per 100 μm^3. At corresponding ages there were 16.0 ± 7.8, 16.9 ± 4.8, 36.2 ± 12 and 34.6 ± 4.7 terminals; 14.7 ± 1.4, 5 ± 1.7, 4.6 ± 1.4 and 0.3 ± 0.1 degenerating profiles; 4.5 ± 0.5, 3.3 ± 1.9, 0.1 ± 0 and 0 growth cone-like profiles per 100 μm^3. Thus, after deafferentation, normal numbers of terminals and synapses develop in the face of altered temporal patterns of degeneration and growth. These are hallmark signs of injury-induced pre- and postsynaptic degeneration and axonal sprouting. The source of the latter remains to be determined, although additional areal fraction data suggest that "replacement" terminals are not of primary afferent origin. Supported by NIH DE07734, NS17763.

694.15

ALTERED PATTERNS OF EYELID MORPHOGENESIS FOLLOWING LESIONS OF THE TRIGEMINAL GANGLIA

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In order to test the hypothesis that the afferent nervous system plays a role in the differentiation of ectodermal derivatives, the left trigeminal ganglion was cauterized in opossum pups at day 1 post-natal. Morphogenetic abnormalities were compared with the equivalent stages of normal development. Neuralectomies were carried out using procedures described by Morohunfola et al (1992 The Anat. Rec., 232, 599). Mother and pups were anaethsetized with halothane (Keller *et al.* 1988 Lab. Anim., 22, 269). Pups were taken for histologic analysis 2-20 days following surgery. Pups were given intraperitoneal nebutal, and fixed in formalin, serial paraffin sections stained with silver. The development of the eyelid neural non-neural constituents occurs post-natally and the lower eyelid lags behind the upper eyelid. Eyelid innervation precedes mesodermal maturation and hair follicle formation suggesting a possible neural role in cutaneous differentiation. Trigeminal ganglionic lesions only involved the sensory component of the nerve sparing the motor root. Trigeminal lesions resulted in altered spatial and temporal patterns in the eyelid that included abnormal eyelash symmetry, delayed follicle formation deletions of eyelashes and delayed dermal maturation. Sham operated pups (skin cauterized but lacking a neural lesion) showed normal development in all parameters of eyelid morphogenesis. These findings support the earlier findings of Morohunfola, *et al*, (1992 a & b), based on lesions of lumbo-sacral dorsal root ganglia and spinal cord. The present study involves a purely sensory lesion and clearly implicates afferent nerves in the formation of their targets in skin, i.e. the cutaneous appendages.

694.16

ISOLATION AND CHARACTERIZATION OF THE CHICK NMDA RECEPTOR cDNA

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A chick brain cDNA library was screened using a ^{32}P radiolabeled oligonucleotide that correspondes to the first transmembrane region of the rat NMDAR1 subunit. A fragment (approx. 0.5 kb) was isolated and subcloned into pGEM 4Z and used for double stranded sequencing (United States Biochemical). Preliminary results show that the first 200 nucleotides of the isolated fragment are homologous (80%) to the rat and human NMDAR1 subunit at the 5' end of the open reading frame. This region corresponds to the amino terminal end of the NMDA receptor and codes for the extracellular domain that preceeds the first transmembrane region. Comparison of the chick cDNA to other NMDA subunits shows no homology. This cDNA fragment has been used to make cRNA probes to determine the normal ontogeny of the NMDA receptor mRNA in the chick spinal cord. Preliminary *in situ* hybridization results show high levels of the NMDA receptor mRNA located primarily in the dorsal horn of the spinal cord (stage 39; E13). We are currently determining the distribution of NMDA receptor mRNA in the spinal cord following administration of curare and various NMDA receptor antagonist.

694.17

TEMPERATURE-DEPENDENT BEHAVIORAL CHANGES AND CEPHALIC WOUNDS IN CAPSAICIN NEONATALLY TREATED RATS.

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Neonatal administration of Capsaicin (CAP) in the rat interferes with eye-opening, scratching frequency and is also associated with skin ulcers emergence. Here we investigate, a) if the wounds emergence is a trophic effect or is produced by the increased cephalic scratching and, b) how the behavior of CAP rats was modified by the warm environmental exposure. We used control and neonatally (day 2 pp) CAP treated (50 mg/kg s.c.) Wistar male rats. In experiment a) rats were using (n=20) or not (n=20) a plastic collar fitted around the neck to prevent head scratching. The control group (n=10) were left undisturbed. In experiment b) the behavioral patterns of control or experimental rats were visually recorded in an isolated chamber at 24 °C or 35 °C of temperature (120 measurements/ 30 min). In both CAP groups the head wounds appeared around the 23 days of age and the progression in the number and extension of the lesions were also similar. Data indicate that wounds emergence is not provoked by the increment in the scratching frequency. Moreover, a significative reduction in self grooming frequency ($p < 0.05$) and relaxed extended posture ($p < 0.01$) in CAP animals was observed. Data showed that neurotoxic is primarily related with trophic skin lesions and has also a long term interference with the behavioral response associated to heat exposure.

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695.1

SYNAPTIC VESICLE PROTEIN 2 (SV2) LOCALIZATION IN THE DEVELOPING HAMSTER PRIMARY VISUAL PATHWAY. AnnaMaria Confaloni* and K. L. Moya. INSERM U334 and CNRS URA 1285, S.H.F.J.-C.E.A., Orsay, France; Istituto Superiore di Sanità, Rome, Italy.

A number of proteins whose synthesis and rapid axonal transport coincide with specific developmental stages of axonal growth have been characterized in vivo. One of these rapidly transported proteins has electrophoretic properties similar to SV2, a synaptic vesicle glycoprotein (Buckley and Kelly, *J. Cell Biol. 100*: 1284, 1985). We used the anti-SV2 monoclonal antibody, 10H, to examine 2-D Western blots containing metabolically labeled rapidly transported proteins of the hamster retinofugal pathway in parallel with immunohistochemical localization of SV2 in brain.

Western blotting showed that the 10H antigen did not correspond to a rapidly transported protein, while immunohistochemistry revealed marked developmental changes in SV2 . In the neonate hamster (day of birth = P0) fiber fascicles in the superficial optic tract (OT) over the hamster lateral geniculate nucleus (LGN) were SV2 immunoreactive (IR). At this age many retinal ganglion cell axons are still elongating over the LGN and superior colliculus (SC). At P1 and P2 immunoreactive fascicles in the position of the internal OT were more evident, and at P5 when retinal axons are arborizing, SV2 IR was more distributed in the neuropil. By P8, few SV2 IR fascicles could be observed. In the adult, the neuropil-like IR had diminished considerably and the OT was devoid of SV2 IR. A similar pattern was observed in the SC: fascicles in the stratum opticum/superficial gray were intensely labeled in the neonate and then the IR shifted to the neuropil at P5 - P6. The retinal origin of these fascicles was confirmed by their disappearance after eye enucleation.

These results show that SV2 is distributed along retinal axons as fibers reach their central targets and begin to arborize. Later, SV2 is distributed in the target neuropil and then diminishes. In addition, it appears that SV2, like other synaptic vesicle proteins such as rab3A and synapsin I, are not transported rapidly and are conveyed to axon terminals in a slow component of axonal transport.

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695.2

FUNCTIONAL PROPERTIES OF OPTIC AXONS REGENERATING INTO THE PRIMARY OLFACTORY CORTEX OF THE FROG. F. Scalia*, M. Reyes, A. Grant and J.Y. Lettvin. Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, New York 11203 and Dept. of Biomedical Engineering, Rutgers University, Piscataway, New Jersey 08855.

When the optic nerve of R. pipiens is cut and deflected into the telencephalon, the regenerating fibers form a coherent terminal field in the primary olfactory cortex [OC] (Scalia, '87, '91). Electrophysiological study 2 mo to 1 yr after surgery showed unit visual activity of types I-IV (Maturana et al., '60) in the superficial neuropil of OC (Layer I). Although the projection did not form a precise map of the visual field, all the units recorded in the neuropil of the more readily exposed anterior end of OC responded only to stimuli in the frontal 45° of the visual field. These stimuli also evoked responses at the level of the OC cell layer deep to the superficial neuropil. No visual responses were observed in OC in normal controls.

At 2 mo, only the small A waveform (as observed in the neuropil of the optic tectum [OT] by Grant and Lettvin, '91) characterized the visual responses in the neuropil of OC. After 5 mo, the B and C waveforms also appeared. They were much larger than any transients found in the neuropil of OC in normal controls, but somewhat smaller than those found in OT. The individual receptive fields in a multiunit receptive field in OC were usually fewer in number and distributed over wider angular areas than in OT.

The novel development of B and C waveforms in the superficial neuropil of OC suggests that the morphology and electrogenic properties of the dendrites are affected by maturation of the aberrant projection. (Supported by NIH Grant EY 05284)

695.3

NEONATAL ADMINISTRATION 5,7-DIHYDROXYTRYPTAMINE RESULTS IN SYNAPTIC REORGANIZATION IN THE SUPERFICIAL GRAY LAYER OF THE HAMSTER'S SUPERIOR COLLICULUS E.A Arce, R.D. Mooney*, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Neonatal subcutaneous administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) to hamsters results in a marked depletion of serotonin (5-HT) in cortex and a dramatic increase in the concentration of this amine in the superior colliculus (SC). In order to determine whether this increase was associated with an alteration in the synaptic organization of 5-HT-containing axons in the superficial grey layer of the SC, immunocytochemistry was combined with electron microscopy. In normal adult hamsters, only 4.4% of 250 5-HT-immunoreactive profiles made synaptic contacts in the superficial grey layer of the hamster's SC. Most of these contacts were axodendritic. In 5,7-DHT-treated animals, examination of 400 individual profiles indicated that 25.5% of 5-HT-positive profiles made synaptic contacts. Like the case in normal hamsters, most (63%) 5-HT-positive profiles in the treated animals were axoaxonic; 35% were axodendritic, and 2% were axosomatic. 5,7-DHT treatment had no significant effect on the diameter of 5-HT-positive profiles in the superficial SC laminae. The average for the normal animals was 0.84 ± 0.36 um and that for the treated hamsters was 0.67 ± 0.24 um. Given the recently demonstrated effect of 5-HT on retinotectal transmission in this species, these results suggest that the functional SC organization may also be markedly altered in animals that sustain neonatal 5,7-DHT administration.

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695.4

EFFECTS OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE TREATMENT ON RETINAL GANGLION CELL AND OPTIC NERVE FIBER NUMBER IN THE ADULT HAMSTER. T.Crnko*, R.S. Crissman, L. Zheng, R.W. Rhoades, and R.D. Mooney. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

A single subcutaneous injections of 5,7-dihydroxytryptamine (5,7-DHT) at birth results in a 50% increase in the concentration of serotonin in the superficial layers of the hamster's superior colliculus and a marked increase in the density of 5-HT-positive axons in this structure. This change is associated with abnormalities in both the crossed and uncrossed retinotectal projections. In the present experiment, Nissl staining, electron microscopy, and retrograde labelling with HRP were employed to compare numbers of neurons in the ganglion cell layer, numbers of optic nerve fibers, and numbers of retinal ganglion cells in normal adult hamsters and adult hamsters that received neonatal 5,7-DHT injections. The average number of neurons in the ganglion cell layer of the normal hamsters (N=5) was 128,383 ± 25,584 and that in the treated animals (N=5) was 150,208 ± 7,455. The average numbers of retrogradely labelled ganglion cells in the two groups (N=7 normal and N=8 5,7-DHT-treated hamsters) were 72,731 ± 11,125 and 74,669 ± 8,120, respectively. The average numbers of axons in the optic nerves of the normal (N=11) and 5,7-DHT-treated animals (N=12) were 102,649 ± 13,660 and 89,992 ± 5,480, respectively. Multivariate analysis of these results indicated that the changes in the retinotectal projections of 5,7-DHT-treated hamsters do not result from effects of this manipulation on retinal ganglion cell or optic nerve fiber number.

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695.5

NMDA RECEPTOR BINDING IN TECTUM OF DEVELOPING XENOPUS. S.B. Udin* & L. G. Murakami. Dept. of Physiology, SUNY, Buffalo, NY 14214.

The ipsilateral visual input to Xenopus tectum comes into register with the contralateral map during development. The initial map is established via chemoaffinity cues, while the final order is determined by a Hebbian mechanism involving binocular visual input. The role of visual input is normally confined to a 3-month critical period starting at about 1 month post-metamorphosis. The role of NMDA receptors in the process of organization of the ipsilateral map is demonstrated by the ability of NMDA receptor blockers to prevent matching of the ipsilateral map to the contralateral map during the critical period. Moreover, application of NMDA itself before or after the critical period confers activity-mediated plasticity during periods when visual input normally has no effect on ipsilateral map formation.

We have investigated whether the changes in plasticity are correlated with changes in NMDA receptor density in the superficial layers of the tectum. These layers contain the axons of the contralateral and ipsilateral visuotectal projections. NMDA receptor numbers in brains from tadpoles and of frogs of ages 1, 3 and 8 months post-metamorphosis were assessed by receptor binding studies in which tritiated glutamate, with or without NMDA as competitor, was applied to tectal slices. Autoradiographs were assessed densitometrically using Image software from NIH. The results show that NMDA receptors are present at all ages.

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695.6

THE ROLE OF RETINOCOLLICULAR AXONS IN THE DEVELOPMENT OF THE CORTICOCOLLICULAR PROJECTION OF MICE. M. Khachab* and L.L. Bruce. Department of Biomedical Sciences, Creighton University, Omaha, NE 68178.

Corticocollicular (CC) axons are believed to use retinocollicular (RC) axons as their primary guiding cue in the development of their topographic specificity in the superior colliculus (SC). Therefore, the development and specification of the CC and RC projections were studied in normal mice and compared to that of the CC projection in anophthalmic (129SV/CPor^{j}) mice. Injections of fluorescent dyes (DiI and DiA) were made in the visual cortex and/or the retina of normal and anophthalmic mice (E15 to adult). In normal mice, both RC and CC axons had reached the rostral pole of the SC by E15. By birth, they had reached the caudal pole of the SC. In mice that completely lacked a RC projection, the CC axons were confined to the rostral pole by P0 and reached the caudal pole by P6. In normal mice, RC and CC axons developed short side branches by P0, as did CC axons in anophthalmic mice. Transneuronal diffusion of DiI, indicating the development of immature synapses, was associated with CC axons of normal and anophthalmic mice at similar ages. Because the growth of the CC projection was delayed in mice that lacked a RC projection, the presence of RC axons may play a critical role in target finding by the CC projection. Furthermore, the elaboration of short side branches and appearance of transneuronal labeling in RC, normal and anophthalmic CC projections at approximately the same age suggests that the initiation of synaptogenesis depends on cues within the colliculus rather than the RC projection.

695.7

EXPRESSION OF CONSTITUTIVELY ACTIVE CaMKII IN XENOPUS TECTAL CELLS MODIFIES RETINAL GANGLION CELL AXON MORPHOLOGY. D.J. Zou*, D. Pettit, R. Malinow and H.T. Cline. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

NMDA receptor activity is essential in the plasticity of the developing frog retinotectal projection. Ca^{2+} entering through the NMDA receptor may act through a Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) pathway. We are studying this possibility by combining recombinant viral technology with *in vivo* imaging techniques. Vaccinia virus containing the DNA sequences encoding truncated constitutively active CaMKII and β-galactosidase (βG) (T.Virus) or βG alone (βG. Virus) was injected into the ventricles of stage 48 albino *Xenopus* tadpoles. Intense infection throughout the CNS, as indicated by the expression of βG, occurred by day 3. To examine RGC axon morphology, a small amount of 0.5% DiI was injected to the retina of stage 46-47 tadpoles. Animals were screened the next day to select those with a single brightly labeled RGC axon. Three days later, when tadpoles were stage 48, the axon morphology was observed *in vivo* by using a confocal microscope. The extent of viral infection was verified by checking βG expression. Single axons were reconstructed. In the animals infected with T. Virus and constitutively active CaMKII was expressed, the average total length of 13 RGC axons was 373 $\pm$ 49 μm ($\pm$ SE) and the average number of axon branches was 13 $\pm$ 2. These values are smaller than those in the control animals with no virus injected (n=15) and those infected with βG Virus (n=12), where the average total axon length was 523 $\pm$ 67 and 616 $\pm$ 116 μm, the average number of axon branches was 27 $\pm$ 3 and 24 $\pm$ 2, respectively. Thus constitutively active CaMKII expressed in the tectal cells reduced the RGC axon total length and the branch numbers in 3 days. These results indicate that CaMKII activity in the postsynaptic tectal neurons is likely to be involved in the control of the growth of presynaptic RGC axons. We are taking time-lapse images of growing axons to study the mechanism by which their morphology is modified.

695.8

Production of monoclonal antibodies to bullfrog brain and localization in optic tectum. Lex C. Towns*, Melissa Stuart, Patricia Sexton, and Nandor Uray. Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

Two thousand hybridoma lines were generated from mice immunized with homogenized brain of *Rana catesbeiana*. Of these lines, 79 were positive by enzyme-linked immunosorbent assay (ELISA) against brain homogenates and subsequently assayed against brain tissue immunohistochemically utilizing a biotin-conjugated secondary antibody and visualized with AEC. Thirteen lines were chosen for further analysis based on tissue staining patterns and the hardiness of the hybridomas under *in vitro* culture and storage conditions. Monoclonal antibodies from all 13 lines were analyzed by ELISA and western blotting against various developmental stages of *R. catesbeiana*. Three interesting lines -- 1B3, 4B8, and 1H12 -- recognized all developmental stages in the ELISA. Western blot patterns of denaturing gels were unique for each antibody, but all three recognized a 100-kDa *R. catesbeiana* component. Antibodies 1B3 and 4B8 reacted with elements in the ependymal/subependymal zone. The labeling was dense adjacent to the entire ventricular lumen in premetamorphic animals, and the labeling became restricted posteriorly in the tectum in older animals. Antibody 1H12 was visualized as a dense, uniform reaction product lying in the middle third of lamina 9 of optic tectum. The antibody labeling in lamina 9 was not continuous across the entire optic tectum at all ages. In early premetamorphic animals, 1H12 labeling of lamina 9 extended from the anterior limit of the optic tectum to approximately mid-tectum. At later premetamorphic ages, the labeling extended further posteriorly; more of lamina 9 was labeled at successively later premetamorphic and metamorphic ages. One speculation is that this labeling in lamina 9 parallels the ingrowth of optic nerve fibers, although this hypothesis awaits experimental analysis.

695.9

EFFECTS OF SEROTONIN ON PATCH-CLAMPED NEURONS IN THE OPTIC TECTUM OF THE FROG. Malayev A.A. and E.A. Debski*. Sch. of Bio. Sciences, University of Kentucky, Lexington, KY 40506.

Recent immunocytochemical studies have shown that serotonergic retinal ganglion cells project to the optic tectum of frogs (Liu & Debski, J. Comp. Neurol. 338:391-404,1993; Liu & Debski, Neurosci. Abstr. 19:452, 1993). The role of serotonin in the functioning and/or development of the tectum is unknown. We are investigating the effects of external application of serotonin on tectal cells in tadpoles and juvenile bullfrogs by applying whole-cell patch-clamping techniques to brain slices. Perfusion of the slices with 100μM serotonin induced a sustained outward current of 3.0±2.0pA/pF (n=4) in 27% of the tadpole cells voltage-clamped at -60mV. Under these conditions 50% of the cells recorded in juvenile frogs to date have shown this serotonin-evoked current (1.1±0.7pA/pF; n=2). Stepping the membrane potential to between -50 and 0mV from a holding potential of -70mV revealed serotonin-induced outward current in 50% of the tadpole cells and in 75% of the juvenile frog cells. Preliminary experiments conducted in current-clamp mode indicated that exposure of the cells to serotonin resulted in a cell hyperpolarization of approximately 10mV. In addition to an evoked outward current, in some cells serotonin also increased the frequency of synaptic inputs. Cells that responded to serotonin also responded to NMDA. External application of 33μM NMDA in the presence of TTX resulted in the activation of an inward current at a holding potential of -60mV. Our data suggest that serotonin may modulate NMDA receptor activity in the optic tectum by changing the potential of the membrane upon which these NMDA receptors sit. Supported by NSF grant IBN-9209651.

695.10

THE DEVELOPMENT OF NORMAL AND ABNORMAL GENICULO-CORTICAL TOPOGRAPHIES IN THE SYRIAN HAMSTER. A.L. Smith, K.Krug and I.D. Thompson (SPON: Brain Research Association). Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK.

The orderly mapping of the lateral geniculate nucleus (LGN) onto area 17 in the adult hamster is disrupted by unilateral eye removal on the day of birth. Ipsilateral to the remaining eye, single injections of tracer into area 17 can produce dual foci of retrogradely labelled geniculate neurons (Trevelyan & Thompson, 1992, Eur. J. Neurosci. 4:1104). We have studied the development of both the normal and the aberrant geniculo-cortical projections.

Separate injections of red and green fluorescent latex microspheres, spaced mediolaterally, were made in visual cortex of neonatal hamsters that had been monocularly enucleated on the day of birth (P0). No topographic order was discernible in animals injected on P2 when a single cortical injection labelled neurons throughout the LGN. With two injections, the populations of red and green labelled cells overlapped completely. At P6, cortical injections contralateral to the remaining eye produced an essentially adult pattern: most of the LGN was unlabelled and there were distinct foci of red and green labelled cells. The pattern in the ipsilateral geniculate was different with more overlap between the populations of red and green labelled cells, however the projection was much more restricted than that seen at P2. The characteristic double focus seen in adult deafferented LGNs appeared later.

The emergence of geniculo-cortical topography between P2 and P6 in the hamster coincides with the invasion of cortical plate by the majority of geniculate axons, as revealed by transneuronal tracing. Preliminary experiments placing MK801-containing Elvax on visual cortex failed to disrupt this early phase of topographic refinement.

This work was supported by the Wellcome Trust, UK.

695.11

THE DEVELOPMENT OF THE RETINO-COLLICULAR MAP IN THE SYRIAN HAMSTER. I.D. Thompson and P.M. Cordery. (SPON: Brain Research Association). Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK.

Previous studies applying DiI to the retinae of foetal rats revealed considerable topographic disorder in the retinal projection to superior colliculus (SC; Simon & O'Leary, 1992, J. Neurosci. 12:1212). We have used two retrograde tracers, red and green fluorescent latex microspheres, to examine the development of topography in the retino-collicular projection of the hamster. Small (25-50 nl) injections of tracers were made in all four quadrants of the SC in neonatal hamsters. Both the separation and the orientation (rostrocaudal vs mediolateral) of the paired red-green injection sites was varied. Single injections of tracer on P2 produced labelling over approximately one third of the retina, even though the injection sites occupied a very much smaller proportion of SC. When injections were separated by 400 μm or less and irrespective of their orientation, there was considerable overlap in the populations of red and green labelled cells. Large (700-800 μm) mediolateral separations resulted in very little overlap between the red and green labelled populations of ganglion cells whereas similarly large rostrocaudal separations produced much more overlap. Similar numbers of ganglion cells were labelled following injections into caudal or into rostral SC. By P6, single injections labelled ganglion cells in much more restricted regions of retina and pairs of injections separated by 400-500 μm produced discrete foci with relatively little overlap. Older animals displayed increasing precision in the retino-collicular projection such that, at P21, injections separated by less than 200 μm could produce two non-overlapping foci of labelled ganglion cells.

These results reveal that a crude retinal topography exists for the mediolateral axis of the SC in neonatal hamsters and that the overall topography refines during the period of ganglion cell death.

695.12

TRANSIENT CHANGES IN THE DEVELOPMENT OF SPONTANEOUS ACTIVITY IN THE NEONATAL RAT SUPERIOR COLLICULUS. S. Fortin, S. Molotchnikoff, and S.K. Itaya*, Dept. Sci. biol., Univ. Montréal, Montréal, Que. Canada H3C 3J7, and Dept. Biomed. Sci., Univ. South Ala., Mobile, AL 36688.

We have found that there are four major events in the functional development of the neonatal rat superior colliculus. (1) Spontaneous discharges are the earliest recordable activity, becoming manifest on postnatal day 5 (P5). (2) The first evoked response to electrical stimulation of the optic nerve is detectable on P10, and (3) the first flash evoked responses in the superior colliculus occur on P12/13. (4) On P14/15, eye opening occurs. Since spontaneous activity occurs first and may influence the development of visual pathways, we examined this function more closely. We measured the frequency of spontaneous spikes at different postnatal ages; interval histograms were constructed between P5-P15. Preliminary data, based on 28 animals, suggests an interesting trend. On P5, P8, P10, and P13, there are unimodal distributions of intervals, indicating clustering of shorter intervals and a relatively high frequency of spontaneous discharges compared to other ages. These ages may be pivotal days, since they precede each of the four major events in the development of the superior colliculus by 24-36 hours. Our data suggests that there is a transient increase in spontaneous activity coinciding with the initiation of each new functional capability. To further characterize the source of the spontaneous activity, we injected 2% xylocaine into the optic nerve head, silencing optic nerve activity at P6-P11. There was no change in the spontaneous activity in the superior colliculus during the blockade. These results indicate that spontaneous discharges in the superior colliculus are independent of the retina. Supported by grants from NATO, NSERC, and USARC.

695.13

A KERATAN SULFATE PROTEOGLYCAN THAT MARKS THE FUNCTIONAL SUBDIVISIONS OF FERRET LGN DURING DEVELOPMENT. E.E. Geisert*1 and J.A. Robson2. 1Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis TN 38163; 2Dept. of Anatomy and Cell Biology, SUNY HSC, Syracuse, NY 13210.

Monoclonal antibody (TED15) identifies a keratan sulfate proteoglycan (ABAKAN) that concentrates along the boundaries between thalamic nuclei during development and blocks cell attachment and neurite outgrowth in culture. In the lateral geniculate nucleus (LGN) of the developing ferret, TED15 transiently labels the borders between laminar subdivisions while retinal axons segregate and laminae form. At birth the LGN is relatively undifferentiated and axons from both eyes overlap. TED15 labeling outlines the nucleus. By postnatal day 10 (P10), retinal axons segregate into eye-specific laminae and interlaminar zones appear. TED15 labeling concentrates in these interlaminar zones. At P20 interlaminar zones are clearly labeled as are the borders between ON and OFF leaflets within laminae A and A1. As development continues labeling becomes relatively uniform throughout the nucleus. To test whether ABAKAN distribution depends on normal laminar development, one eye was removed from young kits causing axons from the intact eye to cross laminar borders into denervated laminae. The animals were killed around P20, the age when TED15 maximally labels laminar borders in normal ferrets. In one-eyed animals the boundaries between laminae are less distinct and TED15 no longer labels interlaminar zones. However, the borders between ON and OFF leaflets within non-denervated laminae remain labeled. These results are consistent with the hypothesis that ABAKAN plays a role in LGN lamination. Supported by the Hendricks Fund (JAR).

695.14

GLYCINE IN THE VISUAL SYSTEM OF YOUNG AND ADULT FERRETS. Kathrin Herrmann*, Lab. Neurophysiology, NIMH, Poolesville, MD 20837, USA.

It is well established that glycine is an inhibitory neurotransmitter in the brainstem and spinal cord. Glycine, however, is also present in retinal ganglion cells both in adult and developing mammals, and glycine receptors are abundant in the early postnatal mammalian neocortex. In the present study the lateral geniculate nucleus (LGN) and the visual cortex were examined in adult and developing ferrets for the presence of glycine employing immunohistochemical procedures using a rabbit anti-glycine antibody. In adults, glycine-positive cells were present in both the LGN and visual cortex. In adults, immunopositive cells in the LGN were mainly large or medium sized neurons, and glycine positive cortical cells were found in all layers, but occurred in greater frequency in the infragranular layers. By birth, glycine-positive cells were already plentiful in the LGN. In the developing cortex, however, glycine-positive neurons were sparse in the cortical plate, although abundant in the subplate (SP) and marginal zone. In addition, the SP/intermediate zone contained a dense plexus of glycine-positive fibers, perhaps representing thalamocortical axons. To determine whether the glycine occurs in relay neurons of the LGN, I injected HRP and/or fluorescent latex beads into visual cortex of adult (≥P85) ferrets. Most if not all retrogradely labeled LGN cells were also glycine-positive. Since the relay neurons of the LGN are likely to use an excitatory neurotransmitter, the presence of glycine in LGN projection neurons strongly suggests that glycine within thalamocortical axons acts on the glycine site of the NMDA receptor, mediating the appropriate excitatory thalamocortical response. The presence of glycine by birth, before the onset of synaptogenesis, might indicate an additional role for glycine perhaps as a neurotrophic factor.

695.15

SIGNAL TRANSMISSION IN THE LATERAL GENICULATE NUCLEUS OF MONKEYS WITH SURGICALLY INDUCED ESOTROPIA. E.L. Smith III*[1], Y.M. Chino[1], H. Cheng[1], Y. Sasaki[1], M.L.J. Crawford[2], R.S. Harwerth[1] and G.K. von Noorden[3]. College of Optometry, University of Houston[1]; University of Texas at Houston[2], Department of Ophthalmology, Baylor College of Medicine, Houston, TX[3].

Early strabismus, a form of abnormal visual experience which often leads to permanent vision anomalies, produces dramatic alterations in the response properties of neurons in the monkey's visual cortex. In addition, the morphology of monkey LGN neurons is clearly altered by strabismus, but it is not known whether the functional status of LGN neurons is also affected. We investigated the effects of early strabismus on the transmission properties of LGN neurons in 3 monkeys reared with surgically induced esotropia by using microelectrode recording techniques to compare the responses of individual LGN neurons with those of their retinal inputs (S-potentials). For well-isolated S-potential and LGN action potential pairs (n=65), we measured orientation, spatial frequency, temporal frequency and contrast response functions using drifting sine-wave gratings. Contrary to our previous findings in strabismic cats, there were no obvious differences between the transfer characteristics of control and treated-eye neurons. The results suggest that, in comparison to the cat, the physiological status of the primate LGN is less susceptible to environmental influences.

695.16

THE EFFECT OF NEONATAL MONOCULAR APHAKIA OR OCCLUSION ON THE EXPRESSION OF CAT-301 ANTIGEN, PARVALBUMIN AND CALBINDIN IN THE LATERAL GENICULATE NUCLEUS OF MACAQUE MONKEYS. N. Jain*, M. Tigges, V. Rema and J. H. Kaas. Dept. of Psychology and Inst. for Developmental Neuroscience, Vanderbilt University, Nashville, TN, and Yerkes Research Center, Emory Univ., Atlanta, GA.

Macaque monkeys (*Macaca mulatta*) were reared from birth to about one year of age either with surgically induced monocular aphakia, resulting in a blurred retinal image, or with a black occluder lens on one eye, which prevents visual experience. Levels of expression of the Cat-301 antigen, parvalbumin (PV) and calbindin (CB) were examined with immunohistochemical techniques in the lateral geniculate nucleus (LGN). Both types of deprivation resulted in a marked reduction of Cat-301 staining in both parvocellular and magnocellular LGN layers connected to the deprived eye, with deprived magnocellular layers retaining a relatively higher level of staining than the deprived parvocellular layers. PV and CB immunoreactivity, however, was reduced only moderately in the deprived layers, with a more pronounced reduction ipsilateral to the deprived eye. These results show that changes in the expression of the Cat-301 antigen, and PV and CB are similar, whether produced by aphakia or occlusion. Remarkably, alterations can be more pronounced in the layers receiving inputs from ipsilateral than the contralateral deprived eye. (Supported by NIH EY02686, EY09737 and RR-00165)

TRANSPLANTATION VI

696.1

MOTOR FUNCTION ANALYSIS OF NORMAL AND TRANSPLANTED MUTANT MICE USING A ROTAROD DEVICE. P.L.Kuhn[1], J.L.Cantrall[1], E.Petrogulakis[2], J.L.Norman*[1] and R.D.McKinnon[2]. California State U., Chico, CA 95926[1] and R.W. Johnson Med. School, UMDNJ, Piscataway, NJ 08854[2].

We examined motor control in normal and shiverer (shi) mutant mice using the rotarod assay, a forced motor activity which tests for balance and coordination. Shiverer mice carry a deletion in the myelin basic protein (MBP) gene, resulting in CNS dysmyelination and characteristic motor dysfunction. Shiverers were evaluated at 6-7 weeks of age in order to avoid the tonic seizures seen in older shi adults, and all animals were acclimated to the test apparatus prior to testing at 3 rpm and 6 rpm each for 1 min. Homozygous mutant mice had a significant increase in cumulative falls (5.05±0.6, n=44) relative to wild-type and heterozygous (shi/+) mice (0.11±0.11, n=9). Comparable results were obtained at speeds of 12 and 18 rpm, 1 min each. Non-acclimated animals showed learning curves with progressive improvement in performance when tested daily for four days, although the shi/shi animals consistently had more falls than normal control animals.

We also examined the potential for the rotarod assay to detect differences in motor performance of shi/shi animals in a transplantation paradigm. Postnatal day 2 animals received thalamic transplants of either rat cortical oligodendrocyte-type 2 astrocyte (O-2A) progenitor cells, or the O-2A line Central Glial-4 (CG-4) cells. Shi/shi transplant recipients showed a significant improvement in rotarod performance (1.72±0.3 falls, n=18) although they continued to demonstrate a gross phenotype, indicating that the recovery measured in the rotarod represents a mild degree of functional restoration. Immunohistochemical analysis of tissue harvested after rotarod analysis revealed the expression of MBP in the CNS of transplanted shi/shi mice. A group of transplant recipients had a decline in motor performance relative to nontransplanted shi/shi mice (9.8±1.8 falls, n=5). RNA analysis of two of these animals showed no evidence of MBP transcripts, consistent with the interpretation that the grafts were not successful in these recipients. Our results demonstrate that the rotarod is a sensitive measure of motor function in dysmyelinated mice, and suggest that transplantation can lead to an improvement in motor function. The cell and molecular basis for this motor recovery in shi/shi transplant recipients remains to be elucidated.

696.2

KINEMATIC AND EMG ANALYSES OF CONTROL AND SPINAL CORD TRANSECTED RATS DURING TREADMILL LOCOMOTION. J.G. Broton*, X.-M. Xu, M.B. Bunge, and B. Calancie, The Miami Project to Cure Paralysis and the Department of Neurological Surgery, University of Miami, Miami, FL.

We previously demonstrated that mid-thoracic spinal cord transections bridged by Schwann cell-filled guidance channels open at both ends (OPEN CHANNEL) promoted a greater number of axons growing into it than do channels closed at the distal end. The following studies were undertaken to determine if behavioral or electrophysiological correlates of this increased growth exist.

Unoperated control rats were studied to determine the normal walking pattern on a treadmill. These data were then compared to spinal cord transected, closed channel and OPEN CHANNEL groups. Rats were studied 1-4 weeks post-surgery. For the kinematic analysis, limb positions were taken at 30 frames/s during 7.5-30s of regular forepaw movement while rats were suspended in a sling over a treadmill which was moving at 0.05-0.15m/s. In conjunction with the kinematic study, EMG experiments were done, in which periods of activity ranging from 7.5-120s were collected from forelimb and hindlimb muscles, digitized at 2000Hz, rectified, and analyzed.

OPEN CHANNEL rats displayed significantly more hindlimb EMG than did rats with closed channels. Further, alternating hindlimb movements were sometimes seen which closely matched appropriate forelimb movements at some treadmill speeds, but which deteriorated at lower or higher speeds. To determine if this movement was under some voluntary control, we also calculated the cross-covariance functions of forelimb vs. hindlimb movements (kinematic analysis) and muscle activations (rectified EMG analysis).

Because involuntary reflex hindlimb movements can confound currently available behavioral measures of motor function, this analysis may prove useful in the assessment of motor deficit and subsequent restoration of movement after complete or incomplete spinal cord injury.

696.3

EFFECTS OF FETAL SPINAL CORD (FSC) IMPLANTS AND EXERCISE ON MUSCLE ATROPHY IN CHRONIC SPINAL RATS. N.B. Reese*, J.D. Houlé, C.A. Peterson, C.M. Gurley, C.L. Berry, R.D. Skinner and E. Garcia-Rill. Depts. of Anatomy and Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205

The potential for interventive procedures to influence the characteristic atrophy of hindlimb skeletal muscle following spinal cord injury was examined. Adult Sprague-Dawley rats sustained a complete spinal cord transection (Tx) by aspiration at the T12 level. Four groups of animals were prepared: Tx only, Tx followed by transplantation of FSC tissue, Tx followed by passive exercise or TX with transplantation and exercise. A fifth group of non-injured animals served as controls. Exercise began 5 days after injury, consisting of two 20-minute sessions on a motorized cycler, 5 days/wk for at least 2 months. Animals were sacrificed after 2-3 mos. and tissue blocks from the gastrocnemius (G) and tibialis anterior (T) muscles were prepared for measurement of cross-sectional area of individual myofibers or for immunocytochemical analysis of myosin heavy chain (MyHC) components. Mean myofiber area was reduced by 45-55% of control in both G and T muscles after Tx alone. With passive exer cise only, the extent of myofiber reduction was less severe (40% in G and 30% in T), while greater maintenance of fiber size was attained with FSC transplantation alone (65% of control for G and 90% of control for T). Combined FSC transplantation and exercise gave similar results to FSC transplantation alone. These results indicate that FSC transplants help maintain muscle fiber size to a greater degree than with exercise alone.

Analysis of MyHC components demonstrated an increase in myofibers expressing type IIb (fast, fatigable) and a decrease in those expressing type I (slow) after TX. With exercise, fewer type IIb fibers were evident, while type IIa (fast, fatigue resistant) fibers were more prominent. These results suggest that the shift in expression towards type IIb fibers caused by spinal cord Tx is reduced by exercise by increasing the proportion of type IIa fibers. Supported by NSF Grant RII 8922103 and NIH Grant NS 29328.

696.4

CERVICAL SPINAL CORD INJURY (SCI) IN THE ADULT RAT: FORELIMB FUNCTION AFTER INTRASPINAL TRANSPLANTATION OF TROPHIC FACTOR-TREATED FETAL SPINAL CORD CELLS. S.M. Onifer', J.F. Rodríquez, J.C. Benitez, D.H. Hesse, D.T. Kim, J.T. Pacheco, J.V. Perrone, D.I. Santiago, A. Martinez-Arizala[1]. The Miami Project, Dept. of Neurological Surgery & [1]Neurology, University of Miami School of Medicine, Miami, FL, 33136.

Traumatic contusion injury to the human spinal cord most frequently occurs at the cervical level and produces varying degrees of chronic tetraplegia. The deficits in the hands and arms result in part from cervical spinal motor neuron degeneration. Using our adult rat model of incomplete cervical SCI and forelimb behavior tests, the present study begins investigations of motor neuron replacement after cervical SCI as a means of ameliorating forelimb dysfunction. Cervical SCI was produced by dropping a 10 gm weight 2 cm onto the C7 spinal cord segment. Between 1-4 weeks post-SCI, SCI rats (n=11) had significant forelimb deficits compared to sham-injured rats (n=4) as shown by: 1) decreased grades for proximal (elbow) and distal (wrist and digits) extension and flexion (grasp) with a modified Tarlov scale and during Forelimb Placing Tests, 2) reduced forelimb grip strengths in the Grip Strength Test, and 3) reduced pellet retrievals and well clearance scores in the food retrieval Staircase Test. At 5-6 weeks, CNTF (Regeneron Pharmaceuticals, Inc., 5 μg/day) (n=4) or saline (n=3) was administered at the C7 segment of SCI rats through an intrathecal cannula. At 5 weeks, some SCI rats received transplants at C7 of diamidino yellow-labeled, E14-15 rostral spinal cord cell suspensions (1.05 X 10^6 viable cells) incubated for 60-90 min in 10.5 μg CNTF (n=2) or saline (n=2). At 10 weeks, forelimb function of all SCI rats had not improved. Nissl-stained cells with motor neuron morphology and ChAT-immunoreactive cells were found in similar small numbers in all transplants. The results show that improving survival and outgrowth of transplanted motor neurons needs to be further examined. Supported by The Miami Project to Cure Paralysis.

696.5

EFFORTS TO REINNERVATE ADULT RAT GASTROCNEMIUS MUSCLE BY EMBRYONIC SPINAL MOTOR NEURONS TRANSPLANTED INTO PREDEGENERATED TIBIAL NERVE. D.E. ERB', L. LIPSON, and R.P. BUNGE, The Miami Project to Cure Paralysis, Un. of Miami School of Medicine, Miami, FL 33136

Motor innervation is required for effective contraction and maintenance of skeletal muscle. If motor innervation is lost the muscle becomes denervated and undergoes progressive atrophy which ultimately leads to degeneration. Efforts to illicit muscle contraction or significantly retard muscle atrophy by direct electrical stimulation of the muscle are relatively ineffective without a nerve supply. Previously, we have reinnervated denervated adult rat gastrocnemius muscle by transplanting a heterogeneous neuronal population of dissociated cells, from E14 ventral spinal cord, into the adult rat tibial nerve, which was then transected proximal to the transplant site (Exp. Neurol. 1993. 124:372-376.). Currently, we seek reinnervation by transplanting into nerve 1,2, and 3 weeks after axotomy. Within six weeks posttransplantation large multipolar cells, resembling alpha motor neurons, were observed within the transplant site. Axons originating from these transplanted cells were identified within the nerve stump and within the previously denervated gastrocnemius muscle. Transplanted motor neurons survived up to 10 weeks (the longest survival) and could be retrogradely labeled with the retrograde tracer fast blue. This study demonstrates that embryonic spinal motor neurons, transplanted into the predegenerated adult peripheral nerve, are able to survive and extend axons into the denervated target muscle. This approach may provide the innervation necessary to artificially control muscle contraction by functional electrical stimulation, thus making previously unavailable therapies available to the muscle. (The Hollfelder Foundation, The Miami Project to Cure Paralysis, NRSA NS09144-01).

696.6

TRANSPLANT MEDIATED BEHAVIORAL RECOVERY OF WEIGHT SUPPORT & INTERLIMB COORDINATION IN NORMAL, SPINAL AND SPINAL+TRANSPLANT RATS.

D.Y.Miya*, B.Clark, A.Tessler and M.Murray. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Transplanted fetal spinal cord tissue into verified lesion sites can support locomotion in newborn spinal cats (Howland et al.,1993). Surgical procedures and a battery of quantitative behavioral tests were developed to extend these results to a neonatal rat model. Blocks of E14 cervical spinal cord tissue were placed into the newly transected rat thoracic spinal cord on/after the day of birth. Reflex and locomotor behavior was studied in normal rats, spinal rats and in spinal rats with a transplant beginning at 4 weeks of age. Tests examined posture, weight support and interlimb coordination and included reflex bipedal and quadrepedal treadmill locomotion, motivated locomotion over a variety of terrains and stair climbing. Transplanted animals demonstrate coordination and weight supported steps on all types of locomotion but also exhibit marked hypermetria in the hindlimbs (HL's). EMG recordings taken from each limb during locomotion also demonstrate intersegmental communication across the graft in transplanted, but not spinal rats. Spinal rats demonstrate coordinated HL stepping only during tests of supported, reflex bipedal locomotion. Stair climbing, a task requiring complex postural adjustments and adaptive changes in weight support and interlimb coordination, was completed differently by each of the 3 groups of trained rats. Supported by NIH NS 24707 and VA Research Service.

696.7

PHARMACOLOGIC MODIFICATION OF TRANSPLANT MEDIATED BEHAVIORAL RECOVERY IN NORMAL, SPINAL AND SPINAL+TRANSPLANT RATS. M.Murray*, A.Tessler, K.Simansky and D.Y.Miya. Dept.of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Fetal tissue transplanted into spinal cord will promote development of locomotor function in cats (Howland et al., 1993) and in rats transected as neonates (Miya et al., this volume). Specifically, interlimb coordination across the graft develops in transplanted but not spinal rats, shown by both behavioral and physiological methods. Transplants may improve interlimb coordination by promoting connectivity across the lesion, by increasing weight support through mechanisms operating at a segmental level or by a combination of both mechanisms. Because regenerating 5-HT axons have been shown to cross grafts in neonates, descending serotonergic systems might contribute to behavioral recovery. Trained normal, spinalized and transplanted rats were studied for the effects of serotonergic agonists. Quadrupedal treadmill locomotion was videotaped both before and after drug administration and analyzed. The non-specific 5-HT receptor agonist quipizine, produced a marked increase in the step cycle duration and augmented markedly the amplitude and duration of hindlimb extensors, flexors and axial muscles in transplanted animals. Quipizine did not efffect either normal or transected rats at the doses used indicating that the descending 5-HT system may be involved in transplant enhanced locomotion. Immunocytochemical and fluorogold results also indicate the possible contribution of both the descending 5-HT and NE systems in the improved function. Supported by NIH NS24707 and VA Research Service.

696.8

COMPARISONS BETWEEN TYROSINE HYDROXYLASE AND PREPROENKEPHALIN mRNA IN RAT ADRENAL MEDULLARY EXPLANTS IN VITRO AND IN CNS TRANSPLANTS. X.-T. Wang*, J. Unnerstall, G.D. Pappas and J. Sagen. Dept. of Anat. & Cell Bio., Univ. of Illinois at Chicago, Chicago, IL 60612.

Our previous work has indicated that pain sensitivity can be reduced by transplanting adrenal medulla into the rat spinal subarachnoid space. To further elucidate the mechanism of this pain alleviation, the expression characteristics of tyrosine hydroxylase (TH) mRNA and preproenkephalin (PPEnk) mRNA were investigated using in situ hybridization in rat adrenal medullary explants in culture and transplants in the spinal subarachnoid space. ^{35}S-labeled oligonucleotide probes and anti-TH monoclonal antibody were applied to 10-μm cryostat sections. The relative mRNA levels and immunoreactivity (IR) were quantified by image analysis. Both TH mRNA and PPEnk mRNA levels in explants started to increase after 4 days in culture, and reached a peak at 7 days. At 14 and 28 days in culture, levels of TH and PPEnk mRNA decreased, but were still significantly higher than that of intact normal adrenal medulla and than at 1 and 4 days in culture. The TH-IR significantly increased by 1 day, and continued to increase up to 4 days in culture. Thereafter, it remained constant at a high level. Similar to adrenal medullary explants, adrenal medullary transplants contained numerous TH mRNA labeled cells at 1, 2 and 12 weeks after transplantation. However, only a few cells expressed PPEnk mRNA by 2 weeks post-transplantation. These results indicate that, while surviving chromaffin cells can still synthesize catecholamines when transplanted to the CNS, the synthesis of opioid peptides may remain low. In addition, these finding suggest that the regulatory mechanisms of TH mRNA and PPEnk mRNA expression in rat adrenal medullary cells differs under the environmental influence of culture and the CNS. Supported by NIH grant NS25054.

696.9

ANALGESIA INDUCED BY IMPLANTATION OF ENCAPSULATED GENETIC ENGINEERED CELLS SECRETING ß-ENDORPHIN.
Y. Saitoh*, T. Taki, N. Arita, T. Ohnishi, T. Hayakawa. Dept. of Neurosurgery, Osaka Univ. Med. Sch., 2-2 Yamadaoka, Suita, Osaka 535, Japan

The purpose of this study was to assess whether genetic engineered, xenogeneic tumor cells immunologically isolated in polymer capsules could survive and continue to reduce pain when transplanted into the spinal CSF space of rats. The mouse neuroblastoma cell line, pro-opiomelanocortin gene transfected Neuro2A (gift from Dr. Boileau, Montreal) which secretes ß-endorphin, was enclosed in polymer capsules (AMICON Co., diameter: 500μm, length: 15mm) at the density of $5x10^6$/ml, and transplanted into the spinal CSF space from the occipito-atlantal juction of male Sprague-Dawley rats. Three analgesiometric tests; tail pinch test, hot plate test, and electrical stimulation test, showed that the rats with encapsulated Neuro2A (n=6) were significantly less sensitive to pain 2 and 4 weeks after transplantation in comparison with control animals (n=8) and pre-transplanted animals (ANOVA; $p<0.05$). The analgesia induced by encapsulated cells secreting ß-endorphin could be attenuated by naloxone (1mg/kg; body weight, i.m.), which suggested the involvement of opiate in mediating this response. Morphological study revealed that the cells in polymer capsules survived a month after implantation in the CSF space. A month after the implantation of capsules in the rat CSF space, some of them were removed and immersed in culture medium. Peptides secretions from encapsulated Neuro2A cells were confirmed. The result of this study suggest that immunologically isolated, genetic engineered xenogeneic tumor cells can secrete opiate in the CSF space, and this method may be applied to the treatment of cancer pain.

696.10

XENOTRANSPLANTATION OF ENCAPSULATED BOVINE CHROMAFFIN CELLS FOR THE TREATMENT OF INTRACTABLE PAIN: A PHASE I CLINICAL STUDY
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Chromaffin cells are known to release a cocktail of analgesic substances such as catecholamines and opioids. Transplantation of allogeneic and xenogeneic chromaffin cells has been shown to alleviate pain in rodent models. To circumvent the problem related to the shortage of human cadaver donors, we are investigating the possibility of transplanting encapsulated xenogeneic cells in humans suffering from intractable pain. Chromaffin cells are isolated from surgically harvested calf adrenal glands and encapsulated in a tubular (5cm long, 950µm OD) semipermeable membrane which allows diffusion of nutrients and neurotransmitters but prevents rejection of the graft by the host immune system. Bacteriologic analysis and capsule catecholamine release studies were performed before implantation.

Ten patients suffering from chronic pain related to advanced cancer or deafferentation were transplanted with cell loaded capsules in the lumbar subarachnoid space (n=8) or lateral ventricle (n=2) using minimally invasive techniques. No evidence of serious complications or significant host tissue reaction related to the capsules was observed. Viable cells positive for tyrosine hydroxylase and methionine enkaphaline immunostaining were observed in all but one of the nine explanted capsules. Seven of the ten patients showed improvement in their chronic pain on the basis of decreased narcotic intake (4/6 opioid responders) or improvement in pain scores (3/3 non-responders to narcotics).

696.11

THE ADRENAL MEDULLAE AUTOTRANSPLANTED INTO THE THALAMI FOR 8 MONTHS STILL PRODUCE ENKEPHALINS
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Enkephalin, an endogenous opioid peptide, plays an important role in pain modulation. As enkephalin is produced by the adrenal medulla, a possible treatment for reduction of refractory pain is by adrenal medullary transplantation. One possible target for this transplantation is within the thalamus, a major site for processing ascending and descending nociceptive information. In the present study, L-enkephalin and M-enkephalin contents were determined in homogeneous adrenal medullae transplants after eight months survival. Male guinea pigs (eight weeks old) were used. Right adrenal medullae were used as control. Following a eight month survival time, animals were perfused with Zamboni's fixative. Thirty micron sections were cut through the thalamic grafts and the left adrenal medulla and the tissue processed immunocytochemically for L-enkephalin and M-enkephalin. Following a eight month survival time, L-enkephalin and M-enkephalin were still detected in the grafts of the thalami of the guinea pigs. This result suggests that L-enkephalin and M-enkephalin were produced by the grafts transplanted into the thalamus after a relatively long period time of survival.

696.12

POLYMER-ENCAPSULATED CELLS GENETICALLY MODIFIED TO SECRETE HUMAN NGF IMPROVE COGNITIVE FUNCTION FOLLOWING INTRAVENTRICULAR IMPLANTATION INTO AGED RATS.
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Exogenous NGF has been reported to improve cognitive function in rats with age-related cognitive deficits. However, as a potential treatment for Alzheimer's disease, concerns have been expressed about the risks involved with supplying NGF to the CNS. Recent work in our laboratories has suggested that the beneficial effects of exogenous NGF may be obtained with doses much smaller than those used previously. In this study, baby hamster kidney cells were genetically-modified to secrete human NGF (hNGF), encapsulated in semipermeable membranes, and implanted intraventricularly. hNGF production was less than 10% of doses previously reported to be effective. Performance in a working-memory version of the Morris water maze spatial learning task was improved in more impaired 20.6 and 26.7 mo rats; yet, in contrast to results with higher doses of NGF, there was no evidence that hNGF impaired performance in the youngest 3.3-5.4 mo rats. Other measures support the safety of this potential treatment. More than 30% of the oldest rats died during the 60 days before being implanted, but analyses of the survival distribution functions failed to detect significant increases in mortality rates related to either the implantation procedure or the hNGF produced by the capsules. Body weights, collected weekly throughout the study, also failed to reveal even transient deleterious effects of the surgical procedure or the hNGF produced by the capsules. Exogenous hNGF administered peripherally produces hyperalgesia, but the intraventricular capsules in the present study did not produce statistically significant effects in the hot plate analgesiometric test. Activity levels monitored throughout the light:dark cycle revealed statistically significant but small increases in activity in the young and middle-aged rats receiving hNGF, but no change in the oldest rats receiving hNGF. Somatosensory function, assessed using von Frey hairs, was not affected by hNGF in the young or middle-aged rats. Old rats are significantly less responsive to light touch than young rats, but hNGF treatment increased the responsiveness of the oldest rats to light touch to the level of young rats. These results suggest that CNS-implanted semipermeable membranes, containing genetically-modified xenogeneic cells continuously produce hNGF that improves age-related cognitive deficits in nonimmunosuppressed rats, and that both the surgical implantation procedure and long-term exposure to low doses of hNGF appear safe in rats.

696.13

EFFECTS OF FETAL SEPTAL GRAFTS ON MEMORY AND LEARNING PERFORMANCE WITH HIPPOCAMPAL ACETYLCHOLINE METABOLISM IN FIMBRIA TRANSECTED RATS.
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The fimbria of Sprague-Dawley rats was transected unilaterally. A group of rats with fimbria lesion received septal graft two weeks after the lesion surgery. The grafts were obtained from ventral forebrain of 13-15 days old fetuses of the same outbred strain. Lesion of the fimbria led to impairments in step-through passive avoidance and Morris' water maze tasks . Such impairments were partially ameliorated by graft tissue but it did not reach to a statistically significant level. Fimbrial lesion produced significant decline in both stimulated acetylcholine (ach) release from the hippocampal slices and in tissue ach levels. Septal grafts both ameliorated hippocampal ach levels ($p<0.01$) and stimulated release of ach ($p<0.01$) although we were unable to show its reflection on behavioral tests appropriately.

696.14

A MODEL OF FETAL NEURAL TISSUE TRANSPLANTATION INTO A SITE OF TRAUMATIC BRAIN INJURY.
G.Sinson, E.S. Flamm*, and T.K. McIntosh. Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104.

Fetal rat cortical cells have been shown to survive when transplanted to the edge of the central core of ischemia induced by middle cerebral artery occlusion. The present study was designed to examine the feasibility of transplanting fetal rat cortical cells into the area of damaged cortex in a clinically relevant model of fluid-percussion head injury in the adult rat. This injury results in a reproducible cavity with glia limitans in the parietal cortex. Male Sprague-Dawley rats (300-400g) received later (parasagittal) fluid - percussion brain injury of moderate severity (2.1-2.3 atmospheres). At 24 hours after injury timed pregnant female Sprague-Dawley rats (E16) were anesthetized (sodium pentobarbital), the fetuses were removed, and the fetal cortical cells ere surgical dissected. These fetal rat cortical cells were stereotactically transplanted with a glass pipette into the site that the injury cavity would form.

Memory testing using the Morris Water Maze and examination of post-traumatic motor dysfunction at 48 hours after transplantation demonstrated that these animals did not have worsened deficits when compared to animals which only received a fluid-percussion injury of similar severity. Histologic evaluation with Nissl stain 48 hours and 7 days after transplantation demonstrated survival of fetal cells. It is concluded that fetal cortical cells can be safely and successfully transplanted into the traumatically injured cortex of the adult rat. (Supported in part by NIH grants NS2618 and NSO8803).

696.15

Host Brain Modulation of Intra-Retrosplenial Transplants of Cholinergic Septal Neurons. *Ying J. Li*, and Walter C. Low*, Depts of Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN, U.S.A.

Previous studies from our laboratory have demonstrated that cholinergic-rich grafts derived from fetal septal nucleus can re-establish the cholinergic innervation in the retrosplenial cortex (RSC) of rats with lesions in both fornix and cingulate pathways. Concurrently, a significant amelioration of spatial memory deficits in these animals was observed. In the present study we postulated that the grafts can become functionally incorporated with the neural circuitry of the host brain, and can thus be regulated by activity from the host brain. To test this hypothesis we evaluated the effect of the animals' performance in a 6-arm radial maze task on high affinity choline uptake (HACU). Three groups of rats were used: 1) normal control rats (NC), 2) rats with lesions of the fornix and cingulate pathways, and 3) lesioned rats with fetal septal grafts in the RSC (RSCsep-TPL). Animals in each group were further randomly divided into two subgroups: 1) rats that performed the maze before the determination of HACU levels (BEH), and 2) rats that were kept in their home cage (NON-BEH) and paired with a BEH mate for HACU assessment. In NON-BEH animals, the HACU values (pmol/4 min/mg protein) were 21.2 $\pm$ 0.9 (mean $\pm$ SEM) for the NC, 9.9 $\pm$ 1.0 for the FX, and 17.7 $\pm$ 0.7 for the RSCsep-TPL animals. The BEH subgroups had HACU values of 29.8 $\pm$ 0.8 for the NC, 9.1 $\pm$ 1.0 for the FX, and 22.9 $\pm$ 0.9 for the RSCsep-TPL. In comparing HACU levels with their NON-BEH counterparts, *t*-test indicated significant increases in the NC ($p < 0.001$) and RSCsep-TPL ($p < 0.005$) groups, but not in the FX animals ($p = 0.565$). These results suggest that fetal septal graft in the RSC can become functionally incorporated with the host neural circuitry, and that the activity of the implanted cholinergic neurons can be modulated by the host brain. (Supported by NIH grant RO1-NS-24464).

696.16

ENHANCED SURVIVAL OF NEURAL XENOGRAFTS AFTER MASKING OF DONOR MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I. P. Pakzaban,*[1,2] T.W. Deacon,[1] L.H. Burns,[1] J. Dinsmore,[3] and O. Isacson.[1,2] Neuroregeneration Laboratory[1], McLean Hospital, Belmont, MA, 02178; Neurosurgery and Neurology Services[2], Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02114; and Diacrin Inc.[3], Charlestown, MA 02129.

To determine the role of major histocompatibility complex class I (MHC-I) in immunological rejection of neural xenografts, fragments of a monoclonal antibody (Fab) directed against porcine MHC-I were used to mask this complex on porcine striatal neuroblasts transplanted into the rat striatum. Adult rats previously lesioned with quinolinic acid in the striatum received stereotaxic intrastriatal injections of cell suspensions prepared from the lateral ganglionic eminence of E35 porcine telencephalon. The presence of MHC-I on the surface of these cells was confirmed by fluorescence-activated cell sorting. Transplanted rats were divided into 4 groups based on immunosuppressive treatment: group I, no immunosuppression; group II, pre-treatment of porcine neuroblasts with anti-MHC-I Fab; group III, daily subcutaneous injections of cyclosporine A (CsA; 10 mg/kg/d); group IV, Fab pretreatment plus CsA administration. Rats were sacrificed after 3 - 4 months, and xenograft survival and integration were assessed by species-specific immunostaining of donor neuronal and glial elements. Xenograft survival rates in the 90 surviving rats were 1/14 in group I, 15/29 in group II, 23/31 in group III, and 13/16 in group IV. MHC-I masking with Fab produced a significant increase in xenograft survival compared to the non-immunosuppressed controls ($P < 0.005$, $\chi^2 = 9.39$, df =1). The difference in graft survival between groups II, III, and IV was not statistically significant, but grafts in group II were smaller and relatively depleted in neuronal elements compared to groups III and IV. Grafts undergoing rejection were infiltrated by host immune cells and were intensely immunoreactive for donor MHC-I. In addition to confirming the importance of MHC-I-restricted immune mechanisms in neural xenograft rejection, these results encourage future application of molecular-level immunoprotection strategies to neural transplantation.

696.17

INDUCED RAPID PATH FORMATION OF FETAL DOPAMINE NEURONS *GRAFTED IN NIGRA AND REINNERVATION OF STRIATUM* WITH FUNCTIONAL IMPROVEMENT. F. C. Zhou*, and Y. H.Chiang, Dept. Anatomy, Indiana Univ. Sch. Med., Indianapolis, IN 46202

Excitatory neurotoxin ibotenic acid (IB) or kainic acid (KA) injection in the brain in one way causes death of excitable neuron, but they also induce a neurotrophic environment for neuronal growth. The lesion effects when confined in a tract, provides a trophic throughway for the axonal outgrowth of transplanted neurons and reinnervation of 5-7 mm distal target in 3 weeks.

Sprague-Dawley rats were unilaterally injected with 6-hydroxydopamine (6-OHDA, 12 ul, 5ug/ul) in medial forebrain bundle. 1-2 weeks later, KA (1ug/ul) or IB (10-20ug/ul), or phosphate buffer (PB) were microinjected through a glass-pipet to make a 5-7mm long tract between nigra and striatum, followed by graft of E-14 brainstem cells into nigra. Animals were either perfused and stained with tyrosine hydroxylase immunocytochemically (TH-im) 3-4 weeks later, or injected with HRP in the striatum and their brains were stained 24 hours later in the graft regions.

The 6-OHDA degenerated DA neurons in nigra and completely denervated the striatum. (n=13). Unilateral turning was evident after amphetamine challenge. Traceable TH fibers from grafts grew distinctly in a bundle along the entire length of KA/IB tract (n=5), but not into the PB tract (n=5). The TH-im fibers did not leave the KA tract when coursing through the globus pallidus, but quickly spread out when reaching the striatum. Reinnervating TH-im fibers were distributed in patchs in the striatum near pallidum but non-discriminately in rostral striatum. HRP tracing reveals labeled neurons in the graft (n=3). Behavioral studies show that amphetamine challenged unilateral turning was reduced by about 40%-50% after the bridging method of transplant.

696.18

PROTEOLYTIC ENZYMES INVOLVED IN GLIAL CELL MIGRATION *FOLLOWING TRANSPLANT OF FETAL RABBIT BRAIN TISSUE INTO MOUSE BRAIN.* M.R. Del Bigio*, J.-L. Tchelingerian, C.M. Jacque INSERM U134, Hopital de la Salpetriere, Paris 75651 France

Following grafting of fetal (E25) rabbit brain fragments into the striatum of shiverer mouse (a mutant lacking MBP) brain, glial cells migrate predominantly along white matter tracts. Differentiated astrocytes can be identified by the antibody TpGFAP1 which recognizes rabbit but not mouse GFAP. Transplanted oligodendrocytes produce myelin which contains MBP. Immunoelectron microscopy shows that transplanted astrocytes are well integrated into host brain in both perivascular and perineuronal positions. To study the role of proteolytic enzymes in the migration of transplanted glial cells 3 days to 4 weeks after transplantation we used immunohistochemistry and non-radioactive ISH. Transplanted astroglial cells are often immunoreactive for tissue plasminogen activator (tPA). Some immature transplant cells at the graft nidus show urokinase PA-like immunoreactivity. In sham-grafted controls, reactive astroglia exhibit tPA and inflammatory cells show UPA. Immature transplanted cells express the mRNA for matrix metalloproteinases (MMP) type 1 and type 3 for up to 4 weeks. Transient expression of MMP3 is occasionally observed at a distance from the graft in sites associated with cell migration. We conclude that some proteolytic enzymes may be associated with the migration of transplanted glial cells. However, they are not unambiguous identifiers of migrating glial cells.

AGING PROCESS

697.1

CHRONIC ADMINISTRATION OF GM1 GANGLIOSIDE ENHANCES CHOLINERGIC PARAMETERS IN THE AGED BRAIN. M. Hadjiconstantinou*, T.G. Fong and N.H. Neff. Depts. of Psychiatry, Pharmacology and The Neuroscience Program. The Ohio State University College of Medicine. Columbus, Ohio 43210

Although the mechanism(s) of action of GM1 ganglioside is unresolved, it has been postulated that it might enhance the action of endogenous trophic factors by augmenting their synthesis and/or release, and/or by altering trophic factor receptor characteristics. We now provide evidence that GM1 has a synergistic action with NGF in the brain of aged animals. Chronic intracerebroventricular administration of GM1 increases choline (Ch) uptake and choline acetyltransferase (ChAT) activity in the striatum and hippocampus of old rats. When a low ineffective dose of GM1 (0.5 mg/day for 4 wks) is co-administered with various doses of NGF (0.2, 0.5 and 1 μg/day for 4 wks) there is enhancement of the response to NGF on Ch uptake and ChAT activity. Our results are consistent with apparent potentiation by GM1 of the effect of NGF on cholinergic parameters in aged animals.

697.2

Cholesterol Protects Against $NO^{\cdot}$- or $OH^{\cdot}$-Induced Oxidative Stress in Perifused Striatal Slices Irrespective of Age. J.A. Joseph*, R. Villalobos, J. Strain and N. Jimanez. ARS-USDA Human Nutrition Ctr. on Aging, Boston, MA 02111.

Previously, cholesterol (Cho) decreased oxotremorine (oxo) enhancement of K^+-evoked dopamine release (K^+-ERDA), suggesting that Cho, which accumulates in membranes during aging alters signal transduction. However, present findings suggest that such accumulation may actually serve to protect neuronal tissue from oxidative damage. Striatal slices (6, 24 mo rats) were incubated in 1 mM cholesterol (30 min) followed by H_2O_2 (7.5 μM, 30 min). Perifusions with 30 mM KCl with, and without 500 μM oxo, indicated no H_2O_2 reductions in K^+-ERDA in Cho-incubated tissue in either age group (e.g., no oxo, young K^+-ERDA Δ from HiKCl alone H_2O_2 = -69.48 $\pm$ 10 Cho+H_2O_2 = 60.34 $\pm$ 14.34 p moles/mg protein, t = 17.9 df = 8 $p < 0.0001$). Similar protection was observed under Oxo conditions as well. Cho also antagonized nitroprusside (NI 150 μM) decreases in K^+-ERDA (young Ni Δ = -79.30 $\pm$ 10.41, Ni + Cho 6.07 $\pm$ 22.96 p moles/mg protein, t = 7.57 $p < 0.001$). Interestingly, Cho comparisons with other antioxidants (e.g., α-tocopherol) on $OH^{\cdot}$ or $NO^{\cdot}$ ductions indicated comparable protection.

697.3

LONG-TERM TREATMENT WITH A MONOAMINE OXIDASE-B INHIBITOR SELEGILINE OF AGEING RATS: EFFECTS ON AMINO ACID AND MONOAMINE NEUROTRANSMITTERS. P. Saransaari*, J. Suhonen, S.M. Lillrank, A. Hervonen and S.S. Oja. Tampere Brain Res. Ctr., Univ. of Tampere, FIN-33101 Tampere, Finland.

Long-term treatment with the monoamine oxidase type B inhibitor L-deprenyl (selegiline) prolongs both mean and maximum survival and improves cognitive functions in aged rats. The effects of this drug on amino acid and monoamine neurotransmitters in different brain regions (cerebral cortex, cerebellum, hippocampus, striatum) were investigated in male Wistar rats treated with selegiline (0.5 mg/kg/d in drinking water) from the third postnatal week up to 10 months or from 12 months to 24 months of age. There were no significant differences between selegiline-treated and control rats in the basal and K^+-stimulated (50 mM) release of labeled preloaded dopamine, noradrenaline and GABA from brain slices. Ageing did not either affect the release parameters. The binding constant K_D of $GABA_A$ sites increased with age in the cerebral cortex and cerebellum but the maximal binding capacity B_{max} for GABA remained about the same. In the selegiline group K_D decreased in the cortex and striatum but increased in the cerebellum during ageing, while B_{max} was affected less. Selegiline treatment thus alters both affinity and number of binding sites. The binding parameters of $GABA_B$ sites remained unaltered during ageing and selegiline treatment until 17 months of age. There were no marked changes in the properties of dizocilpine (MK-801) binding to the cortical NMDA receptor complex. The results show that long-term selegiline treatment affects the receptor binding but not the release of the above transmitters in the brain regions studied.

697.4

METAL-CATALYZED OXIDATION OF BOVINE NEUROFILAMENTS (NF) IN VITRO. JC Troncoso*[1-3], JH Kim[3], AC Costello[3], and GVW Johnson[4]. Departments of [1]Pathology and [2]Neurology, [3]Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD; [4]University of Alabama, Birmingham, AL.

NF are important determinants of the shape and size of nerve cells. The oxidation of NF, potentially relevant to aging, neurodegenerative disorders, and Wallerian degeneration, has not been studied. In this investigation, we combined biochemical and ultrastructural methods to study the metal-catalyzed oxidation (MCO) of bovine NF using an ascorbate/Fe^{+3}/O_2 system. The oxidation of NF proteins was documented by significant increases in carbonyl content, which were time and concentration dependent. Polyacrylamide gel electrophoresis and immunoblot analyses revealed fragmentation of oxidized NF proteins, predominantly NF-H and NF-M. Electron microscopy showed that oxidized NF formed dense aggregates and bundles of laterally aggregated filaments. Finally, we also demonstrated that oxidized NF proteins were more susceptible to calpain proteolysis. In view of the growing evidence to support increased oxidative stress of the nervous system in aging and the report of the Cu/Zn superoxide dismutase mutation in familial motor neuron disease, the oxidative injury of NF may be of relevance to the atrophy and degeneration of nerve cells and the formation of abnormal cytoskeletal structures in neurodegenerative disorders.

697.5

CYSTEAMINE-INDUCED ASTROCYTE CYTOPROTECTION: ROLE OF INTRACELLULAR ANTIOXIDANT DEFENSES. F. Manganaro* and H.M. Schipper. Dept. of Neurology and Neurosurgery, McGill Univ., Bloomfield Centre for Research in Aging, Jewish General Hospital, Montreal, QC, Canada, H3T 1E2

Background: The aminothiol compound, cysteamine (CSH), induces gliosis *in situ* and the appearance of peroxidase-positive cytoplasmic granules in cultured astroglia identical to glial inclusions which progressively accumulate in the aging periventricular brain. In addition CSH-pretreatment protects cultured astroglial from subsequent H_2O_2 and mechanoenzymatic stress. **Objective:** To determine whether changes in antioxidant defenses mediate cytoprotection in CSH-treated astroglia. **Methods and Results:** CSH treatment (880 µM) induced a 6 fold increase in glutathione (GSH) concentrations and a persistent augmentation of manganese superoxide dismutase (MnSOD) activity in cultured astroglia. In contrast, astroglial catalase and glutathione reductase activities and whole cell lipid peroxidation were suppressed by CSH treatment while copper-zinc superoxide dismutase activity remained unchanged. Glutathione peroxidase activity was increased after 3 and 48 hrs of CSH treatment but declined thereafter to below control levels. **Conclusions:** Elevations in intracellular GSH concentrations and sustained augmentation of MnSOD activity may be responsible for astrocyte cytoprotection resulting from CSH exposure. CSH-treated astrocytes may serve as a useful model for stress-related (dys)regulation of MnSOD and other antioxidant defenses which have been documented in the aging and degenerating nervous system. In the latter, astrocyte cytoprotection may facilitate the establishment of reactive gliosis in the face of concomitant neuronal depletion.

697.6

AGE-RELATED IMPAIRMENT OF THE CARDIAC SYMPATHETIC AND VAGAL COMPONENTS OF THE BAROREFLEX ARC IN FISCHER 344 RATS. Eugene W. Shek*, Julianna E. Szilagyi. Department of Pharmacological & Pharmaceutical Sciences, University of Houston, Houston, Texas 77204-5515.

Aging is associated with progressive deterioration of baroreflex sensitivity (BRS). The present study was undertaken to examine whether the impaired BRS observed in aging was due to the change in the relative contribution of the cardiac sympathetic (CS) and cardiac vagal (CV) components of the baroreflex arc (BRA) in 6, 12 and 18-mo-old male Fischer 344 rats. Rats were anesthetized with methoxyflurane and α-chloralose. BRS was determined by calculating the slope of a regression line relating changes of heart period to mean blood pressure in response to randomized doses of phenylephrine (PE). The CS and CV components of the BRA were determined after vagal (2mg/Kg atropine) and sympathetic(2mg/Kg propranolol) blockade, respectively. There was no significant change in basal blood pressure with age. The basal and intrinsic heart rate (HR) of 12 & 18-mo-old rats were significantly lower than 6-mo-old rats. In addition, the 18-mo-old rats had an augmented CS heart rate compared with the younger rats. The CV heart rate appeared to be unaltered with age. The BRS of 18-mo-old rats was significantly attenuated compared with 6 & 12-mo-old rats(1.1 ± 0.1 vs 1.8 ± 0.1 & 1.7 ± 0.1 msec/mmHg, respectively; $p<0.0005$). There was a significant increase in the CS component of BRA with age (12%, 13% & 46%, respectively;$p<0.001$) with only a gradual decline in the CV components (61%, 55% & 49%, respectively). The vascular responsiveness to PE was significantly decreased in 18-mo-old rats compared with 6 & 12-mo-old rats. Thus, these results demonstrated that 1) the reduced basal HR in aged rats was primarily due to the reduced intrinsic HR, 2) the impaired BRS with age was due to the augmented CS contribution of the BRA in respones to an increase in blood pressure, 3) baroreflex function was changed from being primarily a CV mediated response in younger rats to a response equally mediated by CV and CS in the aged rats, and 4) the reduced vascular responsiveness to α-adrenoceptor agonist(PE) suggested a possible change in vascular α-adrenoceptor number, affinity or second messenger system in aged rats. (Supported by: AG09542)

697.7

EFFECTS OF AGE ON THE DISTRIBUTION OF VISUOSPATIAL ATTENTION. S. Panicker*, P.M. Greenwood & R. Parasuraman, Cognitive Science Lab., The Catholic University of America, Washington, D.C. 20064

Assessment of the component operations and brain systems mediating shifts of visuospatial attention, both in normal aging and in dementia (Panicker et al., Soc. Neurosci. Abst., 1993), requires further analysis of the processing of non-informative or neutral spatial cues. To determine age effects in the distribution of visuospatial attention following informative and neutral spatial location cues, young and old Ss performed a cued discrimination task in which choice RT was measured. Cues were informative (60% valid, 20% invalid) or neutral (20%) and preceded discrimination targets ("+" or "x") by 100, 200 or 500 msec. The distance between invalid cues and targets was systematically varied. There were two types of neutral cues: a small diamond in the center of the screen; a large rectangle outlining the periphery of the screen. Old and young produced similarly steep slopes relating RT to invalid cue-target distance and similarly shallow slopes relating RT to neutral cue-target distance for both types of neutral cues. These results indicate: (a) age has no effect on the shifting of attention over a large range of distances; (b) while a narrow focus of attention is induced by informative peripheral cues, a broad and fairly even distribution of attention is induced by both discrete and diffuse neutral cues. This suggests attention is diffusely distributed following a neutral cue, irrespective of the cue's physical attributes.

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697.8

AGE-RELATED DIFFERENCES IN DOPAMINE MEDIATED BEHAVIORS AND FOS IMMUNOREACTIVITY IN FISCHER 344 RATS. C.A. Crawford* and M.S. Levine. Mental Retardation Research Center, University of California, Los Angeles, CA 90024.

Decreases in dopaminergic function are known to occur in the nigrostriatal and mesolimbic systems during aging; however, the neurobiological substrates that underlie these changes have not been determined. Therefore, in the present study, the behavioral and neuronal response to amphetamine were assessed in aged (24 mos) and young (2 mos) Fischer 344 rats. Rats were first habituated for 30 min in a test chamber and then injected with amphetamine (5 mg/kg, i.p.). They were then placed in the testing chamber for 90 min and behavioral data were recorded for a 2 min period at 10 min intervals. Behavioral stereotypies induced by amphetamine were rated on an intensity scale. Low-intensity stereotypes included rearing and line crossing while high-intensity stereotypes included licking and chewing. Following behavioral testing, rats were sacrificed and tissue was processed for Fos immunoreactivity using standard techniques. Behavioral results showed that aged rats had significantly fewer low-intensity stereotypies than young rats. In contrast, the number of high-intensity stereotypies were significantly enhanced in the aged group. Aged rats had fewer Fos-positive nuclei in the lateral neostriatum and nucleus accumbens than young rats. The number of Fos-positive nuclei in the medial neostriatum, piriform cortex and olfactory tubercles did not vary consistently according to age. Supported by AG 10252.

697.9

PARANODAL ACCUMULATION OF LIPOPIGMENTS IN AGING MOTOR AND SENSORY AXONS. K.P. Gatzinsky*, R.H.M. King and P.K. Thomas. Dept. of Neurosci., Royal Free Hospital Sch. of Med., London NW3 2PF, UK.

The aim of this study was to explore if paranodal axon-Schwann cell networks (ASNs), which are entities assumed to take part in removal and degradation of worn-out organelles in large myelinated PNS axons, can serve as depots for non-degradable materials with increasing age. We addressed this issue by examining the occurrence of autofluorescent lipopigments in large myelinated motor and sensory axons of rats aged 2-25 months. After fixation with 4% paraformaldehyde, 10 μm thick longitudinal sections were cut from the lumbar ventral (VR) and dorsal (DR) roots and examined with a Zeiss fluorescence microscope equipped with phase-contrast optics and a FITC filter set. From each root, 200 randomly selected node-paranode regions in nerve fibres 8-12 μm in diameter were searched for autofluorescent granules. Orange-yellow granules situated in close apposition to the paranodal myelin sheaths started to appear in both VR and DR axons from 3-4 months of age. Both the percentage of paranodal regions of this type and the number of granules increased with age. Up to 1 year of age, VR axons showed the highest granular contents. From this age, >90% of the paranodes in both the VR and the DR showed autofluorescent granules. Some paranodes in the oldest animals contained >15 granules, which generally appeared in clumps and often were of larger sizes in VR than DR axons. In both axon types, the vast majority of the granules were situated distal to the nodal mid-level. EM analysis showed that most granules were lipofuscin bodies associated with ASNs. The progressive age-related accumulation of indigestible materials within ASNs may serve a function to shield the aging perikarya mainly of motor neurons from being crammed with retrogradely transported worn-out organelles from the axon.

697.10

REGRESSIVE CHANGES IN STEROID ACCUMULATION IN AGING ANDROGEN-SENSITIVE RAT SPINAL NUCLEI. M.R. Widows, A.K. Finn and D.R. Sengelaub*. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Motoneurons in the rat spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens throughout life. Androgen titers decline with normal aging in male rats, and we have previously reported concomitant regressive changes in SNB dendritic length, soma size, and target muscle weight. Using steroid autoradiography, we determined whether regressive changes in the ability of these motoneurons to accumulate steroids might also occur in aging.

Male rats (12, 19 and 25 months old; n=4 per age) were castrated 48 hours prior to injection with tritiated testosterone (s.c.; 1.5 μCi/g body weight) and killed 1.5 hours later. Accumulation was assessed in the steroid sensitive, sexually dimorphic SNB and dorsolateral nucleus (DLN), as well as in the non-sexually dimorphic retrodorsolateral nucleus (RDLN) from each rat (at least 50 cells per nucleus). The number of SNB and DLN motoneurons reaching criteria (Poisson) was reduced by as much as 21% at 25 months, while no changes were observed in RDLN. The density of labeling also declined with age in all motor nuclei and was particularly pronounced in the SNB. At 12 months of age, 55% of SNB motoneurons were labeled at more than 8 times background levels, but less than 30% were labeled at this density by 25 months. Thus, declines in androgen accumulation by steroid-sensitive motoneurons reflect age-related declines in circulating androgen titers. Regressive changes in motoneuron morphology could result from a reduced ability of androgen to act directly on these motoneurons. (Supported by NIH AG09309)

697.11

EFFECT OF A CONDITIONING LESION ON AXONAL OUTGROWTH RATES IN MATURE AND AGED F344 RATS. Zhong Huang* and Jane M. Jacob. Dept. of Anatomical Science, Univ. of Oklahoma HSC, Oklahoma City, OK 73190.

The conditioning lesion paradigm in which a first, or conditioning, lesion is followed by a second, or testing, lesion, has been shown to accelerate axonal outgrowth rates and to require the cell body reaction in young rats (Jacob and McQuarrie, 1993). While it is known that regeneration rates are slowed in the old animal, it is not known how a conditioning lesion affects axonal outgrowth rates in these animals. In this project, we used the conditioning lesion paradigm to determine if axonal outgrowth rates can be accelerated in old animals in a manner analogous to young animals.

Outgrowth rates were measured in 6 mo and 24 mo male F344 rats 15-19 d after a 20 sec crush lesion (conditioning lesion, CL) of the sciatic nerve at the level of the knee. Tritiated proline was microinjected into the corresponding ventral horn 4 or 8 d after the testing lesion (14 or 18 d after the CL). Rats were killed 1 d later. Using linear regression analysis, outgrowth rates were determined in control and experimental animals. These data suggest 24 mo F344 rats respond less robustly to a conditioning lesion than do 6 mo rats.

Supported by a grant to JMJ from the Presbyterian Health Foundation.

697.12

AGE DIFFERENCES IN MEMBRANE FLUIDITY AND LOW Km GTPase ACTIVITY DUE TO CHOLESTEROL INCORPORATION IN RAT BRAIN. R.Villalobos-Molina* N.D.Jimenez and J.A.Joseph. USDA ARS Human Nutrition Research Center on Aging at Tufts University, 711 Washington St.,Boston, MA, 02111.

Alteration in second messenger generation during aging, after receptor occupancy, has been documented in recent years. Decrements in the production of IP_3 (and cytosolic Ca^{++} mobilization), in cAMP levels and in low Km GTPase activity, have been reported. The suggested mechanism is an alteration in the coupling/uncoupling between the receptor and the G protein in the signal transduction pathway. In order to address if membrane composition alters this mechanism, brain slices or membranes (cortex, hippocampus and striatum), from 6 or 22 mo male Fischer rats, were incubated with cholesterol (water soluble), at 37 C for 60 min. Fluidity (DPH) and carbachol-stimulated GTPase activity were measured after treatment. The results showed that although graded concentrations of cholesterol (up to 1 mM), reduced the fluidity in: cortex [F(3,36) = 5.12, p 0.005],striatum [F93,30) = 3.14, p 0.04], hippocampus [F(3,30) = 3.99 p 0.02], from both young and old tissues to the same extent, the carbachol-stimulated GTPase activity lowered more in the young compared to old striata. The data suggest that cholesterol can alter the coupling/uncoupling between receptor and G protein by modifying the membrane composition.

697.13

LACK OF CORRELATION BETWEEN NGF LEVELS AND ALTERED NERVE FIBRE DENSITY IN PERIPHERAL TISSUES OF AGEING RATS. I.Gavazzi, T.Cowen* and K. A. Crutcher. Dept. of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London, UK and Dept. of Neurosurgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267-0515.

Studies have shown that target tissues undergo age-changes that apparently induce degenerative changes in their innervating sympathetic neurons. In contrast, ageing sympathetic neurons appear to retain a considerable degree of plasticity. In an attempt to identify the target-associated factors that might be responsible for this regulatory role, 2 -site ELISA assays for NGF were carried out on tissues from young (6wk) and old (24m) Sprague Dawley rats. Superior cervical ganglia (SCG) and tissues, some innervated by the SCG, with known and contrasting age changes in their innervation were assayed using established techniques. Cerebral arteries of the Circle of Willis, which exhibit a loss of about 50% in their overall innervation with age (including reductions in sympathetic and sensory nerve fibres), showed no significant alteration in NGF levels expressed as pg/mg wet weight of tissue. The sympathetic innervation of the iris increases slightly with age, whilst the sensory innervation decreases. However, NGF levels increased significantly ($p<0.01$) in the aged iris. The non-innervated common carotid artery showed levels of NGF comparable to those in the Circle of Willis, and exhibited no changes with age. In the old SCG, NGF levels showed a small but significant ($p<0.05$) decrease when NGF levels per ganglion (the preferred method of expression because neuronal numbers are unaltered in the ageing rat SCG) were compared. These results suggest that absolute NGF levels in ageing target tissues may not dictate the density of innervation by sympathetic and NGF-sensitive sensory nerves. Changes in the SCG suggest that neuronal uptake and/or transport of NGF may be impaired with age. Supported by the British Heart Foundation (TC and IG) and the NIH (NS17131, KAC).

697.14

DORSAL ROOT FIBERS IN THE AGED CAT. C. Bertolotto*, J. Yamuy, F. R. Morales, and M. H. Chase. Department of Physiology, Department of Anatomy and Cell Biology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA. 90024-1746.

Nerve impulse conduction in primary afferent fibers is greatly impaired in aged cats as indicated by a decrease in action potential conduction velocity (Exp. Neurol., 100: 583-595, 1988). The present study was designed to examine morphological changes that may underlie this functional disturbance. For this purpose, dorsal roots were studied using light and electron microscopic techniques in two aged cats (17 and 19 years old) and a control cat (2 years old). Many of the dorsal root fibers exhibited clear pathological abnormalities. These included disruption of the myelin sheaths characterized by splitting and ballooning of myelin lamellae which created spaces containing either loops and segments of damaged myelin or a granular matrix. There were abnormal spaces intercalated between myelin and axon cylinders. Retraction of the lateral myelin loops from the paranodal axolemma at the nodes of Ranvier were observed. In addition, axons showed degenerative changes. The fibers which did not exhibit these changes seemed, nevertheless, to also be affected. For example, in spite of the fact that in many fibers the axon appeared to be structurally intact, the myelin was markedly thin. The endoneural space was increased and there was macrophage infiltration; macrophages were filled with myelin fragments and debris. In conclusion, afferent fibers in the aged cat exhibit demyelination and axonal degeneration or an abnormally thin myelin sheath. These morphological changes may be the basis for the marked decrease in afferent fiber conduction velocity. Supported by USPHS Grant AG 04307.

697.15

LOSS OF MOTONEURONS INNERVATING NORMAL AND HYPERTROPHIED MEDIAL GASTROCNEMIUS MUSCLE IN AGED RATS. K. Kanda, K. Hashizume and K. Iwata*. Dept. of Central Nervous System, Tokyo Metropol. Inst. of Gerontol., Tokyo 173, Japan.

Our previous experiments showed that long-term swimming exercise retarded loss of motoneurons in aged rats. To clarify the role of an augmented activity of neuromuscular system itself, we investigated the effect of removal of synergistic muscles, which may cause an increase in activity of remaining muscle with minimal systemic changes, on motoneuron survival in the age rat. Male Fischer 344 rats aged 17 months were anesthetized with pentobarbital sodium (45 mg/kg, i.p.), and the lateral gastrocnemius, soleus and plantaris muscles were removed unilaterally. Unoperated, intact side was served as control. The rats were raised under usual laboratory conditions until the final experiment was performed at the age of 24 or 28 months. Horseradish peroxidase (40%) was injected into the medial gastrocnemius (MG) nerve bilaterally under halothane anesthesia. Two days later, the animal was reanesthetized and perfused transcardially with cold fixative (1.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer, pH 7.4). Serial sections (40 μm in thickness) were cut horizontally from the spinal cord block including L4 to S1 segments, and processed with TMB method. Each labeled motoneuron was identified under a microscope, and counted. There was no sign of contracture or ankylosis in the operated leg, and the MG muscle in the operated side was consistently heavier than that in contralateral, intact side (29.3% for 24-month-old rats and 32.3% for 28-month-old rats). The mean number of labeled motoneurons decreased with advancing age: 126.5 ± 10.5 (n=4) and 116.8 ± 6.6 (n=12) for intact side of 24- and 28-month-old rats. The values for hypertrophied side of 24- and 28-month-old rats were 127.8 ± 7.0 and 115.4 ± 8.4. There was no difference in the numbers of labeled motoneurons between the intact and the operated sides for both age groups (Wilcoxon signed-rank test, p>0.6). The results suggest that an increased motoneuronal activity is not the major factor causing retardation of motoneuronal loss in the exercising rats.

697.16

ELEVATED ADRENOMEDULLARY TYROSINE HYDROXYLASE WITH AGE MAY NOT BE INTRINSIC TO THE GLAND: POSSIBLE ROLE FOR DEXAMETHASONE. N. Tümer* and J.S. LaRochelle. GRECC VA Medical Center and Deptartment of Pharmacology, University of Florida, Gainesville, Florida 32608-1197.

Aging is associated with elevated tyrosine hydroxylase(TH) expression in the rat adrenal medulla(AM). To determine whether this increased TH activity is intrinsic to the gland or dependent on endogenous factors, we assessed TH activity in AM explants from young(3 mo) and old(24 mo) F-344 rats. In addition we examined the ability of dexamethasone(Dex) to stimulate TH. We have previously shown that TH activity in noncultured AM tissues from old was 2-fold higher compared with young rats. Medullas were cultured in RPMI-1640 media supplemented with 5% fetal calf serum, 10% horse serum and 25 μg/ml gentamicin. TH activity at time zero in culture in old AM explants was also 2-fold higher compared with young (119±5 and 59±6 nmol/mgprotein/h, respectively; $p<0.001$) which was similar to our findings in noncultured tissues. After 12h in culture TH activity in explants was no longer different with age (26±2 young and 23±2 nmol/mgprotein/h, old; $p>0.2$). Following 2h with 10 μM Dex, TH activity was not significantly different in young AM explants (4488±435 control vs. 3877±546 nmol/hr/adrenal, Dex; $p>0.40$). In contrast in old AM explants, TH activity was significantly higher compared with aged matched controls (8046±322 control vs. 12057±1253 nmol/hr/adrenal, Dex; $p<0.02$). These data indicate that the increased TH activity in senescent rat AM may be dependent on endogenous factors, and one of those factors may be glucocorticoids. (Supported by VA Med Ctr and Am Heart Assn, Fla Affiliate.)

SYNAPTIC STRUCTURE AND FUNCTION IV

698.1

TWO LOCI ENCODE SNAP-25 IN ZEBRAFISH AND GOLDFISH. C. Risinger*, E. Salaneck, and D. Larhammar. Dept of Medical Genetics, Box 589, Uppsala University, S-751 23 Uppsala, Sweden.

SNAP-25 (synaptosome-associated protein of 25 kDa) is a presynaptic protein involved in vesicle docking and release (Söllner et. al., Nature 362, 1993, and Blasi et. al., Nature 365, 1993), as well as neurite extension (Osen-Sand et. al., Nature 364, 1993). We have previosly shown that SNAP-25 has remained highly conserved from mammals to Drosophila (Risinger et. al., JBC 268, 1993). We initially isolated cDNA clones for SNAP-25 from goldfish because we intended to study this protein in the model system provided by the regenerating nervous system of this species. Although SNAP-25 is evolutionarily conserved we discovered sequence variability within the goldfish. This was found to reflect a gene duplication early in bony fish evolution, leading to *SnapA* and *SnapB*, followed by the tetraploidization 15-20 Myr ago (Risinger and Larhammar, PNAS 90, 1993). However, the tetraploid status of the goldfish makes this animal less appropriate for molecular genetic studies. Instead, the closely related diploid zebrafish provides an excellent system for these purposes. Zebrafish cDNA clones have been isolated for both *SnapA* and *SnapB*. The predicted protein sequences are highly similar between zebrafish and goldfish, and are approximately 90% identical between zebrafish and mammals. Interestingly, the zebrafish clones reveal two alternative splicing variants of exon 5. The existence of these two variants have previously been shown for chicken (Bark, JMB 233, 1993) and humans (Bark and Wilson, Gene 139, 1994). The expression of *SnapA* and *SnapB* and the alternatively spliced exons will be studied in zebrafish.

698.2

THE MAMMALIAN SEC1P-HOMOLOGUE IS A CHROMAFFIN GRANULE-BINDING PROTEIN T. Schaefer, A. Hodel, C. Heuss, A. Matus*, and M. M. Burger. Friedrich Miescher-Institute, PO Box 2543, CH-4002 Basel, Switzerland

Membrane proteins of the synaptic vesicle and the presynaptic plasma membrane together with soluble proteins form a secretory fusion complex conserved from yeast to neurons (Söllner *et al.* (1993) *Nature* 362, 318-324). We have localized three of these membrane proteins in chromaffin cells, which secrete catecholamines stored in chromaffin granules. Syntaxin (1A and 1B) and SNAP-25 are found in a plasma membrane-enriched fraction, whereas VAMP/synaptobrevin is concentrated on the granules. Recombinant syntaxin 1A has been used in an affinity chromatography assay to isolate syntaxin receptor proteins of the chromaffin granules. Solubilized granule membranes contained a single protein with high affinity for syntaxin 1A, the mammalian homologue of Sec1p, mSec1 (Hodel et al. (1994) J.Biol. Chem. 269, 8623-8626). In yeast, this hydrophilic protein acts late in the secretory process. Genetic suppressor analyses predicted the interaction of Sec1p with Sso1p, a yeast homologue of syntaxin 1A, and with Sec4p, a homologue of rab3A (Aalto *et al.* (1993) *EMBO J.* 12, 4095- 4104). Although rab3A is present on chromaffin granules, we did not detect it bound to syntaxin 1A together with mSec1. Western blot analysis of subcellular fractions revealed three pools of mSec1 in chromaffin cells: on plasma membrane vesicles, in the cytosolic protein fraction, and on the chromaffin granule membranes. mSec1 can be detached from the latter by high salt-treatment. It binds back to its receptor upon addition to granules at physiological salt concentrations. The characterization of both the attachment of mSec1 to the granule and its binding to syntaxin 1A should help to elucidate the interaction of transmitter vesicles with the plasma membrane in docking and fusion.

698.3

UNIFORM QUANTAL RELEASE MAY BE REGULATED BY THE SYNAPSE-SPECIFIC CLATHRIN ASSEMBLY PROTEIN F1-20/AP-3. W. Ye and E.M. Lafer*. Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, and *Institute of Biotechnology / Program in Molecular Medicine and Dept. of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, 78245.

We reported the characterization and cloning of the synapse-specific phosphoprotein F1-20. We overexpressed F1-20, and reported that F1-20 is identical to the clathrin assembly protein AP-3. At the nerve terminal, clathrin mediated endocytosis is involved in the biogenesis and recycling of synaptic vesicles. Here we characterize the ability of bacterially expressed F1-20/AP-3 to bind and assemble clathrin cages. We find that bacterially expressed F1-20/AP-3 can bind and assemble clathrin as efficiently as preparations of F1-20/AP-3 from bovine brain. This establishes that the clathrin assembly activity found in F1-20/AP-3 preparations from brain extracts is indeed encoded by the cloned gene for F1-20/AP-3. It also demonstrates that post-translational modification is not required for activation of the clathrin binding or assembly function of F1-20/AP-3. Ultrastructural analyses of the clathrin cages assembled by bacterially expressed F1-20/AP-3 reveals a strikingly narrow size distribution. Because mature synaptic vesicles are derived from precursor clathrin coated vesicles, we hypothesize that an important function of the synapse-specific clathrin assembly protein F1-20/AP-3 is to limit the range of vesicle sizes, and therefore increase the uniformity of quantal release. We also expressed the 33 kD NH_2-terminus of F1-20/AP-3 in *E. coli*, and measured its ability to bind and assemble clathrin. It has been suggested that the 33 kD NH_2-terminus of F1-20/AP-3 constitutes a clathrin binding domain. We found that while the bacterially expressed 33 kD NH_2-terminus of F1-20/AP-3 binds to clathrin triskelia, it fails to bind to preassembled clathrin cages and is not sufficient for clathrin assembly.

698.4

CELLULAR AND SUBCELLULAR LOCALIZATION OF SYNTAXIN IMMUNOREACTIVITY IN THE RAT CORTEX AND STRIATUM. S.R. Sesack* and C. L. Snyder, Departments of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

Syntaxin is a membrane-bound protein that concentrates in axon terminals and may participate in synaptic vesicle docking prior to Ca^{2+}-mediated fusion events. We sought to characterize the cellular and subcellular distribution of immunoreactivity for syntaxin in the rat forebrain using both a polyclonal antibody directed against a bacterially expressed syntaxin protein (Bennett et al., *Science*, 257:255) and a monoclonal antibody against the identical membrane-derived protein, HPC-1 (Barnstable et al., *Dev. Brain Res.*, 20:286). Whether labeled by immunoperoxidase or immunogold methods, syntaxin and HPC-1 proteins were localized exclusively to axons and terminal varicosities in the rat dorsolateral striatum and frontal cortex. Immunoreactive terminals made exclusively asymmetric synapses, primarily on dendritic spines, while unlabeled terminals in the adjacent neuropil made either symmetric or asymmetric synapses on somatodendritic targets. Immunogold labeling for syntaxin or HPC-1 was localized primarily to non-synaptic regions of the plasma membrane. These results suggest that syntaxin is an integral membrane protein localized to a subpopulation of forebrain terminals that use excitatory amino acids as neurotransmitters. The apparent exclusion of syntaxin immunolabeling from the presynaptic active zone must be considered in light of potential limitations in antibody penetration or epitope recognition at junctional specializations. Conversely, the non-synaptic localization of syntaxin implies that its functional role may not be limited to synaptic vesicle docking. This work was supported by USPHS grant MH50314.

698.5

SPECIFICITY AND REGULATION OF PROTEIN INTERACTIONS MEDIATING SYNAPTIC VESICLE DOCKING. Jonathan Pevsner*, Shu-Chan Hsu, Janice E. Braun, Nicole Calakos, Anthony E. Ting and Richard H. Scheller, Dept. of Mol. & Cell. Physiol., HHMI, Stanford U. Med. Center, Stanford CA 94305.

Synaptic vesicle (SV) docking is thought to be mediated by the binding of two SV proteins, VAMP and synaptotagmin, to two plasma membrane proteins, syntaxin and SNAP-25. We have identified n-sec1, a soluble neural-specific 68 kDa protein, and demonstrated its high-affinity binding to syntaxin (K_D=5.7 nM). Recombinant n-sec1 binds syntaxin in a complex distinct from the docked SV complex, based upon immunoprecipitation and glycerol gradient centrifugation studies. N-sec1 inhibits VAMP and SNAP-25 binding to syntaxin immobilized on agarose beads. In the absence of n-sec1, VAMP binds the syntaxin 1 and 4 (but not 2 and 3) isoforms. This VAMP binding to syntaxin 1 (but not syntaxin 4) is potentiated ten-fold in the presence of SNAP-25, suggesting a mechanism for achieving both specificity and high affinity binding of VAMP-containing SVs to the appropriate syntaxin on target membranes. The proposed pathway of SV docking and fusion includes the displacement of synaptotagmin by the soluble protein αSNAP, and subsequent binding of NSF to syntaxin, SNAP-25 and VAMP in a 20S complex in vitro. We have reconstituted this pathway with recombinant proteins binding to immobilized syntaxin.

698.6

AUTOPHOSPHORYLATION OF POSTSYNAPTIC DENSITY-ASSOCIATED CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE. A. Dosemeci* and H. Jaffe. Laboratory of Neurobiology and Protein/Peptide Facility, NINDS, NIH, Bethesda, MD 20892.

The major postsynaptic density protein (mPSDp) is similar or identical to the α-subunit of calcium / calmodulin-dependent protein kinase II (α-CaM Kinase II). Previous studies indicated that when an endogenous phosphatase in the postsynaptic density (PSD) preparation is inhibited, the mPSDp can be autophosphorylated in the presence of calcium to a degree comparable to that observed for the cytosolic CaM Kinase II (Dosemeci and Reese, 1993, J. Neurochem. 61, 550-555). We now show that inhibiting the phosphatase also allows extensive calcium-independent autophosphorylation of mPSDp. In analogy to the cytosolic kinase, prior incubation in calcium, presumably to phosphorylate a distinct site, is necessary for calcium-independent autophosphorylation to occur. In order to identify sites of phosphorylation, peptides generated by endoproteinase Lys-C-digestion of mPSDp were separated by reverse phase HPLC. Comparison of phosphopeptide elution profiles of digests of mPSDp following autophosphorylation in the presence and absence of calcium, showed differences, indicating that certain distinct sites are involved. The major phosphopeptide peak, however, eluted at the same position in both cases. Sequence analysis of this peak revealed a threonine corresponding to Thr-253 of the α-CaM Kinase II as the site of phosphorylation of mPSDp either in the presence or in the absence of calcium. Thus, for the PSD-associated kinase, autophosphorylation of this site appears to be facilitated and is able to continue after the removal of calcium.

698.7

DIFFERENTIAL RESPONSES OF PROTEIN KINASE C SUBSTRATES (MARCKS, NEUROMODULIN, AND NEUROGRANIN) PHOSPHORYLATION TO CALMODULIN AND S100. F.-S. Sheu*, F.L. Huang and K.-P. Huang. NICHD, NIH, Bethesda, MD 20892.

Phosphorylation of three physiological substrates of protein kinase C (PKC), MARCKS, neuromodulin (Nm), and neurogranin (Ng), present in a mixture was analyzed to determine their relative efficacies as substrates of PKC α, β, and γ and relative sensitivities to inhibition by calmodulin (CaM) and S100. The rationale for these experiments was to mimic the *in vivo* conditions where these substrates and inhibitor proteins co-exist. In addition, since PKC and these substrates are all phospholipid-binding proteins, the presence of all these components and phospholipids in the same reaction mixture may create an environment to allow selectivity for each PKC isozyme. Based on the kinetics parameters (V_{max}/K_m) of the phosphorylation of each individual substrate, we estimated the order of efficacy as PKC substrate was MARCKS>Nm>Ng. The rates of PKC α-catalyzed phosphorylation of Nm and Ng in a mixture containing MARCKS were significantly reduced as compared to that phosphorylated individually by this isozyme, especially at a higher level of MARCKS. Although phosphorylation of MARCKS, Nm, and Ng individually by PKC are known to be inhibited by CaM, when present in a mixture, both CaM and S100 preferentially inhibited MARCKS phosphorylation over those of Nm and Ng. Protease-activated catalytic fragment of PKC (PKM) was used to determine the effect of Ca^{2+} on the CaM- and S100-mediated inhibition of PKC substrate phosphorylation. CaM and S100 inhibited the PKM-catalyzed phosphorylation of MARCKS only in the presence of Ca^{2+} while phosphorylation of Nm and Ng by PKM were inhibited more prominently by CaM in the absence than in the presence of Ca^{2+}; S100 was relatively ineffective in inhibiting the phosphorylation of these two substrates even in the presence of Ca^{2+}. The results presented here demonstrate that MARCKS is likely the most preferred substrate of PKC in the brain and its phosphorylation by PKC is most sensitive to inhibition by CaM and S100.

698.8

IDENTIFICATION OF PHOSPHOLIPID BINDING SITES IN SYNAPSIN I USING HYDROPHOBIC PHOTOAFFINITY LABELING. J.J. Cheetham, S. Hilfiker-Rothenfluh, A.J. Czernik, A.C. Nairn* and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Ave., New York, NY 10021

Synapsins Ia and Ib (synapsin I) are peripheral membrane phosphoproteins, associated with the cytoplasmic surface of small synaptic vesicles [Greengard *et al.* (1993) Science, 259, 780-785]. It is thought that synapsin I regulates neurotransmitter release by tethering synaptic vesicles to actin filaments and restricting the availability of vesicles for fusion with the presynaptic membrane. Synapsin I binds to both actin filaments and synaptic vesicles *in vitro*, and these interactions are regulated by phosphorylation of synapsin I by Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II). Synapsin I also binds with high affinity to liposomes containing anionic lipids and induces rapid and extensive liposome aggregation. To further characterize the interaction of synapsin I with membrane lipids, we have attempted to identify the sites in synapsin I that directly interact with phospholipids using a hydrophobic photoaffinity labeling approach. Purified bovine synapsin I was bound to liposomes containing 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine ([^{125}I]-TID). Upon photolysis, membrane-embedded segments of the protein were crosslinked to the [^{125}I]-TID. [^{125}I]-TID -labeled synapsin I was digested with CNBr and peptides were separated using reverse phase HPLC. Radiolabeled peptides were further purified by Tris/Tricine PAGE, transferred to Immobilon and subjected to gas-phase protein microsequencing. Several peptide sequences, each located within the central region of synapsin I (domain C) interact with the phospholipid bilayer. [Supported by USPHS grant MH 39327 to P.G., MRC of Canada Postdoctoral Fellowship to J.J.C. and David Rockefeller Fellowship to S.H.]

698.9

INTERACTION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II WITH MEMBRANES: STUDIES WITH PURIFIED SYNAPTIC VESICLES AND LIPOSOMES S. Hilfiker-Rothenfluh, J.J. Cheetham, A.A. Fienberg*, P. Greengard and A.J. Czernik. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Ave., New York, NY 10021

Purified small synaptic vesicles possess a form of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) which serves as a binding protein for synapsin I [Benfenati *et al.* (1992) Nature, 359, 417-420]. The interaction between synaptic vesicle-associated CaM kinase II and the synaptic vesicle membrane was studied using hydrophobic photoaffinity labeling. Purified synaptic vesicles were labeled with 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine ([^{125}I]-TID). Upon photolysis, synaptic vesicle-associated CaM kinase II was labeled, suggesting that part of the molecule is embedded in the lipid bilayer. To further characterize this interaction, we performed liposome binding studies to determine if purified soluble CaM kinase II could bind to liposomes, using a sedimentation assay. We found that soluble CaM kinase II bound to sucrose-loaded liposomes and that the binding was dependent on the presence in the liposome membrane of anionic lipids, such as phosphatidylserine. Binding of CaM kinase II to liposomes was also dependent on salt concentration, suggesting that electrostatic interactions may contribute to the interaction. Soluble CaM kinase II bound to liposomes was also labeled using [^{125}I]-TID, indicating that some portion of the protein may be embedded in the membrane. [Supported by USPHS grant MH 39327 to P.G., MRC of Canada Postdoctoral Fellowship to J.J.C. and David Rockefeller Fellowship to S.H.]

698.10

SPATIAL DETECTION OF AUTOPHOSPHORYLATION OF CA^{2+}/CaM-DEPENDENT PROTEIN KINASE II IN HIPPOCAMPAL SLICES. S.Kindler*, E.M. Schuman, C. P. Hung, and M.B. Kennedy . Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

Ca^{2+}/calmodulin-dependent protein kinase II (CaM KII) is highly concentrated in mammalian forebrain. About half is located throughout the neuronal cytoplasm; the rest is associated with the particulate fraction, including postsynaptic densities. It has been hypothesized that Ca^{2+} entering through NMDA receptors activated during induction of long term potentiation (LTP) may activate CaM KII. Activation of CaM KII leads to autophosphorylation of a threonine residue neighboring the CaM-binding domain. Even after a decrease of Ca^{2+} levels the autophosphorylated kinase remains in an active state until it is dephosphorylated. This process may enable CaMKII to remain active after a short Ca^{2+} transient for a time that is dependent on local phosphatase activities. Biochemical methods have failed to show strong activation of the kinase during induction of LTP. This finding may reflect the fact that transient rises in Ca^{2+} during induction are restricted to relatively small cell areas. To visualize local changes in CaMKII autophosphorylation we have used a monoclonal antibody that recognizes CaM KII only in its autophosphorylated form, and a polyclonal rabbit serum that reacts only with the non-phosphorylated form (Patton et al. (1993) Mol. Biol. Cell 4, 159). We double-labeled 50μm sections cut from acute hippocampal slices fixed after exposure to different treatments. Antigen distributions were visualized by LS confocal microscopy using two different fluorophores. Images were analyzed with the program MacPhase. When compared to control slices, neurons in slices incubated with EGTA show a dramatically reduced ratio of staining with anti-phosphokinase to staining with anti-dephosphokinase. We are presently examining changes in autophosphorylation produced by activation of specific receptors and by stimulation paradigms that induce LTP.

698.11

NITRIC OXIDE STIMULATES BINDING OF NAD TO GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (G3PD) IN POSTSYNAPTIC DENSITY FRACTIONS FROM PORCINE BRAIN. P. Siekevitz*[1], A.A. Rogalski-Wilk[2], R.S. Cohen[2] and K. Wu[3]. [1]Dept. Cell Biology, The Rockefeller Univ., New York, NY 10021; [2]Dept. Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612; [3]Dept. Neuroscience and Cell Biology, UMDNJ/RWJ Med. Sch., Piscataway, NJ 08854

Nitric oxide (NO), a messenger molecule produced in the brain from the metabolism of L-arginine to L-citrulline, appears to play a key role in a variety of synaptic processes, including long-term potentiation. Recently, both nitric oxide synthase (NOS) (Aoki et al., Brain Res. 620, 97-113, 1993) and G3PD (Rogalski and Cohen, J. Cell Biol. 111, 163a, 1990), enzymes that produce NO and ATP, respectively, were found to be intrinsic components of the postsynaptic density (PSD). NO has been shown to stimulate the apparent auto-ADP-ribosylation of G3PD in whole brain extract (Zhang and Snyder, PNAS USA 89, 9382-9385, 1989). However, the reaction has been found to be binding of the entire NAD molecule to the G3PD (McDonald and Moss, PNAS USA 90, 6238-6241, 1993). We proposed that one locale of this reaction is the PSD. Subcellular fractions of porcine cerebral cortex, including whole homogenate, synaptosomes, synaptic plasma membranes, crude synaptic vesicles and PSDs were incubated at 37°C for 1 hour with (adenylate-^{32}P)NAD in the presence of calmodulin and calcium with and without sodium nitroprusside as the source of NO. The mixtures were then subjected to SDS-PAGE and were analyzed autoradiographically. NO significantly enhanced binding of NAD to a 37 kDa protein in all the above subcellular fractions examined, with the PSD showing the greatest enhancement. Western blot analysis using affinity purified antibodies to human G3PD (Rogalski et al., J. Biol. Chem. 264, 6438-6446, 1989) identified this protein as G3PD. Because G3PD is an actin binding protein and actin is present in the PSD, we propose that NO may alter G3PD-actin associations, thereby leading to structural alterations in the PSDs. (Supported by NIH grants GM 36802 to A.A.R.-W. and HD 24553 to R.S.C.).

PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

699.1

A ROLE OF NITRIC OXIDE IN GANGLIONIC TRANSMISSION OF RAT SUPERIOR CERVICAL GANGLIA. H. Tang* and N. J. Dun. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614

Immunoreactivity to nitric oxide synthase (NOS-I) was localized to nerve fibers opposing the majority of rat superior cervical ganglion (SCG) neurons. NOS-I was either too low to be detected or absent in the postganglionic neurons. The hypothesis that nitric oxide (NO) may modulate synaptic transmission in SCG neurons was examined by evaluating the effects of agents that elevate tissue NO levels on synaptic responses and on depolarizations induced by pressure application of acetylcholine (ACh). Intracellular recordings were made from neurons in isolated rat SCG. Superfusion of L-arginine (10-300 μM) dose-dependently increased the amplitude of excitatory postsynaptic potentials (EPSPs) evoked by stimulation of cervical sympathetic trunk in approximately 50% of ganglion cells studied. The membrane depolarization induced by pressure application of ACh was also increased in some but not in all ganglion cells. D-arginine in comparable conc. caused no significant change of the EPSPs. Sodium nitroprusside (10-300 μM) reversibly increased the EPSPs in a concentration dependent manner. The present study shows that NOS-immunoreactivity is present in nerve fibers presynaptic to post-ganglionic neurons and in SIF-like cells and that NO when released from these neural elements may potentiate synaptic transmission. (Supported by NS18710 & NS24226).

699.2

SPERMINE AFFECTS NEUROTRANSMISSION IN SLICES OF STRIATUM AND HIPPOCAMPUS: EFFECT OF CALCIUM. [1]P.A. Ferchmin*; [2]DiScenna, P.G.;[1]E.M. Rivera;[1]Vesna A.Eterović and [2]T.J. Teyler. [1]*Center for Molecular and Behavioral Neuroscience, Department of Biochemistry, Univ. C. del Caribe, Bayamón, Puerto Rico 00960 and* [2]*Department of Neurobiology, College of Medicine, Northeastern Ohio Universities, Rootstown, OH 44272*

Spermine inhibited neurotransmission in slices from rat striatum and hippocampus. In the latter, spermine increased paired-pulse facilitation in stratum pyramidale and to a lesser extent in s. radiatum of the CA1 area. A similar pattern was observed in the presence of ω-conotoxin (ω-CTX), a blocker of voltage-sensitive Ca^{++} channels (VSCC). In addition, a decreased Ca^{++} concentration in the perfusing solution also increased paired-pulse facilitation. Spermine effect was larger at 1 mM Ca^{++} than at 2 mM and was almost nil at 3 mM Ca^{++}. Addition of APV, an NMDA antagonist, did not interfere with spermine effect on paired-pulse facilitation. These results support the hypothesis that spermine is an endogenous neuromodulator that decreases the release of neurotransmitters by blocking presynaptic VSCC and perhaps also by blocking VSCC on dendrites and soma of pyramidal cells. (Supported by NSF-EPSCoR and NIH-RCMI RR03035)

699.3

PRESYNAPTIC cGMP PRODUCES ACTIVITY-DEPENDENT LONG-LASTING POTENTIATION IN HIPPOCAMPAL CELL CULTURES. O. Arancio*, E.R. Kandel, and R.D. Hawkins. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

Previous results suggest that cGMP is involved in long-term potentiation (LTP) in the CA1 region of hippocampal slices, perhaps as the presynaptic effector of a retrograde messenger (Zhuo et al., 1994). Consistent with this idea, the membrane-permeable analog 8-Br-cGMP also produces activity-dependent long-lasting potentiation of evoked post-synaptic currents (EPSCs) in dissociated cultures of hippocampal neurons (Arancio et al., 1993). This potentiation is paralleled by a decrease in the coefficient of variation and an increase in the frequency but not the amplitude of spontaneous miniature EPSCs, suggesting that cGMP acts presynaptically. However, a postsynaptic increase in cGMP might indirectly produce presynaptic effects. To attempt to distinguish between these possibilities, we injected substances directly into the pre- or postsynaptic neuron. Injection of cGMP into the presynaptic cell paired with weak pre-synaptic activity produced a long-lasting increase in the amplitude of the EPSC. cGMP alone or either vehicle or cAMP paired with activity produced no potentiation. Injection of cGMP into the postsynaptic cell paired with presynaptic activity also did not produce potentiation. Similarly, post-synaptic cGMP did not cause any increase in the amplitude or the frequency of spontaneous miniature EPSCs. In preliminary experiments, injection of the membrane-permeable guanylyl cyclase inhibitor LY83583 into either cell blocked the induction of long-term potentiation by repeated tetanic stimulation. These results demonstrate that a presynaptic increase in cGMP produces activity-dependent long-lasting potentiation that may contribute to LTP.

699.4

DELAYED ENHANCEMENT OF SCHAFFER COLLATERAL-COMMISSURAL SYNAPTIC TRANSMISSION BY PROLINE. S.M. Cohen* and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Many, but not all, glutamate terminals express a high affinity, Na^+-dependent transporter for L-proline. Proline may play a role in synaptic transmission at these sites. This idea was tested by recording Schaffer collateral-commissural synaptic potentials extracellularly in area CA1 during superfusion of hippocampal slices with proline. Two concentrations of proline were used: a concentration typical of normal CSF (3 μM) and a concentration present in CSF of persons with hyperprolinemia type II (30 μM). Neither proline concentration altered synaptic transmission when testing was performed 15 min after its addition to the superfusion medium. However, both concentrations enhanced synaptic transmission when they were continuously present in the medium from the time of slice preparation, as indicated by a statistically significant upward shift in the plot of field EPSP slope against fiber volley amplitude. In another series of experiments, proline was added to control medium and the field EPSP was recorded for the next 4 h. In the presence of proline, EPSP slope began to increase in about 40 min and reached a plateau in 80-90 min. The plateau values of EPSP slope were 24 ± 9% and 35 ± 10% greater than baseline for 3 and 30 μM proline, respectively. In contrast, EPSP slope in control slices declined by 8 ± 10%.

These results suggest that concentrations of proline normally present in CSF enhance transmission at the Schaffer collateral-commissural synapse. The presynaptic proline transporter may serve to regulate this process. Finally, the childhood seizures associated with hyperprolinemia type II may result from excessive facilitation of glutamate transmission.

699.5

PROMINENT DEPRESSION OF DENDRITIC GLUTAMATE (GLU) EXCITABILITY BY COLLATERAL RELEASE OF NOREPINEPHRINE (NE): RECORDINGS FROM LOCUS COERULEUS (LC) NEURONS *IN VITRO*.. A. Ya. Ivanov and G. Aston-Jones.* Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102.

Early evidence *in vivo* indicated that spontaneous and postactivation activity of LC neurons was strongly influenced by collateral release of NE. However, more recent studies in brain slices do not support this view, finding instead that calcium-dependent potassium conductance may be the main mechanism which regulates such activity in LC cells. We examined the possibility of local collateral inhibitory control of LC activity with intra- or extra-cellular recordings from LC neurons in brain slices from rat . Glu (20 mM) was ejected by pressure from a micropipette situated in the rostromedial peri-LC dendritic zone . In all 12 LC neurons tested Glu ejection increased impulse activity; the location of the Glu pipette was critical for this response and small adjustments within in the dendritic zone resulted in no response. This indicates that such Glu-evoked activation was not due to diffusion of Glu to the LC soma area, but apparently reflected direct activation of extranuclear LC dendrites of the recorded cell. Glu-evoked responses were increased in amplitude after blockade of alpha2 receptors with yohimbine (1 μM). A similar increase in Glu-evoked response was obtained with superfusion of 20 mM Mg^{++} and 2 mM Co^{++}. Yohimbine increased the spontaneous activity of 6/12 neurons about 30%, but had no effect on the activity of the other 6 cells. Intracellular recordings revealed that dendritic application of Glu elicited a slow depolarization with impulse generation. After yohimbine, the Glu-evoked depolarization increased in duration resulting in a greater number of impulses generated than after Glu alone. In contrast, the afterhyperpolarization following impulses did not change in amplitude or duration after yohimbine. The slow IPSP evoked by electrical stimulation in the peri-LC area was consistently blocked by yohimbine. Together, these results indicate that NE released from LC neurons is a potent influence on responses of these neurons to excitatory dendritic inputs. Supported by PHS grant NS24698.

699.6

VASOPRESSIN (VP) AND VP 4-8 IN FENTOMOLAR CONCENTRATION AUGMENT EPSPs IN CA1 NEURONS OF THE VENTRAL HIPPOCAMPUS. I.J.A. Urban*,A. N. Chepkova[1], D. De Wied, A.H. Ontskul and G.M.J. Ramakers. Rudolf Magnus Institute, P.O. Box 80040, 3508 AT Utrecht, The Netherlands. 1 Institute of Brain Research, Per. Obukha 5, Moscow, Russia.

Effects of 10^{-10} M vasopressin (VP) and VP(4-8) on passive properties and synaptic excitability of neurons in the CA1 layer in slices from the ventral hippocampus were studied with sharp, KAc or KCl microelectrodes. The medium contained (in mM): NaCl (125); KCl (3.5) $CaCl_2$ (2.5); $MgSO_{4\ (1.3)}$; NaH_2P0_4 (1.2); $NaHCO_3$ (26) and D-glucose (10). Stimuli of 35.9 ± 18.6 μA (S.E.) were applied to the stratum radiatum to evoke EPSPs in CA1 neurons. Nearly 70% of the impaled neurons showed an 10-200% increase in the amplitude and/or the slope of the EPSPs. The EPSPs started to rise at the end of the 15 min VP application. In more than 50% of these neurons the EPSPs increase outlasted the washout of the peptide by more than 60 min. In the remaining cells, the EPSPs declined to the baseline within 60-90 min after the VP application. No treatment-related change was seen in either the resting membrane potential (RMP) and/or input resistance $R_{(IN)}$. However, the threshold for action potentials (APs) by depolarizing current injection (DCI) often decreased and the number of APs generated increased. Neither picrotoxin alone nor pcirotoxin in combination with an NMDA receptor antagonist prevented the effect of VP on EPSPs. Also, VP (4-8) (10^{-10} M) elicited a similar long-lasting increase the amplitude and/or slope of EPSPs without changing the RMP and/or $R_{(IN)}$ of the neurons. The threshold for firing APs was also frequently decreased by VP (4-8). Thus, both peptides appeared to facilitate the excitatory, presumably glutamatergic transmission in the CA1 layer of the ventral hippocampus for a period of time outlasting markedly the peptide application. The mechanism of this long-lasting peptide action may be complex, including changes in transmitter release, postsynaptic glutamate receptors and/or postsynaptic conductances.

699.7

PRESYNAPTIC DEPRESSION OF GABA-MEDIATED SYNAPTIC RESPONSES OF CA1 PYRAMIDAL CELLS BY METABOTROPIC GLUTAMATE RECEPTORS IN RAT HIPPOCAMPAL SLICES. A. Jouvenceau, P. Dutar* and J.M. Billard. INSERM U161, 75014 Paris, France.

Evidence indicate that the glutamatergic synaptic transmission is modulated by presynaptic metabotropic glutamate receptors ($mGlu_R$) but a participation of $mGlu_R$ in the control of GABAergic neurotransmission in the CNS remains poorly documented. To address this question, we studied the sensitivity of the GABA-mediated responses to the $mGlu_R$ agonists trans-ACPD (50-200 μM) or 1S-3R-ACPD (10-50 μM) using intracellular recordings from CA1 neurons in the rat hippocampal slices. Bath application of $mGlu_R$ agonists rapidly caused a membrane depolarisation, an increase in membrane resistance and a loss of the postburst afterhyperpolarization. Stimulation of the *str. radiatum* elicited an early excitatory postsynaptic potentials (EPSPs) followed by fast $GABA_A$ (fIPSP) and slow $GABA_B$ (sIPSP)-mediated inhibitory postsynaptic potentials. At low concentration, trans-ACPD (50-100 μM) or 1S-3R-ACPD (10 μM) reduced the EPSP by 49% and 42% respectively. The fIPSP was slightly affected (15% and 12% depression) while the sIPSP was consistently decreased (41% and 28%). Increasing the concentration of the $mGlu_R$ agonists to 200 μM and 50 μM respectively further depressed the EPSP (65% and 79%) and dramatically reduced both IPSPs (70% for the fIPSP and 79% for the sIPSP for both agonists). Monosynaptic IPSPs evoked in the presence of APV (30 μM) and CNQX (10 μM) were also depressed by the $mGlu_R$ agonists. In contrast, preliminary experiments suggest that the discharge frequency of spontaneous miniature IPSPs is rather increased. Besides, the postsynaptic hyperpolarization caused by the $GABA_B$ agonist baclofen was not affected in the presence of the $mGlu_R$ agonists.

These results suggest that besides the control the excitatory neurotransmission, $mGlu_R$ also modulate GABA release through the activation of receptors located on GABAergic terminals. This mechanism may contribute to the facilitatory effect of $mGlu_R$ in the induction of long term potentiation.

699.8

METABOTROPIC GLUTAMATE RECEPTORS ATTENUATE INSPIRATORY-MODULATED SYNAPTIC CURRENT IN PHRENIC MOTONEURONS. X-W. Dong*, D. Morin & J.L. Feldman. Systems Neurobiology Lab., Dept of Physiological Science, UCLA, Los Angeles, CA, 90024-1527

Metabotropic glutamate receptors (mGluRs) modulate synaptic transmission at many glutamatergic synapses. We examined the effects of activation of mGluRs on synaptic transmission of endogenous excitatory amino acid-mediated inspiratory drive to phrenic motoneurons (PMNs) in the isolated brainstem-spinal cord preparation of neonatal rat. PMN activity was recorded under whole cell patch-clamp conditions. Drugs were applied via pressure ejection from micropipettes positioned over the PMN pool. The mGluR agonist 1S,3R-ACPD (0.5 mM, 3 sec) reversibly reduced PMN peak inspiratory-modulated synaptic current (I_{insp}) to 76±3% (n=5) of control and increased excitability via a tonic inward current (see Morin *et al.*, this volume). This effect on I_{insp} was antagonized by the mGluR antagonist (R,S)-α-methyl-4-carboxyphenylglycine (MCPG). To elucidate underlying mechanisms, we examined records from cells with few enough events during inspiration that unitary peaks could be resolved. ACPD produced a prominent decrease in the number of unitary events; this finding suggests a decrease in transmitter release. We had observed a similar action of AP4 (Liu *et al.*, J Neurophysiol., 64:423, '90). MCPG antagonized the action of AP4, indicating that the action of AP4 is mediated by mGluRs. However, whether the effects of the agonists ACPD and AP4 are mediated by same or distinct receptors remains unclear. We conclude that presynaptic mGluRs modulate transmission of endogenous inspiratory drive to PMNs, consistent with our hypothesis of an autoregulatory site for glutamate release modulating inspiratory drive to PMNs. Supported by NIH Grant NS 24742.

699.9

PRESYNAPTIC INHIBITION OF INHIBITORY SYNAPTIC TRANSMISSION MEDIATED BY METABOTROPIC GLUTAMATE RECEPTORS IN MONO–SYNAPTICALLY ISOLATED PAIRS OF CULTURED HIPPOCAMPAL NEURONS. R. Maki*[1] and M. A. Dichter.[1,2] [1]David Mahoney Institute of Neurological Sciences, [2]Depts. of Neurology and Pharmacology, Univ. of Pennsylvania School of Medicine and Graduate Hospital, Philadelphia, PA 19104.

Whole-cell patch clamp recordings were performed to elucidate the effects of the metabotropic glutamate receptor (mGluR) agonist ACPD and the recently described mGluR antagonist (RS)-α-methyl-4-carboxyphenylglycine (MCPG) on inhibitory synaptic transmission in low density cultures of hippocampal neurons. Application of ACPD (100 μM) resulted in a reversible decrease in the amplitude of IPSCs (33.6±24.6% decrease; n=9). Coapplication of MCPG (500 μM) completely reversed the effects of ACPD (n=7); MCPG alone had no effect on IPSC amplitude. Examination of miniature IPSCs (n=5) indicated that changes in postsynaptic $GABA_A$ receptor sensitivity did not account for the decrease in evoked IPSC amplitudes; therefore, inhibition of the IPSCs was due to a presynaptic mechanism. Application of the glutamate uptake inhibitor L-*trans*-PDC (250 μM) led to a reduction in 8 out of 13 pairs tested; these 8 inhibitory pairs also exhibited an increase in baseline NMDA-receptor mediated noise, indicating an increase in the ambient concentration of glutamate. The reduction of IPSC amplitude by application of L-*trans*-PDC could also be blocked by the mGluR antagonist MCPG (1 mM). Based on these and other previously reported results (Maki, Robinson and Dichter, J Neurosci, in press) we concluded that (1) all inhibitory neurons in our very low density cultures express functional mGluRs responsive to ACPD, and (2) inhibition of glutamate uptake by L-*trans*-PDC results in the activation of mGluRs by increased ambient glutamate concentrations, and that this occurs only in the cases where there was a source for glutamate. Modulation of the activation of presynaptic auto- and heteroreceptors may serve to tightly regulate the balance of inhibitory and excitatory synaptic transmission in the CNS. Supported by GM-34781 (MAD) and AG-12003-01 (RM).

699.10

ACPD-INDUCED LONG-TERM DEPRESSION IN IMMATURE CA1 REQUIRES BOTH mGluR AND NMDAR ACTIVATION L. S. Overstreet, J. F. Pasternak, N. T. Slater, J. W. Cozzens, B L. Trommer* Pediatric Neurology Research Laboratory, Northwestern Univ. Medical School, Evanston Hospital, Evanston, IL 60201

Activation of the mGluR by 1S,3R-ACPD produces long-term potentiation (LTP) in mature hippocampal area CA1, and long-term depression (LTD) in immature CA1. The latter is blocked by the mGluR antagonist (+)MCPG (500μM). We studied the effects of NMDA receptor (NMDAR) blockade on ACPD-induced LTD. Field EPSPs were recorded in the stratum radiatum of CA1 in response to stimulation of the Schaffer collateral path in hippocampal slices from 9-12 day rats (n=13). In the presence of the NMDAR antagonist D-AP5 (20 μM), bath application of ACPD (10μM, 20 min) did not produce LTD, although a reversible depression (-18%) of EPSP slope was seen in 8 slices. After ACPD wash, EPSP slope remained near baseline (n=8) or showed LTP (+27%, n=5). With subsequent AP5 wash (20, 60, or 120 minutes following ACPD wash), a persistent decrease (-20%) in EPSP slope occurred in all slices. Subsequent ACPD application produced further decrease in EPSP slope (-25%); subsequent tetanus reversed LTD and/or produced LTP (+31%). Thus LTP, the dominant effect in mature CA1, is seen in immature CA1 only with NMDAR blockade; when both mGluR and NMDAR are active in immature CA1, LTD is produced. Moreover, the depression of synaptic activity in immature CA1 consists of 2 components; one that is reversible and depends only on mGluR activation and a second that is long-lasting and requires concomitant, but not necessarily coincident, NMDAR activation. mGluR activation alone creates a "depression primed" state that is subsequently expressed as LTD after return of the NMDAR-associated current produced by background synaptic activity. It is likely that this "priming" effect results from a long-lasting change in intracellular 2nd messenger system modulation induced by mGluR activation in developing brain/CA1.

699.11

DISTRIBUTION AND PHARMACOLOGICAL CHARACTERIZATION OF DOPAMINE RECEPTORS IN THE SUBTHALAMIC NUCLEUS OF THE RAT. A. E. Johnson*, H. Coirini, L. Källström and F. -A. Wiesel. Department of Psychiatry, Ulleråker Hospital, Uppsala University, S-75017 Uppsala, Sweden.

The display of neuroleptic-dependent movement disorders is thought to be due to a disruption in the balance of neurotransmission between the "direct" and "indirect" pathways connecting the striatum with the major output nuclei of the basal ganglia. Classical neuroleptics are thought to initiate this imbalance through the blockade of striatal dopamine (DA) D_2-receptors resulting in a cascade of neurochemical events throughout the basal ganglia and thalamo-cortical motor circuitry. However, classical neuroleptics may also interfere with DA transmission within a key intermediate structure of the indirect pathway, the subthalamic nucleus (STh). While DA receptors are located in the STh, their distribution and pharmacological identity is unclear. The purpose of the following experiments was to describe the distribution and to pharmacologically characterize the DA-receptors in the STh. For these experiments, brain sections were incubated with either D_1- or D_2-receptor selective ligands (^{125}I-SCH23982 or ^{125}I-NCQ298, respectively) in the presence or absence of a wide range of unlabelled dopaminergic compounds and binding was analyzed using receptor autoradiographic techniques. Results of distribution experiments showed that ^{125}I-NCQ298 exhibited moderately high binding in the STh and that the binding was uniform throughout the region. Contrary to previously published data, ^{125}I-SCH23982 binding was not observed in the STh. However, DA displaceable ^{125}I-SCH23982 binding was observed ventral to the STh in the internal capsule. Subsequent competition studies confirmed that STh DA receptors are of the D_2-subtype. These data suggest that D_2-receptor mediated DA neurotransmission may be an important modulator of STh neural activity and that the blockade of STh D_2-receptors may influence the display of neuroleptic-dependent movement disorders. Supported by grant #8318 from the Swedish MRC.

699.12

REGULATION OF [3H]RX821002 BINDING TO ALPHA-2 ADRENOCEPTORS BY G-PROTEIN IN RAT BRAIN J. Chung, G. Chen, H. K. Manji, J. Crawley*, W. Z. Potter, D. Pickar Experimental Therapeutics Branch, NIMH, NIH, MD 20892.

In recent years, it has become increasingly clear that for a number of neurotransmitter systems, receptors can exist in a precoupled to G-proteins state even in the absence of agonists. This has been demonstrated by the effects of antagonists or GTPase activity. We have sought to examine the coupling of alpha-2 adrenoceptors in rat brain by the use of the highly selective alpha-2 antagonist, [3H]RX821002. Saturation experiments showed that [3H]RX821002 binding to alpha-2 adrenoceptors is saturable, highly specific and best fitted to a one-site model by iterative Scatchard analysis. Non-specific binding defined by the addition of 0.1 mM epinephrine was less than 10 % of total binding. Interestingly, we found that the addition of 0.1 mM Gpp(NH)p increased both the affinity and the density of sites recognized by the antagonist [3H]RX821002 (paired T-test, $P < 0.01$ and $P < 0.05$, respectively). Similar effects on the density of alpha-2 sites were observed by pretreatment of rat membranes with pertussis toxin. Taken together, these results suggest that a percentage of alpha-2 adrenergic receptors exists in a precoupled state in rat cortex, and guanine neucleotides regulate antagonist affinity to these sites as well.

These findings of the coupled state of empty alpha-2 adrenoceptor in rat cortex have important implications for basic and clinical studies using alpha-2 antagonists.

699.13

PROPERTIES OF MIPSCS IN CULTURED RAT SPINAL CORD AND MEDULLARY NEURONS. C. A. Lewis* and D. S. Faber. Dept. of Anatomy and Neurobiology, Med. College of Penn., Philadelphia, PA 19129.

We previously concluded there are at least 3 populations of inhibitory receptors present on cultured rat embryonic neurons, those activated only by glycine or GABA or by either one (Neurosci. 52:83-96, 1993). Also, whole-cell current responses to bath-applied agonist have both desensitizing and nondesensitizing components, with the former being more sensitive to block by antagonists.

To determine which of these postsynaptic receptors are involved in inhibitory synaptic transmission, whole-cell patch recordings were obtained from neurons voltage clamped at -70 mV and perfused with NaCl Ringer's solution containing 300 nM TTX, 10 μM APV, and 8 μM CNQX. Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were digitized at 2kHz and analyzed using a peak detection program (Ankri, N., et. al. Neurosci. Meth., in press), which calculates the peak amplitude and an exponential decay time constant (τ) for each event.

Amplitude distributions were skewed, and frequently showed no discernible peaks. The effects of different antagonists on the amplitude distributions were complex and did not provide insights concerning the involved inhibitory receptors. On the other hand, the decay distributions were consistently best fitted by the sum of 4 Gaussians with τs of : $\tau_1 = 8.11 \pm 0.96$ (± S.D., n = 22), $\tau_2 = 13.6 \pm 2.8$ (n = 24), $\tau_3 = 25.9 \pm 2.3$ (n = 22) and $\tau_4 = 49.3 \pm 6.8$ (n = 25) ms. These 4 classes of decay times have different pharmacological sensitivities to antagonists. The results of experiments using different concentrations of strychnine and bicuculline lead to the following conclusions: τ_1 is mediated by the nondesensitizing GABAR, τ_2 by the nondesensitizing GlyR, and τ_3 and τ_4 by the desensitizing Gly/GABAR. However, since all four decay components are seen in cell-attached patches that exhibit mIPSCs, it is possible the responses are generated by one receptor with complex binding affinities and sensitivities to block by antagonists.

699.14

VOLTAGE DEPENDENCE OF THE EPSC AND DESENSITIZATION OF AMPA RECEPTORS BY TRANSMITTER. S. Zhang* & L. Trussell, Dept. of Neurophysiology, Univ. Wisconsin - Madison, 53706.

Chick VIIIth nerve fibers form somatic synapses in the nucleus magnocellularis and generate large EPSCs. A previous study of brain slices showed that the neurotransmitter, presumably glutamate, activates and then desensitizes AMPA receptors (Trussell et al., Neuron 10,1185). The kinetics of glutamate and kainate responses in these cells are voltage dependent (see abstract by Otis, Raman, & Trussell, this meeting). We examined changes in the time course of the EPSC with postsynaptic potential to see whether voltage sensitivity might reveal further information about the transmitter's lifetime and receptor desensitization. The decay of the AMPA receptor EPSC could be described by two exponentials, of 0.7-1 ms and 6-40 ms. With depolarization from -65 to +125 mV, both components slightly lengthened, but the relative magnitude of the slow component increased 5-fold, resulting a marked overall slowing of the EPSC. Such a large slow component could not be explained by a long channel burst duration or desensitization time course. The increase with depolarization in the magnitude of the slow phase is consistent with an increased receptor affinity. We propose that there is a prolonged phase of transmitter removal from the synaptic cleft. Synaptic depression, monitored with EPSC pairs, was reduced at positive holding potentials. Since receptor desensitization is reduced by depolarization, depression may in part result from desensitization, a consequence of the slow clearance of transmitter.

699.15

SYNAPTIC INHIBITION AND FACILITATION IN RABBIT PANCREATIC GANGLIA. J.A. Love* and C. Owyang. Dept. of Internal Medicine, University of Michigan, Ann Arbor, MI 48109.

The intrinsic ganglia of the pancreas receive innervation from vagal, sympathetic, myenteric and primary afferent nerves while providing the postganglionic parasympathetic innervation to pancreatic endocrine and exocrine cells. Presently little is known of the synaptic mechanisms and neurotransmitters which regulate the firing of pancreatic neurons. We have performed the first intracellular recordings from rabbit pancreatic ganglia to study these questions. Portions of the pancreas, with or without the duodenum attached, were removed and intracellular recordings were made from neurons in single or interconnected ganglia. Stimulation of nerve fibers elicited multiple nicotinic fast EPSPs (fEPSPs) and action potentials. At low stimulus frequency (0.5 Hz) fEPSP activity decreased with time until frequent synaptic failures were observed. This phenomenon was antagonized by atropine. At higher stimulus frequencies (1-10 Hz), facilitation of fEPSP activity, resulting in synaptic action potentials, was observed. Conditioning (10Hz) trains also delayed the onset of synaptic failures observed at low frequency stimulation. Superfusion with norepinephrine (NE) or the α_1 agonist phenylephrine (5-10μM) depolarized (8 ± 1 mV) 8/19 neurons and facilitated synaptic transmission while 7/15 neurons responded to NE with a hyperpolarization (8 ± 1 mV) and synaptic inhibition. Thus, the post-ganglionic output to the endocrine and exocrine pancreas can be extensively regulated at the level of pancreatic ganglia by intrinsic synaptic mechanisms as well as by cholinergic and adrenergic neurotransmitters. Supported by NIH.

699.16

CHOLINERGIC MODULATION OF POSTSYNAPTIC RESPONSES ELICITED FROM RAT MAGNOCELLULAR CHOLINERGIC BASAL FOREBRAIN NEURONS. J.A.Sim[1]*, W.H.Griffith[2] & D.A.Brown[1]. [1]Dept. of Pharmacology, Univ. Coll. Lond., London WC1E 6BT & [2] Dept. of Medical Pharmacol. and Toxicol., Coll. of Medicine, Texas A & M Univ., College Station, TX 77843.

Basal forebrain nuclei, which form the main cholinergic projection to cortical and subcortical regions of mammalian brain, receive afferents from various brain regions. In the present study, a thin-slice preparation was used to study the postsynaptic currents of basal forebrain nuclei. Coronal sections (200 μm) were prepared from 10-14 day-old rat pups, incorporating the horizontal limb of the Diagonal Band of Broca and substantia innominata. Whole-cell recordings were made from visually identified magnocellular neurons, using a potassium acetate-based internal solution. Neurons with diameter > 20 μm were selected, as they stained positive with acetylcholinesterase histochemistry, and thus were considered to be cholinergic. In most cells, spontaneous synaptic responses could be recorded, displaying sensitivity to either bicuculline (Bic) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). These events were not readily blocked by tetrodotoxin (TTX). Following bipolar stimulation within a 50-100 μm radius of the recorded cell synaptic responses, both CNQX- and bicuculline-sensitive evoked-responses could be recorded. The mean time constant for decay (τ) for CNQX-sensitive epscs was approximately 3.03 + 0.31 ms and was 27.5 + 2.4 ms for Bic-sensitive ipscs (as measured at 20-23 °C). Carbachol (CCh: 0.3-10 μM) reduced the amplitude of these epscs in a dose-dependent manner. In addition to reducing the amplitude of these postsynaptic events, inhibition by CCh was frequently observed as failures of synaptic events.

In summary, synaptic connections within basal forebrain nuclei have been preserved in thin-slice preparations and that excitatory and inhibitory afferents to the magnocellular cholinergic basal forebrain express presynaptic muscarinic receptors. [Supported by Wellcome Travel Award (W.H.G) and M.R.C. (JAS,DAB)].

699.17

SYNAPTIC CONNECTIONS BETWEEN CELL PAIRS IN RAT HIPPOCAMPAL SLICE CULTURES.

D. DEBANNE*, N.C GUÉRINEAU, B.H. GÄHWILER and S.M. THOMPSON.
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Pairs of individual neurons were recorded in rat hippocampal slice cultures with sharp electrodes or in whole-cell configuration. Unitary synaptic connections were studied between pre- and postsynaptic cells in CA3 and CA1 hippocampal fields by eliciting single action potentials in the presynaptic cell. Monosynaptic excitatory connections were highly probable between CA3 and CA1 pyramidal cells (p=0.76, n=125) with almost no feed-forward inhibition (p=0.024). By contrast, disynaptic IPSPs, blocked by CNQX or bicuculline, were observed with a very high probability (p=0.43) between CA3 pyramidal cells. Monosynaptic excitation was found in 56% of cases between CA3 neurons (n=91) but only between 16% of CA1 pyramidal cell pairs (n=25). AMPA- and NMDA-components of EPSPs could be identified pharmacologically at both CA3/CA3 and CA3/CA1 cell synapses, with kinetics similar to those seen with extracellular stimulation. Single action potentials evoked in interneurons resulted in monosynaptic, bicuculline sensitive IPSPs only in pyramidal cells within the same region. Paired-pulse facilitation of unitary EPSPs and paired-pulse depression of unitary IPSPs, were observed. Probability of transmitter release, studied in recordings where failures could be unambiguously distinguished from spontaneous or small PSPs, was found to be close to 100% at both excitatory and inhibitory synapses. Bath application of the inhibitor of glutamate release, adenosine, reduced the amplitude of unitary EPSPs in a graded manner, indicating that the release at excitatory synapses is multiquantal.

699.18

IDENTIFICATION AND CHARACTERIZATION OF PROTEIN TYROSINE PHOSPHATASES AT THE NEUROMUSCULAR JUNCTION. L. Mei* and R.L. Huganir. Department of Neuroscience, HHMI, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Protein tyrosine phosphorylation is prevalent throughout the nervous system suggesting that it may be involved in the regulation of neuronal function. The phosphorylation of proteins is regulated by the balance of protein kinases and protein phosphatases. Although protein tyrosine kinases in the brain have been chatacterized, much less is known about neuronal protein tyrosine phosphatases. We have been using the neuromuscular junction as a model to study the role of protein tyrosine phosphatases in the regulation of synaptic transmission and platicity. Twelve individual protein tyrosine phosphatases were isolated from the rat skeletal muscle based on the consensus sequence of previously characterized tyrosine phosphatases, including three novel ones. Previously we have shown that PTP1D, a cytoplasmic phosphatase containing two SH2 domains, appeared to be abundant in the brain and muscle at the mRNA level and its phosphatase activity was down-regulated by a four amino acid insert generated by RNA splicing. Using affinity-purified antibodies, PTP1D was identified as a 68-kDa protein most abundant in the brain and heart, moderate in the skeletal muscle, lung, and liver, least in the kidney. Its expression in the brain appeared to increase continually during the development: from barely detectable on embryonic day 10 to maximal on postnatal day 60. The catalytic domain of PTP1D could be phosphorylated by protein kinase C but not by cAMP-dependent kinase. Phosphoamino acid analyses indicated that PKC phosphorylation of PTP1D was on serine residues. GST fusion proteins containing SH2 domains of PTP1D were made to study its interaction with other proteins. The SH2 domains were found to bind to several tyrosine-phosphorylated proteins *in vitro*, including nicotinic acetylcholine receptors in the skeletal muscle. Studies are under way to investigate the effects of PKC phosphorylation of PTP1D on the phosphatase activity and to identify the proteins that interact with PTP1D.

699.19

CHARACTERIZATION OF INTRACELLULAR CALCIUM OSCILLATION IN RAT HIPPOCAMPAL NEURONS IN THE PRESENCE OF MAGNESIUM IONS. T.Tanaka*, H.Saito, and N.Matsuki. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., Univ. of Tokyo, Tokyo 113, Tokyo

It is widely known that the embryonic neurons can develop morphologically and functionally in culture and form the synaptic network. In Mg^{2+}-depleted condition the cultured neurons show spontaneous and periodical oscillations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which are synchronized among the cells. In the present study the spontaneous oscillatory changes of $[Ca^{2+}]_i$ were observed even in the presence of physiological concentration of magnesium and characterized pharmacologically. The hippocampal cells from embryonic day 18 rats were cultured for 10-16 days and changes in $[Ca^{2+}]_i$ of single cells were measured by a microfluometrical technique with fura-2. The spontaneous oscillation was blocked completely by application of 1 μM tetrodotoxin. CNQX (10 μM) and nicardipine (10 μM) blocked the oscillation completely while APV showed only a partial depression of the increase in $[Ca^{2+}]_i$. This contracts with the results in Mg^{2+}-free condition, in which either APV or CNQX alone inhibits the oscillation completely. The inhibitory neurotransmitter GABA (10μM) suppressed the spontaneous oscillation, whereas bicuculline (3 μM), a $GABA_A$ antagonist, slightly enhanced the amplitude. Basic fibroblast growth factor (bFGF) also had depressant effect on the oscillation, and this effect was encountered by suramin. These results suggest that excitatory and inhibitory transmission is involved in the formation and maintenance of the intracellular Ca^{2+} oscillation.

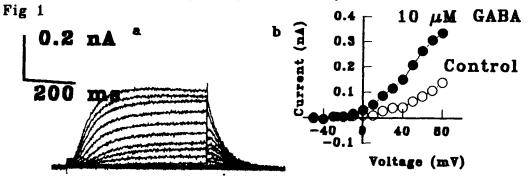
CHLORIDE AND OTHER CHANNELS

700.1

GABA-MEDIATED OUTWARD CURRENTS IN *HERMISSENDA* PHOTORECEPTORS. E. Yamoah* and A. Kuzirian. Marine Biological Laboratory, Woods Hole, MA 02543.

GABA is the inhibitory neurotransmitter at the mechanosensitive-hair cell-photoreceptor synapse in *Hermissenda*. In addition, GABA induces a transient rise in intracellular Ca^{2+}. It has been proposed that such induction may play a role in plasticity in *Hermissenda*. Here we report GABA-induced outward currents in photoreceptors of *Hermissenda* using whole-cell patch-clamp technique. Individual photoreceptors were isolated as previously described. Inward Na^+ and Ca^{2+} currents were minimized by substitution of Na^+ with choline and using low external Ca^{2+} (1 mM). Outward K^+ currents were blocked by 5 mM 4-aminopyridine and 100 mM tetraethylammonium. BAPTA (10 mM) was added to the internal solution to block $I_{K,Ca}$. Under these condition, minimal inward or outward currents were recorded. However, an outward current could be recorded upon the application of 10 nM GABA (fig1a & b). Desensitization to GABA occurred after prolonged exposure. Current was blocked by bicucculine (100 μM), a known blocker of $GABA_A$ receptor. The properties of the GABA-mediated outward current were consistent with those of Cl^- current: (1) The current magnitude and the estimated reversal potential were altered dependent on the external and internal Cl^- concentrations. (2) The current was reduced by DIDS (100 μM), a known blocker of Cl^- current. The inhibitory post synaptic potential at the hair cell-photoreceptor synapses may be mediated by the Cl^- current.

Fig 1



700.2

HYPERPOLARIZING AND DEPOLARIZING ACTIONS OF GLYCINE ON RAT ROSTRAL VENTROLATERAL MEDULLA NEURONS. H. H. Lin* and N. J. Dun. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614

Intracellular recordings were made from rostral ventrolateral medulla (RVLM) neurons of brain stem slices (500 μm) of immature (12-16 days) rats. Pressure application of glycine from a micropipette containing 100 mM glycine to 37 RVLM neurons evoked 3 types of responses: a depolarization (n=24), a hyperpolarization (n=6) and a biphasic response consisting of a hyperpolarization followed by a depolarization (N=2). The hyperpolarizing and depolarizing responses were associated with a marked decrease of membrane resistance. Increasing and decreasing the membrane potential reduced and increased the glycine hyperpolarization; the reversal potential was between -60 and -70 mV. The glycine depolarization was reduced by depolarizing and increased by hyperpolarizing the membrane from the resting potential of -60 mV. Strychnine (1 μM) blocked the glycine-induced hyperpolarization, whereas it partially suppressed the glycine-induced depolarization in 3 cells and had no effect in the other 5 cells. The results indicate that there are two pharmacologically distinct types of glycine receptor, the activation of which initiates a strychnine-sensitive hyperpolarization and a depolarization that is less susceptible to blockade by strychnine in the immature rat RVLM neurons. (Supported by NS18710).

700.3

ACTIVATION OF A CATIONIC CONDUCTANCE BY METABOTROPIC GLUTAMATERGIC AND MUSCARINIC AGONISTS IN CA3 CELLS IN HIPPOCAMPAL SLICE CULTURES.

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Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

An increase in a membrane cationic conductance in response to metabotropic glutamatergic or muscarinic agonists was characterized in CA3 pyramidal neurons using the patch-clamp technique. When $[K^+]_o$ was raised above 5 mM, 1S,3R-ACPD (50 μM) or methacholine (MCh, 5 μM) induced a biphasic inward/outward current. The initial inward component was associated with an increase in membrane conductance, displayed no apparent voltage sensitivity over the range -120 to -50 mV, and had a mean extrapolated reversal potential in 16 mM $[K^+]_o$ of -9.1 ± 21.2 mV and -3.3 ± 24.0 mV for 1S,3R-ACPD and MCh, respectively. Decreasing $[K^+]_o$ or $[Na^+]_o$ reduced the current amplitude and shifted the reversal potential to more negative values. The response to 1S,3R-ACPD or MCh was reduced by extracellular Ba^{2+} (1 mM), Cd^{2+} (200 μM), Mg^{2+} (10 mM) or TEA (20 mM) whereas extracellular Cs^+ (2 mM) or loading cells with BAPTA (20 mM) or Cs^+ (140 mM) did not affect the current. The effects of 1S,3R-ACPD persisted unchanged in the presence of 20 μM CNQX and 40 μM DAPV, but were reversibly inhibited by bath-applied MCPG (1 mM). The outward component of the 1S,3R-ACPD- and MCh-induced response was associated with a decrease in membrane conductance, reversed close to E_{K+}, varied linearly with membrane potential and may correspond to $I_{k,leak}$. Thus, in addition to a reduction in K^+ conductance, both 1S,3R-ACPD and MCh activate a non-selective cationic conductance mainly permeable to K^+ and Na^+ ions that is more prominent upon elevating $[K^+]_o$.

700.4

PROPERTIES OF A NOVEL VOLTAGE-DEPENDENT CATION CURRENT IN RAT NEOCORTICAL NEURONS. C. Alzheimer*. Department of Physiology, University of Munich, Pettenkoferstr. 12, D-80336 Munich, Germany.

Pyramidal neurons acutely isolated from rat sensorimotor cortex were found to express a nonlinear cation current (I_{CAT}) which could not be explained using established categories of channel classification. The ionic nature and the voltage dependence of this current were investigated using the whole-cell configuration of the patch-clamp technique. Experimental conditions were chosen to block Na^+ currents (1 μM TTX), Ca^{2+} currents (0.4 mM Cd^{2+}), and K^+ currents (internal Cs^+ instead of K^+). Recordings were performed at room temperature.

In symmetrical high Cs^+ solutions, depolarizing pulses applied from a holding potential of -70 mV evoked sustained currents that were inward between -40 and 0 mV (peak amplitude -300 to - 400 pA at -20 mV) and became outward at positive command potentials. I_{CAT} displayed outward rectification in the depolarizing direction. Upon repolarization to -70 mV, I_{CAT} slowly decayed producing a prominent inward tail current (τ: 40 - 60 ms). At a given potential, the time constant of tail current decay was independent of prepulse potential. In contrast, when repolarization potentials were varied along the voltage axis (-120 to -60 mV) after a prepulse to 30 mV, deactivation kinetics of I_{CAT} showed a strong voltage dependence, the decay time constant substantially increasing with depolarization.

Ion substitution experiments revealed that the channel producing I_{CAT} was permeable to a number of monovalent cations including K^+, Cs^+, Na^+, and choline, but not to the anion Cl^-. I_{CAT} was reduced by TEA at high concentrations (35 mM), but not by 4-AP, and proved to be insensitive to cation channel blockers such as Cs^+, amiloride, or gadolinium. Activation of I_{CAT} did not require Ca^{2+} influx nor cytosolic Ca^{2+} rise.

Due to its slow deactivation kinetics, I_{CAT} is likely to contribute to the sequence of afterpotentials following a depolarizing event in electrically active neurons.

Supported by the Deutsche Forschungsgemeinschaft (DFG, Al 294/3-1,2).

700.5

CALCIUM PERMEABILITY AND CYCLIC NUCLEOTIDE MODULATION OF THE HYPERPOLARIZATION ACTIVATED CATION CURRENT, I_h, IN N1E-115 NEUROBLASTOMA CELLS.

C. Mathes* and S.H. Thompson. Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

We recorded I_h in a neuronal cell line derived from mouse sympathetic ganglion. This time and voltage dependent current is activated at hyperpolarized potentials and tail current measurements give a reversal potential of -30 mV. The instantaneous (I_{inst}) and steady-state (I_{ss}) I(V) curves are inwardly rectifying. The activation curve of I_h is fit by a single Boltzmann function with V½ = -85 mV and the slope factor z = 3. Like $I_{h(f)}$ in cardiac and other cells, the inward current is inhibited by Cs but not by Ba which distinguishes this inward current from the fast inward rectifying K current. I_h amplitudes increase by about 144% when $(Ca)_{out}$ is elevated and when Mn or Co are added. These results suggest that I_h is permeable to divalents, including Ca, Mn and Co. Single channel currents have been measured with Ca as the carrier that have a similar I(V) curve and extrapolated reversal potential to I_h. The slope conductance is ~ 50 pS. In nystatin patch experiments, cAMP (1 mM 8-bromo cAMP) increases I_h by 400% (I_{inst}) and 151% (I_{ss}) at -95 mV. Cyclic GMP (1 mM 8-Br cGMP) inhibits I_h by 36% (I_{inst}) and 46% (I_{ss}) at -95 mV. Cyclic AMP increases I_h amplitudes at all voltages, while the inhibition of I_h by cGMP occurs only at negative potentials. This cyclic nucleotide modulation of I_h may be the mechanism by which muscarinic receptors suppress I_h. Muscarinic agonist application reduces I_h amplitudes by 35% at -90. Muscarinic inhibition of I_h should decrease spike frequency and counteract the slow EPSP during synaptic transmission. [NIH NS14519 to S.H.T., MH10425 and AFAR to C.M.]

700.6

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF AN INWARDLY RECTIFYING CONDUCTANCE IN RAT BRAIN. R.L. Smith, K.J. Staley, G.H. Clayton, and C.L. Wilcox* Dept. of Neurology, UCHSC, Denver, CO 80262 and Dept. of Microbiology, CSU, Fort Collins, CO 80523

A voltage sensitive inwardly rectifying chloride channel is present in hippocampal pyramidal neurons but not dentate gyrus neurons. This conductance has electrical properties similar to a chloride channel (designated ClC-2) that has been cloned and functionally characterized in a xenopus expression system, and has been shown to have a significant role in modulation of neuronal responses to $GABA_A$-mediated mechanisms (K. Staley, JNP 7/94; in press). Therefore, defining the CNS distribution of ClC-2 is of interest to understanding the neuron-specific mechanisms that can modulate $GABA_A$ response. Using *in situ* hybridization methods to detect the ClC-2 mRNA, we have demonstrated that expression of ClC-2 mRNA was restricted to the same population of neurons in the hippocampus that contain a voltage sensitive inwardly rectifying Cl^- conductance. In rat brain, expression of ClC-2 was restricted to neurons and choroid plexus but was not detectable in glial cells. Although ClC-2 mRNA was widely expressed in cortical neurons, significant differences in signal intensity were observed regionally and in different cell layers. In subcortical structures ClC-2 expression was restricted to specific regions and neuronal cell types. These results suggest that in brain ClC-2 may have neuron specific functions in modulation of the chloride gradient that differ significantly from the proposed role in volume regulation of non-neural tissues.

700.7

ACTIVATION, PERMEABILITY AND INHIBITION OF THE LARGE-CONDUCTANCE SWELLING-INDUCED ANION CHANNEL IN ACUTELY ISOLATED RAT CORTICAL ASTROCYTES. T.O.Jalonen*, C.J.Charniga, A.J.Popp, and H.K.Kimelberg. Department of Surgery, Albany Medical College, Albany, N.Y. 12208.

We have used GFAP(+) cortical astrocytes within 1-8 hours after their isolation from postnatal day 3 to day 28 rats to study the characteristics of a large-conductance (over 300 pS), swelling-induced anion channel (LA^-) found in astrocytes. Hyposmotic solutions activate this channel and we have now found that both isosmotic and hyperosmotic 50 mM EtOH solutions also induce its activation. Since hyperosmotic EtOH does not swell the cell, this also indicates a direct, osmolarity-independent effect of ethanol on channel activity. Contrary to results in rabbit kidney cells (Mills et al.,1994;J.Exp.Zool. 268:111), cytochalasin D (0.3-1 uM in DMSO, added into the bath in a cell-attached recording configuration) did not activate the astrocytic LA^- channel, indicating that possible disruption of actin filaments during swelling is not responsible for this channel's activation in astrocytes. Though Gray and Ritchie earlier showed that in normal (non-swollen) conditions glutamate does not permeate astrocyte Cl^- channels in whole-cell recordings from primary astrocytes (1986; Proc. R. Soc. Lond. B. 228:267), we now find in single-channel recordings that high concentrations of glutamate (20-137 mM) permeate the LA^- channel when the channel is first activated by hyposmotic solutions. This supports the idea that this is one possible pathway for amino acids to permeate the membrane during astrocytic swelling. The anion transport inhibitor, L-644,711, therapeutic in animal models of head injury and ischemia, blocks the LA^- channel at 0.1-0.6 mM concentrations, but has no inhibitory effect on K^+ channel activities. These findings give new information on possible functions of the anion channel in astrocytic swelling during traumatic head injury and ischemia.
(Supported by BRSG NIHSO7RR05394-31 (T.O.J.), H.Schaffer Foundation (A.J.P.) and NS 30303 (H.K.K.)).

700.8

RAPID AND REVERSIBLE INHIBITION OF GAP JUNCTIONAL DYE TRANSFER USING 18α-GLYCYRRHETINIC ACID AND 18α-CARBENOXOLONE IN CELL CULTURE. G.S. Goldberg, J.F. Bechberger, and C.C.G. Naus*. Dept. of Anatomy, University of Western Ontario, London, ON N6A 5C1, CANADA.

Gap junctions are intercellular conduits, made up of proteins called connexins, through which molecules may pass from one cell into another - a process referred to as gap junctional intercellular communication (GJC). GJC appears to play crucial roles in the control of cell growth and differentiation. Therefore, the ability to effectively inhibit GJC, without causing cytotoxic or other side effects, would be extremely useful for investigating the functional significance of GJC in a variety of systems. Others have shown the glycyrrhetinic acid family of compounds to have great promise in this respect. We describe here a simple method for determining concentrations of 18α-glycyrrhetinic acid and 18α-carbenoxolone which are able to completely and reversibly inhibit GJC in cell culture, as assayed by dye transfer, with no detectable cytotoxic effects. This procedure should prove useful in many other cell types and systems.

701.1

SENSITIVITY TO OSMOTIC CHANGES OF VOLTAGE-GATED CALCIUM CURRENTS IN RAT ANTERIOR PITUITARY CELLS.

O. Matzner & I. Nussinovitch*. Dept. of Anatomy, Hebrew University Sch. of Med., Jerusalem, Israel.

In a recent study it has been shown that voltage gated calcium currents, in pituitary cells, are flow sensitive (Ben-Tabou, Keller and Nussinovitch, J. Physiol. 476.1, 1994). This finding raised the possibility that local deformation of pituitary cell membranes can affect influx through voltage sensitive calcium channels. We tested this possibility by exposing pituitary cells to solutions with different osmolarities, causing deformation of pituitary cell membranes by swelling or shrinking. In this study, pituitary somatotrophs were exposed to hypotonic solutions (165-200 mosmol) or hypertonic sucrose containing solutions (400-520 mosmol) by bath perfusion. The osmolarity of the standard extracellular solution was 320 mosmol. Calcium currents were recorded with the whole-cell mode of the patch-clamp technique.

Exposure to hypo-osmotic solutions resulted in two major effects: 1. Block of T-type calcium currents with no change in their voltage dependence of activation. 2. Negative shift (of about 10 mV) in the activation curve for high voltage activated (HVA) calcium currents. HVA calcium currents which were activated at negative test potentials (-50 mV to -10 mV) increased in size whereas those activated at more positive test potentials(0 mV to +30 mV) decreased in size. Similar results were observed when the cells were exposed to hyper-osmotic solutions except that the block of HVA currents was not always associated with a shift in activation curve. These findings suggest that deformation of pituitary cell membrane by osmotic changes can regulate influx through voltage sensitive calcium channels.

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701.2

CALCIUM-DEPENDENT INHIBITION OF HUMAN N-TYPE CALCIUM CHANNELS.

C. J. Grantham , P.S. Chard, P.T. Toth, J. Jordán†, G. Ghadge, R. Roos, R.J. Miller and D. Bleakman. Lilly Research Centre Ltd. Surrey GU20 6PH U.K. and Depts. of Pharmacol. Physiol.. Sci. & Neurology, Univ. of Chicago, Chicago, Illinois 60637, U.S.A.

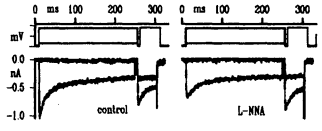
Voltage-dependent calcium channels (VDCC) can undergo both voltage and current-dependent inactivation. We have investigated the modulation of human N-type VDCC by changes in $[Ca^{2+}]i$. Ca^{2+} and Ba^{2+} currents were measured in HEK 293 cells which had been transfected with the human neuronal $\alpha_{1B-1}\alpha_{2b}\beta_{1-3}$ subunits. Using whole cell voltage clamp recordings, prepulse protocols were employed to examine current-dependent reductions in N-type VDCC currents. Voltage-depenedent inactivation was minimized (<10%) by utilizing a double pulse protocol with P1 (Vh= -90mV, Vt= +10mV, dur.= 200ms) and P2 (Vh= -90mV, Vt= +10mV, dur.= 200ms) separated by a 1200ms pulse interval (at Vh=-90mV). Under low Ca^{2+} buffering conditions (100μM Fura-2 in the patch pipette), the P2/P1 ratio was significantly reduced when Ca^{2+} ions replaced Ba^{2+} ions as the charge carrier (0.48±0.28, n=6 & 0.83±0.07, n=6 respectively. p<0.02). However, when 15mM BAPTA was added to the patch pipette, the P2/P1 ratio for Ca^{2+} ions was significantly increased (0.89±0.01, n=6, p<0.01).

HEK 293 cells were infected with a replication defective calbindin D_{28K} (AdCABP-1), or β-galactosidase (AdβGAL), expressing adenovirus. 24 hrs following infection, immunocytochemical staining confirmed expression of these proteins. Ca^{2+}-dependent inhibition was also attenuated in calbindin D_{28K} expressing cells. Using simultaneous electrophysiological (nystatin perforated patch)/ fura-2 based microfluorimetric recordings, we assessed increases in $[Ca^{2+}]i$ (per pC charge influx) for the AdCABP-1 and AdβGAL infected HEK 293 cells. Increases in $[Ca^{2+}]i$ (per pC charge influx) were significantly lower in AdCABP-1 infected cells compared to AdβGAL infected cells and abolished in BAPTA containing cells. These experiments demonstrate that N-type VDCC can be inhibited by a process involving increases in $[Ca^{2+}]i$. †Supported by NATO grant.

701.3

NITRIC OXIDE SYNTHASE (NOS) DOES NOT MEDIATE CURRENT-DEPENDENT INACTIVATION OF N-TYPE Ca^{2+} CHANNELS BUT IT DOES SOMETHING TO I_{Ca}. J.L. Kenyon* Dept. of Physiol., Univ. of NV School of Med., Reno, NV 89557

I tested the hypothesis that Ca^{2+} entry via I_{Ca} inhibits I_{Ca} by stimulation of NOS and production of nitric oxide (NO). N-type I_{Ca} was measured in chick dorsal root ganglion neurons using amphotericin perforated patches at 35-36°C. $I_{Cl(Ca)}$ was reduced by low $[Cl^-]_o$ and 100 μM niflumic acid. Inactivation of I_{Ca} during 250 ms conditioning steps (-70 to +40 mV) was monitored by 50 ms test steps (-8 mV). 10 s intervals separated the conditioning steps. As conditioning voltages were made less negative, test I_{Ca} decreased then recovered as expected for current-dependent inactivation of I_{Ca} (Tillotson, PNAS 76:1497, 1979). Inactivation was not reduced by 100 μM Nitro-L-arginine (L-NNA) suggesting that synthesis of NO by NOS is not required for this process. Curiously, I_{Ca} during the conditioning step to -8 mV was larger than I_{Ca} in the first test step (4 of 5 cells, left panel). This effect was abolished by L-NNA (right panel) implying an NO-dependent potentiation of I_{Ca} during the train of conditioning and test steps. NS 32144



701.4

PROTON-INDUCED CURRENT CHANGES IN ION SELECTIVITY DURING CULTURE OF EMBRYONIC RAT SPINAL CORD NEURONS.

Y.X. Li, A.E. Schaffner, H.R. Li, R. Nelson*, and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, Maryland 20892

Rapid increases in protons (H^+) induce Na^+ conductance in a variety of cell types(Gen.Physiol.Biophys.11:39,1992). Here we report that H^+ can trigger a cation selective conductance in cultured embryonic(E) rat spinal cord neurons that changes over time in culture. Experiments were performed on neurons dissociated at E15 and cultured for 1-4 weeks. All recordings were carried out at 22-25°C with conventional whole-cell patch clamp techniques using CsCl-filled pipettes. An inward current exhibiting a rapid decay phase (≤6seconds) in many of cells and requiring several minutes to recover after complete inactivation, was triggered by brief application of saline buffered to pH≤6.5. More modest decreases in pH, which by themselves failed to activate this current, depressed those triggered by effective changes in pH. The currents recorded from the cells cultured for up to one week could be abolished completely in the absence of Ca^{++} and persisted in Na^+-free solution and in Ba^{++}-, Mn^{++}- and Co^{++}-containing solutions. The currents were blocked by 1mM Ni^{++} or 2mM Mg^{++}. The reversal potential was about +55mV, similar to the Ca^{++} equilibrium potential, consistent with the Ca^{++}-dependency of the current. After ~10 days in culture protons induced an inward current with similar kinetic properties, but Na^+-dependency. Thus, proton-activated cation conductance changes ion selectivity in culture.

701.5

PHARMACOLOGY OF LOW-THRESHOLD CALCIUM CURRENTS IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS.

R.B. Avery* and D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Hippocampal neurons possess multiple types of voltage-gated calcium channels (Fisher et al., *J. Neurophys.* 64:91). The contribution of specific channel types to cellular functions, however, is not well-established. Low-threshold calcium currents are thought to support complex firing patterns in CA3 pyramidal cells. Since the pharmacology of low-threshold calcium channels varies among neurons, we wish to screen potential blockers of these channels in CA3 cells.

Recordings were made from CA3 pyramidal neurons acutely isolated from rats 7 to 14 days old. Cell-attached patches confirmed the presence of low-threshold calcium channels on the somata of these cells. In 110 mM barium, single channel openings could be evoked from potentials as low as -60 mV. The slope conductance of single channels was less than 10 pS. Patches with several channels exhibited a rapidly inactivating macroscopic current.

To screen putative blockers of these channels, we made whole-cell recordings in 5 mM calcium. From hyperpolarized holding potentials, calcium currents activated at potentials more positive than -60 mV. We assayed low-threshold calcium currents with test pulses to -40 mV from a holding potential ≤ -80 mV. The current at -40 mV was slowly activating and inactivating. The amplitude was about a third of the peak whole-cell current, which occurred between -10 and 0 mV. High-threshold currents were isolated by holding at -50 mV to inactivate low-threshold channels. Steps from -50 mV to 0 mV elicited a slightly inactivating current. The amplitude was about half that compared to currents evoked from a holding potential of -80 mV. 50 or 100 μM nickel strongly reduced the low-threshold component but only slightly blocked the high-threshold currents. (MH10473, MH44754, MH48432, NS11535)

701.6

REGULATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS OF MOUSE DRG NEURONS BY CHRONIC ELECTRICAL STIMULATION IN CULTURE.

MX. Li, M. Jia, R.D. Fields*, and P.G. Nelson. NICHD and NINDS, NIH, Bethesda, MD 20892.

Previous research has shown that influx of calcium through voltage-sensitive calcium channels is responsible for the arrest of growth cone motility in mouse DRG neurons in culture. After chronic electrical stimulation, growth cones recover and become insensitive to repeated stimulation. (Biphasic stimulation was delivered at 6V in 0.5 s bursts at 10 Hz every 2 s for 40 - 70 hrs.) To understand the mechanism of growth cone accommodation, whole cell voltage clamp experiments and calcium imaging using fura-2 were performed on mouse DRG neurons cultured in multi-compartmental chambers. The electrically-induced increase in calcium was significantly slower in the growth cones and cell bodies of DRG neurons after chronic stimulation (tau = 3.2s vs. 1.4s, $p < 0.001$). High-threshold-activated (HVA) whole-cell calcium currents were reduced in chronically stimulated neurons from 3.2 nA to 0.59 nA ($p < 0.02$), measured at the end of a 200 ms stimulation pulse. Low-threshold-activated (LVA) were reduced from 0.74 nA to 0.095 nA ($p < 0.02$). The membrane potential of the peak current was shifted from -15 mV in the control neurons to +9.1 mV ($P < 0.0002$). There was no difference in the mean diameter of neurons in the control and stimulated groups. The HVA calcium currents inactivated more rapidly in the stimulated group. Our results suggest that development of the nervous system could be influenced by activity-dependent regulation of calcium conductance through VSCC, by ensuing effects on growth cone motility, and other calcium-dependent processes.

701.7

MODULATION OF RYANODINE RECEPTORS BY MITOCHONDRIAL CALCIUM IN DORSAL ROOT GANGLION NEURONES. D. Bowie*, P. Feltz and R. Schlichter, *Laboratoire de Physiologie Générale, Université Louis Pasteur, Strasbourg, France.*

An extensive repertoire of ionic channels are modulated by changes in the concentration of cytosolic calcium ($[Ca^{2+}]_i$) which, in many cases such as for ryanodine receptors[1], exerts a bell-shaped dependency on channel activity. As yet, these observations have not been demonstrated in an intact neurone nor has the Ca^{2+}-signalling pathway which modulates receptor function been identified. In single, intact nociceptive sensory neurones pre-loaded with fura-2, we have attempted to identify the Ca^{2+}-signal which modulates ryanodine receptors using a variety of receptor agonists which elevate $[Ca^{2+}]_i$ through known pathways[2]. The sensory neurone excitant, capsaicin (500nM-2μM) and 50mM KCl evoked biphasic elevations in $[Ca^{2+}]_i$ consisting of a peak and plateau component. The peak Ca^{2+}-rise was due to entry of extracellular Ca^{2+} which was attenuated and sustained at a plateau level for long periods by the mitochondria; identified using 10μM FCCP to selectively inhibit Ca^{2+}-uptake into the organelle. The amplitude of the plateau was greater following application of capsaicin than 50mM KCl. Activation of ryanodine receptors by 10mM caffeine elicited transient Ca^{2+} rises that were reversibly inhibited during the Ca^{2+}-plateau evoked by capsaicin but unaffected by the plateau to 50mM KCl. Selective inhibition of the mitochondrial Na^+/Ca^{2+} exchanger with 2μM CGP37157 lowered the plateau amplitude observed with capsaicin and restored the Ca^{2+} rises evoked by caffeine. These observations support a novel role of the mitochondria as a long-term modulator of intracellular Ca^{2+} stores as well as a prospective regulator of other Ca^{2+}-dependent events in the cytoplasm.

(1) Bezprozvanny *et al*, (1991) *Nature*, 351, 751-754.
(2) Bowie *et al* (1994) *Neuroscience*, 58, 141-149.

D.B. was funded by an ULP-CNRS-Lilly postdoctoral fellowship.

701.8

THAPSIGARGIN DISCHARGES INTRACELLULAR Ca^{2+} ($[Ca^{2+}]_i$) STORES IN MOUSE CORTICAL ASTROCYTES. I.A. Edwards*, H.H. Wolf and H.S. White. Anticonvulsant Drug Development Program, Department of Pharmacology and Toxicology, University of Utah, SLC, UT 84112.

Thapsigargin stimulates Ca^{2+} release from $[Ca^{2+}]_i$ stores by selectively inhibiting the Ca^{2+}-ATPase on the intracellular storage membrane. By poisoning the Ca^{2+} pump thapsigargin treatment produces a release of Ca^{2+} from the thapsigargin-sensitive Ca^{2+} stores. The present investigation was initiated to assess the $[Ca^{2+}]_i$ storage capacity of mouse cortical astrocytes. In an attempt to deplete $[Ca^{2+}]_i$ stores varying concentrations of thapsigargin (10, 30 & 100 nM) were bath applied to a confluent monolayer of astrocytes for 10 minutes. Changes in the $[Ca^{2+}]_i$ of type-1 mouse cortical astrocytes (21-28 days in culture) were measured using an indo-1 based microfluorometry imaging system. Treatment with thapsigargin resulted in a dramatic increase (417, 646 & 621% at 10, 30 & 100 nM, respectively) in the $[Ca^{2+}]_i$ over basal levels. This effect was only partially reversible upon subsequent wash-out with drug-free buffer. Of particular interest was the finding that the increase in $[Ca^{2+}]_i$ was highly dependent on the concentration of $[Ca^{2+}]_o$. These results suggested that depletion of $[Ca^{2+}]_i$ stores was producing Ca^{2+} influx across the plasma membrane. Additional studies with dantrolene and ryanodine (to block $[Ca^{2+}]_i$ release) and nimodipine (to block voltage-sensitive Ca^{2+} channels) were unable to attenuate the thapsigargin-induced influx of Ca^{2+} and subsequent increase in $[Ca^{2+}]_i$. These data support the hypothesis that the decrease in Ca^{2+} stores may release some type of intracellular messenger (e.g., calcium influx factor) which would initiate refilling of $[Ca^{2+}]_i$ stores. (Supported by NIH grant 2-RO1-NS-22200 and NIH/NINDS contract NO1-NS-9-2328).

701.9

CALCIUM INFLUX THROUGH P-TYPE CHANNELS ACTIVATES CLOSELY ASSOCIATED CALCIUM DEPENDENT POTASSIUM CHANNELS AT THE MOUSE MOTOR NERVE TERMINALS. D.A. Protti and O.D. Uchitel*. Faculty of Medicine , University of Buenos Aires, Paraguay 2155 (1121), Buenos Aires, Argentina.

Transmitter release is commonly shortened by the repolarization of the membrane, due to the onset of diverse potassium currents. At the mammalian motor nerve terminals, two main potassium conductances have been described, a voltage dependent K^+ current, blocked by 3,4-diaminopiridine (3,4-DAP) and a calcium activated potassium current ($IK_{(Ca)}$) sensitive to TEA and to Charybdotoxin. We have previously reported that FTX and ω-Aga-IVA, two P-type calcium channel blockers, inhibit neuromuscular transmission, diminish quantal content and block calcium currents at the mouse motor nerve terminals (PNAS 89,3330-3333, 1992; Neuroreport 5, 333-336, 1993).

We studied the activation of the ($IK_{(Ca)}$) in the levator auris muscle-nerve preparation of the mouse by means of perineural recordings in the presence of 3.4-DAP (1mM). We have found that the $IK_{(Ca)}$ is abolished in the presence of FTX (1:10,000) and ω Aga-IVA (10 nM).This $IK_{(Ca)}$ was also inhibited after loading the nerve terminals with the fast calcium chelators BAPTA-AM and DM-BAPTA-AM (25 μM) but not when loaded with EGTA-AM (25 μM), a slow calcium chelator.

These results indicate that calcium influx through P-type calcium channels is responsible for the activation of the calcium-dependent potassium channels. Moreover, the ability of BAPTA-AM and DM-BAPTA-AM of preventing $IK_{(Ca)}$ activation and the lack of effect of EGTA-AM suggest that both types of channels are closely associated at the release sites.

701.10

SINGLE CHANNEL BASIS OF THE MULTIPLE FUNCTIONAL COMPONENTS OF MACROSCOPIC L-TYPE Ca^{2+} CHANNEL CURRENT IN CLONAL PITUITARY CELLS. D. M. Fass* & E. S. Levitan. Depts. of Neuroscience and Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

In the presence of the dihydropyridine agonist BAY K 8644 (1 μM), whole cell L-channel Ba^{2+} currents in GH_3 cells consist of two functional components. These two components differ in their rate of deactivation, voltage dependence, sensitivity to BAY K 8644, and sensitivity to inhibition by the neuropeptide TRH. The simplest hypothesis which may account for these two components is one which invokes two populations of L-channels. To test this hypothesis, single channel recordings were obtained from cell-attached patches. To control the potential of the membrane outside the patch, the K^+ ionophore valinomycin (10 μM) was applied to the cells. This treatment stabilized Vm at ~-60 mV. In addition, rundown was much less pronounced (channel activity remained stable for hours) than when the typical high K^+ external solution was used. Ensemble tail currents derived from recordings from multi-channel patches were fit by two exponentials, indicating that the two components were detectable in the presence of valinomycin. Two exponentials were also required to fit the ensemble tail current derived from one of two single-channel patches obtained thus far. The other single-channel patch had an ensemble tail fit well by one exponential. Obtaining more single channel patches will allow us to determine whether individual L-channels display the behaviors of one or both components of macroscopic L-current.

701.11

STIMULUS-INDUCED SECRETION AND VESICLE ACIDIFICATION IN SEROTONERGIC PARANEURONS (PARAFOLLICULAR CELLS): THE ROLE OF VOLTAGE-GATED Ca^{2+} CHANNELS. H. Tamir*, K.P. Liu, M. Heath, M. Adlersberg, and M. D. Gershon, . D. Anat. & Cell Biol., & D. Anesth. Columbia Univ. P&S New York, NY 10032

Thyroid parafollicular (PF; "C") cells are neural crest-derived endocrine cells that resemble neurons. These cells synthesize 5-HT and co-store it with calcitonin in secretory vesicles. The pH within these vesicles is regulated by gatable membrane Cl^- channels. These channels open and permit the vesicles to acidify in response to secretogogues, which include $\uparrow[Ca^{2+}]_e$ and $\uparrow[K^+]_e$. We now report that a Cl^- channel (p64), identical to that found in epithelial cells, is present in the membrane of PF vesicles. The opening of this channel is associated with an increase in its state of phosphorylation. Secretogogues, in concentrations that also cause secretion, induce an increase in $[Ca^{2+}]_i$. Secretion in response to $\uparrow[Ca^{2+}]_e$ was partially inhibited by the L-type channel blocker, nimodipine (NIM; 0.5 μM) and by tetrodotoxin (TTX; 10 μM), which were additive when combined. Secretion in response to $\uparrow[K^+]_e$ was unaffected by TTX, but abolished by NIM. Antagonism of secretion by NIM was reversed by the L-type channel agonist, Bay K8644 (10 μM). In contrast to NIM, neither the N-type channel blocker, Ω-conotoxin (0.1 μM), nor the P-type channel blocker, Ω-agatoxin (0.1 μM), affected secretion. Vesicle acidification in response to $\uparrow[Ca^{2+}]_e$ was partially inhibited by both NIM (0.5 μM) and Ω-conotoxin (0.1 μM), but was unaffected by Ω-agatoxin (0.1 μM). Vesicle acidification induced by $\uparrow[K^+]_e$, was inhibited by NIM (1.0 μM), Ω-conotoxin (0.1 μM), Ω-agatoxin (0.1 μM), and by TTX (1.0 μM). These data suggest that Ca^{2+} entry through L-type voltage-gated Ca^{2+} channels is involved in coupling stimulation to both secretion and vesicle acidification; however, N-type channels (and P-type channels when cells are stimulated with $\uparrow[K^+]_e$) contribute only to vesicle acidification. Supported by grants DK19743, NS07062 and NS12969.

701.12

LEAD ATTENUATES L-TYPE CALCIUM CHANNEL INACTIVATION IN BOVINE CHROMAFFIN CELLS. L. Sun, J. B. Suszkiw, and N. Sperelakis*. Dept. of Physiology and Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267-0576.

Using whole-cell voltage clamp, we investigated the effects of inorganic lead (Pb^{2+}) on the voltage-gated calcium channel currents recorded from bovine adrenal chromaffin cells. Currents were evoked by 200ms voltage step pulse to +10mV from a holding membrane potential of -80mV. Extracellularly-applied Pb^{2+} (0.05, 0.1, 0.5, 1.0 μM) depressed calcium currents in a dose-dependent manner and acted as a potent and reversible blocker of Ca^{2+} channels, which is consistent with a competition by Pb^{2+} with Ca^{2+} at a binding site within the calcium channel. However, intracellular application of Pb^{2+} (0.001, 0.01, 0.1 μM) showed a facilitation of calcium currents. This effect was dose-dependent, could be blocked by 10 μM nifedipine, and appears to reflect reduced rate of calcium current run-down. It has been indicated that the Ca^{2+}-dependent inactivation of L-type channels involves an activation of protein phosphatase and the channel dephosphorylation. These results suggest that Pb^{2+} may attenuate Ca^{2+} channel inactivation by maintaining Ca^{2+} channel in phosphorylated status.

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701.13

ACUTE EXPOSURE TO LOW LEVELS OF LEAD ALTERS CALCIUM CURRENT AMPLITUDES IN RAT PC-12 CELLS. C.C. Hegg & V. Miletic*. Dept. Comp. Biosci. & Environmental Toxicology Center, Univ. Wisconsin, Madison, WI 53706.

Lead is a known neurotoxin with varied and poorly understood mechanism(s) of action. We employed the whole-cell patch-clamp technique to examine the effects of low levels of lead on high-threshold voltage-dependent calcium currents in rat pheochromocytoma (PC-12) cells. PC-12 cells were placed in NGF-containing media for 4, 8, or 12 days prior to recording. During recordings, the cells were first perfused for 5 minutes with lead-free solution, then switched to a 1 or 10 μM lead-containing solution for another 5 minutes, and finally washed again with lead-free solution. As previously reported, acute low-levels of lead caused a decrease in calcium current amplitude without an obvious change in activation or deactivation kinetics in some cells (n=7). However, unlike previous reports, acute exposure to the same low levels of lead also produced increases in calcium currents in other cells (n=12). In both situations, the effect of lead was only partially reversible with washing. There was no systematic difference in the effects of lead among the 4, 8 or 12 day cultures. These data suggest complex interactions of lead with calcium channels in PC-12 cells. (Supported by NIH NS21278).

701.14

CALCIUM MOBILIZATION IN RESPONSE TO IRON, HEMIN AND PROTOPORPHYRIN IX: MULTIPLE PATHWAYS OF ACTIVATION. DL Kraemer*, AH Cornell-Bell. Neurosurgery* and Cell Biology Yale University School of Medicine, New Haven, CT 06510

Intracranial hemorrhage is associated with stroke, reperfusion injury, trauma, and epilepsy. Abnormalities in calcium metabolism have been reported with all of these disease processes, and have been attributed to iron-induced Fenton chemistry. The relative contribution of inorganic and organic blood breakdown products upon intracellular calcium physiology was investigated to determine whether multiple factors might be involved. Cultured rat hippocampal astrocytes were exposed to varying concentrations of $FeCl_2$, hemin and protoporphyrin IX. Relative changes in intracellular calcium were measured using Fluo-3 and time-lapse video confocal laser microscopy. $FeCl_2$ induced intracellular oscillations and long-range regenerating calcium waves in a dose dependent manner (r=0.85, EC_{50}=1μM). Hemin produced similar changes (r=0.95, EC50=5μM), except that a late, sustained calcium elevation was also noted. Protoporphyrin IX induced a slow rise in intracellular calcium, with minimal wave generation. Removal of extracellular calcium from the bathing medium abolished the response seen to 50μM $FeCl_2$, ($p<.0001$), but had no effect on the calcium rise induced by 50μM protoporphyrin IX. ($p>0.5$, NS). These results suggest that blood breakdown products mobilize different sources of calcium, and that multiple pathophysiologic mechanisms may be induced after hemorrhage.

701.15

SELECTIVE MODULATION OF T- and L-TYPE CALCIUM CURRENTS BY STIMULATION OF m3 AND m4 MUSCARINIC RECEPTOR SUBTYPES IN TRANSFECTED 3T3 CELLS. K.E. Pemberton* and S.V.P. Jones. Molecular Neuropharmacology, Departments of Psychiatry and Molecular Physiology and Biophysics, University of Vermont College of Medicine, Burlington, VT 05405.

Calcium currents were recorded from NIH 3T3 cells transfected with the m3 and m4 muscarinic receptor subtypes. Whole cell patch clamp recordings were made with (mM): NaCl 150, $MgCl_2$ 1, $BaCl_2$ 20, Hepes 10 and Glucose 5. Patch pipettes (4-6MΩ) were filled with (mM) NMGCl 120, $MgCl_2$ 4, EGTA 10, Hepes 10, ATP 4, GTP 0.1, creatine phosphate 5, and creatine kinase 50 units/ml. Using a variety of stimulation protocols two types of voltage dependent calcium current were observed. A low voltage activated, transient, nifedipine-insensitive calcium current (T-type) and a slowly inactivating, high voltage activated calcium current that was dihydropyridine-sensitive (L-type). Stimulation of cloned m4 receptors resulted in a pertussis toxin-sensitive reduction in amplitude of the L-type calcium current but appeared to be without effect on the amplitude of the T-type calcium current. Conversely, activation of the m3 muscarinic receptor produced an increase in the amplitude of the T-type calcium current. The respective mechanisms of action of these muscarinic receptor subtypes will be addressed.

701.16

ACTIVATION OF $GABA_B$ AND μ-OPIOID RECEPTORS MODULATE DIFFERENT TYPES OF Ca^{2+} CURRENTS IN RAT NODOSE GANGLION NEURONS. K.I.Rusin* and H.C.Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109-0622.

Whole cell patch clamp recordings were obtained from nodose neurons acutely dissociated from 10-24 day old rats to characterize the Ca^{2+} channel types that are modulated by $GABA_B$ and μ-opioid receptors. Five components of high-threshold current were distinguished on the basis of their sensitivity to blockade by ω-CgTxGVIA, nifedipine, ω-AgaIVA and ω-CTx MVIIC. Administration of the μ-opioid agonist H-Tyr-D-Ala-Gly-Phe(N-Me)-Gly-ol (DAGO, 0.3-1μM) or the $GABA_B$ agonist baclofen (30μM) suppressed high-threshold Ca^{2+} currents by 51.5±2.7% (n=51) and 20.7±2.4% (n=24), respectively. Blockade of N-type channels by ω-CgTxGVIA eliminated current suppression by baclofen in all cells tested (n=11). The inhibitory effect of DAGO was completely abolished by ω-CgTxGVIA in 12 of 30 neurons and partially reduced in the remaining 18 neurons. Administration of ω-Aga IVA in saturating concentrations failed to completely eliminate the μ-opioid sensitive current component which persisted after blockade of N-type channels. This ω-CgTxGVIA/ω-AgaIVA-resistant component of the μ-opioid sensitive current was blocked by ω-CTxMVIIC in 9 neurons. The remaining neurons (n=9) contained an unidentified component of Ca^{2+} current that was resistant to blockade by ω-CgTxGVIA, dihydropyridines, ω-AgaIVA and ω-CTxMVIIC. This toxin-resistant component was reduced by DAGO. Dihydropyridine-sensitive (L-type) current was unaffected by μ-opioid or $GABA_B$ receptor activation. These data suggest that μ-opioid receptors in nodose ganglion neurons are negatively coupled to N, P, Q and unidentified toxin-resistant Ca^{2+} channels, whereas $GABA_B$ receptors are coupled only to N-type channels. (Supported by DA03365 to HCM).

701.17

Noradrenergic potentiation of a low-voltage-activated calcium current in dissociated nucleus basalis neurons. S. Williams*, A. Pegna, M. Mühlethaler and L. Bernheim *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland.*

Nucleus basalis (NB) neurons provide an important cholinergic input to the cerebral cortex and receive a prominent innervation from noradrenergic (NA) neurons of the locus coeruleus. Previous studies have indicated that cholinergic neurons of the NB are endowed with the capacity to emit action potentials in bursts. These bursts are thought to be mediated by a potent low-voltage-activated (LVA) calcium current. We have studied the NA modulation of the LVA current in dissociated NB neurons using whole-cell voltage-clamp recordings. In the presence of TTX (0.5 μM), TEA (20 mM) and 4-AP (4 mM), NB neurons recorded with an internal solution containing (in mM) 130 Cs acetate, 20 Cs Cl, 5 HEPES, 5 $MgCl_2$, 0.1 BAPTA, 3 Na_2ATP and 0.1 NaGTP, consistently displayed a transient LVA current. From a V_H of -90 mV, the LVA current was activated at potentials near -50 mV, had a peak amplitude (measured at -30 mV) of 223 + 60 pA (n=8, mean ± SD) and was reduced by amiloride (300 μM, n=3) or cadmium (200-400 μM, n=8). Perfusion of NA (50 μM) produced a long lasting (>15 min) increase of 44 ± 32 % (n=4) in the peak amplitude of the LVA current. The addition of prazosin (1μM, n=3), an α_1 receptor antagonist, prevented this effect. Perfusion with L-phenylephrine (40 μM), an α_1 receptor agonist, mimicked the NA induced potentiation, producing an increase of 52 ± 23 % (n=7). These results suggest that NA, through the activation of an α_1 receptor, can potentiate the LVA current and could therefore in certain conditions, favour the propensity of NB neurons to generate rhythmic bursts. *(Canadian MRC and Swiss FN).*

701.18

Serotonergic and GABAergic modulation of HVA calcium currents in dissociated nucleus basalis neurons A. Pegna, L. Bernheim, M. Mühlethaler, M. Serafin*, and S. Williams, *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland.*

A major source of cholinergic input to the cerebral cortex arises from nucleus basalis neurons (NBn). In addition to a low-voltage-activated Ca^{2+} current, NBn exhibit an important high-voltage-activated (HVA) Ca^{2+} current. The inhibition of the HVA current by baclofen and 5-HT was studied in guinea-pig acutely isolated NBn using the whole-cell voltage-clamp technique. The HVA current was isolated with TTX (0.5 μM), TEA (20 mM) and 4-AP (4mM), and recorded with patch pipettes containing (in mM), 130 CsAc, 20 CsCl, 5 HEPES, 5 $MgCl_2$, 0.1 BAPTA, 3 Na_2ATP and 0.1 NaGTP. A voltage step to 0 mV for 40 ms, from a V_H of -90 mV, activated a HVA current with a peak amplitude of 1.46 ± 0.47 nA (n=9, mean ± SD). The HVA Ca^{2+} current could be dissociated pharmacologically into three components (L, N and P). The total HVA current was reduced by 20 ± 14 % (n=9) with the application of nifedipine (3-5 μM), an L-type blocker, by 38 ± 20 % (n=10) with ω-conotoxin GVIA (100 nM), an N-type blocker, and by 35 ± 20 % (n=5) with ω-aga-IVA (100 nM), a P-type blocker. The application of 5-HT (10 μM) or the $5\text{-HT}1_A$ agonist 8-OH-DPAT (10 μM) reduced the peak amplitude (at 0 mV) of HVA currents by 34 ± 8 % (n=3) and 29 ± 3 %, respectively. In the presence of ω-conotoxin GVIA (100 nM), 8-OH-DPAT (n=2) did not affect remaining HVA currents suggesting that 8-OH-DPAT reduces only the N-type Ca^{2+} current. The $GABA_B$ agonist baclofen (10 μM) produced a 33 ± 10 % (n=5) reduction of HVA currents. We are presently determining which HVA current(s) is (are) modulated by baclofen. Taken together, these results suggest that, in NBn, the HVA Ca^{2+} current is composed mainly of types L, N and P. Serotonin and GABA may selectively modulate only one component of the HVA current.
(Supported by Canadian MRC and Swiss FNRS)

701.19

SEROTONIN (5HT) MODULATES HVA CALCIUM CURRENTS IN RAT NEOCORTICAL PYRAMIDAL NEURONS. Fochring, R. C*., Dept. of Anatomy & Neurobiology, Univ. of Tenn., Memphis, TN 38163.

Neocortical pyramidal cells were acutely-dissociated from the sensorimotor cortex of rats (P7-adult) and whole cell voltage-clamp recordings were obtained. 5HT reduced HVA currents in a reversible and dose-dependent manner. In a given cell, 10-40% of the whole cell current was modulated by 5HT, with no change in voltage-dependence. The 5HT effect was mimicked by the 5HT1 agonist 5-CT and by the 5HT1A agonist R(+)-8OHDPAT. The 5HT effect was blocked by the 5HT1A antagonist NAN-190, but not by the 5HT2 antagonists mianserin or ketanserin. These data suggest that the modulation is mediated by 5HT1A receptors. Under our standard internal Ca^{2+} chelation conditions (10 mM EGTA or 20 mM BAPTA), the modulation was unaffected by 5 μM nifedipine and partially occluded by saturating doses of ω-CgTx GVIA (1 μM) or ω-AgTx IVA (100 nM). The combination of these two agents completely blocked the modulation, suggesting that N- and P-type channels were modulated. The modulation of N- and P-type current by 5HT was rapid in onset (τ of several hundred ms) and partially reversed by prepulses to +120 mV. In the presence of GTPγS, the modulation was irreversible. These data indicate the involvement of a G protein in the modulation and suggest utilization of a membrane-delimited pathway. When the internal solution contained 0.1 mM BAPTA as the only chelator, L-type current was reduced in addition to N- and P-type. This modulation appeared slower in onset than that of N- and P-type currents. Under all chelation conditions, 5HT reduced the slow tail currents generated by L-type channels in the presence of Bay K 8644. Supported by NINDS grant #R29NS27180. ω-AgTx IVA was a gift from Pfizer, Inc.

701.20

BIOPHYSICAL PROPERTIES OF PHARMACOLOGICALLY-DEFINED HVA Ca^{2+} CURRENTS IN NEOCORTEX. Lorenzon, N.M.*, and Fochring, R. C., Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163.

Neocortical pyramidal cells were acutely-dissociated from the sensorimotor cortex of rats (P7 or adult) and whole cell voltage-clamp recordings were obtained. We defined L-, N-, and P-type Ca^{2+} currents as those blocked by saturating doses of nifedipine (5μM), ω-CgTx GVIA (1 μM), and ω-AgTx IVA (100 nM), respectively. Currents were isolated by subtracting the current obtained in the presence of one of the blockers from control records. We measured: (1) The voltages at which currents were first observable and at peak current. (2) Time-to-peak current activation (at -10 mV). (3) Deactivation time constant (at -55 mV). (4) The steady-state voltage-dependence of activaton was estimated by transformation of cuurents obtained with a voltage ramp (the GHK equation was used to generate a driving force term) or from the amplitude of tail currents (at -50 mV) following steps to various potentials. The conductance vs. voltage relationship was fit by a single Boltzmann function. We found good agreement between the two methods. (5) Time constant of inactivation (at -10 mV), and (6) Percent inactivation at 2.7 s (at -10 mV). In cells from adult rats, we found no significant differences bewtween L-, N-, and P-type currents for any of these parameters. Similarly, in cells from 1 week-old animals (P6-10), we found no significant differences between the various current types. The voltage-dependence of activation and percent inactivation differed between immature and adult cells. Currents in young cells activated at voltages ~5-10 mV more positive than in adult cells and exhibited slightly more inactivation. Supported by NINDS grant #R29NS27180 (R.C.F.) and an NIMH predoctoral fellowship to N.M.L. ω-AgTx IVA was a gift from Pfizer, Inc.

EXCITATORY AMINO ACIDS: PHARMACOLOGY V

702.1

INDUCTION OR PROTECTION OF LIMBIC SEIZURES IN MICE FOLLOWING INTRACEREBRAL INJECTIONS OF METABOTROPIC GLUTAMATE RECEPTOR AGONISTS. J. P. Tizzano*, K. J. Griffey, J. Goldsworthy, S. R. Baker, and D. D. Schoepp, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140.

Metabotropic glutamate receptors (mGluRs) are a heterogeneous family of receptors coupled to multiple second messenger systems via G-proteins. We have previously described that the mGluR selective agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) induces limbic seizures in mice. In this study, other mGluR selective agonists were evaluated for effects in this model. 3,5-dihydroxyphenylglycine (DHPG), which like ACPD activates phosphoinositide (PI) coupled mGluRs (mGluR1 and/or mGluR5), was found to be about equipotent to ACPD in inducing seizures. Other mGluR agonists such as L-2-amino-4-phosphonobutanoate (L-AP4) and L-serine-O-phosphate (L-SOP) which are highly selective for the negatively-linked cAMP coupled mGluR subtypes (mGluR4, mGluR7) were inactive at doses of up to 3200 nmol. In contrast, these agonists blocked seizures induced by either ACPD or DHPG in a stereoselective manner. (2S,3S,4S)a-(carboxycyclopropyl) glycine (L-CCG I) which has some selectivity for cAMP linked mGluRs (mGluR2 and mGluR3), also protected against ACPD or DHPG at lower doses. However, higher doses of this agonist induced seizures that were qualitatively similar to ACPD or DHPG. These data suggest that induction of mGluR mediated limbic seizures in mice are a result of activating PI linked in situ mGluRs. In contrast, mGluR agonists which act to decrease cellular cAMP do not induce seizures, but can functionally antagonize the effects of other mGluR agonists which induce seizures through activation of the PI pathway.

702.2

KETAMINE-INDUCED ALTERATION OF NITRIC OXIDE SYNTHETASE ACTIVITY IN RAT CAUDATE NUCLEI. R.A. Mueller, M.D., Ph.D.*, and R.D. Hunt, B.S., Departments of Anesthesiology and Pharmacology, UNC-Chapel Hill, Chapel Hill, NC 27599-7010

It has previously been reported that pretreatment with inhibitors of nitric oxide synthetase (Type 1) (NOS) can antagonize the anesthetic effects of ketamine in rats, but the effect may be due to decreased delivery of ketamine to its site of action. In the present study in vivo NOS synthetase activity was used to assess caudate NOS during the onset, maintenance and disappearance of ketamine anesthesia.

Rats (60-120 g) given saline (2 ml/kg) or ketamine (75 mg/2 ml/kg) i.m. at zero time were used to study NOS activity in vivo 15 (onset) 30 (maintenance) or 60 (disappearance) minutes after IM saline or ketamine. Each rat received 5-10 μc of ^{3}H-arginine i.v. and as killed by decapitation 5 minutes later, with rapid removal of plasma, liver, parietal cortex, cerebellum, and caudate nuclei samples. ^{3}H-citrulline was measured by ion exchange and HPLC with all tissue values corrected for contamination of the tissue by plasma ^{3}H-citrulline calulated to be within the tissue.

Synthesis of caudate ^{3}H citrulline was increased 50% in rats that received ketamine 15 minutes previously ($p < 0.05$), but not at 30 or 60 minutes. No significant changes were noted in the cerebellum or parietal cortex.

These results suggest that after ketamine, the NOS activity is briefly increased acutely but returns to normal even though analgesia persists. NOS activity changes do not parallel the time course of anesthetic effects.

702.3

FUNDAMENTALLY DIFFERENT MECHANISM UNDERLIE THE LOCOMOTOR STIMULATORY ACTIONS OF DOPAMINE AGONISTS AND MK-801. A. Mele*, D.N. Thomas and A.Pert, Dip. Biol. Molec., Univ. Roma " La Sapienza", P.le Aldo Moro, 5, 00185, Roma and Biological Psychiatry Branch, NIMH, Blg. 10, Rm 3N212, Bethesda, MD 20892

The actions of dopamine (DA) agonists in the nucleus accumbens (Acb) are thought to be translated into locomotor activation through decreases in ventral pallidal GABA. Blockade of glutamate function in the Acb by non-competitive glutamate antagonists such as MK-801 also increases locomotor output. Some have suggested that MK-801 produces locomotor stimulation through mesolimbic DA, although support for this proposition is equivocal. For example, we have previously failed to find significant increases in Acb DA overflow by pharmacologically relevant doses of MK-801. The present behavioral studies also failed to provide support for a role of Acb DA in the stimulatory effects of this glutamate antagonist. DA depleting lesions of the Acb significantly attenuated the locomotor activating effects of amphetamine applied focally to this structure while the stimulatory actions of MK-801 were not altered. The second series of studies was directed at evaluating the role of pallidal GABA in mediating the motoric actions of DA agonists and MK-801. Using in vivo Microdialysis procedures, apomorphine was found to decrease pallidal GABA while MK-801 failed to have an effect despite producing pronounced increases in locomotor activity. Injections of muscimol (10ng) into the ventral pallidum prevented the excitatory effects of amphetamine but not MK-801. The locomotor activating effects of MK-801 do not appear to involve Acb DA nor pallidal GABA.

702.4

PHARMACO-EEG PROFILES OF SELECTIVE LIGANDS FOR DIFFERENT BINDING SITES ON THE NMDA-RECEPTOR COMPLEX. D.F. Sisson*, D.H. Snyder, J.M. Goldstein, Zeneca Pharmaceuticals Group, Wilmington, DE 19897.

Selective ligands for sites on the NMDA-receptor were administered to unanesthetized, unrestrained rats by infusion into chronic, indwelling, jugular catheters. Drugs were infused at a rate of 1 ml·hr^{-1} for four hours. Each rat received one dose of 5, 10 or 20 mg·kg^{-1}·hr^{-1} of either the competative NMDA antagonist CGS19755, the non-competative NMDA antagonist CNS1102 or the glycine-site antagonist ACEA1021. EEG activity was monitored from chronic transcranial screws implanted bilaterally over frontal and parietal cortices. CGS19755 produced a profound elevation of EEG spectral power in the 0-4 hz (delta) band starting 15 min into the infusion and reaching a plateau in 30-60 min. A further increase in delta-band power was observed at 4 hr with the 10 mg·kg^{-1}·hr^{-1} dose. Stereotypy and ataxia were observed after about 3 hr infusion at 10 mg·kg^{-1}·hr^{-1}. CNS1102 produced a profound increase in delta activity 15 min into infusion, reaching a phasic peak at 30 min and declining to a plateau by 60 min. Almost full recovery was observed by 60 min post-infusion. Ataxia and stereotypy were observed starting about 45 min into the infusion at 10 mg·kg^{-1}·hr^{-1}. The effects of ACEA1021 on EEG were subtle. Rats maintained a sleep wake cycle throughout the infusion, although sleep was somewhat disrupted in the first hour of infusion. This is reflected in a decrease in delta band power at 30 min. At a dose of 10 mg·kg^{-1}·hr^{-1}, this decrease continued for the duration of the infusion; at the higher dose, delta activity exhibited an initial decrease and then an increase. Mild ataxia was observed during infusion; no stereotypy was observed. Quantitative EEG is a useful tool not only in determining the time course of central action of NMDA ligands, but is also capable of discriminating between ligands for different sites on the NMDA-receptor complex.

702.5

ANTAGONISM OF STRIATAL QUINOLINATE TOXICITY BY L-4-CL-KYNURENINE IN VIVO. P. Guidetti*, H.-Q. Wu and R. Schwarcz. Md. Psych. Res. Ctr., Baltimore, MD 21228.

7-Cl-kynurenic acid (7-Cl-KYNA), a selective and potent antagonist of the glycine co-agonist site of the NMDA receptor, can be produced from L-4-Cl-kynurenine (4-Cl-KYN) by the action of kynurenine aminotransferase, a glial enzyme which catalyzes the biosynthesis of kynurenic acid from L-kynurenine (KYN) (J. Med. Chem. 37: 334, 1994). The anti-excitotoxic effect of 4-Cl-KYN was now studied in the rat striatum using glutamic acid decarboxylase (GAD) as a marker of neuronal death. Infusion of 120 nmol quinolinic acid (QUIN) into the striatum (over 2 hrs) resulted in a 45 ± 3% decrease in GAD in the injected striatum after one week (N=11). Combined pre- and co-infusion with a total of 135 nmol 4-Cl-KYN (over 4 hrs) prevented the QUIN-induced decrease in GAD activity. The production of 7-Cl-KYNA from 4-Cl-KYN was verified by HPLC analysis of brain extracts. 181 ± 15 pmol/mg prot 7-Cl-KYNA were recovered from a normal striatum infused with 135 nmol 4-Cl-KYN for 4 hrs (N=14). The production of 7-Cl-KYNA was *decreased* by 51% after astrocytic impairment by fluorocitrate (1 nmol/1 µl, i.str.; N=13), but was *increased* by 204% in the astrogliotic striatum, i.e. seven days after an intrastriatal QUIN (300 nmol) injection. These data indicate that astrocytes can produce neuroprotective quantities of 7-Cl-KYNA from 4-Cl-KYN in the rat striatum in vivo. Supported by a grant from the Huntington's Disease Society of America (to P.G.).

702.6

COMPETITIVE NMDA ANTAGONISTS DECREASE STRIATAL 3-METHOXYTYRAMINE LEVELS IN THE MOUSE. M.L. Carlsson*. Dept. of Pharmacol., Univ. of Göteborg, Medicinareg. 7, S-413 90 Göteborg, Sweden.

The present study was aimed at investigating the effect of the competitive N-methyl-D-aspartate (NMDA) receptor antagonists D-CPPene and CGS 19755 on mouse brain 3-methoxytyramine levels, as an indirect measure of dopamine release. In some experiments, the animals were treated with the monoamine oxidase inhibitor pargyline as to accumulate 3-methoxytyramine. The mice were sacrificed at a time point when the NMDA antagonist-induced locomotor stimulation in previous studies has been shown to be maximal. D-CPPene and CGS 19755 were found to decrease mouse striatal 3-methoxytyramine levels, whereas in general 3-methoxytyramine levels in the limbic forebrain were not significantly altered. Both D-CPPene and CGS 19755 have been observed to produce psychotic symptoms in man. The present results suggest that these symptoms are not a result of enhanced central dopaminergic transmission, but rather depend on the primary action of these compounds, ie an interference with central glutamatergic neurotransmission.

The results obtained in the present study are in agreement with observations made in rats receiving CGS 19755 (N Waters, personal communication): In vivo microdialysis experiments showed that CGS 19755 decreased extracellular dopamine levels in the striatum.

702.7

LEARNING IMPAIREMENT INDUCED BY CHRONIC INFUSION OF QUINOLINIC ACID - PROTECTION BY MEMANTINE. W. Danysz*[1], M. Misztal[1], R. K. Filipkowski[2], L. Kaczmarek[2] and J. Skangiel-Kramska[2]. Dept. Pharmacol., Merz+Co, 60318 Frankfurt /Main, Germany[1]; Nencki Institute, 02-093 Warsaw, Poland[2].

A few studies only have aimed to mimic the chronic nature of neurodegeneration, involving possibly glutamate receptors, which is believed to take place in senile dementia of the Alzheimer's type (SDAT). In the present study, NMDA agonist quinolinic acid (9 mM), was infused icv by ALZET osmotic minipump for 2 weeks (Yamada et al. 1990, Neurosci. Lett., 1118, 128-131). This treatment produced a persistent, short-term memory deficit in T-maze, developing over 2 weeks. In the hippocampus, induction of c-fos mRNA was observed 1 day, but not 1 hour and 3 or 7 days after the start of quinolinic acid infusion. Autoradiography revealed a decrease in the density of muscarinic receptors and choline uptake sites. Parallel s.c. infusion of an uncompetitive NMDA antagonist memantine (1-amino-3,5-dimethyladamantane), 20 mg/kg/day by another minipump prevented the learning deterioration induced by quinolinate. The treatment with memantine resulted in steady-state plasma levels of 1.2 µM which should assure inhibiton of NMDA receptors and is similar to levels seen in demented patients treated with this agent. Hence, if the glutamate hypothesis of dementia is accepted the neuroprotective activity of memantine in SDAT patients might be expected.

702.8

NON-AMINERGIC MECHANISMS INVOLVED IN NMDA-INDUCED MOTOR ACTIVATION IN RODENTS. E. Martínez, L. Giménez-Llort and S. Ferré*. Dept. of Neurochemistry, C.S.I.C., 08034 Barcelona, Spain.

The systemic administration of NMDA in mice induced an initial motor depression followed by a sustained dose-dependent increase in motor activity. With similar doses (25-100 mg/kg i.p.), NMDA induced a dose-dependent motor activation in reserpinized mice (reserpine 5 mg/kg s.c. 20 h prior to NMDA administration), which was counteracted with a dose of the non-competitive NMDA antagonist MK-801 which was associated with motor activation in non-reserpinized mice (0.5 mg/kg i.p.). On the other hand, MK-801 induced motor activation in reserpinized mice at very high and toxic doses (about 2 mg/kg i.p.), which were associated with motor depression in non-reserpinized mice. The NMDA-induced motor activation was still present after a stronger cathecolamine-depleting pretreatment (reserpine 10 mg/kg s.c. plus αmethyl-*p*-tyrosine 200 mg/kg s.c.). These results suggest that, instead of NMDA receptor antagonists, as it is being recently claimed in the literature, NMDA receptor agonists could counteract motor deficits in dopamine-dependent motor disorders.

A more specific and long-lasting effect of NMDA on motor activity was found when studying exploratory activity in non-convulsant rats. Fast movements (FM), slow movements (SM) and rearings (R) were analyzed during both the light and dark periods of the light-dark cycle. A single systemic administration of NMDA (100 mg/kg i.p.) produced an acute short-lasting depressant effect on FM, SM and R, followed during the next two days by a long-lasting increase in exploratory activity, only significant for FM during the dark period. These results could represent a behavioural correlate of the NMDA-induced changes in synaptic plasticity. (Supported by FISS-93/0350).

702.9

NMDA, BUT NOT AMPA, BLOCKADE REDUCES MONOSODIUM GLUTAMATE (MSG) INDUCED DAMAGE IN NEONATAL RATS. B. Milburn, J. Parks, T. Bambrick, R. Telford, D. Bannerman and M. Saari*. Neuroscience Research Unit, Nipissing University, 100 College Dr., North Bay, Ontario, Canada, P1B 8L7.

In two experiments we investigated whether NMDA and/or AMPA receptor blockade mitigates the physiological and behavioural damage associated with neonatal MSG injections in the rat. In the first experiment, Wistar rat pups received two injections on postnatal days 2, 3, 5, 7 and 9. The first injection was either saline (SAL) or MK-801 (0.1 mg/kg; sc.) while the second injection was either saline or MSG (4 g/kg; sc.). This yielded 3 treatment groups: MK-801/MSG, SAL/MSG and SAL/SAL. In the second experiment, we tested the protective effects of MK-801 and/or NBQX injection prior to MSG administration. Behavioural testing revealed the expected SAL/MSG induced learning deficit in the water maze and reduced pain sensitivity as measured by the tail flick test. Postmortem analyses revealed organ weight reductions in MSG treated rats. We conclude that MK-801, but not NBQX, protects against damage associated with neonatal MSG treatment. ACKNOWLEDGEMENT: The authors thank NOVO NORDISK for their contribution of NBQX.

702.10

MUSCLE RELAXANT ACTION OF FLUPIRTINE: EVIDENCE FOR AN INVOLVEMENT OF ALPHA$_2$ ADRENERGIC AND N-METHYL-D-ASPARTATE (NMDA) RECEPTORS? M. Schwarz[1], F. Block[1], G. Pergande[2], J. Noth[1]* [1]Department of Neurology, RWTH, 52057 Aachen, Germany; [2]ASTA Medica, 60314 Frankfurt, Germany

The aim of the present study was to characterize the pharmacological mechanisms involved in the central muscle relaxant action of flupirtine, a novel non-opioid centrally acting analgesic agent. For this purpose in urethane-chloralose anaesthetized rats the action of flupirtine was investigated on the mono-synaptic Hoffmann-(H) reflex recorded from plantar foot muscles and on polysynaptic flexor reflexes recorded from tibialis muscle. Intraperitoneal (1-10 mg/kg) and intra-thecal (33-330 nmol) administration of flupirtine de-pressed poly-synaptic flexor reflexes in a dose-dependent manner without affecting monosynaptic H-reflexes. The effect of intrathecal injection of flupirtine was prevented by co-administration of the mixed alpha$_1$-alpha$_2$ adrenergic antagonist yohimbine (10 nmol) and the excitatory amino acid N-methyl-D-aspartate (NMDA; 1nmol), but neither by co-administration of the alpha$_1$ adrenergic antagonist prazo-sin (10 nmol), the GABA$_A$ antagonist biculline (1 nmol), the GABA$_B$ antagonist phaclofen (100 nmol) and the non-NMDA agonist amino-3-hydroxy-5-tert-butyl-4-isoxazole-propionic acid (ATPA; 0.1 pmol) nor by pre-treatment with the benzodiazepine antagonist flumazenil (5 mg/kg). These observations suggest that alpha$_2$ and NMDA receptors are involved in the mediation of the muscle relaxant activity of flupirtine.

702.11

EFFECTS OF MK 801 AND FLUPIRTINE ON ISCHEMIC RETINAL DYSFUNCTION. F. Block[1], M. Schwarz[1], G. Pergande[2], S.W. Schoen*[1]; 1 Dept. of Neurology, RWTH, 52057 Aachen, Germany; 2 ASTA Medica, 6o314 Frankfurt, Germany.

Electrophysiological data suggest that flupirtine exerts a muscle relaxant effect via antagonisitc action at the NMDA receptor (Schwarz et al, present meeting). Transient occlusion of both common carotid arteries (24 minutes) in normotensive rats induces retinal ischemia as reflected by a reduction in amplitude of the b-wave of the electroretinogram (Neurosci. Lett. 144:124-126; 1992). In the present study the effect of flupirtine on the reduction in amplitude during ischemia and on the recovery during reperfusion of the b-wave was compared to that of the NMDA antagonist MK 801. The electroretinogram was recorded from pentobarbital-anesthetized rats before, during and after transient occlusion of both common carotid arteries. The drugs were administered intrapertioneally 20 minutes before or at the onset of occlusion. MK 801 (1 mg/kg) significantly reduced the depression of the b-wave during occlusion and accelerated the recovery during reperfusion when given 20 minutes before or at the onset of occlusion. Flupirtine (5 mg/kg) led to an attenuation of the reduction of the b-wave during occlusion and to an enhancement of the recovery during reperfusion at either time point of application. The present results demonstrate that the NMDA antagonist MK 801 and flupirtine which seems to interact also with the NMDA receptor provide protection against ischemic retinal dysfunction.

702.12

KETAMINE-INDUCED DISTRACTIBILITY & THOUGHT DISTURBANCE REDUCED BY HALOPERIDOL IN HEALTHY SUBJECTS. J.H. Krystal*, L. Karper, A. Bennett, A. Abi-Dargham, D.C. D'Souza, K. Morrissey, D. Abi-Saab, D.S. Charney. Dept. Psychiatry, Yale Univ. Sch. of Med., VA Medical Center, West Haven, CT 06516

The NMDA antagonist, ketamine, produces psychosis and cognitive impairments in humans. Because ketamine effects resemble endogenous psychoses, the effects of antipsychotic pre-treatment on ketamine responses might provide insights into glutamate-dopamine interactions relevant to psychosis and its treatment. This study evaluated the effects of haloperidol pretreatment on ketamine effects in healthy humans. METHODS: In an ongoing study, healthy subjects (n=8) completed 4 test days under double-blind conditions in which haloperidol (placebo or 5 mg. p.o.) was administered prior to ketamine (placebo or 0.26 mg/kg i.v. bolus & i.v. infusion of 0.65 mg/kg/hr). Behavioral ratings were made periodically during the test day. Plasma prolactin, cortisol, and HVA responses were assessed. RESULTS: Ketamine produced psychosis and "negative symptoms" on the Brief Psychiatric Rating Scale, increased distractibility on a continuous performance test, impaired learning, increased perseverative error on the Wisconsin Card Sorting Test, and produced bizarre and concrete proverb interpretations in the healthy subjects. Haloperidol had few behavioral effects on its own. However, it reduced ketamine-induced distractibility and bizarre and concrete proverb interpretations.

702.13

WITHDRAWN

GABA RECEPTORS: FUNCTION III

703.1

AN OPEN PROBABILITY ESTIMATE FOR CNS $GABA_A$ CHANNELS. M.V. Jones* and G.L. Westbrook. The Vollum Institute for Advanced Biomedical Research, Portland, OR 97201.

Inhibitory synaptic transmission in the CNS is shaped, in part, by the probability (P_O) that postsynaptic $GABA_A$ receptor channels will open once they have bound GABA. In the past, P_O has been estimated by examining the proportion of time spent open during long bursts in steady-state single channel records. (Newland et al., 1991). At synapses, however, the GABA concentration is not at steady state. Dissociation of GABA and receptor desensitization may play a role in determining time-dependent changes in P_O that shape the synaptic current. To assess P_O after pulses of GABA, we examined the variance of GABA-activated currents (by the method of Sigworth, 1980). Outside-out patches from cultured postnatal rat hippocampal neurons were voltage-clamped, and currents were activated by rapid applications of a saturating GABA concentration (10mM, 1 to 100ms duration, <1ms solution exchange time, 25°C). Sampled currents (f_c=2kHz, 100µs/point) activated rapidly (~2ms, 10-90%), showed multiphasic desensitization (τ=~20ms and ~1s) and multiphasic deactivation (τ=~10, ~50 and ~200ms). For a series of up to 100 currents in each patch, the variance of each sampled data point about its mean was calculated using local averaging of every three currents to compensate for rundown. Plots of variance (σ^2) *vs* mean (I) were fit by $\sigma^2 = iI - I^2/N$, where N is the number of independent channels and i is their apparent conductance. i, but not N, varied with potential (0.74±0.1pA (n=5), 1.6±0.3 (8), 2.9±0.8 (5) at -30,-60 and -90mV), giving a slope conductance of 30pS, close to the 26-28pS main conductance obtained by direct single channel measurements from these neurons. The mean peak current divided by the maximum current possible (I_{peak}/Ni) gave a mean P_O between 0.56 and 0.67, independent of potential. Our estimate of P_O at the peak of responses to saturating pulses of GABA is ~25% lower than that found within long bursts of steady-state single channel activity (Newland et al., 1991). These results predict that inhibitory postsynaptic currents in the CNS represent the opening of not more than ~60% of the channels that are present opposite the sites of GABA release.

Supported by NIH grants T32-DA07262 (M.V.J.) and NS26494.

703.2

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF RECOMBINANT GABAR ISOFORMS COMPRISING THE MAIN CEREBELLAR GABARS EXPRESSED IN L929 MOUSE FIBROBLAST CELLS. N.C. Saxena* and R.L. Macdonald. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI-48104.

Recent imunoprecipitation studies using mixtures of subunit-specific antibodies have provided valuable evidence for the subunit composition of GABAR isoforms in the rat cerebellum (C.I. Ragan et al. 1993. Biochem. Soc. Trans. 21:622-626). Based on their findings, three main GABAR isoforms comprise approximately 90 % of total rat cerebellar GABARs. These are $\alpha1\beta x\gamma2L$ (28% of total cerebellar GABARs), $\alpha6\beta x\gamma2L$ (36% of total cerebellar GABARs) and $\alpha6\beta x\delta$ (23% of total cerebellar GABARs). This study seeks to characterize the electrophysiological and pharmacological properties of corresponding recombinant cerebellar GABAR isoforms expressed in L929 cells transfected with $\alpha1$, $\alpha6$, $\beta3$, $\gamma2L$ and δ subunits. Transfections with all three subunit combinations yielded a high percentage of GABA-responsive cells suggesting that GABAR isoforms predicted by immunoprecipitation studies of Ragan et al do indeed constitute functional GABARs in these cells. The concentration-response (CR) curves for $\alpha1\beta3\gamma2L$, $\alpha6\beta3\gamma2L$ and $\alpha6\beta3\delta$ GABAR isoforms indicate that the substitution of the $\alpha1$ subunit for the $\alpha6$ subunit and the substitution of the γ subunit for the δ subunit decreases the EC_{50} values for GABAR isoforms. $\alpha1\beta3\gamma2L$ whole-cell currents were enhanced by diazepam and pentobarbital while the $\alpha6\beta3\gamma2L$ and $\alpha6\beta3\delta$ whole-cell currents were insensitive to diazepam but enhanced by pentobarbital. The three different GABAR isoforms displayed differences in their response to divalent and polyvalent cations and to the inverse agonist DMCM but displayed similar block by picrotoxin. Therefore, cerebellar GABAR isoforms show different electrophysiological and pharmacological properties depending on subunit composition and could offer potential therapeutic specificity in the action of drugs in the cerebellum. Single channel characteristics of these GABAR isoforms are under investigation.

703.3

γ-AMINOBUTYRIC ACID INCREASES INTRACELLULAR CALCIUM CONCENTRATION IN CULTURED RAT CORTICAL NEURONS.

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Recent reports suggest that a major inhibitory neurotransmitter, γ-aminobutyric acid (GABA) increases intracellular calcium concentration ($[Ca^{2+}]_i$) in neonatal hippocampal neurons. In this study, we examined GABA-induced Ca^{2+} mobilization in cultured rat cortical neurons by using calcium indicator, fura-2/AM. Frontal cortex was isolated from Wistar rat pups at embryonic day 18 and cultured for 5-6 days. $[Ca^{2+}]_i$ in single cells was measured with fluorescence microscope/video camera systems. GABA and $GABA_A$ agonist, muscimol, applied for 30 sec transiently, increased $[Ca^{2+}]_i$ dose-dependently. The increase was inhibited by $GABA_A$ antagonists, bicuculline and picrotoxin, but not by $GABA_B$ antagonist, phaclofen. Because the $[Ca^{2+}]i$ increase was inhibited by EGTA and a Ca^{2+}channel blocker, nifedipine, the $[Ca^{2+}]i$ increase could be explained by Ca^{2+}influx through voltage-gated Ca^{2+}channel. These results suggest that GABA increases $[Ca^{2+}]i$ chiefly through $GABA_A$-receptor and voltage-gated Ca^{2+}channel in immature cortical neurons .

703.4

CHLORIDE-DEPENDENT EXCITATORY ACTION OF γ-AMINOBUTYRIC ACID ON ISOLATED IDENTIFIED NEUROSECRETORY CELLS.

U. García*, J. Garduño, E. Becerra and H. Aréchiga º. Dept. Fisiología, Biofísica y Neurociencias, CINVESTAV, IPN México and ºDivisión de Estudios de Postgrado e Investigación, Facultad de Medicina, UNAM, México.

A sub-population of neurosecretory cells in the X organ of the crayfish was identified by using a monoclonal anti-Leu-enk antibody (Amersham). Only eight cell bodies with diameters between 35 to 50 μ located in the most superficial layer of the X organ were immunopositives. These cells may be the same found to react to an antibody against the Crustacean Hiperglycemic Hormone (Van Herp & Van Buggenum, Experientia 35: 1527, 1979).

The isolated X organ cells were kept in culture several days. GABA was applied in micropulses onto different regions of the neuron. A dose-dependent depolarization was elicited, resulting in the firing of action potentials. This effect was reversively blocked by Picrotoxin and Bicuculline and was mimmicked by Muscimol. Under voltage-clamp conditions, the reversal potential for the GABA-induced current was found at -20 mV. When chloride was substituted by methanosulfonate in the external solution, the reversal potential for the GABA-induced current was shifted to more positive values. This suggest that chloride mediates the effect of GABA on X organ cells.

This work was supported by CONACyT grant number 1402-N9206

703.5

GABA-A MEDIATED CALCIUM RESPONSE IN GT1-7 NEURONS IS DEPENDENT UPON THE CHLORIDE GRADIENT ACROSS THE MEMBRANE.

S. Yagodin*, Q-Y. Liu, L.A. Holtzclaw,J. L. Barker and J. T. Russell. LCMN, NICHD, and LN, NINDS, NIH, Bethesda, MD 20892

It is known that activation of GABA-A receptors in LHRH secreting GT1-7 neurons results in membrane depolarization and calcium entry via voltage dependent calcium channels resulting in a calcium signal. We have analysed this calcium signal using fura-2 based calcium imaging and the perforated patch-clamp technique. Instantaneous replacement of chloride ions in the medium with isethionate, resulted in a significant increase in the muscimol-induced calcium response. This increase, however, was transient and after 15 min of perfusion in zero chloride medium, the signal amplitude was only 50% of the normal chloride condition. Replacement of chloride with gluconate dramatically reduced the calcium response since gluconate chelated extracellular calcium ions. Under current clamp, muscimol application induced a depolarizing response of 26 ± 10.0 mV with a few TTX-sensitive action potentials. In the absence of external chloride, the amplitude of muscimol-induced depolarization was smaller but the frequency of spikes was increased. Muscimol-induced depolarization and calcium increase were abolished in cells treated with furosemide (0.1mM) for 20 min. Ethacrynic acid (0.3mM) also reduced the calcium signal by 70%. Depletion of intracellular calcium stores by thapsigargin (100 nM) decreased the calcium response by 60% suggesting that release from stores contributes to the GABA-A mediated cellular calcium response. The data demonstrate that a cooperative action of furosemide- and ethacrynic acid-sensitive Cl-pumps maintain outwardly directed driving force for chloride ions.

703.6

GABA-INDUCED GABA RELEASE IN CULTURED EMBRYONIC RAT THALAMIC NEURONS IS CA_o^{2+}-DEPENDENT AND TTX-INSENSITIVE.

Q.Y. Liu*, J. Vautrin, A. Schaffner, K.M. Tang, W. Ma and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bldg 36/Rm 2C02, Bethesda, M.D. 20892.

In embryonic and early postnatal stages, GABA has excitatory effects on most if not all neurons expressing functional $GABA_A$ receptor-coupled Cl^- channels. Due to a depolarizing Cl^- gradient, activation of $GABA_A$ receptor-coupled Cl^- channels depolarizes neurons, which in turn may activate voltage-dependent Ca^{2+} channels and lead to increases in cytosolic Ca^{2+} (Ca_c^{2+}). But in some cells GABA induced increase in Ca_c^{2+} does not appear to be mediated by classical Cl^- channel-coupled $GABA_A$ receptors. In culture embryonic (E17) rat thalamic neurons, we found that 10 μM GABA induced or increased the frequency of miniature synaptic-like transients (minis) superimposed on persistent Cl^- current response. The minis reversed polarity at E_{Cl}, were blocked by bicuculline and decayed with kinetics similar to GABA-activated Cl^- channels suggesting they were GABAergic. The agonist concentration that induced minis was lower than that required to activate Cl^- channels. Interestingly, the $GABA_A$ receptor/Cl^- channel agonist muscimol readily induced Cl^- current response but failed to mimic GABA in triggering minis. GABA-induced GABA release was dependent on extracellular Ca^{2+} and insensitive to 2 μM TTX. We conclude that GABA can trigger GABA transients in cultured embryonic rat thalamic neurons through an action of GABA that does not involve classical $GABA_A$ receptor/Cl^- channels.

703.7

CHARACTERIZATION OF $GABA_A$ AND $GABA_C$ RECEPTORS IN TURTLE RETINAL GANGLION CELLS.

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We recently reported that turtle retinal ganglion cells express both $GABA_A$ and $GABA_C$ receptors coupled to Cl^- channels. Experiments were carried out to further characterize the kinetic and pharmacological properties of these channels. Standard whole-cell patch clamp recordings were made from isolated, cultured turtle retinal ganglion cells. Test agents were applied by a superfusion system. Application of GABA (1~100 μM) induced a prominent inward current with two components in all ganglion cells tested. The decay of the GABA response could be perfectly fit with a single exponential time course plus a steady state. The time constant of the transient current is about 3 sec, while the steady state current is about 1000 pA. Cis-4-aminocrotonic acid (CACA, 100 μM~1 mM) activated only a sustained current, which is very similar to the steady state current induced by GABA. Bicuculline (10~100 μM) inhibited the transient but not the sustained current induced by GABA. Picrotoxin (PTX, 100 μM) totally blocked both components of GABA response. Surprisingly, when PTX was washed out, subsequent application of GABA only activated the sustained current. It took another application of GABA to induced both components. To further test if PTX blocks the two types of GABA receptors via different mechanisms, lower concentration of PTX (1~10 μM) was applied twice (interval, 2 min). It was found that the second application of PTX inhibited more transient current as compared to the first application of PTX. This is consistent with the mechanism of open channel block. On the other hand, the percentage of the sustained GABA response inhibited by PTX is constant regardless of how many times PTX was applied, which indicates that the inhibition of the sustained GABA response is mediated by some other mechanisms than open channel block. Our data suggest that the GABAA and GABAC receptors in turtle retinal ganglion cells are coupled to Cl^- channels with different kinetic and pharmacological properties. Moreover, we first report that PTX may regulate the $GABA_A$ and $GABA_C$ receptors via different mechanisms.

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703.8

SELECTIVE SUPPRESSION OF INTRINSIC BUT NOT AFFERENT FIBER SYNAPTIC TRANSMISSION BY BACLOFEN IN THE PIRIFORM CORTEX.

Akaysha C. Tang* & Michael E. Hasselmo. Dept. of Psychology, Harvard Univ., Cambridge, MA 02138.

The $GABA_B$ agonist baclofen has been shown to suppress synaptic transmission in subregions of the hippocampus and in the piriform (olfactory) cortex. Here we report a laminar selectivity of suppression of synaptic potentials in the olfactory cortex. In brain slice preparations (n=37), baclofen suppresses extracellularly recorded field potentials at the intrinsic fiber synapses proximal to the superficial pyramidal cell bodies (layer Ib) while leaving the afferent fiber synaptic potentials recorded at the distal dendrites (layer Ia) little affected. The suppression of synaptic potentials was significantly stronger in layer Ib (n>=5) than in layer Ia (n>=3) at each of the following concentrations: 0.1, 1.0, 10, 100 and 500 μM. At the concentration of 100 μM, suppression in layer Ia was 5.1 ± 6.3% (n=5) ; suppression in layer Ib was 68.8 ± 6.7 % (n=5). Suppression of intrinsic fiber synaptic transmission in layer Ib had an IC_{50} = 9.2 μM . This dose dependent selective suppression of intrinsic fiber synaptic transmission is also correlated with an increase of paired-pulse facilitation. These results suggest that afferent and intrinsic synaptic inputs may be differentially modulated by the activation of $GABA_B$ receptors and that this selective suppression is at least partially mediated via a presynaptic mechanism.

703.9

A LATE, $GABA_A$ RECEPTOR-MEDIATED, BICARBONATE-DEPENDENT, DEPOLARIZING SYNAPTIC POTENTIAL IN CA1 PYRAMIDAL CELLS K.J. Staley*, W. R. Proctor, and P. J. Whiting, Neurology and Pharmacology Depts, University of Colorado Health Sciences Center, Denver, CO 80262

In the many reports describing a depolarizing component of the $GABA_A$ receptor-mediated synaptic response in hippocampal pyramidal cells, two consistent experimental features have been 1) the use of extracellular (EC) HCO_3^- / CO_2 buffer, and 2) prolonged $GABA_A$ receptor activation.

Only a monophasic hyperpolarizing membrane potential response was seen during 4-second $GABA_A$ receptor activation by muscimol application to patch-clamped adult rat CA1 pyramidal cells when both the electrode and EC solutions were buffered with HEPES (n=12) and electrode $[Cl^-]$ was 8 mM. However, when HCO_3^- / 5% CO_2 was used to buffer the incubating and EC solutions, a large, late depolarizing component was consistently seen with onset 65 ms after the start of the hyperpolarizing response and peak amplitude of 9.4 mV at 1.8 seconds (n=7).

Monophasic responses to GABA were also seen in voltage-clamped fibroblasts expressing α_1 β_1 γ_{2L} $GABA_A$ receptor subunits when using EC HEPES buffer and electrode solutions containing $KHCO_3$ / HEPES (pH 7.2 using mM ratios 0:10, 16:36, and 32:72) and 8 mM Cl^- (n=9). No significant shifts in the reversal potential were seen during 10-second GABA applications. The ratio of HCO_3^- to Cl^- permeability calculated from the HCO_3^- effect on the GABA reversal potential was 0.6.

These results indicate that an outward HCO_3^- flux is responsible for the late depolarizing component of the $GABA_A$ synaptic response, which may be important in activity-dependent disinhibition. The kinetics of the response are not due to an interaction between the HCO_3^- anion and the $GABA_A$ receptor. Supported by NIH NS15173 and HD27827.

703.10

DEPOLARIZING AND HYPERPOLARIZING GABA RESPONSES IN THE *XENOPUS* SPINAL CORD ARE MEDIATED BY $GABA_A$ RECEPTORS. J. Rohrbough and N.C. Spitzer* Department of Biology, UCSD. La Jolla, CA 92093.

Rohon-Beard (RB) sensory neurons in the *Xenopus* spinal cord are depolarized by GABA throughout their development, unlike the majority of other GABA-sensitive CNS neurons. The GABA reversal potential (E_{Rev}) recorded intracellularly from RB cells is near -30 mV, which is not at an equilibrium potential expected for any single ion. While brief reduction in external $[Cl^-]$ was not observed to shift the E_{Rev} of whole cell responses to bath applied GABA (Rohrbough & Spitzer, 1993), subsequent Na^+ and K^+ substitutions also did not produce expected changes in E_{Rev}.

We have reexamined the Cl^- dependence of the response to iontophoretically applied GABA in voltage clamped neurons in the spinal cord. Altering either external or pipette $[Cl^-]$ in whole cell recordings shifted the GABA E_{Rev} in RB cells by 52 mV per 10-fold change in the $[Cl^-]_o/[Cl^-]_i$ ratio, consistent with dependence upon Cl^-. Bicuculline (50-100 μM) reversibly blocked 65% to 85% of the current activated by GABA. GABA responses recorded from dorsolateral interneurons for comparison had a similar Cl^- dependence and pharmacology. However, in perforated patch configuration, in which internal Cl^- is not dialysed by the pipette, the E_{Rev} for these interneurons was ~ 15 mV more negative in normal external $[Cl^-]$ than for RB cells. These results indicate that $GABA_A$ receptors mediate the GABA responses in RB cells, as well as in other classes of differentiating spinal neurons. A simple explanation of the depolarizing GABA response in intact RB cells is an elevated level of internal $[Cl^-]$, as has been proposed for other developing neurons. Interestingly, replacement of external Na^+ in perforated patch recordings from RB cells shifted E_{Rev} by as much as -10 mV, raising the possibility of cation-dependent $[Cl^-]$ modulation. We are interested in mechanism(s) contributing to this response and in the potentially excitatory role of GABA in RB cells during this period of development.

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703.11

GABA ACTIVATES TWO DISTINCT $GABA_B$ POSTSYNAPTIC RESPONSES IN CA1 PYRAMIDAL CELLS OF RAT HIPPOCAMPAL SLICES. T.M. Pham* and J.-C. Lacaille, Center for Research in Neurological Sciences and Department of Physiology, University of Montréal, Montréal, Qc, Canada H3C 3J7.

The aim of this study was to characterize pharmacologically and electrophysiologically the non-$GABA_A$ postsynaptic responses elicited by GABA in rat hippocampal CA1 pyramidal cells. GABA (5mM) and CGP11973A (L-baclofen [BAC]; 0.25mM) were applied by micropressure (drop diameter 50-100 μm) in s. radiatum to dendrites of CA1 pyramidal cells during intracellular recordings. In the presence of antagonists of GABA uptake and of $GABA_A$, NMDA and non-NMDA receptors, GABA hyperpolarized cells (-4.2 ± 0.1mV [mean ± sem], n=29) and reduced membrane resistance (n=5). The equilibrium potential (E_{rev}) of GABA responses was -90.1 ± 3.1mV (n=9). GABA responses were heterogeneous: a component was antagonized by Ba^{2+} (1mM, n=5), leaving a residual Ba^{2+}-insensitive response (amplitude 59.5 ± 5.6% of control; n=5) with an E_{rev} of -75.7 ± 3.0mV (n=3). Ba^{2+}-sensitive and insensitive components were mediated by $GABA_B$ receptors because GABA responses were totally blocked by the $GABA_B$ antagonist CGP55845A (5μM, n=7). BAC hyperpolarized cells (-3.9 ± 0.2mV, n=38) and reduced membrane resistance (n=5). BAC responses reached equilibrium at -88.4 ± 1.9mV (n=8) and were completely blocked by Ba^{2+} (n=15). GABA elicited hyperpolarizations with KCl-filled (3M, n=7) electrodes, and depolarizations with CsCl (3M, n=9) and LiCl (3M, n=7) electrodes. These results suggest that GABA activates 2 distinct postsynaptic $GABA_B$ responses coupled to G proteins in hippocampal cells: the first response, also elicited by baclofen, involves a Ba^{2+}-sensitive K^+ conductance, and the second response, only induced by GABA, is mediated by a Ba^{2+}-insensitive K^+ conductance.

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703.12

THE INHIBITION OF PRIMATE SPINOTHALAMIC TRACT NEURONS PRODUCED BY STIMULATION IN PERIAQUEDUCTAL GRAY IS REDUCED BY SPINAL BICUCULLINE. Q. Lin*, Y.B. Peng and W.D. Willis, Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77555-0843.

GABA is well established as one of the inhibitory substances in the spinal cord, involved in antinociception arising from supraspinal centers. However, it is still not clear that whether the descending inhibitory effects are mediated by $GABA_A$ or $GABA_B$ receptors at the spinal level. Experiments were performed on anesthetized young adult monkeys (Macaca fascicularis). Spinothalamic tract (STT) cells were identified by antidromic activation and recorded using a carbon filament electrode. The inhibitory effects of electrical stimulation in periaqueductal gray (PAG) on cutaneous mechanical and thermal stimuli were tested before, during and after perfusion of the spinal cord with bicuculline ($GABA_A$ antagonist) or phaclofen ($GABA_B$ antagonist) through a microdialysis fiber placed in the dorsal horn. Infusion of bicuculline or phaclofen resulted in an increase both in background activity and responses to cutaneous mechanical and thermal stimuli. PAG-induced inhibition was significantly reduced in most of cells tested during intra-spinal administration of bicuculline. The reduction was mainly on the PAG-induced inhibition of responses to noxious mechanical stimuli. In contrast, infusion of phaclofen did not significantly alter the PAG-induced inhibition. These results indicate that GABAergic inhibitory interneurons in the dorsal horn exert a tonic control of the activity of STT neurons by acting both on $GABA_A$ and $GABA_B$ receptors. Stimulation of PAG results in activation of GABAergic interneurons, which may contribute to descending antinociceptive actions via $GABA_A$ receptors in the spinal cord. This work was supported by NIH Grants NS09743 and NS11255.

703.13

STIMULUS-INDUCED INHIBITORY POSTSYNAPTIC POTENTIALS IN REGULAR-SPIKING NEURONS OF THE HUMAN ASSOCIATION NEOCORTEX IN VITRO. GGC Hwa*, A. Olivier, J-G Villemure and M. Avoli, Montreal Neurological Inst., McGill Univ., Montreal, Canada

Intracellular recordings with K-acetate-filled microelectrodes were made from regular-spiking neurons in slices of temporal and frontal neocortex that was obtained during epilepsy surgery. Electrical focal stimuli were applied within the neocortical layers. At resting membrane potential of -56 to -72 mV, single stimuli could induce: (i) an initial excitatory postsynaptic potential (EPSP), (ii) an early hyperpolarizing inhibitory postsynaptic potential ($IPSP_{early}$, peak latency 10-25 ms), (iii) an intermediate depolarizing component (peak latency 50-100 ms) and (iv) a late, hyperpolarizing IPSP ($IPSP_{late}$). Increasing the stimulus strength by small increments demonstrated that the EPSP was the first potential to appear, followed by the $IPSP_{early}$; the $IPSP_{late}$ and the depolarizing component were seen only at high-strength stimulation. Application of the NMDA antagonist APV (100μM) reduced the amplitude of both hyperpolarizing IPSPs to a greater extent than the amplitude of the initial EPSP. A response similar to that recorded in control could be seen in the presence of APV by increasing the stimulus strength. Application of CNQX (4μM) blocked the initial EPSP and also caused a reduction of the $IPSP_{early}$, depolarizing component, and $IPSP_{late}$. Under this experimental condition both $IPSP_{early}$ and depolarizing component were reduced and eventually blocked by the $GABA_A$-receptor antagonist bicuculline methiodide (10μM). These results indicate that human neocortical cells generate an inhibitory postsynaptic sequence that includes both hyperpolarizing IPSPs and a depolarizing component. We propose that as in the rat hippocampus, the latter potential might be caused by an outward-directed Cl^- movement due to the activation of $GABA_A$ receptors located on the dendrites of regular-spiking neurons. Supported by MRC of Canada.

703.14

GAMMA-HYDROXYBUTYRIC ACID INCREASES INTRACELLULAR Ca^{2+} CONCENTRATION AND NUCLEAR CRE- AND AP-1 DNA-BINDING ACTIVITIES THROUGH $GABA_B$ RECEPTORS IN CULTURED CEREBELLAR GRANULE CELLS. Y.Ito*, K.Ishige, E.Zaitsu and H.Fukuda, Dept. of Pharmacology, Coll. of Pharmacy, Nihon University, Funabashi, Chiba 274, Japan.

In primary cultures of mouse cerebellar granule cells, a brief stimulation by γ-hydroxybutyric acid (GHB, 0.1-3 mM) significantly increased intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in a concentration-dependent manner. In addition, gel-mobility assay showed that exposure of the cells to GHB also increased nuclear DNA-binding activities specific for the cAMP responsive element (CRE) and activator protein 1 (AP-1) transcriptional element. The concentration range of GHB to increase nuclear CRE- and AP-1 DNA-binding activities was the same as that of GHB to elicit an increase in $[Ca^{2+}]i$. The GHB-induced increases in $[Ca^{2+}]i$ and nuclear DNA-binding activities were antagonized by CGP 35348, a specific $GABA_B$ antagonist. In addition, thapsigargin (1 μM) suppressed GHB-induced increase in nuclear DNA-binding activities. Caffeine, which has been shown to activate Ca^{2+}-induced Ca^{2+} release store sites, increased $[Ca^{2+}]i$ in a concentration-dependent manner, and this effect was also blocked by thapsigargin. Furthermore, GHB inhibited [^{3}H]baclofen binding to cultured cerebellar granule cells and mouse cerebellar membranes. These results suggest that stimulation of the $GABA_B$ receptors by GHB activates intracellular Ca^{2+} store sites and that increased $[Ca^{2+}]i$ by releasing stored Ca^{2+} plays an important role in a increase in nuclear CRE- and AP-1 DNA-binding activities in primary cultures of cerebellar granule cells.

703.15

EVALUATION OF FLUORESCENCE AND ELECTROCHEMICAL DETECTION OF γ-AMINOBUTYRIC ACID IN MICRODIALYSIS SAMPLES

J. Kehr*, W.T.O'Connor and U. Ungerstedt. *CMA/Microdialysis and Dept. of Pharmacology, Karolinska Inst., Stockholm, Sweden.

A technique of in vivo microdialysis has been used in number of studies to characterise extracellular release and uptake of γ-aminobutyric acid (GABA) in the brain. It was shown that at least 50% of GABA overflow in the striatum of freely moving rats is of neuronal origin. Determination of GABA requires an ultrasensitive analytical method, since GABA levels in a typical microdialysis sample are often in the range of 0.1-0.5 pmol (5-50 nmol/l). Previously we developed an isocratic HPLC method based on electrochemical (EC) detection of GABA derivatized with OPA/t-butylthiol[1]. The limit of detection was 50 fmol GABA. However, the excess of thiol which is oxidised on the electrode and a high acetonitrile (50%) in the mobile phase are most probable causes of shortened life-time of the electrode, gradually increasing background signal and noise levels. The result is that a substantial portion of "research time" is spent on system maintenance.

Fluorescence detection of OPA derivatized amino acids is a more common way to determine physiological amino acid in body fluids. Normally, by using gradient elution and OPA/mercaptoethanol derivatization the limit of detection for GABA is only 0.5-1 pmol. To achieve GABA detection of 50 fmol we developed a new method based on microbore columns and optimized isocratic separation with a step gradient. The step gradient is achieved by a low pressure switching valve installed at the pump inlet. The whole system is fully automated allowing analysis of up to 80 samples/day.

Correlation between these two techniques was studied for microdialysis samples from the rat. Advantages and limitations of both methods are discussed. Ref.1. Kehr J.and Ungerstedt U., J. Neurochem. (1988) 51, 1308.

703.16

GABA$_A$-MEDIATED INHIBITION IN NEOCORTEX IS EVOKED VIA AMPA (BUT NOT NMDA) RECEPTORS ON INHIBITORY INTERNEURONS.

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Previous studies from our laboratory have suggested that recruitment of GABA$_A$-mediated inhibition is limited in the neocortex, regardless of the level of excitation. The specific mechanisms underlying this effect are unknown. Whole-cell patch recordings of layer V neurons in coronally sectioned slices (400μm) of somatosensory cortex were used to examine the excitatory amino acid receptor subtypes involved in the recruitment of evoked GABA$_A$-inhibitory postsynaptic currents (IPSCs). Stimulus-response characteristics of evoked, isolated IPSCs were recorded by holding cells at the excitatory postsynaptic current (EPSC) reversal potential (0 mV), while stimulating in either layer VI or deep white matter.

Under physiological conditions, the fast IPSC reached a maximum magnitude at a stimulus strength of approximately 1.5-2X the threshold stimulus. Modulation of NMDA-mediated transmission by exposure of slices to either magnesium-free solution or to CPP (10μM) did not affect the maximal IPSC. However, blockade of AMPA receptors with CNQX (5μM) reduced the magnitude of the maximal IPSC by >50%, with subsequent blockade or enhancement of NMDA-mediated activity having no effect. From the results of this study, we conclude that neocortical interneurons are activated by glutamatergic afferents terminating on AMPA, but not NMDA, receptors. (Supported by NS01386 and NS09534)

703.17

HETEROGENEITY OF ALLOSTERIC COUPLING IN GABA$_A$ RECEPTORS REVEALED BY α SUBUNIT SPECIFIC ANTIBODIES.

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GABA$_A$ receptors are composed of multiple binding domains which are allosterically coupled with each other in a functionally relevant manner. Differential degrees of coupling observed in different brain regions have been interpreted as the existence of receptors with diverse subunit combinations. In the present study, the subunit specificity of allosteric interaction between GABA and benzodiazepine binding sites was studied using α subunit specific antibodies. In the TX-100 soluble extract from rat whole brain, GABA$_A$ receptors immunoprecipitated with α1 antibodies had a significantly lower maximal enhancement of [^{3}H]flunitrazepam binding by GABA than those immunoprecipitated with α2 or α3 antibodies (35 +/- 2 % for α1, 75 +/- 4 % for α2 and 63 +/- 1 % for α3). On the other hand, receptors containing α1 or α3 subunits had higher affinities for the enhancement (32 +/- 10 nM and 36 +/- 20 nM, respectively) as opposed to those containing α2 subunits (142 +/- 27 nM). These differential coupling efficiencies endow unique functional and structural properties to different α subunit containing receptors in addition to the previously identified benzodiazepine pharmacology. It would be of interest to see if this heterogeneous binding profile can also be translated into the differential coupling with the channel gating domain. Supported by NIH grant NS 28772.

OPIOID RECEPTORS: TOLERANCE AND REGULATION

704.1

ALTERATION OF MU-OPIOID RECEPTORS AND G-PROTEINS IN BRAINS OF MORPHINE TOLERANT RATS.

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Previous studies detected high affinity opioid binding sites in synaptic plasmamembrane (SPM) and microsomal (MI) fractions of rat brain. Since they were found to be uncoupled from G-proteins in the latter, they were postulated to be desensitized sites. To explore this hypothesis, female Wistar rats were s.c. injected by increasing doses of morphine twice daily for 5 days. Western-blotting with specific antisera, pertussis toxin catalysed ADP-ribosylation and photoaffinity labeling studies all showed that prolonged exposure to morphine elevated the amount of G-proteins which was calculated 63% and 30% in SPM resp. MI in [^{35}S]GTPγS binding studies. The B_{max} for the μ-opioid agonist [^{3}H]DAMGO nearly doubled in MI after morphine treatment. These up-regulated sites displayed profound sensitivity to GTP unlike the μ-sites detected in MI of untreated rats. These results suggest that regulation of G-proteins represent part of the molecular changes that underlie morphine effects in brain, and further emphasize the significance of intracellular events.

Supported by OTKA-895 research grant.

704.2

REGIONAL CNS DISTRIBUTION OF MU OPIOID RECEPTOR (MOR) mRNA IN NAIVE, MORPHINE TOLERANT AND MU DEFICIENT RODENTS.

C.E. Inturrisi*, M. Brodsky, K. Elliott and A. Hynansky. Dept. of Pharmacology, Cornell U. Med. Coll., New York, NY.

The steady-state levels of MOR mRNA were measured by solution hybridization in samples microdissected from rodent brain and spinal cord. In adult male rats, a 7 day treatment with morphine (2 x 75 mg pellets, implanted sc on days 1 and 4), which produces an 11-fold shift in morphine dose-response curve, did not alter MOR mRNA levels in the dorsal horn of spinal cord (SpC), nucleus raphe magnus (NRM), periaqueductal gray (PAG), medial thalamus (Thal), hypothalamus (Hyp) or somatosensory cortex (Ctx) of treated rats as compared to placebo controls. The MOR mRNA levels ranged from 0.7 pg/ug RNA in Ctx to 15.0 pg/ug RNA in Thal. The CXBK (MU deficient) mice were found to be 7-fold less sensitive to systemic morphine than CD-1 mice, as assessed using the tailflick test (ED50 estimates were 29.4 and 4.2 mg/kg sc, respectively). CXBK mice were also found to have lower MOR mRNA levels than CD-1's in SpC (by 28%), NRM (by 43%), PAG (by 35%), Hyp (by 49%) and Thal (by 15%), but not in Ctx. The CD-1 MOR mRNA levels ranged from 0.04 pg equiv./ug RNA in Ctx to 0.52 pg equiv./ug RNA in Thal. Thus, while no change in MOR mRNA levels occurs in morphine tolerant rats, the low levels in CXBK mice may result in fewer opioid binding sites and contribute to the functional difference between CD-1 and CXBK strains. Supported by DA07274, DA01457, DA00198 and VZV Research Foundation.

704.3

CHRONIC OPIOID AGONIST TREATMENT DOES NOT ALTER DELTA OPIOID RECEPTOR (DOR) mRNA LEVELS IN THE CNS OF MICE. B. Kest*, S. Jenab, M. Brodsky, K.J. Elliott and C.E. Inturrisi. Dept. of Pharmacology, Cornell U. Med. Coll., NY, NY. 10021.

The steady-state levels of DOR mRNA were measured in male CD-1 mice rendered tolerant to the antinociceptive effects of the selective DOR opioid [D-Ala^2]Deltorphin II (DELT II) and morphine (MOR). After tid administration of MOR (10-40 mg/kg, sc) for 3 days or DELT II (10 ug, icv) for 4 days, the ED_{50} values for MOR and DELT II were increased 5- and 8-fold, respectively, relative to saline controls. DOR mRNA levels were measured by solution hybridization in samples obtained by microdissection of CNS regions of tolerant and control mice. DOR transcript levels, in pg/ug of total RNA, were highest in the caudate/putamen (0.42) and frontal cortex (0.32). Moderate levels were observed in n. accumbens (0.26), olfactory cortex (0.20), PAG (0.2), and hippocampus (0.14). The medial thalamus (0.05) and cerebellum (0.04) displayed relatively low levels of DOR mRNA. The DOR mRNA levels in the CNS regions of MOR or DELT II tolerant mice did not differ significantly from controls in any CNS region studied. These data suggest that altered steady-state DOR mRNA levels are not one of the adaptive changes observed in mice tolerant to the antinociceptive effects of MOR or DELT II. Supported by DA07274, DA01457, DA00198, the VZV Research Foundation and a grant from the Aaron Diamond Foundation.

704.4

MOLECULAR LESIONING OF OPIOID DELTA-NCX BINDING SITES IN RAT BRAIN BY ANTISENSE OLIGODEOXYNUCLEOTIDES. X.Y. Cha1, H. Xu1, K.C. Rice2, F. Porreca3, J. Lai3, and R.B. Rothman1*. 1CPS, IRP, NIDA, NIH Baltimore, MD 21224. 2LMC, NIDDK, NIH, Bethesda MD 20892. 3Department of Pharmacology, University of Arizona, Tucson AZ 85724.

Previous studies support the existence of two delta receptor subtypes called delta-cx and delta-ncx. Other studies have delineated the existence of additional subtypes termed delta-cx-1 , delta-cx-2 , delta-ncx-1 and delta-ncx-2. These sites are assayed with [3H]DADL (10 mM TRIS-HCl, pH 7.4, 100 mM NaCl, 3 mM MnCl2, 2 µM GTP and 5 mM 2-mercaptoethanol) using rat brain membranes depleted of delta-ncx sites by pretreatment with the delta-ncx-selective acylating agent, (+)-trans-SUPERFIT or depleted of delta-cx sites by pretreatment with the delta-cx-selective acylating agent, BIT. The recent cloning of a rat brain delta receptor raises the issue of the relationship between the multiple binding sites defined on the basis of our ligand binding studies and the molecularly defined delta receptor. To determine this, the antisense oligodeoxynucleotides complementary to the 5' end of the coding sequence of the cloned delta opioid receptor (nucleotides 7 to 26) and the corresponding sense oligodeoxynucleotides were administered i.c.v. twice a day for three days. Our data demonstrate that the antisense DNA selectively inhibited [3H]DADL binding to the delta-ncx site by 50%. Studies underway will determine effect of the antisense DNA on the two subtypes of the delta-ncx binding site.

704.5

Modulation of Rat Brain Opioid Receptors by the Novel Pyrrole Resorcylate, (±) RTI-14: An Autoradiographic Study. C.B. Goodman[1], B. Emilien[1-2], J.S. Partilla[1], J.L. Cadet[2], F.I. Carroll[3], B. Blough[3], K.F.A. Soliman[4*] and R.B. Rothman[1]. [1]CPS and [2]MPS, NIH/NIDA, Addiction Research Center, Baltimore, MD 21224, [3]Research Triangle Institute, Research Triangle Park, NC 27709-2194, [4]Florida A&M University, College of Pharmacy and Pharmaceutical Sciences, Tallahassee, FL 32307.

The utilization of various agents to pharmacologically manipulate the opioid receptor system has been extensively studied using several radiolabeled techniques. In the present study, in vitro receptor autoradiography was used to characterize the anatomical distribution of [125I]-DAMGO binding sites in discrete brain nuclei of rats treated chronically i.c.v. with (±)RTI-14, a novel PCP site 2 ligand with moderate amine uptake inhibition activity, and of other potent amine uptake inhibitors. ALZET 2002 osmotic minipumps (0.5 µl/hr for 13 days) were filled with saline, (±)RTI-14 (6 mg/ml), cocaine hydrochloride (5 mg/ml) or imipramine (5 mg/ml). Regional brain sections (14 µm) at the striatal level were used to assess treatment effects on the mu opioid receptors. Initial findings demonstrate that (±) RTI-14 significantly decreased [125I]-DAMGO binding distribution in each specific nuclei measured. Chronic cocaine increased [125I]-DAMGO binding in caudate patches and matrix, while the imipramine-treated animals showed no changes from controls. Studies are in progress to confirm and extend these observations.

704.6

µ OPIATE RECEPTOR: DEVELOPMENTAL PROFILE AND REGULATION. *Yasuo Imai[1], Jia-Bei Wang[1], Akiyoshi Moriwaki[1] and George R. Uhl[1,2,3*]* [1]Molecular Neurobiology, [2]Office of the Director, Intramural Research Program, NIDA, NIH and [3]Dept. of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21224

The developmental profile and regulation by morphine treatment of rat µ opiate receptor (µOR) mRNA was investigated using a ribonuclease protection assay.

µOR was most abundantly detected in thalamus, followed by hypothalamus, midbrain, and spinal cord in cortex, striatum, brainstem, hippocampus, but not in cerebellum or peripheral tissues in adult rats. Total RNA was isolated from whole brain of fetal rats of 11-20 days gestation, from cortex, mesencephalon, brainstem, cerebellum, spinal cord of neonatal rats, and from adult rat brain tissues dissected after 5 days morphine pellet s.c. implantation and/or withdrawal. µOR mRNA was detectable as early as 14 days gestation, and gradually increased to plateau levels at 2-3 months after birth in cortex or mesencephalon. No mRNA was detected in cerebellum in all periods. Preliminary data reveal modest effects of withdrawal from chronic morphine treatments.

704.7

DEVELOPMENTAL EXPRESSION OF THE DELTA OPIOID RECEPTOR IN THE MOUSE Y. Zhu*, B. Anton, M. Zheng, C. J. Evans, J. E. Pintar. Dept. Neurosci. & Cell Biol., UMDNJ Robert Wood Johnson Med. Sch., Piscataway, NJ 08854 and Dept. Psychiatry., UCLA Sch. of Med., Los Angeles, CA 90024.

To characterize the establishment of opioid systems during development, we have begun to examine the distribution of δ receptor mRNA at early postnatal stages of mouse development using in situ hybridization. In the developing nervous system, we have thus far detected restricted δ receptor gene expression as early as postnatal day 1 (p1) in the pons, caudate-putamen, and nucleus accumbens, and have localized particularly intense hybridization to a band of cells near the boundary between the superior colliculus and tegmentum. By p4 and p5, δ receptor expression has expanded to include subpopulations of neurons in the cerebral cortex (especially in the retrosplenial cortex), amygdala, hippocampus, olfactory bulb, thalamus, hypothalamus, and substantia nigra. The cellular resolution afforded by in situ hybridization has provided additional anatomical detail that complements prior receptor binding studies. In the hypothalamus at p4, for instance, δ receptor expression is detected in specific cells of the lateral hypothalamic area, in addition to the expression in the ventromedial hypothalamic nuclei similar to reported results from opioid binding studies. In addition, the pattern of high δ receptor expression in subgroups of adult CA3/CA4 hippocampal neurons is already established at early post-natal ages. Although the levels of δ mRNA/cell are significantly lower in most, if not all, cell groups than in adult brain sections hybridized equivalently, the initiation and significant increase in δ receptor expression observed in the perinatal period indicate a rapid maturation process for the δ receptor system in early postnatal development. Supported by DA-08622 and DA-05010.

704.8

PERIPHERAL µ- OPIOID RECEPTORS DURING INFLAMMATION: ENHANCED SYNTHESIS AND ACCESSIBILITY INCREASE ANTINOCICEPTIVE EFFICACY OF OPIOIDS. M. Schäfer[1], Y. Imai[2], S. Mousa[3], I. Antonijevic, G.R. Uhl[2,4,5], C. Stein[3*,1]Preclin. Pharmacol., [2]Mol. Neurobiol.,[4]Off. of the Director, ARC,NIDA,NIH,[3]Dep. of Anesth., [5]Dep. of Neuroscience, Johns Hopkins Univ., Baltimore, MD 21224

This study examined the magnitude of peripheral opioid antinociception in relation to 1) the development of inflammation; 2) µ-opioid receptor (µOR) gene expression in dorsal root ganglia (DRG);3) the number of peripheral µOR and 4)leakage of the perineurium of peripheral nerves. After Freund's adjuvant induced unilateral hindpaw inflammation in Wistar rats volume and temperature of the inoculated paws and, in parallel, antinociceptive effects of DAMGO (a µ-selective opioid agonist) increased significantly at 6 hrs, reached a maximum at 24 hrs and did not significantly change until 96 hrs. Doses of i.pl. ß-funaltrexamine, an irreversible µ-receptor antagonist, required to antagonize the antinociceptive effects (IC50 values) increased linearly up to 96 hrs of inflammation. Using a ribonuclease protection assay, µOR-mRNA levels were detectable in DRG L3-L5 of noninflamed limbs and displayed a trend towards an increase on the inflamed side by 24 hrs. Perineurial leakage, assessed by horseradish peroxidase histochemistry, was apparent both at early (12 hrs) and late stages (96 hrs) of inflammation. In conclusion, gene expression, number and accessibility of µ- opioid receptors appear to be "up-regulated" during inflammation, resulting in a greater efficacy of peripherally acting opioids.

704.9

CHRONIC MORPHINE DOWN-REGULATES μ-OPIOID RECEPTORS IN GUINEA-PIG MEDIOBASAL HYPOTHALAMUS (MBH). G. Zhang*, M. A. Bosch and M.J. Kelly. Dep. of Physiology, Oregon Health Sciences Univ., Portland, OR 97201.

Electrophysiological data have shown that chronic morphine decreases the potency and efficacy of μ-agonist DAMGO mediated hyperpolarization in the subpopulation of arcuate neurons in MBH. The effect of chronic morphine on μ-opioid receptor density in MBH was investigated in the present study. MBH were dissected from ovariectomized females implanted s.c. with morphine- or placebo-pellets (4x75 mg for 2 days plus 6 more for a total of 7 days). The P_2 membrane fraction was prepared, pre-incubated in 50 mM Tris with NaCl+GTP+ EDTA and subjected to several washings to eliminate opiates from opioid binding sites. Saturation of μ-opioid receptors was performed using [^{3}H]diprenorphine as an opioid antagonist radioligand in the presence of DPDPE and U50488 to occupy δ- and κ-receptors, respectively. $B_{max} \pm$ S.E. was 276.4 $\pm$ 16.3 fmol/mg protein in placebo animals (n=6). For morphine-treated animals (n=6), the μ receptor density was significantly ($p < 0.05$) decreased (B_{max}=216.9$\pm$8.7 fmol/mg protein) without a change of the antagonist affinity (K_d: 0.38$\pm$0.03 nM vs 0.37$\pm$0.05 nM). Preliminary data from competition of [^{3}H]diprenorphine by DAMGO showed no shift in the fraction of μ-receptors between high and low affinity states in morphine-treated animals. Solution hybridization assays are presently in progress to evaluate the μ-receptor mRNA expression in MBH. Therefore, this 20% down regulation of μ-receptors in MBH appears to contribute to the reduced efficacy of DAMGO in arcuate β-endorphin cells among morphine-tolerant neurons (supported by DA05158).

OPIOID RECEPTORS: LOCALIZATION

705.1

TOPOGRAPHICAL DISTRIBUTION & ULTRASTRUCTURAL LOCALIZATION OF δ-OPIOID RECEPTOR IMMUNOREACTIVITY IN RAT LIMBIC CORTEX: RELATIONSHIP WITH LEU^5-ENKEPHALIN. A.L. Svingos*, P.Y. Cheng, C.L. Clarke, C.E. Inturrisi, & V.M. Pickel, Cornell University Medical College, Dept. of Neurol. & Neurosci. & Dept. of Pharmacology, NY, NY, 10021

Mediation of opioid reinforcement has been associated with δ-opiate receptors in cortical-limbic structures. We utilized an antiserum shown to be specific for the δ-opiate receptor to determine whether topographical or ultrastructural localization could demonstrate a selective cellular substrate for these actions. Light microscopic examination among cortical areas revealed immunoreactivity (IR) in limbic-associated cortex to be distinguished by 1) lack of superficial laminar staining, 2) heavier deep laminar labeling and 3) sparse to moderate labeling of varicosity-laden fibers. Ultrastructural analysis of insular cortex showed δ-opiate receptor labeling localized predominantly in small unmyelinated axons and terminals and in distal processes of astrocytes. In terminals, plasma membranes and synaptic vesicles were most intensely labeled. Additionally, dendritic labeling was detected and was most prominently associated with plasma membranes, smooth endoplasmic reticulum and occasionally postsynaptic densities. In a second portion of the study we examined the anatomical relationship between the δ-opiate receptor and endogenous opioids by combining immunoperoxidase and immunogold-silver methods, for localization of antisera directed against the δ-opiate receptor and leu^5-enkephalin (ENK) in single sections. ENK labeling was principally seen in large dense core vesicles (DCV) within axon terminals. Terminals appeared more homogeneously distributed as compared to the δ-opiate receptor. In the regions thus far examined neither axon terminals nor dendrites in contact with ENK neurons have shown detectable δ-opioid IR. We conclude that in limbic cortex, δ-opiate receptors 1) are prominently presynaptic modulators and 2) may be activated by opioid peptide released more distally from DCV. Supported by DA04600, DA07274, DA01457, DA00198 and Aaron Diamond Foundation.

705.2

δ-OPIOID RECEPTOR IMMUNOREACTIVITY IN RAT SPINAL CORD: ULTRASTRUCTURAL DISTRIBUTION AND RELATION TO LEU^5-ENKEPHALIN. P.Y. Cheng, A.L. Svingos, C.L. Clarke, J. Chan*, & V.M. Pickel., Dept. of Neurol. and Neurosci., Cornell Univ. Medical College, New York, NY 10021.

δ-opioid receptors in the dorsal horn are thought to mediate the antinociceptive effects of enkephalins. We examined the ultrastructural localization of antiserum raised against a 15 amino acid fragment, from the amino terminus of the δ-opioid receptor in sections through the cervical spinal cord. By light microscopy, δ-opioid receptor-like immunoreactivity (δ-ORLI), observed using immunoperoxidase, was intensely localized to varicose processes in lamina I of the dorsal horn. Electron microscopy further demonstrated that δ-ORLI was mainly localized in unmyelinated axons and terminals. The most intense peroxidase product in axons was localized to the plasma membrane, smooth endoplasmic reticulum (ER), and synaptic vesicles. Less frequent, but equally intense peroxidase activity was selectively accumulated at postsynaptic densities and ER of dendrites. In sections dually labeled for the δ-opioid receptor (peroxidase) and Leu^5-enkephalin (immunogold), we established that the δ-opioid receptor was present in axon terminals both with and without detectable Leu^5-enkephalin-like immunoreactivity (ELI). Less commonly, the δ-opioid receptor was seen in terminals presynaptic to neurons showing ELI. We conclude that the δ-opioid receptor is mainly localized so as to modulate the presynaptic release of enkephalin and other neurotransmitters in lamina I of the spinal cord.

(supported by an Aaron Diamond Fellowship to PYC and NIDA grant DA 04600 to VMP)

705.3

STUDIES TOWARD IDENTIFYING TISSUES CONTAINING δ1 OPIOID RECEPTOR SUBTYPES. L. Fang*, I. De Leon, R. J. Knapp, E. Malatynska, E. Varga, W. Roeske, and H.I. Yamamura. University of Arizona Dept. of Pharmacology , Tucson, AZ 85724.

Delta opioid receptor heterogeneity has been well established by pharmacological studies. Recently, we cloned a human δ opioid receptor by screening human cDNA libraries. It is suggested that this human δ opioid receptor matched the δ_2 subtype, since it showed higher affinity to the δ_2 selective ligand (NTB) than that to the δ_1 selective ligand (BNTX). Other cloned mouse and rat δ opioid receptors also showed δ_2 receptor ligand binding properties. Thus, the δ_1 subtype has not been cloned in any species.

In order to obtain the δ_1 opioid receptor subtype molecular structure, one of the strategies is to find tissues containing δ_1 rather than δ_2 opioid receptor subtypes. Therefore, three interesting tissue membranes have been studied. First is the CXBK mouse brain membrane, which was suggested to have a predominant population of supraspinal δ_1 opioid receptors, rather than δ_2 opioid receptors by Raffa et al. (1992). The second is the guinea pig brain membrane, in which the δ_1 selective antagonist BNTX showed 100-fold greater affinity (Ki = 0.1 nM) for [^{3}H]DPDPE binding sites (δ_1) relative to those of [^{3}H]DSLET (δ_2) (Portoghese et al., 1992). The third is the rat olfactory bulb membrane, in which the stimulation rather than inhibition of adenylate cyclase activity was observed (Oliana and Onali, 1992). Radioligand binding studies were performed in these three tissues, and the data will be presented. Supported in part by NIDA grants.

705.4

DISTRIBUTION OF DELTA (δ)- AND MU (μ)-OPIOID RECEPTORS IN SMALL SENSORY NEURONS AND AXONS. C.N. Honda*, R.J. Dado, M. Riedl, J-H. Lee, U. Arvidsson, M.W. Wessendorf, H.H. Loh, P.-Y. Law, R. Elde. Departments of Cell Biology & Neuroanatomy and Pharmacology, University of Minnesota, Minneapolis MN 55455.

We have demonstrated previously that a population of small neurons in dorsal root ganglia as well as afferent fibers and terminals in the spinal cord are immunoreactive (ir) for delta (δ)-opioid receptors (Neuroreport 5:341,1993). Presently, we report that in part, mu (μ)-opioid receptors share a similar distribution, and that both opioid receptors are restricted to a population of sensory neurons and axons not immunoreactive for neurofilament protein.

Rabbit antisera produced against synthetic δ (DOR)- or μ (MOR)-opioid receptors were combined with a mouse monoclonal antibody (RT-97) directed against the 200 kD neurofilament protein subunit, which is a cytochemical marker for large sensory neurons with rapidly conducting axons. Dual color immunofluorescence was employed to test for the coexistence of opioid receptor- and RT-97-ir in sensory cells and axons. Within the DRG, DOR- and MOR-ir were confined almost exclusively to a population of small diameter cells and axons. In peripheral nerve, dorsal rootlets, and spinal gray matter, opioid receptor-ir was localized to small fibers. RT-97-ir was distributed to larger DRG cells and axons, and in peripheral nerve and spinal cord was found in large and small caliber fibers. Rarely did DOR- or MOR-ir coexist with RT-97-ir within the same neuronal elements, supporting the hypothesis that opioid receptors are localized to the terminals of nociceptive primary afferent neurons. Supported by NIH grant NS25658 and grants from NIDA.

705.5

THE RELATIVE DISTRIBUTIONS OF mRNAs ENCODING FOR MU AND DELTA OPIOID RECEPTORS AND FOR GLUTAMIC ACID DECARBOXYLASE (GAD) IN THE VENTRAL MESENCEPHALON. Darragh P. Devine*, Stanley J. Watson and Huda Akil. University of Michigan, Mental Health Research Institute, Ann Arbor, MI, USA 48109-0720

Radioactive *in situ* hybridization was used to characterize the distributions of mRNAs that encode for mu and delta opioid receptors, and for glutamic acid decarboxylase (GAD) in rat ventral mesencephalon. Riboprobes complementary to the mu, delta, and GAD mRNAs were labeled with ^{35}S, and applied to serial sections of rat brain. GAD mRNA was most abundant in the interpeduncular nucleus (IPN). A moderate amount of GAD mRNA was found in the Substantia Nigra pars reticulata (SNpr), and a low abundance of GAD mRNA was found throughout the Substantia Nigra pars compacta (SNpc) and Ventral Tegmental Area (VTA). Mu receptor mRNA was most abundant in the IPN, and low levels of mRNA were found in the SNpr, SNpc, and VTA. Delta opioid receptor mRNA was also most abundant in the IPN, with low levels throughout the SNpr, SNpc, and VTA. We are currently examining the possibility of cellular colocalization of these three mRNAs using double-labeled radioactive (^{35}S) and non-radioactive (digoxigenin) *in situ* hybridization.

705.6

μ OPIATE RECEPTOR: IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION. A. Moriwaki#*, P.S. Johnson@, D. Walther#, J.-B. Wang# and G.R. Uhl#@. #Molecular Neurobiology Branch, Office of the Director, Intramural Research Program/NIDA, NIH; @Pharmacology Research Associate Program, NIGMS; 1Department of Neurology and Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland 21224.

A rabbit polyclonal antiserum was raised against a hemocyanin conjugate of the C-terminal 18 amino acids of the rat μ opiate receptor. The antiserum produced high titers (1:40,000) when analyzed by ELISA, recognized purified μ receptor protein in Western immunoblots, and stained COS cells expressing the μOR cDNA. Microscopic examination of immunostained brain and spinal cord sections revealed that μ-opiate receptor immunoreactivity was present in fiber-and terminal patterns in amygdala, nucleus accumbens, globus pallidus, hypothalamus, thalamus, medial habenula, substantia nigra, ventral tegmental area, periaqueductal gray, nucleus raphe magnus, nucleus tractus solitarius, zona spongiosa and substantia gelatinosa, while little was observed in neocortex or hippocampal formation. Neural cell bodies were immunostained in areas including hypothalamus. In situ hybridization studies of brain regions including thalamus reveal hybridization over neurons which display little somatic immunoreactivity. No immunoreactivity was observed when preimmune or preadsorbed sera were used. These results directly show the distribution of the immunoreactive receptor in the rat central nervous system.

705.7

ULTRASTRUCTURAL EVIDENCE FOR PRESYNAPTIC KAPPA OPIOID RECEPTORS IN GUINEA PIG HIPPOCAMPUS. CT Drake*#, TA Patterson^, ML Simmons^, C Chavkin^, and TA Milner#. #Dept. of Neurology & Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021 and ^Dept. of Pharmacol., Univ. of Washington, Seattle WA 98195

Activation of kappa (κ) receptors diminishes excitatory transmission in the dentate gyrus and CA3 regions of the guinea pig hippocampal formation. Although many biochemical and physiological studies have suggested that κ receptor effects are mediated presynaptically, the anatomical localization of κ receptors to pre- or post-synaptic processes has not yet been reported. We examined the distribution of a polyclonal rabbit antibody raised against a peptide from the C-terminal portion of a mouse κ receptor. Specificity of the affinity-purified antibody was determined by Western blotting and immunolabeling of κ receptors expressed in *Xenopus* oocytes. The antibody was localized immunocytochemically in acrolein-fixed brain sections. Sections incubated in antibody preadsorbed with the antigenic peptide had no labeling by light or electron microscopy. By light microscopy, κ receptor-like immunoreactivity (κ-LI) was present in varicose processes in the dentate gyrus inner molecular layer, granule cell layer and hilus, and in CA3 stratum lucidum and radiatum. By electron microscopy, κ-LI was found in small unmyelinated axons and terminals, and was associated with large vesicular structures, cytoplasmic surfaces of small vesicles and plasma membranes. In the dentate gyrus, axons and terminals with κ-LI contacted large dendrites and perikarya of presumed granule cells. In hilus and in stratum radiatum of CA3, axons with κ-LI were apposed to unlabeled dendrites or other axons. In stratum lucidum, axons with κ-LI were found occasionally in bundles of mossy fibers. Thus our ultrastructural data support physiological and biochemical evidence that κ opioids modulate transmitter release from axons in guinea pig hippocampus. Supported by DA08259, DA07274, DA04123.

705.8

A MONOCLONAL ANTIBODY TO THE KAPPA$_3$ OPIATE RECEPTOR A.I. Brooks, K.M. Standifer, G.W. Pasternak.* The Cotzias Laboratory of Neuro-Oncology, Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY U.S.A. 10021

Monoclonal antibodies (mab) were raised against Be(2)-C human neuroblastoma membranes which contain μ, κ_3, and δ opiate receptors. Over 5000 hybridoma cell lines were screened, with 2000 hybridomas testing positive against the Be(2)-C membranes. Screening against another neuroblastoma line lacking opioid receptors, left 98 positive for Be(2)-C only. Several hybridomas showed high immunoreactivity to the Be(2)-C membranes, but only five of these clones, (8D8$_1$, 25G11, 15F3, 7E1, and 22H1) inhibited binding to the κ_3 opioid receptor. Mab 8D8$_1$ inhibited up to 90% of ^{3}H-NalBzoH (κ_3) binding with no effect on ^{3}H-DAMGO (μ) or ^{3}H-DPDPE (δ) binding, illustrating the selectivity of mab 8D8$_1$ for the κ_3 subtype. Mab 8D8$_1$ recognized κ_3 receptors in mouse, rat, and calf brain homogenate binding assays. In addition, mab 8D8$_1$ blocked the inhibition of cAMP accumulation by NalBzoH but not morphine. On Western blots, mab 8D8$_1$ labeled proteins consistent with the putative κ_3 receptor. Mab 8D8$_1$ also recognized a novel opioid receptor clone corresponding to the κ_3 receptor. In immunocytochemical studies the mab effectively labeled Be(2)-C cells but not cells lacking κ_3 receptors. Mab 8D8$_1$ also has been effective in labeling brain slices, demonstrating its utility in establishing the distribution of κ_3 receptors in the nervous system. Further characterization and purification of mab 8D8$_1$ is ongoing.

705.9

DELTA OPIOID RECEPTOR- AND ENKEPHALIN-IMMUNOREACTIVITY ARE SPATIALLY RELATED IN THE MOUSE CNS. I-H Lee*, TC Brelje, RJ Dado, HH Loh, P-Y Law and R Elde. Departments of Cell Biology & Neuroanatomy and Pharmacology, University of Minnesota,, Minneapolis, MN 55455

The cloning of a δ-opioid receptor (DOR) has permited the raising of antisera which recognize this protein. We previously reported that immunostaining for DOR in the dorsal horn of the spinal cord is strikingly restricted to axons and their terminals, and that these terminals are closely apposed by enkephalin-immunoreactive (-ir) boutons (Dado et al., NeuroReport 5:341-344, 1993). In the present study, we have examined the spatial relationship of DOR and ENK-ir nerve fibers in other regions of the central nervous system of mice using digitally-rendered montages produced by confocal microscopy. Nerve fibers and terminals with prominent DOR-ir were observed, for example, in the islands of Calleja and olfactory tubercle, the caudate-putamen, the globus pallidus and entopeduncular nucleus (n), amygdaloid nuclei (nn), mamillary nn, substantia nigra, interpeduncular nn, n raphe pontis, parabrachial nn, n of the solitary tract, lateral reticular n and spinal trigeminal n. DOR- and ENK-ir nerve fibers and terminals were interspersed in most of these regions, however only rarely was DOR-ir found on the same elements as ENK-ir. Thus, DOR is unlikely to be an autoreceptor. In some regions, DOR-ir was abundant in areas which did not contain ENK-ir, and vice versa. However, these regions of complementary staining were in many instances immediately adjacent to each other, suggesting that the opioids may participate in interneuronal communication over significant distances. Supported by grants from NIDA.

705.10

COLOCALIZATION OF SUBSTANCE P AND A DELTA OPIOID RECEPTOR IN THE RAT CENTRAL NERVOUS SYSTEM. M Riedl*, U Arvidsson, RJ Dado, J-H Lee, MW Wessendorf, HH Loh, P-Y Law and R Elde. Departments of Cell Biology & Neuroanatomy and Pharmacology, University of Minnesota, Minneapolis, MN 55455

Antibodies to the δ-opioid receptor (DOR) and the μ-opioid receptor (MOR) were developed and used to map DOR- and MOR- immunoreactivity (-ir) in the central nervous system (CNS) of adult mice and rats (Lee et al., this meeting; Arvidsson et al., this meeting). In the present study, these antibodies were used to examine spatial relationships between these receptors and neuropeptides and other transmitters at the cellular level. Sera from rabbits immunized against amino acids 3-17 (Dado et al., NeuroReport 5:341-344, 1993) of the predicted sequence of DOR were used in conjunction with a monoclonal antibody to substance P (SP) for two color immunofluorescent staining and analysis. A high degree of colocalization between DOR and SP was seen in axons and terminals in many regions of the brain and spinal cord. Extensive colocalization was evident, for example, in the islands of Calleja and olfactory tubercle, the shell of n accumbens, ventral pallidum, lateral septal n, amygdaloid nn, several hypothalamic nn, interpeduncular n, substantia nigra, n of the solitary tract and the spinal trigeminal n. Controls suggested that the apparent colocalization was not artifactual. Interestingly, it has been suggested that μ-opioid receptors occur on noradrenergic fibers originating from locus coeruleus. However, a high degree of colocalization was not observed for molecules which mark these systems. In summary, a surprisingly constant relationship was observed between DOR- and SP-ir in axons and terminals, suggesting a presynaptic role for this receptor in regulating the release of SP and transmitters which coexist with it, in a wide range of systems. Supported by NIDA.

706.1

RECONSTITUTION OF A PURIFIED μ OPIOID BINDING PROTEIN WITH PURE G-PROTEINS IN LIPOSOMES

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A functionally active μ opioid binding protein (OBP), purified to homogeneity from bovine striatal membranes, has been reconstituted with purified G-proteins into liposomes containing the phospholipid, phosphatidylcholine. Liposomes were formed by precipitating the lipid-protein mixture with PEG and resuspending the liposomes in buffer. Most experiments were done with a highly purified bovine brain G-protein mixture, but individual purified G_i and G_o-proteins were also found to be effective. Functional reconstitution was evaluated by measuring stimulation of the GTPase activity of G-proteins by opioid agonists and high affinity agonist binding. Low-K_m GTPase was stimulated by the μ agonists DAGO, morphine, and levorphanol (max. 100% over basal), but not by DPDPE or U50,488H. Stimulation was reversed by naloxone and both stimulation and naloxone reversal were stereospecific. The [^{3}H]-DAGO binding observed in the liposomes with purified G-protein mixture and OBP had a K_d= 8nM, an affinity slightly lower than that seen in bovine striatal membranes, and was sensitive to inhibition by GTPγS and sodium. No high affinity delta or kappa binding was observed in the reconstituted system. The evidence from these studies supports our previous results suggesting that OBP is a μ receptor. (supported by grant DA-00017 from NIDA to EJS).

706.2

INVOLVEMENT OF GUANOSINE 5'-TRIPHOSPHATE IN μ-OPIOID RECEPTOR-ACTIVATED POSTSYNAPTIC RESPONSES IN SUBSTANTIA GELATINOSA NEURONS.

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The involvement of the nucleotide 5'-triphosphates GTP and ATP in mediating μ-opioid actions on substantia gelatinosa (SG) neurons was investigated using tight-seal whole-cell recordings from superfused transverse slices of hamster spinal cord. Bath application of the μ-opioid receptor agonist [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO; 1-5 μM) caused a hyperpolarization and decrease in neuronal input resistance (R_N) in 62% of SG neurons when the electrode internal solution contained 100 μM GTP and 2 mM ATP. However, at bath concentrations up to 10 μM DAMGO had little or no effect on the transmembrane potential or R_N of SG neurons using electrodes without GTP and ATP. DAMGO-activated responses were attenuated in SG neurons after dialysis with internal solution containing 2 mM ATP but no GTP. DAMGO activated a non-reversing membrane hyperpolarization in SG neurons recorded with electrodes containing 100 μM GTP-γ-S, a non-hydrolyzable GTP analogue. SG neurons were less responsive to DAMGO following intracellular application of the analogue GDP-β-S (500 μM) which blocks G-protein activation. We conclude that intracellular GTP is necessary for μ-opioid receptor-mediated responses in SG neurons, consistent with the idea that antinociceptive effects of opioids are mediated through receptor-coupled second messenger systems involving G-proteins. The results resolve why DAMGO does not always evoke postsynaptic effects in superficial dorsal horn neurons in whole-cell recordings. *Supported by NINDS grant NS25771 (S.P.S.), NIDA grant DA04420 (A.R.L.), and NIDDK grant DK475901 (E.R. Perl).*

706.3

AGONIST-INDUCED DESENSITIZATION OF THE MU OPIOID RECEPTOR COUPLED POTASSIUM CHANNEL (KGA).

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Application of mu-opioid agonists evoked an increase in K^+ conductance in Xenopus oocytes expressing the rat mu receptor and the G-protein-gated, inwardly rectifying K^+ channel (KGA). The amplitude of the response decayed during agonist exposure with a $\tau_{1/2}$ of 8 ± 2 min. In oocytes co-expressing the mu and 5HT1A receptors, stimulation of either receptor resulted in heterologous desensitization of the subsequent response to the other. Injection of GTPγS (1 mM) increased the peak response to agonist but did not affect the rate of desensitization. Basal channel activity in the absence of agonist also desensitized at the same rate when the oocytes were clamped at -80 mV and bathed in high K^+ (96 mM) solution. The above results indicate that the desensitization of the response occurred downstream of the receptor, possibly at the channel. Response desensitization occurred at the same rate if the cells were clamped either at 0 mV or with agonist in low K^+ buffer (2 mM). The rate of desensitization was not significantly altered by any of the following treatments: removal of external Ca^{2+}, preloading the oocytes with BAPTA-AM (1-10 uM), elevation of cAMP levels, treatment with phorbol esters (1 uM), staurosporine (0.5 uM), or cytocholasin B (0.5 uM). These results suggest that desensitization requires open channels but may not involve a calcium or phosphorylation-dependent mechanism or internalization of components of the transduction pathway. Supported by DA04123.

706.4

KAPPA OPIOID RECEPTORS COUPLE TO INWARD RECTIFIER K^+ CHANNELS WHEN COEXPRESSED IN XENOPUS OOCYTES.

D.J. Henry,[1]* N.F. Lim[2], H.A. Lester[2], N. Davidson[2] and C. Chavkin.[1,3] Dept. Pharmacol[1]., Univ. of Washington, Seattle, WA and Division of Biology[2] and CNS Program,[3] California Institute of Technology, Pasadena, CA.

Xenopus oocytes expressed kappa (κ) opioid specific binding sites 5-14 days following injection of mRNA prepared from a clone of the mouse κ opioid receptor (provided by David Grandy, Vollum Inst., Portland, OR). Peak expression of κ receptor (870 fmol ^{3}H-U69,593/mg membrane protein) was observed 7 days after injection of 5 ng mRNA/oocyte. The κ receptor was previously shown to negatively couple to N-type Ca^{2+} channels. We find additionally that coinjection of κ receptor mRNA with mRNA coding for an inward rectifier channel (KGA) resulted in oocytes that expressed κ agonist-gated K^+ currents. The κ agonist U69,593 activated a large (1.0-1.5 μA) inwardly rectifying K^+ current with an EC50 of 220 nM. This agonist-gated K^+ conductance was not observed in oocytes injected with mRNA for the κ receptor or KGA channel alone, and was blocked by either 300 μM Ba^{2+} or the opioid antagonists norbinaltorphimine (1 μM) or naloxone (1 μM). When mRNAs (1-2 ng/oocyte) coding for G-protein subunits were coinjected with KGA and κ receptor mRNA, only $G_{\alpha i2}$ (but not $G_{\alpha i3}$, $G_{\alpha z}$, $G_{\alpha oA}$, $\beta_1\gamma_2$ or $\beta_2\gamma_2$) significantly potentiated the maximum response to U-69593. The acute response to U-69593 desensitized rapidly, with a $t_{½}$ of 4 minutes following peak response. Supported by DA 04123 and DA 05610.

706.5

ONTOGENY OF μ-OPIATE INHIBITION OF ADENYLATE CYCLASE AND GTP MODULATION OF μ-RECEPTOR BINDING IN RAT BRAIN.

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Signal transduction pathways play a critical role in opiate receptor function, especially after chronic exposure. To understand the consequences of perinatal opiate exposure, it is necessary to characterize the coupling of opiate receptors to their respective signal transduction pathways during development.

The present study investigated the inhibition of basal and forskolin-stimulated cAMP accumulation by DAMGO, a selective μ-agonist, in striatal membranes from 10 and 60 day old rats. The ability of GppNHp to shift striatal μ-receptors from high to low affinity at these ages was also assessed.

DAMGO (0.1-10 μM) significantly inhibited basal and forskolin-stimulated cAMP accumulation by 25%, and 22%, respectively, in 60 day old rats. In contrast, DAMGO failed to inhibit significantly cAMP accumulation in 10 day old rats. Approximately 30% of striatal μ-receptors were shifted to low affinity by GppNHp in 60 day old rats, whereas only 4% were shifted to low affinity in 10 day old rats. These data suggest that μ-receptors in 10 day old rats are not coupled as tightly to effector systems as in 60 day old rats. These ontogenetic differences in μ-receptor coupling may prevent receptor function from adapting to chronic opiate exposure, making neonates especially vulnerable to the adverse consequences of chronic administration. (Supported by DA-02739 and MH-15177)

706.6

CHP-212, A HUMAN NEUROBLASTOMA CELL LINE, EXPRESSES A HETEROGENEOUS POPULATION OF δ OPIOID RECEPTORS

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Identification of two δ opioid receptor subtypes, termed δ_1 and δ_2, has been greatly facilitated by the use of specific agonists (DPDPE and deltorphin II, respectively) and specific antagonists (BNTX and naltriben, respectively). We have recently reported that the human neuroblasoma cell line, CHP 212, expresses δ opioid receptors, but does not demonstrate binding to μ (^{3}H-DAMGO), κ_1 (^{3}H-U69,593) or κ_3 (^{3}H-NalBzoH) specific ligands (Babey, et al., 1993). Competition binding assays in this cell line raise the possibility that the δ receptor population in these cells is comprised of both δ_1 and δ_2 subtypes. The K_i values for the subtype-specific agonists and antagonists against ^{3}H-naltrindole were: DPDPE 3.45 ± 0.56; deltorphin II 1.67 ± 0.33; BNTX 0.92 ± 0.24; naltriben 0.21 ± 0.04; while the Hill coefficients were all less than one. In order to characterize the receptor proteins, ^{125}I-β-endorphin was chemically cross-linked to CHP-212 membranes using BSCOES. SDS PAGE of the covalently labeled membranes reveals several specific bands. This cell line may be a useful tool in the study of the interaction between the two δ receptor subtypes.

706.7

FUNCTIONAL EXPRESSION OF THE MOUSE DELTA OPIOID RECEPTOR IN A PITUITARY TUMOR CELL LINE. E. Piros*, P. Zaki, R.H. Edwards, C.J. Evans & T.G. Hales Depts Psychr., Neurol. & Anesth., UCLA, LA., CA. 90024

Cloning of the delta opioid receptor offers a unique opportunity to characterize its functional coupling with effector systems in different cell lineages. The rat growth hormone and prolactin secreting GH3 cell line expresses somatostatin (SRIF) receptors, G-proteins, adenylate cyclase and Ca^{2+} and K^+ channels, but lack opioid receptors, assessed by ligand binding and cAMP assays. Therefore, GH3 cells would appear to be suitable for the expression of opioid receptors and for the investigation of their coupling to second messenger systems, channels and hormone release.

A plasmid containing the entire coding region of the mouse delta opioid receptor (DOR) was transfected into GH3 cells. Stable expression of DOR was verified by immunocytochemistry and radioligand binding with ^{125}I-D-Ala^2-D-Met^5-enkephalin (DADLE). Twelve positive clones were analyzed for their ability to bind delta-specific ligands. One of these clones (GH3-DOR-22) was used in further analysis. [D-PEN 2,5]-enkephalin (DPDPE), etorphine and DADLE (1-1000 nM) all dose-dependently displaced ^{3}H-diprenorphine bound to GH3-DOR-22 cells.

The ability of the transfected delta opioid receptors to inhibit adenylate cyclase was assessed by measuring cAMP levels in GH3-DOR-22 cells. Etorphine (1-1000 nM) dose-dependently inhibited adenylate cyclase in GH3-DOR-22 cells and the effect was blocked by naloxone. cAMP levels were also reduced by SRIF (0.01-5 μM) in both control and DOR transfected GH3 cells.

The receptor's ability to modulate Ca^{2+} channels was investigated using the whole-cell voltage-clamp recording technique in both NG108-15 cells (from which DOR was cloned) and in GH3-DOR-22 cells. While both DADLE and SRIF inhibited voltage-activated Ca^{2+} channels in NG108-15 cells, neither was effective in the GH3-DOR-22 cell line. In addition, both ligands failed to modulate K^+ conductances in GH3-DOR-22 cells. It is concluded that the delta opioid receptor can couple to adenylate cyclase in transfected GH3 cells, although this cell line may not be a suitable model for studying cloned opioid receptor coupling to ion channels. (NIDA #DA-05010)

706.8

PROTEIN KINASE C INVOLVEMENT IN HOMOLOGOUS DESENSITIZATION OF OPIOID δ-RECEPTOR IN GUINEA PIG STRIATAL MEMBRANES AND *XENOPUS* OOCYTE EXPRESSION SYSTEM. H. Ueda*, T. Miyamae, N. Fukushima and Y. Misu. Dept. Pharmacol. Yokohama City Univ. Sch. of Med, Yokohama 236, Japan.

DSLET, an opioid δ-agonist showed a weaker stimulation on GTPase activities in striatal membranes of young (4-week old) guinea pigs than in mid-old (16 weeks) ones or in rat striatal ones. The responses in young guinea pigs were markedly enhanced by the addition of 0.1 μM K252a or calphostin C, protein kinase C inhibitors, but not by 0.1 μM KT5720, a protein kinase A inhibitor. On the other hand, the evoked currents by 0.1 μM DSLET through an action of Gi1α and phospholipase C in *Xenopus* oocytes injected with mouse δ-receptor (DOR1) and Gi1α RNAs (Febs Lett.(1993) **333**, 311-314) were constant upon repeated challenges of the agonist, and markedly attenuated by a bath-application of phorbol ester (10 nM, 10 min) in a protein phosphatase 2B-reversible manner. The currents by repeated challenges of higher concentration (3 μM) of δ-agonist in the oocytes injected with DOR1, Gi1α and M2 muscarinic receptor RNAs were rapidly desensitized, while there was no cross desensitization with M2-responses. Such a homologous desensitization was completely rescued by a bath-application with 2 μM calphostin C, but not with 2 μM KT5720. These results suggest that δ-opioid receptor is homologously desensitized through an action of protein kinase C.

706.9

CHARACTERIZATION OF μ AND δ OPIOID RECEPTOR BINDING DOMAINS: SENSITIVITY TO N-ETHYLMALEIMIDE. M. Shahrestanifar and R.D. Howells*. Department of Biochemistry and Molecular Biology, UMD-New Jersey Medical School and Graduate School of Biomedical Sciences, Newark, NJ 07103

It has been shown previously that opioid receptor binding is sensitive to alkylation by the sulfhydryl-specific reagent, N-ethylmaleimide (Smith and Simon, PNAS 77: 281-284, 1980). As part of a more general project aimed at identifying specific amino acid residues that contribute to the opioid receptor ligand binding site, a series of experiments have been initiated to locate the cysteine residues that are involved in the inhibition of receptor binding following treatment with N-ethylmaleimide. For these studies, human embryonic kidney 293 cells were transiently transfected with μ and δ receptor expression plasmids by the calcium phosphate method, and cell membranes were harvested 48 h later. The δ receptor expression plasmid, DOR-1, has been reported previously (Evans et al., Science 258: 1952-1955, 1992). The μ receptor expression plasmid was constructed by subcloning a 1.4 kbp *Hin*dIII fragment containing the open reading frame of the μ receptor from a 4.8 kbp cDNA insert in pBluescript (Chen et al., Mol. Pharmacol. 44: 8-12, 1993). Membranes from μ and δ receptor-transfected 293 cells bind tritiated diprenorphine and bremazocine with low nanomolar affinity and high capacity (20 pmoles/mg protein), while cells transfected with expression plasmids lacking inserts did not exhibit specific binding. When μ receptor-transfected membranes were preincubated at 37°C for 15 min with 0.5 mM N-ethylmaleimide, specific binding was reduced to 25% of controls; δ receptor binding was somewhat less sensitive. Kinetic analysis of the inhibition indicated a half-life of inactivation of 8 min. Preincubation with opioid alkaloid agonists, peptide agonists, and alkaloid antagonists prior to treatment with N-ethylmaleimide partially protected against receptor inactivation, with a rank order of potency that correlated well with competitive binding affinities. We are currently using site-directed mutagenesis to identify the relevant cysteine residues that are subject to N-ethylmaleimide alkylation. Supported by DA 05819 and the Foundation of UMDNJ.

706.10

Gi1α AND Gi2α, BUT NOT Gi3α INVOLVEMENT IN RECEPTOR-MEDIATED PHOSPHOLIPASE C ACTIVATION IN *XENOPUS* OOCYTE EXPRESSION SYSTEM. T. Miyamae*, H. Ueda, N. Fukushima and Y. Misu. Department of Pharmacol. Yokohama City Univ. Sch. of Med., Yokohama 236, Japan.

Involvement of Giα in phospholipase C activation by metabotropic receptors in *Xenopus* oocyte expression system was studied. The currents evoked by 0.1 μM DSLET, a δ-opioid agonist or by 10 μM ACh in the *Xenopus* oocytes injected with mouse δ-opioid receptor (DOR1) or M2 muscarinic receptor (M2) RNA were rapidly desensitized by repeated challenges of the agonist. Such a rapid desensitization was rescued by the addition of RNA of Gi1α and Gi2α, but not Gi3α, Goα, Gqα, G11α nor G14α. In the oocyte injected with Gi1α and DOR1 or M2, the agonist-evoked currents were constant in amplitude upon at least 6 repeated challenges and these were completely abolished by the intracellular injection of 100 pmol of EGTA or 1 pmol of inositol 1,4,5-trisphosphate. When the chloride ion-concentration was reduced to one fourth, the I-V curve (or reversal potential) was shifted to the right (from -25 to 0 mV). When AlF_4^-, an activator of universal heterotrimeric G-proteins was used instead of the receptor stimulation, the rapid desensitization and its rescue by Gi1α RNA injection as well as Gqα or G11α RNA were also observed. These results provide the evidence that α-subunits of some of pertussis toxin-sensitive G-proteins are also involved in the receptor-mediated phospholipase C activation.

706.11

EFFECT OF FORSKOLIN/IBMX AND ROLIPRAM ON ∂-OPIOID RECEPTOR mRNA LEVELS IN NEUROBLASTOMA X GLIOMA NG108-15 HYBRID CELLS. K.H. Gylys*, K. Magendzo, P. Zaki, P. Y. Law, and C.J. Evans. Dept. of Psychiatry & Biobehavioral Sciences, University of California, Los Angeles CA 90024, and Dept. of Pharmacology, University of Minnesota, Minneapolis, MN.

Forskolin, an adenylyl cyclase activator, together with the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) have previously been shown to induce cell differentiation and down-regulation of ∂-opioid receptor mRNA in NG108-15 cells (Bergsbaken et al., 1993; Magendzo et al., 1993). IBMX and other xanthine-derived PDE inhibitors are notorious for their lack of selectivity; therefore, we have compared the effects of forskolin/IBMX with the selective PDE inhibitor Rolipram on ∂-opioid receptor transcripts by Northern analysis. NG108-15 cells were exposed to drugs for varying lengths of time before harvesting and isolating total RNA. Rolipram treatment does not result in a similar down-regulation of ∂-opioid receptor mRNA; however, like the forskolin/IBMX treated cells, all transcripts were regulated in a parallel fashion. No differential regulation was observed between the previously identified multiple ∂-opioid receptor transcripts in NG108-15 cells. These results suggest that the increased selectivity of Rolipram may make it a appropriate agent to study cAMP effects in this system.

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706.12

CO-LOCALIZED OPIOID AND GLUTAMATE RECEPTORS ON ASTROGLIAL CELLS - POSSIBLE REGULATORS OF SYNAPTIC TRANSMISSION. T. Thorlin[1], P. S. Eriksson[1,2], M. Nilsson[1], E. Hansson[1,3] and L. Rönnbäck*[1,2], Institute of Neurobiology[1] and Department of Neurology[2] University of Göteborg and Department of Cell Biology, Faculty of Health Sciences, University of Linköping[3], Sweden.

Neurons and astroglial cells from the cerebral cortex in primary culture respond differentially to opioid receptor stimulation using selective agonists. Cultured neurons were shown to respond with a substantial decrease in the cytoplasmic free calcium after stimulation of μ, δ and κ receptors. On the other hand, stimulation of δ and κ receptors, on astroglial cells in culture using DADLE or U-50,488H resulted in a substantial increase in the cytoplasmic free calcium. Glutamate receptors were shown to be co-localized with opioid receptors on cultured astroglial cells. These effects were visualized through the use of the fluorescent calcium indicator fura-2. Astroglial cells in culture and acute dissociated astrocytes were identified by immunostaining with antibodies against glial fibrillary acidic protein (GFAP). The presence of δ-receptor mRNA was verified in cultured astroglial cells using RT-PCR and Northern blot. The present study suggest that cultured neurons from the cerebral cortex express μ, δ and κ-receptors. Cultured astroglial cells from the cerebral cortex express co-localized glutamate, δ- and κ-receptor. A method for acute dissociation of astrocytes and is under development to further investigate functional δ-receptors (i.e. calcium activating) to validate the relevance of the in vitro system. This might represent a novel mechanism contributing to the depressant action of opioids on synaptic transmission via decreasing the availability of calcium.

706.13

OPIOID RECEPTORS ARE DIFFERENTIALLY TARGETED IN THE NERVOUS SYSTEM AND IN A CELLULAR EXPRESSION SYSTEM. RJ Dado*, AH Nakano, S Chakrabarti, J-H Lee, M Riedl, U Arvidsson, M W Wessendorf, HH Loh, P-Y Law and R Elde, Departments of Cell Biology & Neuroanatomy and Pharmacology, University of Minnesota, Minneapolis, MN 55455

The δ-opioid receptor (DOR) that has been cloned appears to be enriched in axons and terminals in spinal cord, where it may function as a presynaptic receptor (Dado et al., NeuroReport 5:341-344, 1993). Further examination suggests that DOR may be almost exclusively targeted to axons. In contrast the μ-opioid receptor (MOR) that has been cloned appears to be targeted to the plasma membrane of dendrites and cell bodies, and, in some cases, to axons (Arvidsson et al., this meeting). Immunofluorescence studies were used to characterize this distinction more completely. COS-7 cells were electroporated with constructs which expressed DOR, MOR and the cloned κ-opioid receptor (KOR) behind a 9 amino acid epitope tag (TAG) followed by a 4 amino acid thrombin site. Cells were fixed 72 hrs after electroporation and stained with a monoclonal anti-TAG antibody. With both MORTAG- and KORTAG-transfected cells, TAG immunoreactivity (-ir) was observed in the endoplasmic reticulum, the golgi and the plasma membrane. In contrast, in DORTAG-transfected cells, TAG-ir was restricted to the endoplasmic reticulum. The targeting of these receptors in axons was examined in sciatic nerves of rats which had been previously ligated under general anesthesia. DOR- and MOR-ir accumulated both proximal and distal to ligation sites at survival times ranging from 2 - 8 hrs. Taken together these results suggest that there are consistent differences among opioid receptors in their targeting. Supported by NIDA.

706.14

NUCLEAR MATRIX OPIOID BINDING IS ABOLISHED UNDER DESENSITIZATION CONDITIONS. M.M. Belcheva[+], S. Gucker[§], D.-M. Chuang[§], W.G. Clark[+], R.J. McHale[+], G. Toth[#] A. Borsodi[#], C.J. Coscia[+*], [+]Dept. Biochem. & Mol. Biol., St. Louis Univ. Sch. Med., St. Louis, MO, [§]Biol. Psych. Branch, NIMH, Bethesda, MD, [#]Biol. Res. Center, Szeged, Hungary.

Opioid binding sites were found in nuclear matrix preparations from NG108-15 neurohybrid cells. The opioid antagonist, ^{3}H-naltrindole, and ^{3}H-diprenorphine, display high affinity binding, while agonists bind with low affinity, if at all, to nuclear matrix preparations. Gpp(NH)p insensitivity of agonist binding and the absence of adenylyl cyclase activity suggest the occurrence of G protein uncoupled opioid sites in this fraction. Opioid inhibition of basal and forskolin-stimulated adenylyl cyclase activity was found in nuclear membrane preparations. *In vitro* preincubation with protein kinase A or desensitization of cells with etorphine elicited similar binding alterations in each of four subcellular fractions. Both treatments abolished nuclear matrix opioid binding, without affecting antagonist binding densities in membranes sedimenting at 20,000 g (P_{20}) or nuclear membrane preparations. A decrease in agonist binding affinities and densities in P_{20} fractions was also observed under these conditions, whereas treatment of cells with forskolin potentiated both P_{20} and nuclear matrix binding. Taken together our results support the hypothesis that nuclear membrane opioid receptors are newly synthesized molecules enroute to the cell surface, whereas nuclear matrix contains internalized δ-sites.

706.15

PROPERTIES OF A KAPPA OPIOID RECEPTOR EXPRESSED IN CHO CELLS: ABILITY TO ACTIVATE MULTIPLE G-PROTEINS IS SIMILAR TO OTHER OPIOID RECEPTORS. P.L. Prather*, T.M. McGinn, P.A. Claude, L. Y. Liu-Chen+, H.H. Loh and P.Y. Law. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455 and +Dept. of Pharmacology, Temple Univ., Philadelphia, PA 19140.

The purpose of the present study was to determine if the coupling pattern of a recently cloned κ-opioid receptor stably transfected in CHO cells to individual G_α subunits was different than that observed previously for δ- and μ-opioid receptors. Data presented in the current study indicate the successful stable expression of a κ-opioid receptor in CHO cells. This is supported by experiments which revealed that ligands with selectivity for κ-, but not δ- or μ-opioid receptors demonstrated high affinity for the expressed receptor and were able to potently and efficaciously produce inhibition of adenylyl cyclase activity. In addition, only κ-opioid agonists were able to induce dose-dependent increases in the incorporation of [^{32}P]-azidoanilido-GTP into four G_α subunits, three of which were identified as $Gi3_\alpha$, $Gi2_\alpha$ and $Go2_\alpha$. Further, the amount of κ-opioid agonists required to induce 50% maximal labeling of any individual G_α subunit was not different. Although κ-opioid agonists produced equivalent maximal labeling of $Gi3_\alpha$, $Gi2_\alpha$ and $Go2_\alpha$, significantly less agonist-induced labeling was observed for an unknown G-protein designated as $G?_\alpha$. These results are similar to that observed previously for both δ- and μ-opioid receptors and suggest that although all opioid receptors interact with multiple G-proteins, this coupling is not selective for any individual G_α subunit.

706.16

KAPPA OPIOID INHIBITION OF STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IS NOT MEDIATED BY A DECREASE IN INTRACELLULAR CALCIUM. Dennis Paul*, Lerna Minor and Naili Duan. Department of Pharmacology and Centers for Neuroscience and Alcohol and Drug Abuse, LSU Medical Center, New Orleans, LA 70112.

Kappa opioid agonists close N-type Ca^{2+} channels and attenuate stimulated phosphoinositide (PI) hydrolysis in neural tissue. PI hydrolysis is Ca^{2+} dependent. Accordingly, we assessed the possibility that the kappa attenuation of stimulated PI hydrolysis is due to reduced intracellular Ca^{2+}. Rat hippocampal slices labelled with ^{3}H-myoinositol were incubated with or without the Ca^{2+} ionophores, BayK 8644 (10 μM) or A23187 (10 μM). These doses of ionophores blocked the nifedipine induced attenuation of PI hydrolysis. KCl (27.5 μM) or NE (30 μM) were added to stimulate PI hydrolysis. In addition, various concentrations of the kappa agonist U-50,488 were added. One hour later, the assays were stopped and PI hydrolysis was measured as the ratio of dpm released / dpm incorportated. U-50,488 dose dependently attenuated KCl-, NE- and carbachol stimulated PI hydrolysis. Assurance of high intracellular Ca^{2+} levels with BayK 8644 or A23187 did not affect kappa agonist attenuation of stimulated PI hydrolysis. Additional studies assessed kappa agonist effects on heart ventricle slices, a tissue in which kappa agonists increase intracellular Ca^{2+}. EKC and U-50,488 inhibited NE-stimulated PI hydrolysis dose-dependently. Together, these results provide evidence that kappa agonists do not attenuate PI hydrolysis by reducing intracellular Ca^{2+}.

706.17

IDENTIFICATION OF TRANSCRIPTS ENCODING OPIOID RECEPTORS IN IMMUNE CELLS. Claire Gavériaux-Ruff, Frédéric Simonin, Katia Befort and Brigitte Kieffer*, ESBS, Univ. Louis Pasteur, 67 Strasbourg, France.

Numerous in vivo and in vitro studies have shown that opioids modulate the immune response. A direct effect of opioids on immune cells has been postulated, however pharmacological studies to demonstrate the existence of opioid receptors on these cells have been poorly convincing. We, and others, recently isolated cDNAs encoding neuronal opioid delta, mu and kappa receptors. The cloning of these receptors has provided molecular tools for the identification of the expression of opioid receptors in immune cells. We designed opioid receptor subtype-specific primers, based on the known sequence of the cloned receptors, and used Reverse Transcriptase-Polymerase Chain Reaction followed by Southern hybridization, for the detection of opioid receptor transcripts. We have tested both mouse and human cells representative of the different components of the immune system. Our results indicate that kappa receptor mRNA is undetectable in several mouse T cell lines. We found low level of delta receptor mRNA in some human T and monocyte cell lines. In all the human B cell lines tested, substantial amounts of kappa receptor transcript, as well as low level of delta and mu receptor transcripts are present. Our data suggest that the cloned mu, delta and kappa receptor subtypes are not only present in nervous tissues, but also are expressed in immune cells.

706.18

OPIOID-DEPENDENT TYROSINE PHOSPHORYLATION IN NEURONS. D. A. Mangoura*. Department of Pediatrics, The University of Chicago Chicago, IL 60637.

In primary neuronal cultures derived from 6-day-old chick embryo cerebral hemispheres (E6CH) met-enkephalin elicits a transient activation of phospholipase D (PLD), which precedes a 2-fold increase in PKC-ε activity. Opioid-dependent PLD activation is blocked by the tyrosine kinase (TK) inhibitor herbimycin A, strongly indicating that the mechanism of opioid-dependent PLD activation involves tyrosine phosphorylation. Western blot analysis and immunoprecipitations with anti-phosphotyrosine antibodies revealed that 10^{-7}M met-enkephalin caused increases in protein tyrosine phosphorylation in E6CH. Among the reactive protein bands, three proteins of Mr 170K, 125K and 35K had the highest level of phosphorylation. Immunocytochemical analysis with anti-phosphotyrosine antibodies revealed that neurons are stained throughout their cell body, processes, and growth cones and the bases of filopodia in particular. In an effort to identify these TK substrates we have initiated studies on a tyrosine kinase, localized in focal adhesions, termed FAK. FAK is tyrosine-phosphorylated by neuropeptides that act through G protein-linked receptors. In our studies we compared abundance and level of tyrosine phosphorylation between E6CH neuronal cultures and total tissue of corresponding age, using immunoprecipitation with the 2A7 mAb to FAK. Neuronal cultures were enriched in FAK (>3fold by blot densitometry). In addition, immuno-reactivity for FAK was present in all cell bodies, neurites, varicosities and growth cones, in a homogeneous way. Following post fixation permeabilization, the nuclei were no longer visible, indicating a general cytosolic localization of FAK. These data suggest that opioid peptides are linked to tyrosine kinase and possibly FAK activation (supported by HD 09402).

707.1

PERIPHERAL EFFECTS OF NALOXONE IN MICE WITH INTESTINAL INFLAMMATION. O. Pol and M.M. Puig*. Anesthesiology Research Unit, IMIM, Universidad Autónoma de Barcelona, Barcelona, Spain, E-08003.

The aim of the present study is to evaluate the effects of opioid antagonists on gastrointestinal (GI) transit in mice with diarrhoea associated with intestinal inflammation.

Diarrhoea was induced by p.o. administration of croton oil (CO), and demonstrated by weight loss ($P < .01$), and increased GI transit ($P < .05$) when compared to animals treated with saline (SS). The presence of inflammation in CO, was demonstrated by electron microscopy. GI transit was evaluated with a charcoal meal used as a marker. Administration of 0.1 mg/kg i.p. naloxone (NX) or NX-methiodide, induced a significant increase ($P < .05$) in GI transit in CO but not in SS animals. The inactive enantiomer (+)NX had no effect either in CO or SS animals. The delta antagonist naltrindole (3 mg/kg) also induced an increase in transit ($P < .01$) in CO animals, while MR-2266 (kappa) had no effect.

Our results suggest that CO diarrhoea induces a local release of endogenous opiates (EO) which play an inhibitory role in the physiological response to intestinal inflammation in mice. It is postulated that the EO´s released, could be a mu or delta agonist.

707.2

EVIDENCE FOR ENDOGENOUS OPIOID-MEDIATED INHIBITION OF MEDULLARY NEURONS THAT MODULATE NOCICEPTIVE TRANSMISSION. Z.Z. Pan* and H.L. Fields. Dept. of Neurology & Physiology, UCSF, San Francisco, CA 94143.

There are significant numbers of endogenous opioid-containing cell bodies and terminals in the rostral ventromedial medulla (RVM) and the midbrain periaqueductal gray (PAG) which control nociceptive transmission at the spinal cord. Five-barrel micropipettes were used for extracellular recording of the RVM neurons and iontophoresis. On-cells (cells that increase firing just prior to tail flick (TF)) and Off-cells (cells that pause in firing before TF) were identified in the RVM in lightly anesthetized rats *in vivo*. Microinjection of bicuculline (30-40ng) into ventrolateral PAG inhibited >95% of the spontaneous firing of the ON-cell and blocked the TF. Iontophoresis of naloxone at current levels (20-30nA) which blocked the inhibition of the On-cell firing induced by iontophoretic morphine (20-30nA), consistently reversed most of the inhibitory effect on the On-cell firing produced by bicuculline or morphine (5μg) microinjected into the PAG. Naloxone was ineffective in the inhibition of the On-cell firing induced by iontophoretic clonidine (10-20nA), an α_2 adrenoceptor antagonist. The Off-cell firing was increased to >190% of control by PAG bicuculline and was not affected by iontophoretic naloxone. These results suggest that activation of the PAG neurons (probably through disinhibition by bicuculline) that produces antinociception could elicit the release of an endogenous opioid peptide(s) (most likely enkephalin) in the RVM and that the released opioid peptide(s) selectively inhibits a specific class of RVM neurons (On-cells) which has been shown to receive enkephalin-immunoreactive inputs and has a putative permissive or facilitating action on spinal nociceptive transmission. Supported by NIDA grant 1949 to HLF.

707.3

ACTIVATION OF BRAIN OPIOIDERGIC STRUCTURES IMPROVES ADAPTATION TO CHRONIC EXERCISE STRESS IN MICE

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Injection of opioids, direct electrical stimulation of the periaqueductal grey substance and transcranial electrical stimulation (TES-"electroanalgesia") increase velocity of nerve regeneration and wound healing in rats (Ilyinsky et. al.'85;86;89). Naloxone blocked the effects. Recently we demonstrated that TES improved the response to another type of stress: acute exercise stress (Mechavkin et al.'93). In this study, we investigated the effects of TES on exhaustive swim performance after chronic exercise training, consisting of 21 days of swimming 30 min/day. 72 mice (BALB/c) were divided into: Group A (n=25) received 21 days of swim training and 3 days of TES (0.6mA, 30 min) prior to a swim to exhaustion, Group B (n=23) received training and sham stimulation and Group C (n=24) received training only. The results demonstrated that swim times for mice receiving TES was 143.3±12.8 min vs. sham - 100.1±7.0 ($p<0.01$), vs. control - 80.3±4.1 ($p<0.001$). So, TES improved the adaptive response to chronic exercise stress. Further work revealed that the timing of TES, influenced the results. Mice (n=23) that received the last TES 30 hrs prior to swim, swam shorter than those receiving TES 6 hours prior to swim but longer than sham (n=24) or control (n=23) mice ($p<0.01$). Difference in swim time between sham and control, which existed after 6 hrs ($p<0.01$), disappeared after 30 hrs Naloxone (0.5 mg/kg ip., prior to TES) blocked the effects of TES. We concluded that brain opioidergic structures participate in the adaptation to chronic exercise training.

707.4

SPINAL CORD AND BRAIN OPIATE RECEPTORS: DIFFERENTIAL EFFECTS ON LARGE-FIBER VERSUS SMALL-FIBER SOMATOSENSORY PATHWAYS. A.D. Legatt*, C.E. Schroeder, I.B. Hollinger, and L.P. Seimon. Departments of Neurology, Neuroscience, Anesthesiology, and Orthopedic Surgery, Montefiore Medical Center and the Albert Einstein College of Medicine, Bronx, New York, 10467.

During spinal instrumentation and fusion surgery for scoliosis, morphine sulfate may be injected into the spinal subarachnoid space to provide postoperative analgesia, and bolus doses of intravenous narcotics may be given as part of the anesthesia regimen. Somatosensory evoked potentials (SEPs) to posterior tibial nerve stimulation are monitored to detect spinal cord compromise during this operation. We examined the effects of the narcotics on the cortical SEPs and on the SEPs recorded over the neck. The latter, often called "cervical SEPs", are generated near the cervicomedullary junction.

Bolus doses of intravenous narcotics did not alter the cervical SEPs, but did cause transient waveshape changes and attenuation of the cortical SEPs. In contrast, intrathecal narcotics affected neither the cortical nor the cervical SEPs, though they did produce analgesia.

These SEPs predominantly reflect activity in the large-fiber (dorsal column/lemniscal) somatosensory pathways. The differential narcotic effects on the cortical SEPs demonstrates that the large-fiber afferent somatosensory pathways are influenced by opiate receptors in the brain but not by those in the spinal cord. Since intrathecal narcotics produce analgesia, spinal cord opiate receptors affect the small-fiber (pain), but not the large-fiber, somatosensory pathways within the spinal cord.

707.5

ULTRASTRUCTURE OF DYNORPHIN IMMUNOREACTIVE PERIKARYA AND AXON TERMINALS IN THE NUCLEUS ACCUMBENS: RELATIONS TO DOPAMINE AND SUBSTANCE P. E.J. Van Bockstaele*, K.N. Gracy and V.M. Pickel., Dept. of Neurol. and Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Dynorphin (DYN) facilitates conditioned place aversion and reduces locomotor activity through mechanisms potentially involving direct activation of target neurons or presynaptic release of catecholamines or substance P (SP) in the nucleus accumbens (Acb). We examined the ultrastructural substrates underlying these actions by combining, in single sections of rat tissue, immunoperoxidase and gold-silver labeling for dynorphin 1-8 and either the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH) or SP. DYN-labeled perikarya were spherical in shape, contained unindented nuclei and were closely apposed to other perikarya and dendrites, some of which also contained DYN immunoreactivity. Smooth endoplasmic reticulum and coated vesicles could also be identified in the cytoplasms on either side of the apposed membranes. The DYN neurons received afferent input from unlabeled as well as DYN and/or SP labeled terminals. Terminals containing one or both peptides were similar in their content of small and large dense core vesicles and their prominent formation of symmetric synaptic specializations. TH-labeled terminals formed primarily symmetric synapses with small dendrites and spines. In some cases, single dendrites were postsynaptic to both DYN and TH terminals. Additionally, presynaptic interactions were suggested by close appositions between DYN terminals (n= 370) and other terminals that were unlabeled (41%), TH-labeled (10%), DYN-labeled (14%) or SP-labeled (11%). We conclude that DYN neurons may (1) communicate with other neurons through non-synaptic appositional contacts, (2) are most likely inhibited by synaptic input from terminals containing DYN and/or SP and (3) may interact presynaptically through contacts with axons either containing catecholamines or SP. Supported by NARSAD to EJVB and MH 40342 and DA 04600 to VMP.

707.6

REGULATION OF DYNORPHIN (DYN), TYROSINE HYDROXYLASE (TH) AND [Met^5]ENKEPHALIN (ME) IN NEURONAL/GLIAL CO-CULTURES FROM RAT HYPOTHALAMUS G.-C. Wu*, M.K. McMillian and J.-S. Hong
LMIN/NIEHS/NIH, RTP, NC 27709

Hypothalamic neurons contain the highest concentration of dynorphin in the brain and this neuropeptide may be an important modulator of feeding behavior as well as contribute to regulation of pituitary hormone secretion. Using a mixed hypothalamic cell culture model (confluent glia from PN7 pups, neurons from E16 fetuses), we have examined the second messenger pathways involved in regulation of DYN, and have compared DYN responses to changes in TH-immunopositive (TH+) cell number and to ME release. Elevating cAMP with forskolin (FSK,10^{-6} M) increased DYN levels and also increased TH+ cell number. Dexamethasone (DEX,10^{-7} M) increased dynorphin production, but did not affect ME release or TH+ cell number. FSK+DEX produced a near additive effect on DYN, but had a more pronounced effect on ME release (previously shown to be glia-derived). Depolarization with KCl (45 mM) more effectively increased TH+ cell number than DYN, as did activation of PKC with phorbol ester (10^{-7} M). DYN is regulated differently than TH and ME in these hypothalamic cultures.

707.7

DEVELOPMENT OF OPIOID TOLERANCE IN THE nTS IS ASSOCIATED WITH GENERAL CHANGES IN NEURONAL RESPONSIVENESS. C.J. Malanga*, W.W. Fleming and D.A. Taylor. Dept. of Pharmacology, West Virginia University School of Medicine, Morgantown, WV 26506-9223.

Chronic morphine exposure results in non-specific changes in the sensitivity of guinea-pig nucleus tractus solitarius (nTS) neurons to inhibitory agonists. While a 5.6-fold rightward shift of the dose-response curve to muscimol was observed, no significant shift of the dose-response curve to morphine was seen with cumulative drug applications. One explanation for the lack of altered sensitivity to morphine is acute desensitization, which can be avoided by application of single drug concentrations to spontaneously-active nTS neurons. Using standard extracellular electrophysiological techniques in brainstem slice preparations from vehicle- and morphine-pellet implanted guinea pigs, application of morphine (1 μM) resulted in an 80±7% inhibition of firing in control and a 47±13% inhibition of firing in chronically morphine-exposed neurons, indicating a significant ($P<0.05$) 41% reduction in response to morphine in the morphine-treated group. Similarly, application of muscimol (0.3 μM) to the same nTS neurons inhibited firing 57±11% in controls and 30±8% in morphine-treated preparations, indicating a significant ($P<0.05$) 47% reduction in response to muscimol in morphine-treated animals. Furthermore, elevation of extracellular K^+ (normal aCSF=5.0 mM $[K^+]_O$; depolarizing aCSF=7.28 mM $[K^+]_O$) resulted in an excitation of 58±13% in control neurons and an excitation of 146±47% in neurons from the morphine-treated group. Together, these findings suggest that 1) the *in vitro* nTS/brainstem slice preparation is an appropriate model system in which to study the development of opioid tolerance, and 2) the development of opioid tolerance in the nTS is associated with a non-specific, general change in the physiology of these neurons. Supported by PHS grant DA 03773.

707.8

OPIATES SUPPRESS FORSKOLIN-INDUCED EXCITATION OF GABAERGIC NEURONS IN THE MEDIAL SEPTUM/DIAGONAL BAND OF BROCA (MS/DBB).

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This study explored the electrophysiological actions of opiates in the MS/DBB as receptor autoradiography and in situ hybridization studies show that the MS/DBB is rich in the μ type of opiate receptor and its mRNA (Mansour et al, 1988, 1993). Since, opiates have been shown to inhibit adenylate cyclase in various brain regions, this study focussed on interactions between opiates and the cAMP pathway. In extracellular recordings, the opioid peptide met-enkephalin (ENK) decreased spontaneous firing and blocked forskolin (an activator of adenylate cyclase)-induced excitation in a sub-population of MS/DBB neurons. In intracellular recordings from cholinergic and non-cholinergic type cells (Griffith, 1988), forskolin (10 μM) or 8-Br-cAMP (2 mM) increased the number of bicucculine-sensitive, depolarizing synaptic potentials (i.e. reverse GABAergic IPSPs) seen with KCl-containing electrodes. ENK decreased the forskolin-induced IPSPs in a reversible manner. A subpopulation of MS/DBB neurons were directly excited by forskolin and 8-Br-cAMP; these cells most likely represent the GABAergic neurons that give rise to IPSPs in both cholinergic and non-cholinergic cells.

These results show that opiates inhibit forskolin-induced excitation of GABAergic neurons in the MS/DBB, presumably via inhibition of adenylate cylase.

707.9

PROOPIOMELANOCORTIN EXPRESSION IS REDUCED BY AN ANTISENSE OLIGONUCLEOTIDE

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Gene expression in mammalian cells can be suppressed by oligonucleotides complementary to the target mRNA. This strategy was explored as means of arresting translation of the prohormone precursor proopiomelanocortin (POMC). The synthesis of the POMC-derived peptides adrenocorticotropin (ACTH) and β-endorphin (β-END) was markedly reduced by an oligodeoxynucleotide (ODN) complementary to a region of β-END mRNA in AtT-20 cells, which retain many of the differentiated phenotypes of corticotrophs; this treatment did not affect the steady-state levels of POMC mRNA. Antisense ODN was stable in cell culture medium for 24 h and cellular uptake was low (≈2.5% of the added ODN); however, the intracellular levels of the ODN were sufficient to form a ribonuclease-resistant duplex with complementary cellular mRNA. Addition of ODN to the cell culture did not affect cell viability and proliferation. Microinfusion of the antisense ODN in the rat hypothalamic arcuate nucleus (0.625 μg/h/60 h) , where the majority of POMC-positive brain perikarya are located, significantly reduced ACTH-immunopositive neurons in the medio-basal hypothalamus (1430±87 vs 2590±90 in vehicle-treated rats, $p<0.01$; $n=7$) and antisense ODN-treated rats showed substantially less of the grooming behavior usually observed in a novel environment. Changes of nociceptive threshold, body temperature and of plasma levels of anterior pituitary hormones in rats exposed to antisense oligonucleotide will be presented.

707.10

NORADRENERGIC DRIVE ON OXYTOCIN NEURONES DURING NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL. N.P.Murphy, §C.H.Brown, §G.J.Munro, G.Leng, R.G.Dyer*, §J.A.Russell. Dept. Neurobiology, Babraham Institute, Cambridge, CB2 4AT, U.K. §Dept. Physiology, Medical School, University of Edinburgh, Edinburgh EH8 9AG, U.K.

Oxytocin neurones in the supraoptic nucleus (SON) develop dependence on morphine given intracerebroventricularly (i.c.v) over five days (up to 5μg/h) as revealed by naloxone (NLX) precipitated morphine withdrawal which increases oxytocin neurone firing rate by 3.5 fold. We have studied the importance of the noradrenergic input for the expression of this withdrawal excitation. (1) NLX (5mg/kg) precipitated withdrawal-induced Fos expression in tyrosine hydroxylase neurones in the nucleus tractus solitarii. These neurones are known to project to the SON and mediate the response of oxytocin neurones to systemic cholecystokinin-8S (CCK). (2) Electrophysiological extracellular recording was carried out in urethane anaesthetized rats. Oxytocin neurones were identified by firing pattern, antidromic activation and response to CCK. The excitatory response to i.v. CCK injection was similar before (mean±S.D.: 1.06±0.39 Hz increase, over 5 mins) and after NLX in morphine-infused rats (1.18±0.46 Hz, n=11), though smaller than in morphine naive animals. (3) Clonidine (2.5 mg/kg i.v.), an α_2 adrenoceptor agonist, attenuated the rise in oxytocin secretion after withdrawal (mean±S.E.M., vehicle: 702±186 pg/ml vs. + clonidine: 303±113 pg/ml after 10mins, n=7, $p<0.05$, one-way t-test). (4) Electrical activity in oxytocin neurones after withdrawal was reduced by both acute (25μg) and continuous (0.32 to 1.62 μg/min for >20 min) administration of i.c.v. α_1 adrenoceptor antagonists such as benoxathian. This effect could be reversed by simultaneous infusion of the α_1 adrenoceptor agonist phenylephrine. These results indicate that an excitatory, or permissive, noradrenergic input to oxytocin neurones drives the expression of opiate withdrawal-induced excitation.

707.11

THE OPIOID-INDUCED CURRENT IN RAT LOCUS COERULEUS (LC): ELECTROTONIC CONSIDERATIONS. R.A. Travagli, M. Wessendorf, T.V. Dunwiddie and J.T. Williams*. Vollum Institute, OHSU, Portland, OR 97201.

We utilized whole cell and intracellular recordings from slices of rat brainstem to study the effects of opioids on LC cells. The amplitude of the [met^5] enkephalin-induced outward current (I_{ME}) was larger and E_{ME} was more negative in slices cut in the horizontal than in the coronal plane. Morphological analysis with neurobiotin indicated that cells from horizontal slices had a more extensive dendritic arborization than from the coronal plane. When the pipettes were filled with Cs gluconate, I_{ME} was still outward, did not reverse polarity even at strongly negative potentials and was blocked by $BaCl_2$. When recordings were made from 2 neurones, $BaCl_2$ and TTX induced synchronous oscillations ion membrane potential. LC basic properties were not affected by carbenoxolone, a gap junction blocker which reduced I_{ME}, shifted the reversal potential to the right and prevented the Ba-induced oscillations. The results suggest that I_{ME} is mediated by an increase in gK only. Deviation from Nernst predictions for K^+ result from poor space clamp which may be due to electrotonic coupling among LC cells.

707.12

PAIN-PRODUCING SUBSTANCES AUGMENT I_h IN NODOSE GANGLION NEURONS. S. L. Ingram*[1] and J. T. Williams[2], Department of Pharmacology[1] and Vollum Institute[2], Oregon Health Sciences University, Portland OR 97201.

Our previous work in the nodose ganglion has shown opioid inhibition of forskolin-stimulated I_h in a small subpopulation of nodose neurons. Pain-producing substances which are known to increase cAMP levels (PGE_2, bradykinin, and 5-HT) also elicit an inward current (at -60 mV) in a subpopulation of neurons. The mean amplitude of the inward currents are as follows: PGE_2 (1μM), 120 ± 39 pA (n = 4); bradykinin (1μM), 263 ± 88 pA (n=2); and 5-HT (10μM), 122 ± 12 pA (n =5). Cs^+(2 mM) blocked the currents in 7/7 neurons regardless of drug applied. Thus, we have preliminary evidence that agents other than forskolin are effective in augmenting I_h. In an attempt to further identify responsive neurons on the basis of conduction velocities, intracellular recordings from guinea pig isolated nodose ganglion preparations were made. In 75% of neurons with conduction velocities <1.5 m/s (n = 28), only 35% expressed an I_h current. In contrast, 83% of the neurons with conduction velocities >1.5 m/s had an I_h current. These results suggest that Aδ neurons may be regulated by both pain-producing substances and opioids through opposite actions on adenylyl cyclase. (Supported by NIDA Grants DA00141, DA08163, and DA07262.)

707.13

INTERACTION OF MK-801 WITH THE DEVELOPMENT OF MORPHINE TOLERANCE AND DEPENDENCE IN THE ISOLATED SPINAL CORD OF THE NEONATAL RAT. J.A. Bell* and C.L. Beglan. Neuroimaging and Drug Action Section, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD 21224.

Chronic co-treatment of rats with opioids and MK-801 (MK) inhibits the development of tolerance and physical dependence in intact animals. The mechanism of this phenomenon is obscure. Use of the isolated spinal cord from neonatal rat eliminated the potential interference from behavioral effects. Newborn rats were treated with either morphine (M) or MK + M in incrementally increasing doses for 3 days. The spinal cord was set up in a superfusion bath. Nociceptive reflexes elicited by electrical stimulation or capsaicin were inhibited by M (10 nM - 10 μM) in controls (18 ± 10.5% S.E.M. depression of capsaicin response at 10 nM to 81 ± 3.4% at 10 μM). In cords of neonates treated with chronic M, additional doses of M did not depress nociceptive reflexes indicating the development of tolerance. Combination of MK (0.3 mg/kg) with M did not inhibit the development of tolerance. The development of dependence evidenced by increases in naloxone-induced spontaneous activity was not affected by co-treatment with MK. Thus, MK combined with chronic treatment with M failed to block the development of tolerance and physical dependence in this model.

We conclude that the spinal cord has to be mature and/or act in synergy at least with the periphery (spinal) for MK inhibition of development of tolerance and physical dependence to be realized.

707.14

NEUROPROTECTIVE ACTIVITY OF THE SELECTIVE δ-OPIOID SNC-80 *IN VITRO*. F. Tortella*, K. Klette, S. Calderon[1], K. Rice[1] and M. DeCoster. Walter Reed Army Inst. Res., Washington, DC 20307 and [1]LMC, NIH, Bethesda, MD 20892.

SNC-80 is a new, highly selective non-peptide δ-opioid (Calderon et.al., J. Med. Chem. In Press, 1994). Unlike other peptide and non-peptide opioids, high i.c.v. doses of SNC-80 (up to 800 μg) fail to elicit EEG seizures or convulsant activity in rats. In contrast, SNC-80 causes a complex behavioral response consisting of initial, brief periods of stupor followed by prolonged episodes of intense hyperactivity associated with marked EEG theta-driving (unpublished observations). We have begun to assess the effects of SNC-80 in a neuronal culture model of CNS excitotoxicity. In the present study SNC-80 was added to primary rat cortical cultures immediately prior to 80 μM glutamate. Neurotoxicity and/or neuroprotection was assessed 18 h later. LDH measurements and morphological evaluation revealed that SNC-80 produced a concentration dependent neuroprotection. Compared to glutamate alone, 5, 20, and 40 μM SNC-80 protected neurons to the extent of 16, 30, and 60%, respectively. Intracellular calcium ($[Ca^{2+}]_i$) was monitored as an indicator of glutamate-induced neuronal injury in these same cultures. While glutamate consistently produced a sustained increase in $[Ca^{2+}]_i$ in 83% of the neurons analyzed, treatment with SNC-80 (50-100 μM) resulted in a shift in the $[Ca^{2+}]_i$ dynamics such that greater than 80% of the neurons demonstrated only biphasic or transient increases in $[Ca^{2+}]_i$, an event previously shown to correlate with less toxic concentrations of glutamate (DeCoster et.al., NeuroReport, 1992). These results demonstrate that SNC-80 is neuroprotective *in vitro* and suggest a potential utility of selective δ-opioids for attenuating CNS injury.

707.15

EFFECTS OF DAMGO, NALOXONE AND ACETYLCHOLINE ON THE FIRING OF MEDIAL PREFRONTAL CORTICAL NEURONS. J. L. Giacchino* and S. J. Henriksen. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Extracellular recordings were made from fifty-six spontaneously firing neurons of the medial prefrontal cortex (mPFC) in halothane-anesthetized rats. Areas investigated include the dorsal anterior cingulate, prelimbic, and infralimbic components of the prefrontal cortex. Previous work in our laboratory demonstrated that systemic morphine decreases neuronal activity in the mPFC, and that this effect can be reversed by systemic naloxone. As the mPFC is suggested to function in reinforcement pathways and drug-related behaviors in the rat, the action of opiates, specific opioid receptor agonists and antagonists at the cellular level is of interest. Microiontophoretic application of the selective mu agonist DAMGO (1mM) decreased firing of most mPFC neurons studied. This drug effect was typically slow in onset and often prolonged (approx. 3-10 min). This effect could be blocked or reversed by naloxone (2mM). Iontophoresis of naloxone alone had no effect on most mPFC neurons. As expected, acetylcholine (1M) increased firing in the majority of spontaneously active mPFC cells. In addition, ACH pulses elicited transient increases in cell firing during DAMGO-induced depression, and also during concurrent application with naloxone. Even when mPFC neuronal firing was completely suppressed following DAMGO, ACH was able to elicit firing, implying a receptor specific effect. These results suggest that activation of mu receptors may hyperpolarize mPFC neurons and/or may function presynaptically to inhibit excitation (Supported by SDAC #3-5-94271 and NIDA #3-5-94219)

707.16

DISTRIBUTION OF DYNORPHIN mRNA IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. P. Cianchetta, D.E. Lennard, D. Bronstein & I. Merchenthaler*. LMIN, NIEHS, NIH, RTP, NC 27709.

The distribution of dynorphin mRNA in brain and spinal cord of adult male rats was studied with *in situ* hybridization histochemistry. Intact or colchicine-treated animals were either (a) perfused with paraformaldehyde (PF) and sectioned with a vibratome or (b) frozen on dry ice, cut on a cryostat, thaw-mounted, and post-fixed with PF. Free-floating vibratome sections and mounted slides were treated with triethanolamine, then dehydrated with ethanol. While vibratome sections were rehydrated, cryosections were airdried after dehydration. Sections were hybridized with a ^{35}S-UTP-labeled probe (bp 100-835 of dynorphin cDNA), incubated overnight at 55 C and stringently washed to remove nonspecific label. Subsequently, vibratome sections were mounted on gelatin coated slides. Slides were dipped in liquid photographic emulsion and exposed for ten to twenty days. Evaluation of autoradiograms revealed that the location of dynorphin mRNA was in good agreement with the location of the peptide detected by immunocyto- chemistry. However, we were able to detect mRNA in several regions of the central nervous system in which no peptide was reported. These include the olfactory bulb, the island of Calleja, posterior thalamic nuclei, CA1 and CA2 regions of the hippocampus, optic tectum, spinal trigeminal nucleus, etc. In addition, the number of mRNA-containing perikarya was higher then the number of peptide-containing perikarya previously observed in cerebral cortex. The use of 30 μm vibratome sections resulted in a shorter exposure time and less background than the use of 16 μm cryosections. Colchicine-treatment had no significant effect on the level of dynorphin mRNA.

707.17

HILAR MEDIATED EXCITATION AND LTP OF THE DENTATE GRANULE CELL. GW Terman*[1], ML Simmons[2] and C Chavkin[2]. Departments of Anesthesiology[1] and Pharmacology[2], University of Washington, Seattle, WA 98195.

Kappa agonists inhibit excitatory neurotransmission and long-term potentiation (LTP) in the hippocampus via a decrease in amino acid release from both hippocampal perforant path and mossy fiber terminals. We now report a third site of kappa opioid inhibition of excitatory neurotransmission in the hilar region of the dentate gyrus.

Extracellular recordings were made in the granule cell layer of the dentate gyrus of the guinea pig hippocampal slice. The hilar pathway was selectively stimulated by making a knife cut through the molecular layer to sever the perforant path and placing the stimulating electrode in the molecular layer across the cut from the recording electrode.

In the presence of bicuculline (10μM), stimulation (0.3 μs, 50-300μA) of the hilar pathway elicited granule cell population spikes whose amplitudes were decreased by the kappa$_1$ agonist, U69593 (1μM) in a norbinaltorphimine (100nM) reversible manner. LTP in this pathway was blocked by the NMDA antagonist, APV (25μM), and by U69593. Without bicuculline, no population spikes were elicited from the hilar path and no LTP could be induced. However, following LTP, population spikes were evident with or without bicuculline.

Thus kappa opioids modulate neural excitability in at least three distinct regions of the hippocampus. Although the hilar pathway studied here is normally under strong GABA$_A$ control, plastic changes appear capable of overriding GABA inhibition - perhaps leading to hyperexcitable states. Supported by DA04123 and the Foundation for Anesthesia Education and Research with a grant from Abbott Labs.

708.1

IMPLICATION OF CCK IN THE POTENTIATION OF THE NMDA RESPONSE INDUCED BY SIGMA LIGANDS IN THE RAT DORSAL HIPPOCAMPUS.
N. Lavoie*, B. Gronier and G. Debonnel. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada, H3A 1A1.

Sigma (σ) ligands modulate the response of pyramidal neurons to NMDA in the CA_3 region of the dorsal hippocampus. It has also been found that CCK is involved in the effects induced by σ ligands on colonic motility. The present experiments were undertaken to determine if this interaction is also present in the CNS. Using five-barrelled glass micropipettes for extracellular recordings and microiontophoresis, we evaluated the effects of different CCK_A and CCK_B receptor antagonists on the modulation of the NMDA response, induced by the intravenous administration of low doses of the σ ligands DTG, (+)pentazocine and JO-1784 on rat CA_3 dorsal hippocampus pyramidal neurons. The potentiation of the NMDA response induced by these σ ligands was abolished by the specific CCK_A receptor antagonist SR 27897, but not by the CCK_B antagonist CI-988. CCK-8S, applied with a low current, insufficient to induce an increase of the firing activity by itself, markedly potentiated the response of NMDA, without affecting that of QUIS. SR 27897 applied during the potentiation of the NMDA response by CCK-8S, markedly reduced this potentiation. In contrast, the selective CCK_B receptor antagonits CI-988 applied microiontophoretically, and PD 135158 administered intravenously (3mg/kg), failed to abolish the effect of CCK-8S on the NMDA response.

Our results suggest that the potentiation of the NMDA response of hippocampal pyramidal neuron by σ ligands involves the activation of CCK_A receptors. Since CCK-8S, by itself, potentiates markedly and selectively the NMDA response, these results also suggest that, in the CA_3 region, σ ligands might induce their modulatory effect on the NMDA response by promoting the release of CCK-8S.

708.2

NEUROMODULATORY EFFECTS OF SIGMA LIGANDS IN THE RAT NUCLEUS ACCUMBENS. G. Debonnel* and B. Gronier. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada, H3A 1A1.

The present study was undertaken to investigate *in vivo* the effects of sigma (σ) ligands on neuronal firing activity in the dorsomedial part of the rat nucleus accumbens. Using extracellular recording and microiontophoresis, the effects of applications, or intravenous (i.v.) administration, of the selective σ ligands (+)pentazocine and JO-1784, on the neuronal firing activity induced by NMDA, were assessed. Microiontophoretic application, as well as i.v. administration (20 μg/kg) of JO-1784 had no significant effect on the spontaneous firing activity, but produced a marked increase of the activation induced by NMDA. This potentiating effect was reversed by the i.v. administration of the selective σ antagonist JO-5220 (150 μg/kg). (+)Pentazocine, administered i.v. at 10 or 30 μg/kg, failed to modify significantly the NMDA-induced excitation. Since the nucleus accumbens receives a rich dopaminergic (DA) innervation, we have also explored the effects JO-1784 on the DA modulation of the NMDA response. Microiontophoretic applications of low currents of DA significantly reduced the excitatory response of accumbens neurons to NMDA. JO-1784, simultanously applied with DA, enhanced significantly its inhibitory effect.

These findings suggest that the σ ligand JO-1784 exert a modulatory effect on NMDA-induced activation in the rat nucleus accumbens, in keeping with our previous observations in the CA_3 region of the dorsal hippocampus. The lack of effect of (+)pentazocine is in contrast with our results in the dorsal hippocampus and constitutes another argument suggesting that (+)pentazocine acts on a different subtype of σ receptor than JO-1784, not present in the nucleus accumbens. In addition, the fact that JO-1784 increases the neuronal sensitivity to DA in rat nucleus accumbens neurons suggests the existence of a functionnal interaction between the σ and DA receptors.

708.3

ENDOGENOUS DYNORPHIN RELEASE IN THE GUINEA PIG DENTATE MOLECULAR LAYER IS MEDIATED BY L-TYPE CALCIUM CHANNELS.
M. L. Simmons*[1], G. W. Terman[2], S. M. Gibbs[1], and C. Chavkin[1]. Depts. of Pharmacology[1] and Anesthesiology[2], University of Washington, Seattle, WA 98195.

In the dentate molecular layer, dynorphins are released from granule cell dendrites and inhibit glutamate release via kappa1 opioid receptors on perforant path afferents. In the CA3 region, dynorphins are released from, and act upon, mossy fiber terminals. In both regions, dynorphins also have been shown to inhibit the induction of LTP. The present study investigated the roles of Ca^{2+} channel subtypes in mediating the release of endogenous dynorphins. High frequency stimulation was applied to granule cells to induce dynorphin release, which was detected as a >20% depression of the field EPSP that lasted 2-15 minutes. In the dentate molecular layer, the L-type Ca^{2+} channel blockers nifedipine (10 μM) and PN200-110 (5 μM) inhibited the dynorphin-mediated depression. These drugs did not alter the effect of kappa receptor activation by exogenous agonist (1 μM U69593) application, nor did they affect the field EPSP responses themselves. Therefore, the L-type channel blockers inhibited dynorphin release without inhibiting the action of dynorphin or the release of glutamate. Furthermore, nifedipine and PN200-110 facilitated the induction of LTP in the dentate gyrus. This result is consistent with our previous studies showing that the kappa opioid receptor antagonist, norbinaltorphimine, also facilitates the induction of LTP. In contrast to the effects observed in the dentate gyrus, the L-type channel blockers had no effect on dynorphin release from mossy fibers in CA3. Supported by DA04123.

708.4

ENDOGENOUS OPIOID PEPTIDES MEDIATE THE SUCKLING INDUCED PROLACTIN INCREASE IN POST-PARTUM FEMALE RATS. S. Klosterman, D. Prunty, P. Callahan and J. Janik*, Miami University, Dept of Zoology, Oxford, Ohio 45056.

We have reported that β-endorphin is a potent stimulus for prolactin secretion in virgin female rats (Kehoe, et al., Neuroendo 57:875, 1993). Doses as low as 25 ng were capable of producing a prolactin secretory response that mimicked the prolactin response to suckling in post-partum female rats. In addition, antiserum to β-endorphin totally abolished the suckling-induced Prolactin increase in post partum, lactating female rats. The purpose of this study was to determine whether or not endogenous opioid peptides (EOP) from the enkephalin or dynorphin family were involved in the regulation of Prolactin secretion during lactation.

Lactating, female, Sprague-Dawley rats between days 8-12 post-partum were used in all experiments. Animals were surgically implanted with chronic intraventricular (ivt) cannula into the lateral ventricle on day 2 post-partum. After at least a 5 day recovery period and one day prior to the experiment, animals were implanted with chronic jugular cannula to facilitate blood withdrawal. On the day of the experiment, dams were separated from their pups for six hours prior to ivt administration of specific antisera to met-enkephalin or dynorphin. Blood samples were withdrawn immediately prior to antiserum injection and at 15 minute intervals, up to 1 hour, after the onset of suckling.

Antiserum to each one of the EOP effectively blocked the suckling-induced Prolactin increase. These results indicate that met-enkephalin and dynorphin are both important in the suckling-induced Prolactin increase. The mechanism(s) responsible for this action is not known. It is also not clear which receptor subtype mediates this response or if there is interaction among the different EOP and/or their receptor subtypes. (This work was supported by NIH grant # 1 R15 HD30375-01 to JJ and PC).

708.5

EFFECTS OF MORPHINE ON LIVER ORNITHINE DECARBOXYLASE (ODC) ACTIVITY AND PLASMA INSULIN AND GLUCOSE LEVELS IN RAT PUPS. B. Alicke, M.B. Bartolome and J.V. Bartolome*. Department of Pharmacology, Duke University, Durham, N.C. 27710

We have previously reported that central, but not peripheral, administration of β-endorphin (BE) to infant rats markedly decreases basal levels of ODC (a key growth regulatory enzyme) activity throughout the body, as well as tissue ODC responsiveness to classical trophic factors. Based on these and other observations we have hypothesized that endogenous CNS BE plays a prime role in controlling postnatal development. The question remains as to whether perinatal exposure to exogenous opiates causes a pattern of developmental effects similar to those produced by BE.

In the current study we found that, like BE, intracerebroventricular (i.c.v.) administration of 2 ug of morphine to 6-day-old rats decreased basal levels of liver ODC activity. In addition, hepatic ODC responsiveness to subcutaneously (s.c.) administered insulin (I) or growth hormone, two important trophic hormones, was almost totally arrested in pups pretreated i.c.v. with morphine. Also similar to BE, i.c.v. morphine increased the half-life of exogenously administered I. As expected, hypoglycemia in rats injected with morphine i.c.v. plus I s.c. was more marked than in pups given I alone. Equivalent results were obtained in animals given morphine s.c.

The data obtained suggest that the developmental disabilities commonly observed in infants born to opiate-addicted mothers could result, at least in part, from an exacerbation of the effects of endogenous opioids on tissue ODC expression and on insulin/glucose metabolism. (Supported by USPHS Grant NS25738)

708.6

MORPHINE TOLERANCE/DEPENDENCE: REVERSAL FROM INHIBITION TO ENHANCEMENT OF cAMP FORMATION. **L. Wang* and A.R. Gintzler. Dept. of Biochemistry, SUNY Hlth. Sci. Ctr. at Brooklyn, N.Y. 11203**

This laboratory has previously demonstrated that sufentanil can produce a naloxone-reversible increase or decrease in the stimulated formation of cAMP in the myenteric plexus, depending on the concentration of opioid employed. Low doses of opioid (10^{-10} M) enhance whereas higher concentrations (10^{-6} M) inhibit the magnitude of cAMP formation. On the basis of these results, we suggested that μ-opiate receptors are positively as well as negatively coupled to adenylyl cyclase. In the present study, the effect of chronic *in vivo* morphine exposure on the balance between opioid excitatory and inhibitory actions was investigated. In chronically morphine-treated preparations, the magnitude of stimulated cAMP formation, while in the presence of an inhibitory concentration of sufentanil, is indistinguishable from that which occurs in opiate naive preparations (in the absence of exogenous opioid). This indicates the development of tolerance to the negative modulation of stimulated enteric cAMP formation by sufentanil. However, in 'addicted', tissue the magnitude of increase in cAMP formation produced by electrical stimulation, in the presence of a previously inhibitory concentration of sufentanil, is significantly larger than in 'addicted' tissue that had been washed in morphine-free Krebs' or Krebs' solution containing naloxone. Thus, the equivalence between the magnitude of stimulation-induced increase in cAMP formation observed in naive vs 'addicted' tissue in the presence of sufentanil, is due to the ability of an originally inhibitory concentration of opioid to enhance or facilitate stimulated formation of cAMP. It is suggested that tolerance/dependence to the opioid inhibition of stimulated cAMP formation results not only from the loss of inhibitory potency but from the reversal to enhancement.

708.7

THE EFFECT OF HALOPERIDOL ON PREPROENKEPHALIN GENE EXPRESSION IN THE RAT ANTERIOR PITUITARY. F. Tang* and T.S.M. Lau, Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong.

To study the effect of haloperidol on the gene expression of preproenkephalin in the anterior pituitary, male SD rats weighing 250-300g were injected for 3 weeks with 2mg/Kg haloperidol i.p. The anterior pituitary was homogenized in AT buffer using RNasin as RNase inhibitor. Cytoplasmic RNA was obtained after digestion with proteinase K and the equivalent of about one-sixth of an anterior pituitary was assayed for preproenkephalin mRNA using solution hybridization - RNase protection assay. Standards and P-32 labelled riboprobes were prepared from linearized plasmid DNA's (courtesy of Dr. J. Hong), using SP6 polymerase. An actin probe (gift of Dr. D. Autelitano) was used to normalize the values, which are expressed as pg preproenkephalin mRNA per pg actin mRNA. It was found that haloperidol treatment resulted in a significant decrease of preproenkepkhalin mRNA contents in the anterior pituitary, and this correlates well with the decrease of met-enkephalin levels as measured by RIA. Dopamine receptor blockade may therefore lower the met-enkephalin level in the anterior pituitary through a decrease in preproenkephalin synthesis.

708.8

ROLE OF BRAIN OPIOIDS IN THE DEVELOPMENT OF HYPERTENSION IN STRESSED BORDERLINE HYPERTENSIVE RATS. A.A. Houdi* and M. Welch College of Pharmacy and THRI, University of Kentucky, Lexington, KY 40546.

Opioid peptides have been implicated in modulating autonomic function under basal and stressful conditions, but their role in regulating cardiovascular function remains unclear. The borderline hypertensive rat (BHR) is a unique and useful animal model of neurogenic hypertension because its genetic background makes it susceptible to the hypertensive effect of stress. In this study, we examined the effect of a time limited period of restraint stress in the development of hypertension and the role of brain opioids on the development and maintenance of hypertension in 8-9 week old BHR. We found that restraint stress, 2 hr daily, 5 days a week for 5 weeks, caused a significant rise in systolic blood pressure one week after beginning stress treatment. This increase in blood pressure (BP) continued throughout the period of stress (5 weeks). Moreover, stressed BHR continued to have higher blood pressure compared to the control group 10 weeks after the end of stress (182 ± 8.1 mmHg vs 158 ± 3 mmHg, $P < 0.05$, n=10). Pretreatment with naloxone (1 mg/kg, intra-arterially) attenuated the increase in blood pressure due to acute restraint stress (10 min) applied 5 min after naloxone. Intracerebroventricular (icv) administration of the mμ-opioid receptor agonist DAMGO (5 nmole), produced an increase in BP (Δ MAP= 16.6 ± 2.3 mmHg) and heart rate (Δ HR= 89.6 ± 13.2 bpm). This increase in BP and HR peaked at 5-10 min and remained elevated. ICV administration of the delta opioid agonist DPDPE (50 nmole) or saline (4 μl) had no effect on BP or HR. Restraint stress in saline-treated rats evoked an increase in BP and HR. Rats pretreated with DAMGO showed no further alteration in BP or HR responses to stress applied 20 min after drug treatment. These data support the involvement of mμ-opioid receptors in the development and maintenance of hypertension due to stress. Supported by UKMC & KTRB.

708.9

TREATMENT WITH ANTISENSE OLIGODEOXYNUCLEOTIDE TO THE CLONED MOUSE CCK_B RECEPTOR PRODUCES NALTRINDOLE-SENSITIVE ENHANCEMENT OF MORPHINE ANTINOCICEPTIVE POTENCY. T.W. Vanderah, P. Peterson, R. Horvath, J. Lai, H.I. Yamamura and F. Porreca* Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

CCK may function as an endogenous anti-opioid. Thus, intracerebroventricular (*i.c.v.*) L365,260 (CCK_B receptor antagonist) increases the antinociceptive potency of *i.c.v.* morphine. In mice, the modulatory effect of CCK_B antagonists(but not the direct morphine antinociception) is sensitive to naltrindole (NTI, δ antagonist), suggesting the involvement of the δ opioid receptor (DOR). Furthermore, treatment with antisera raised against [Leu^5]enkephalin, but not [Met^5]enkephalin, blocks the L365,260-modulation of morphine. Here, the possible involvement of the DOR in this modulatory effect was further evaluated using treatments with 20-base oligodeoxynucleotides (oligos) directed towards the N-terminal portion of the cDNA of the mouse cloned CCK_B receptor. Male, ICR mice (20-35 g) received twice daily *i.c.v.* injections of saline, antisense or mismatch oligo (12.5 μg) for 3 days. Nociceptive thresholds in control or *i.c.v.* morphine-injected mice were determined on day 4 using the 55°C warm-water tail-flick assay. Treatment with the oligos did not alter nociceptive threshold alone. Antisense, but not mismatch, oligo displaced the morphine dose-effect curve to the left by 4.9-fold. NTI (19 mg/kg, *s.c.*) also did not alter nociceptive threshold alone and did not affect morphine antinociception. NTI pretreatment on day 4, however, blocked the leftward shift of the morphine dose-effect curve in antisense oligo treated mice. Decreases in CCK_B receptor number were shown using [^{3}H]SNF8702 (CCK_B ligand) only in midbrain and striatum. These data suggest a tonic inhibition of enkephalin release by CCK via CCK_B receptors. The subsequent enhancement of morphine antinociceptive potency may result by actions of enkephalins (possibly [Leu^5]enkephalin) at the DOR.

708.10

DIFFERING ULTRASTRUCTURAL RELATIONSHIPS BETWEEN ENKEPHALIN AND GABA CONTAINING NEURONS WITHIN THE HIPPOCAMPAL FORMATION. K. Commons*, and T.A. Milner, Dept. of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021.

Electrophysiological studies have suggested that the excitatory actions of opioids in the hippocampal formation are mediated by inhibition of GABA interneurons; however, an anatomical basis for this interaction has never been demonstrated. Thus, we sought to determine the ultrastructural relationship of leu^5 enkephalin (LE)-containing terminals and GABAergic neurons using double labeling immunohistochemistry throughout the hippocampal formation. In the s. lacunosum-moleculare of CA1, LE-containing terminals (n = 99) occasionally contacted GABA labeled perikarya and dendrites (15%) and were often in apposition to GABA labeled (13%) axon terminals. In the outer molecular layer of the dentate gyrus, LE-containing terminals (n = 65) almost exclusively contacted unlabeled dendrites, although they were often in the same field as GABA containing terminals. In contrast, many small round LE-containing terminals and larger LE-labeled mossy fiber boutons in the infragranular hilus of the dentate gyrus (n = 119) often synapsed on GABAergic perikarya and dendrites (40%), but were rarely in apposition to GABA containing terminals. However, LE-containing mossy fiber boutons in the s. lucidum (n = 85) most often contacted unlabeled dendrites while GABA-labeled terminals were in the same field. These results suggest in that in CA1 enkephalin may modulate GABAergic neurons directly through contacts with both dendrites and terminals, while in the hilus enkephalin may primarily modulate GABAergic neurons through contacts on dendrites. In contrast, the primary effects of enkephalin in the outer molecular layer of the dentate gyrus and in s. lucidum of CA3 may not be meditated through an interaction with GABAergic interneurons. (Supported by DA08259.)

708.11

KAPPA RECEPTOR mRNA LOCALIZATION IN THE GUINEA PIG BRAIN: COMPARISON TO [^{3}H]BREMAZOCINE AND [^{3}H]U69,593 BINDING. A. Mansour, C.A. Fox*, S. Burke, G. Xie, H. Akil and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Three opioid receptor types have been identified in the CNS that are referred to as μ, δ, and κ. Given the recent cloning of the guinea pig κ receptor (Xie et al, Proc. Natl. Acad. Sci. USA, in press), this study examines the mRNA distribution of the κ receptor in the guinea pig brain and compares it to the kappa binding sites defined either by [^{3}H]U69,593 (4.3nM) or [^{3}H] bremazocine (0.6nM, in the presence of a 300 fold excess of DAMGO and DPDPE) using *in situ* hybridization and receptor autoradiographic techniques. Kappa receptor mRNA was identified with a S^{35}-cRNA probe generated to Eco R1-Bam H1 fragment of the guinea pig receptor (602-1334) that spans from extracellular loop 1 to near the C-terminal end of the protein coding region. An excellent correspondence is observed between κ receptor binding sites and mRNA in the deep layers of cortex, caudate-putamen, nucleus accumbens, olfactory tubercle, dentate gyrus, and cerebellum. Differences were observed, however, in such brain regions as the substantia nigra and ventral tegmental area, where κ mRNA is localized in the substantia nigra, pars compacta and ventral tegmental area, while κ binding is predominantly in the pars reticulata. This suggests κ receptors may be synthesized in the striatum and transported to the substantia nigra, pars reticulata, or alternatively, may represent binding sites on dendrites of pars compacta neurons. Similarly, κ receptors are synthesized in the substantia nigra and ventral tegmental area and transported to the striatum. Species comparisons to the rat κ mRNA distribution will be discussed.

708.12

MULTIPLE EXPRESSION OF OPIATE TOLERANCE IN HYPOTHALAMIC ARCUATE NEURONS. M.J. Kelly*, G. Zhang, A. H. Lagrange and O. K. Ronnekleiv. Department of Physiology, Oregon Health Sciences U., Portland, OR 97201

To determine if hypothalamic arcuate (ARC) neurons develop tolerance to μ-opioids, intracellular recordings were made with biocytin-filled electrodes in ARC neurons in hypothalamic slices from placebo and chronic morphine-treated (4 x 75 mg pellets for 2 d + 6 more pellets for a total of 6-8 d), ovariectomized female guinea pigs. A dose-response curve was generated measuring the hyperpolarization in response to the selective μ-opioid agonist DAMGO. β-endorphin (βEND) neurons were identified using double labeling. Chronic morphine caused both a decreased potency (EC_{50} = 245 ± 22 nM, N= 12 vs. 63 ± 3 nM in controls, N=50), a decreased efficacy (ΔV_{max} = - 7.1 ± 1.1 mV vs. -10.7 ± 0.6 mV) and a 60% decrease in Δg_{DAMGO} in all βEND neurons (N=5) and other ARC neurons (N=7). In another population of tolerant ARC neurons from morphine-treated animals (N=11), DAMGO was less potent (EC_{50} = 110 ± 4 nM) without a change in efficacy. A third population of neurons (N=5) did not exhibit any signs of tolerance with morphine treatment. Morphine-tolerant neurons also exhibited a higher incidence of dye-coupling versus cells from controls (20% vs. 4% , χ^2, $p < 0.01$). Therefore, chronic morphine uncouples μ-opioid receptors from K^+ channels and increases dye-coupling in ARC neurons. Since βEND neurons show widespread projections throughout the forebrain, this would not only affect neuroendocrine but also homeostatic and reward circuits. (supported by PHS grant DA05158)

708.13

KAPPA OPIOID RECEPTOR ACTIVATION MODULATES EXCITATORY SYNAPTIC RESPONSES OF THE RAT SPINAL DORSAL HORN NEURONS. Lj. Kojic*, G. Cheng and M. Randic, Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011.

To determine how κ-opioid receptor agonists affect the primary afferent neurotransmission, we examined the actions of dynorphin A_{1-17} (Dyn_{1-17}), *trans*-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide (U-50,488H) and (5α,7α,8ß)-(+)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4.5)dec-8-yl]-benzeneacetamide (U-69,593) on synaptic transmission between dorsal root afferents and neurons in the superficial laminae of the spinal dorsal horn (laminae I-III) by using intracellular recording in transverse slice preparation of rat (17-26 days) spinal cord. The strength of primary afferent neurotransmission was assayed by recording the size of presumed monosynaptic EPSPs that result from stimulation of L3-L6 dorsal roots with electrical shocks of 2-40V (0.01-0.5ms duration) intensity. Bath application of Dyn_{1-17} (0.01-1μM for 10 min) caused hyperpolarization (-4.0±0.8mV, mean±SEM, 11/22 cells) or depolarization (7.1±3.4mV, 7/22 cells) of the resting membrane potential accompanied by a decrease (to 79.3±4.1% of control, 6/21 cells) or an increase (by 15.9±3.6%, 9/21 cells) in membrane input resistance. During the superfusion of 10nM Dyn_{1-17}, the peak amplitude of the presumed monosynaptic EPSPs was reversibly depressed (to 67.4±5.5% of control, 7/11 cells) or enhanced (by 25.2±8.2%, 3/11 cells), whereas 1μM Dyn_{1-17} induced only potentiation (by 44.5±6.2, 5/5). The Dyn_{1-17}-induced depression and enhancement of the EPSPs remained under conditions in which NMDA-, $GABA_A$- and glycine-mediated synaptic responses were blocked by selective antagonists D-2-amino-5-phosphonovaleric acid, bicuculline and strychnine, respectively. Similar dual modulation of EPSPs was observed with U-50,488H (n=6) and U-69,593 (n=11). The Dyn_{1-17}-induced depression of synaptic responses was reduced or abolished by a claimed selective κ_1-receptor antagonist nor-binaltorphimine (0.1μM, 5/5 cells). The results indicate that distinct modulation of synaptic efficiency can be induced at primary afferent synapses with neurons in the superficial laminae of the spinal dorsal horn by κ-receptor activation and that these changes may be physiologically relevant for transmission and integration of sensory information, including pain. (Supported by NS-26352 and IBN-9209462).

708.14

DYNORPHIN A MODULATES EXCITABILITY IN AMPHIBIAN AND MAMMALIAN NERVES. M.R. Luna and M.F. Pacheco*. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Colima, Col. 28045 México.

The effects of dynorphin A 1-17 (DYN) on nerve excitability were studied in isolated sciatic nerves of the frog and rat. The amplitude of recorded diphasic action potential was gradually reduced by increasing concentrations of locally applied DYN (10 - 100 μM). Amplitude inhibition attained, at each DYN concentration, was not modified by increasing stimulation frequency up to 10 Hz, however, higher stimulus rates reduced the degree of inhibition. DYN effect on action potential amplitude was concomitant with a decrease in conduction velocity and a 1 to 3 fold increase in threshold strength **input-output curve**; reaching maximum effects after 15 to 35 min of DYN application, and recovering completely (in 86% of the experiments) after 25 to 700 min of drug washout. DYN actions were antagonized by nor-binaltorphimine (50 μM), therefore, our data are indicative of a widespread action of DYN on nerve conduction, which results in an excitability decrease through an specific interaction with κ1-opioid receptors. Finally, these results provide an additional mechanism in order to explain reversible flaccid hindlimb paralysis and loss of reflexes, observed in rats, after intrathecal administration of DYN (Stewart & Isaac, Brain Res. 543:322-326, 1991).
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708.15

INHIBITORY ACTIONS OF OPIOIDE ON SYNAPTIC TRANSMISSION IN THE RAT NEOSTRIATUM. B. Schlösser, M. Kudernatsch, B. Sutor and G. ten Bruggencate*. Department of Physiology, University of Munich, D-80336 Munich, Germany

In the neostriatum, opioid receptors exist at a high concentration. Furthermore, enkephalin is colocalized with GABA in spiny stellate cells indicating that opioids may regulate the activity in striatal synaptic circuitry. In the present study, we investigated the influence of opiate-receptor activation on neostriatal neurons in vitro using intracellular recordings.

Experiments were performed on rat neostriatal slices (400 μm) with maintained corticostriatal connection. In the recording chamber the slices were kept submerged in artifical cerebrospinal fluid (ACSF). Synaptic activity was evoked by intracortical or intrastriatal electrical stimulation. Drugs were applied by addition to the ACSF.

Application of the opioid receptor agonist D-Ala^2-D-Leu^5-enkephalin (DADLE) and the selective δ-recptor agonist D-$Pen^{2,5}$-Enkephalin (DPDPE) (0,1-1 μM) produced a dose-dependent decrease in the amplitude of both striatally or cortically evoked synaptic potentials. The opioids did not affect membrane potential or current-voltage relation. The effects were blocked by the opioid antagonist naloxone (1 μM) and persisted when GABA-ergic components were abolished by the $GABA_A$-receptor antagonist bicuculline (20 μM).

The data demonstrate, that activation of δ-opioide-receptors decreases synaptic transmission in the rat neostriatum mainly by reducing glutamatergic excitation. (Supported by the BMFT, 01 KL9001).

708.16

EFFECT OF OPIATE ON THE IN VITRO RELEASE OF NEUROPEPTIDE FF FROM THE RAT SPINAL CORD. H.-Y.T. Yang* AND J. Zhu. Lab. Biochem. Genetics, NIMH, Neuroscience Center at St. Elizabeths, Washington DC 20032

Neuropeptide FF (FLFQPQRFamide) is an FMRFamide-like peptide with opiate modulating activity. Neuropeptide FF (NPFF) is highly localized in the spinal cord where there are also specific NPFF receptors. Furthermore, there have been studies indicating that NPFF may participate in the regulation of pain threshhold in the spinal cord. Thus, in this study, possible relationships between opiates and NPFF were investigated by studying the effects of various opioid receptor ligands on the K^+-evoked release of NPFF immunoreactive material (IR) from rat spinal cords. NPFF release experiments were carried out by using an in vitro superfusion of isolated intact rat spinal cords. The κ-opioid agonist U50488H decreased the K^+-evoked release of NPFF-IR in a dose dependent manner. In contrast, the μ-opioid agonist, Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol (DAGO), was inactive and δ-opioid agonist, Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE), exerted only a very slight inhibitory effect. The inhibitory effect of U504884H was antagonized by the κ-opioid antagonist nor-binaltorphimine (NBI). NBI was found to enhance the K^+-evoked release of NPFF probably resulting from the concomitant release of endogenous dynorphin by the high K^+. In view of the role of dynorphin in the antinociception, the results of this study further support that spinal cord NPFF participates in the regulation of opiate mediated antinociception.

708.17

INHIBITION OF ADENYLYL CYCLASE ACTIVITY IN RAT CAUDATE PUTAMEN BY DYNORPHIN A_{1-17}. L.H. Claye, E.M. Unterwald, A. Ho, and M.J. Kreek*, The Rockefeller University, New York, NY 10021.

Adenylyl cyclase activity is used as one functional measure of opioid receptor-mediated signal transduction. The naturally occurring opioid peptide, dynorphin A_{1-17}, is thought to be the endogenous ligand of the κ opioid receptor. The aim of the present study was to investigate the effects of dynorphin A_{1-17} on adenylyl cyclase activity *in vitro*. Adenylyl cyclase activity was determined by measuring cAMP production in membranes prepared from caudate putamen of naive male Fischer 344 rats using a cAMP radioligand binding assay. Dynorphin A_{1-17}, 10^{-9} to 10^{-4}M, produced a significant dose-dependent inhibition of adenylyl cyclase activity with a maximum inhibition of 67%. The ability of norbinaltorthimine, a selective κ receptor antagonist, and naloxone to attenuate dynorphin A_{1-17}-inhibition of cyclase was determined to investigate if this effect of dynorphin A_{1-17} is opioid receptor mediated. Preliminary findings show that norbinaltorthimine, 10^{-5} and 10^{-6}M, attenuated dynorphin A_{1-17}-inhibition of cAMP production at dynorphin A_{1-17} concentrations of 10^{-9} to 10^{-6}M. Similarly, naloxone, 10^{-6} and 10^{-4}M, reversed the effects of dynorphin A_{1-17} at concentrations of 10^{-9} to 10^{-6}M. Neither norbinaltorthimine nor naloxone inhibited the actions of higher concentrations of dynorphin A_{1-17}, 10^{-5} and 10^{-4}M. These results suggest that dynorphin A_{1-17}-inhibition of cyclase activity at low concentrations is mediated by κ opioid receptors, but may not be opioid receptor mediated at higher concentrations. [Supported by The Aaron Diamond Foundation and NIDA Research Center Grant, P-50-05130 (MJK)]

709.1

LACK OF NMDAR1 EXPRESSION IN LUTEINIZING HORMONE-RELEASING HORMONE NEURONS OF THE MALE SYRIAN HAMSTER. G. Munro,* V. Garyfallou, S.G. Kohama and H.F. Urbanski. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

There is substantial evidence demonstrating that excitatory amino acid (EAA) receptors are involved in luteinizing hormone-releasing hormone (LHRH) secretion both *in vivo* and *in vitro*. Since immortalized LHRH neurons (GT1 cells) express the NMDAR1 gene it has been proposed that EAAs may stimulate the secretory activity of LHRH neurons directly. We have used immunocytochemistry combined with *in situ* hybridization histochemistry (ISHH) in an attempt to confirm whether this particular facilitatory influence of EAAs is indeed mediated by such a direct neuronal pathway. Adult male Syrian hamsters were perfused with heparinized saline under ether anesthesia and their brains fixed with 4% paraformaldehyde. Coronal Vibratome sections (50 µm) containing the medial preoptic area were then processed initially for immunocytochemistry using a monoclonal antibody to LHRH (HU4H) and stained with diaminobenzidine. Subsequent ISHH for NMDAR1 mRNA using a ^{35}S-labeled 450-bp antisense riboprobe (the DNA template was excised from NMDAR1 cDNA using *PstI* and *EcoRI*) was performed on these same sections and also cultured GT1 cells. After dipping in photographic emulsion, silver grain deposition was examined microscopically to determine colocalization of NMDAR1 mRNA within the immuno-stained LHRH neurons. As expected, GT1 cells clearly demonstrated hybridization of the riboprobe. In the brain sections, a high density of silver grains was localized in the outer fringe of the cerebral cortex and within the hippocampus but not in the vicinity of the LHRH perikarya; only 1 neuron from a total of 103 neurons/3 animals showed possible colocalization. These results suggest that although GT1 cells may have an intrinsic capacity to express the NMDA receptor, in the intact animal excitation of LHRH neurons by EAAs is more likely to be mediated indirectly, by a pathway that involves interneurons.

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709.2

IMMUNOHISTOCHEMICAL STUDIES ON VASOTOCIN INNERVATION OF AROMATASE-CONTAINING REGIONS IN THE MALE QUAIL FOREBRAIN. C.Viglietti-Panzica°*, P. Absil†, G.C.Panzica°, N.Aste°, and J. Balthazart†. °Dept. Human Anatomy and Physiology, Univ. Torino, I-10126 Torino, Italy and †Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium.

In the male quail forebrain, aromatase-immunoreactive (ARO-ir) elements are clustered within the sexually dimorphic medial preoptic nucleus (POM), the nucleus striae terminalis (nST), the nucleus accumbens (Ac), and the ventromedial (VM) and tuberal hypothalamus. Previous studies demonstrated that ARO-ir cells are sensitive to testosterone (T) and its metabolites: their number and size increase after exposure to these steroids. In a recent study, we demonstrated that the vasotocinergic system is also sensitive to T: the density of the vasotocin- immunoreactive (VT-ir) fiber networks located within the POM and the lateral septum is greatly reduced in gonadectomized male birds and restored to intact level by a treatment with T. We analyzed here the anatomical relationships between ARO-ir elements and VT-ir fibers. The study was performed on brains perfused with Zamboni fixative and serially sectioned with a cryostat. Sequential staining for VT, ARO, or VT+ARO was performed on adjacent 30 µm-thick sections. High concentrations of VT-ir fibers were observed within the POM, the nST, the lateral septum, the VM and the tuberal region. In double-labelled sections, all clusters of ARO-ir cells, with the exception of those localized in the Ac, were embedded in a dense network of VT-ir fibers. Many of the VT-ir terminals appeared to end in the neuropile surrounding ARO-ir elements rather than directly on their cell bodies. However, there was a very close correspondence between the extension of the ARO-ir cells and of VT-ir fibers. This study hence supports the idea that the T-dependent ARO system is also directly innervated by a T-dependent peptidergic system. ARO could therefore be modulated by steroids both directly and indirectly through the VT system. Further studies will be needed to determine whether this VT innervation has a functional significance for the ARO-ir cells and whether these two neurochemical systems (ARO and VT) affect reproduction in an independent or coordinated manner. *Supported by grants of ESF, CNR, MURST, FRFC. P.A. was a fellow of the Ministero per gli Esteri.*

709.3

FOS EXPRESSION IN GNRH NEURONS WITHIN PREOPTIC AREA (POA) GRAFTS IN HYPOGONADAL (HPG) MICE: EFFECT OF PROGESTERONE AND SEXUAL BEHAVIOR M.J. Gibson*, T.J. Wu, G.M. Miller, and A.-J. Silverman. Dept. of Medicine, Mount Sinai School of Medicine, NY, NY 10029,and Dept. of Anatomy and Cell Biol., Columbia University, New York, NY 10032 .

Expression of FOS, the protein product of the immediate early gene c-*fos*, is widely accepted as a marker for neuronal activation. We have shown that in normal steroid-primed, ovariectomized mice paired with an ejaculating male, FOS expression occurs in greater than 40% of GnRH neurons and is maintained at this level for a longer time than in similarly treated females paired with an unsuccessful male (Endocrinol 131:2045, 1992). HPG mice lack a functional GnRH gene and are infertile. After receiving intraventricular POA grafts containing GnRH neurons, females may enter persistent estrus, and have the capacity to ovulate reflexively or, more rarely, ovulate in response to a progesterone challenge. The present study of HPG/POA mice examined the effects of both progesterone treatment (P; 500 µg; 0930h) and sexual behavior (introduced to an experienced male at 1400 h) on the expression of FOS in grafted GnRH neurons. In response to sexual behavior, 47.5±10.2% of GnRH neurons of P-treated HPG/POA mice expressed FOS in contrast to 0.9±0.6% of GnRH neurons in those mice that did not receive P. P treatment alone did not increase FOS expression in GnRH neurons (0.4±0.4%). These results suggest that in the presence of P, sexual behavior may serve as a stimulus of FOS expression in GnRH neurons. In addition, the present study supports the hypothesis that at least a portion of the graft-host connectivity that underlies aspects of reproductive competence in HPG/POA mice is steroid sensitive.

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709.4

EFFERENT CONNECTIONS OF THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IN THE FEMALE RAT. G.B. Gu* and R.B.Simerly. Div. of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The anteroventral periventricular nucleus of the hypothalamus (AVPV) is thought to be a nodal point in the forebrain circuits mediating hormonal feedback on gonadotropin secretion. It contains nearly an 8-fold greater number of neurons that project to the arcuate nucleus of the hypothalamus, as well as greater numbers of tyrosine hydroxylase and dynorphin B immunoreactive neurons in female rats, relative to males. The present study was designed to clarify the organization of projections from the AVPV in female rats to facilitate a full understanding of it possible role in neural pathways underlying reproductive function. *Phaseolus vulgaris* leucoagglutinin (PHA-L) was injected iontophoretically into the AVPV and the distribution of labeled fibers evaluated immunohistochemically. The AVPV sends ascending projections through peri-ventricular and dorsolateral pathways with dense terminal fields in the ventral part of the lateral septal nucleus, the parastrial nucleus, and the vascular organ of the lamina terminalis (OVLT), including the region adjacent to the OVLT where a subpopulation of gonadotropin releasing factor (GnRH)-containing neurons are located. The majority of projections from the AVPV pass caudally through the periventricular zone and provide extremely dense terminal fields to all of the periventricular nuclei of the hypothalamus, most parts of the paraventricular nucleus, the arcuate nucleus of the hypothalamus, and the medial part of the medial preoptic nucleus. Thus, the organization of projections from the AVPV in female rats suggests that sexually dimorphic populations of neurons in this nucleus may regulate the activity of GnRH cells directly, or indirectly via projections to regions of the hypothalamus that contain hypothalamic releasing factors such as β-endorphin or NPY. Work in progress utilizing double and triple fluorescence labeling methods and confocal microscopy will confirm whether tyrosine hydroxylase and dynorphin B immunoreactivities are expressed in these pathways.

709.5

THE SEXUAL DIMORPHISM OF THE RAT MEDIAN EMINENCE : AN INVESTIGATION ON A MECHANISM OF DIFFERENTIATION. P. Ciofi[1]*, D. Deneux[1], D. Croix[1], J.E. Krause[2], J.C. Beauvillain[1], A.R. Bello[3], G. Tramu[3] and M. Mazzuca[1]. (1) U.156 INSERM, Place de Verdun, 59045 Lille (France); (2) Dept. Anat. & Neurobiol., Wash. Univ., St. Louis, Miss. 63110; (3) Lab. Neurocyto. Fonct., URA CNRS 339, Univ. Bordeaux I, 33405 Talence (France).

The hypothalamic arcuate nucleus contains a neuronal population immunoreactive (ir) for prodynorphin- (DYN) and protachykinin B- (TKB) derived peptides and giving a sexually differentiated projection to the median eminence : to the subependymal zone in females, to the gonadoliberin (GnRH)-rich regions of the palisadic zone in males (Soc. Neurosci. Abs. 17:713, 1991). This DYN/TKB neuronal system was studied in the two sexes using immunohistochemistry, (1) during normal postnatal development and (2) following alterations of the neonatal gonadosteroidal milieu.

If at birth (day 0, d0) this system ir for TKB only appears immature, not different between sexes, it seems to develop faster in females in which it becomes progressively ir for DYN (d20) and seems to be established at puberty (±d40). In the male, the system appears to develop slowly over the first month of life and becomes ir for DYN at puberty (±d50). In the two sexes, gonadectomy (GX) at d0 results in adults (d120) treated or not with estradiol (d90-120) in a female type of organization, whereas in adult testosterone (T)-treated (d90-120) d0-GX animals the organization appears identically hermaphrodite in the two sexes. In males GX at d10, d15 or d20, and T-treated as adults (d90-120), the organization appears similarly hermaphrodite, ressembling that of d0-GX+T males and that of females GX+T (30 days) as adults. In adults of the two sexes, both the number and the distribution in the arcuate nucleus of cell bodies ir for DYN/TKB are identical, with or without adult gonadectomy.

At this point, results suggest a postnatal androgen-driven, "non numerical", mechanism of differentiation for this neuronal system of the mediobasal hypothalamus whose organization evokes a direct involvement in the auto- or retro-control of GnRH release.

709.6

EFFECTS OF INTRAUTERINE POSITION ON METABOLIC CAPACITY OF THE HYPOTHALAMUS IN THE GERBIL: A CYTOCHROME OXIDASE STUDY D. Jones.* F. Gonzalez-Lima, D. Crews, B.G. Galef[1] and M.M. Clark[1], Dept of Psych and Zoology, Univ of Texas, Austin TX, 78712 USA and [1]Dept of Psych, McMaster Univ, Ontario L8S 4K1, Canada.

Neural metabolic correlates of known effects of intrauterine position on behavior in the Mongolian Gerbil were investigated. Hypothalamic regions were compared in female gerbils that developed *in utero* between two female fetuses (2F) or between two male fetuses (2M) to assess differences in metabolic capacity. Taking advantage of quantitative image analysis systems we measured the cytochrome oxidase (C.O.) activity in histochemically stained brain slices of the gerbil. Due to its limiting role in oxidative metabolism, C.O. is an excellent marker of metabolic capacity: increased activity of neurons in a brain region lead to increased C.O. content in their mitochondria. The C.O. staining procedure involved metal intensification through cobalt preincubation, as well as oxygenation and heating of the reaction medium. Optical density (O.D.) of ten hypothalamic regions, including the sexually dimorphic area, were assessed using grey to white matter O.D.. Significant group differences in the metabolic capacity of three hypothalamic regions were revealed. Increases in C.O. in the anterior portion of the lateral hypothalamic area, the posterior portion of the anterior hypothalamic area and the sexually dimorphic area were seen in 2M as compared to 2F females. To our knowledge, this is the first demonstration of functional changes in hypothalamic areas related to the differences in intrauterine position. (Supported by RO1 MH43353 and OGP0037338).

709.7

THE DISTRIBUTION OF AROMATASE mRNA IN THE BRAIN OF ADULT MALE AND FEMALE RATS USING *in situ* HYBRIDIZATION

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Many of the effects of gonadal steroid hormones in the male brain are due to the actions of the testosterone metabolite, estradiol, which is synthesized by the actions of the P450 enzyme, aromatase. Aromatase activity has been demonstrated, through the use of *in vitro* enzyme activity assays, to be present in regions of the preoptic area, hypothalamus and limbic system. Levels of aromatase activity are much higher in males than in females in some brain regions. The present study examined the distribution and cellular localization of aromatase mRNA in adult male and female rat brains using *in situ* hybridization. A 113-base ^{35}S-labelled cRNA probe, complementary to the aromatase mRNA just upstream from the heme-binding domain, was synthesized from the rat aromatase cDNA inserted into pGEM3Z (generous gift from J. Richards, Baylor College of Medicine, Houston TX), using a BstN1 fragment and SP6 polymerase. Following the hybridization procedure, slides were dipped in nuclear track emulsion in order to detect aromatase mRNA at the cellular level. In the male, many heavily labelled cells were found in the encapsulated bed nucleus of the stria terminalis and the medial amygdala. A smaller number of heavily labelled cells were seen in the periventricular preoptic nucleus, the medial preoptic nucleus, the ventromedial nucleus, the cortical amygdala and the subfornical organ. Lightly to moderately labelled cells were observed in the horizontal diagonal band, the magnocellular preoptic nucleus, the median preoptic nucleus, the anterior hypothalamus, the arcuate nucleus, the piriform cortex and nuclei of the thalamus. Aromatase mRNA was found in the same brain regions in females. Sex differences and the effect of castration in males on levels of aromatase mRNA will be discussed.

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709.8

EFFECTS OF TRH ON THE SEMI-CIRCADIAN RHYTHMS OF DA RELEASE IN THE MBH AND PRL LEVELS IN PLASMA DURING PSEUDOPREGNANCY IN AWAKE RATS. W. Timmerman*[1], R.T. Poelman[1], B.H.C. Westerink[2] and G.A. Schuiling[1]. Departments of [1]Obstetrics & Gynaecology and [2]Medicinal Chemistry, University of Groningen, 9713 EZ, 9713 AW Groningen, The Netherlands.

The tuberoinfundibular dopaminergic (TIDA) neurons inhibit the prolactin (PRL) secretion from the adenohypophysis. Thyrotropin-releasing hormone (TRH) is a PRL-releasing hormone, but under certain conditions it suppresses PRL secretion. We tested the hypothesis that the inhibitory effect of TRH on PRL secretion is mediated via a stimulatory effect of TRH on DA release from TIDA neurons. To simultaneously study DA release and plasma PRL concentrations, a microdialysis probe was implanted in the mediobasal hypothalamus (MBH) together with a permanent heart cannula in pseudopregnant female rats. These animals display a diurnal PRL peak (between 1300-1800 h) and a nocturnal peak (between 0100-1200 h), with an interphase (between 1800-0100 h) during which PRL levels are low for 10 consecutive days. Dialysis experiments were performed with nomifensine (5 µmol/l) in the dialysate. This infusion increased extracellular DA to 350% of basal values. During the interphase, DA levels in the MBH were ~4 fmol/min, while during the nocturnal PRL surge period DA levels were ~1.6 fmol/min. Plasma PRL concentrations were ~35 and ~240 ng/PRL-RP-2/ml in the inter- and nocturnal phase, respectively. When the TRH analogue CG 3703 (500 and 2500 µg/kg iv) was administered at 0000 h, the PRL surge was postponed dose-dependently, which could be related to the dose-dependent increase in DA release in the MBH. These data suggest that (1) DA levels in the MBH and plasma PRL levels are inversely correlated during pseudopregnancy, and (2) the inhibitory effect of the TRH analogue on PRL secretion was mediated by a stimulatory effect of the drug on DA release from TIDA neurons. [Supported by a grant form the De Cock Foundation].

EXTRASTRIATE VISUAL CORTEX: FUNCTIONAL ORGANIZATION IV

710.1

DISTRIBUTION OF THE CALCIUM-BINDING PROTEINS PARVALBUMIN, CALBINDIN AND CALRETININ IN THE MACAQUE MONKEY VISUAL CORTEX. P.R. Hof, C.A. Sailstad, N. Archin, A.M. Edwards, W.G.M. Janssen and J.H. Morrison*. Fishberg Research Center for Neurobiology, Mount Sinai Medical Center, New York, NY 10029.

Immunohistochemical studies localizing the calcium-binding proteins (CaBPs) parvalbumin (PV), calbindin (CB), and calretinin (CR) in the monkey and human visual areas V1 and V2 have shown that these CaBPs are present within morphologically distinct groups of neurons that display specific regional and laminar distribution patterns. In order to further characterize the cellular organization of the primate visual system, we performed a quantitative analysis of the distribution of CaBPs-immunoreactive (ir) neurons in several divisions of the magno- (M) and parvocellular (P) visual pathways in the macaque monkey. Area V1 was characterized by very high numbers of PV- and CB-ir cells, while CR-ir neurons were less numerous. Areas V2, V3 and V3A displayed lower numbers than area V1 for the three neuronal populations. However, cell counts markedly increased in areas at higher levels in the cortical hierarchy. In particular, areas MT, VIP, V4 and TEO were characterized by very high densities of stained neurons. In addition, a subset of CB-ir pyramidal neurons located in layer III exhibited differential distribution among the cortical areas investigated, with the highest densities in areas V4 and MT and slightly lower cell counts in areas TEO, TE, VIP and LIP. These results indicate that CaBPs define neuronal populations that are differentially represented among cortical regions of known function in the M and P pathways in the primate visual system. (Supported by NIH AG06647, MH52154 and the Brookdale Foundation)

710.2

SELECTIVITY OF AREA V2 OF THE MACAQUE TO KINETIC- AND OTHER TYPES OF BOUNDARIES. V.L. Marcar*, D.-K. Xiao, S.E. Raiguel & G.A.Orban. Lab. Neuro- en Psychofysiologie, Medical School, KULeuven, B-3000 Leuven, Belgium, EEC

We have reported the presence of cells in area V2 that can signal the orientation of a boundary defined by motion cues only (Marcar *et al*, Soc. Neurosci. Abstr. 1992, **537.5**). We have extended our investigation of V2 by including boundaries defined by additional fourier and non-fourier cues.

Luminance contrast stimuli were used to determine the preferred orientation, centre of the receptive field and the optimum stimulus length. Cells in which the preferred orientation differed by less than 23° between the parallel and the orthogonal motion condition were considered orientation selective for kinetic boundaries. About 10% (9/94) of V2 cells fell into this category. Histological reconstruction placed these cells in or bordering layer V. For these boundary cells we compared the orientation selectivity and orientation preference for luminance, texture and the three types of anomalous edges. We have found that in some cells the preferred orientation and sensitivity to all types of edges was very similar.

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710.3

RESPONSES TO A MOVING KANIZSA SQUARE IN AREAS V2 AND V4 OF THE MONKEY. J. I. Nelson*, P. De Weerd, R. Desimone and L.G. Ungerleider, Lab Neuropsychology, NIMH/NIH 491B80, Bethesda MD 20892

Prior physiological studies of subjective contours used patterns which induce bars or edges by line ends or corners (van der Heydt et al., *Science* 224:1260,1984). By contrast, we employed a more complex figure in which a Kanizsa square is induced and placed in apparent motion. The Kanizsa square moved back and forth along a double row of inducers ("PAC-Men"). This illusion is achieved by appropriately orienting neighboring inducers in successive frames of a movie and randomly orienting inducers elsewhere (Bravo et al., *Vision Res.* 28:861,1988).

At eccentricities equal to those tested cellularly, six observers perceived the illusory square as a darker, bounded object moving against the background. If the open quadrants of the inducers were closed by thin circular arcs, no such object was seen (control stimulus). We recorded from cells in areas V2 and V4 of one fixating rhesus monkey and sought a cellular correlate of this perceptual illusion. We attempted to place all inducing elements outside the classical receptive field.

In V2, 6/59 cells (eccentricity 3-4 deg) responded to the moving Kanizsa square but gave a reduced response or no response at all to the control stimulus. In preliminary data from V4 (eccentricity 0.5-1.5 deg), 4/13 cells showed similar behavior. Response peaks corresponded to the presence of the induced object centered on the receptive field.

Some of these cells were clearly color selective and responded to patterns in which inducers and background colors were photometrically isoluminant. In some cells we reversed inducer/background colors and could abolish the subjective square response. In another case, increasing luminance contrast to 19% but rendering background and inducers achromatic did not evoke an increased response.

These results confirm the presence of illusory contour responses in V2 and extend these findings to a more complex stimulus. In addition, we have preliminary evidence for similar responses in V4. Our results suggest the existence of figural completion mechanisms for chromatic patterns similar to the boundary completion mechanisms shown earlier for achromatic patterns.

710.4

MECHANISMS OF CONTOUR PROCESSING IN MONKEY PRESTRIATE CORTEX INCLUDE INFORMATION ON DIRECTION OF GRAVITY.

X. M. Sauvan, V. Henn, and E. Peterhans (SPON: European Neuroscience Association), Dept. of Neurology, University Hospital Zurich, CH-8091 Zurich, Switzerland.

Form perception requires a generalized representation of contours with regard to the quality defining them (e.g. luminance, color, motion, or texture) and their spatial position and orientation. We report here the effect of body tilt on mechanisms of contour processing in the visual cortex of the alert rhesus monkey.

The responses of single neurons were studied while the monkey performed a visual fixation task in either an upright position, or with its body tilted about the naso-occipital (roll) axis by ± 25-30 deg. We recorded from 106 orientation selective neurons both in striate and prestriate cortex (areas V2 and V3/V3A). For 44 neurons we could compare the preferred orientation and the position of the response field for light and dark bars or edges at 2 or 3 body positions. In striate cortex, most neurons (19/21) were of a non-compensatory type showing a change in the preferred orientation and position of the response field according to the body tilt and the estimated counterrolling of the eye (see Vision Res, 32:1341-1348,1992). By contrast, 40% of the neurons in prestriate cortex (8/20) were of a compensatory type. These cells, although showing a change in field position, preferred similar stimulus orientations at all body tilts. This indicates that the orientation selectivity of the compensatory neurons was invariant with respect to the direction of gravity.

In conclusion, information about the direction of gravity is implemented in monkey visual cortex first at the level of area V2. This suggests that otolithic signals contribute to mechanisms of contour processing at early stages of visual processing.

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710.5

TOPOGRAPHIC PATTERN OF CORTICAL CONNECTIONS TO DORSAL AND VENTRAL V2 IN MACAQUE MONKEYS. I. Stepniewska* and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

We studied the connections of V2 with other extrastriate visual areas in macaque monkeys (*Macaca mulatta*) to better determine their extent and visuotopic organization. Multiple injections of different fluorescent tracers and WGA-HRP were made in dorsal V2 representing the lower central vision and in ventral V2, representing the upper central vision. After 4-7 days survival, animals were anesthetised and perfused, and visual cortex separated from the rest of the brain. Cortex was manually flattened and cut parallel to the surface. Transported label was related to architectonic boundaries identified from adjacent myelin- and cytochrome oxidase-stained sections. Injections in both dorsal and ventral V2 labeled neurons in the adjacent portion of primary visual area (V1), in the dorsomedial (DM, dorsal V3) and dorsolateral (DL, V4) visual areas as well as middle temporal area (MT, V5) in the superior temporal sulcus. This suggests that all these areas, including DM, contain complete representations of the contralateral visual hemifield. Two separate groups of labeled neurons were found in DL, indicating that this region consists of rostral (DLr) and caudal (DLc) fields, each with its own visuotopic organization. The pattern of connections suggests that DL extends less far ventrally than described in previous studies. Other areas weakly connected with V2 were located in the superior temporal (MST, FST), and intraparietal sulci. Most connections of V2 were topographically organized so that dorsomedial V2 was connected with dorsomedial locations in other extrastriate areas, and ventrolateral V2 was connected with ventrolateral portions of extrastriate areas. These results provide further information about the retinotopic organization of extrastriate areas and suggest that much of the area designated as dorsal V3 in macaque is a homoloque of DM in owl monkey. (Supported by NEI Grant EY-O2686)

710.6

THE DISTRIBUTION OF CORTICO-COLLICULAR PROJECTION NEURONS CORRELATES WITH THICK CYTOCHROME OXIDASE STRIPES IN VISUAL AREA V2 OF MACAQUE MONKEY. P.L. Abel*, B.J. O'Brien, B. Lia and J.F. Olavarria. Depts. of Psychology, Physiology and Biophysics, and Ophthalmology, Univ. of Washington, Seattle, WA 98195.

Several lines of evidence suggest that the three classes of CO stripes observed in V2 (CO-dense thick and thin stripes, and pale interstripes) are related to specialized functional streams in visual cortex. One line of evidence comes from the pattern of connections that CO stripes have with ipsilateral cortical visual areas. Thus, in accordance with the idea that thick CO stripes are part of the magnocellular processing stream, these stripes have been shown to project to areas involved in the analysis of motion and spatial attributes, such as area MT. Exploring the pattern of V2 connections with subcortical nuclei that have magnocellular functions offers another opportunity for investigating the specificity of projections originating from V2 stripes. For instance, the superior colliculus (SC) receives a strong projection from V2, and available information indicates that the corticotectal pathway in the monkey is predominantly magnocellular in nature. We have therefore correlated the distribution of V2-SC projection neurons with the array of CO V2 stripes.

Guided by recordings of visually evoked potentials, multiple injections of either HRP or fluorescent tracers were made into the SC of adult monkeys (*Macaca fascicularis*). Three-four days later, area V2 was sectioned coronally or tangentially to the cortical surface, and the distribution of labeled cells in layer V was compared to the CO staining pattern in overlying cortex. The identification of stripes was aided by analyzing the distribution of CAT-301 positive cells.

We found that labeled cells accumulate in finger-like clusters which extend perpendicularly from the V1/V2 border across the width of V2. These clusters are preferentially distributed within areas of infragranular cortex underlying thick CO stripes, with fewer cells underlying thin and interstripe regions. Our results thus support current notions about the functional specificity of pathways originating from V2 stripes. Supported by NIH EY09343 and T32 EY07031.

710.7

ANALYSIS OF COLOR AND MOTION IN MACAQUE AREA V3. Gegenfurtner, K.R.*[1], Kiper, D.C.[2] and Levitt, J.B.[3] MPI for biological Cybernetics, 72076 Tübingen[1], Germany; Inst. of Anatomy, University of Lausanne[2], Switzerland; Inst. of Ophthalmology, London[3], UK.

Extrastriate area V3 may represent an important site for integration of visual signals. It receives inputs from both magno- and parvocellular pathways and has prominent projections to both areas MT and V4. Therefore we investigated the representations of color and motion in V3. We recorded from single cells in area V3 of macaque monkeys (*Macaca mulatta*) using standard acute recording techniques. After measuring each cell's spatial and temporal properties, we tested its chromatic and motion properties using sinewave gratings, colored bars and plaid stimuli.

All V3 neurons were orientation selective. About 50% of our sample was selective for direction of motion. Contrary to previous reports, we also found a high proportion (about 40%) of cells that were selective for color. These two subpopulations overlapped to a large extent, and we found a significant proportion of cells highly selective for both of these stimulus attributes. An analysis of the responses of directionally selective cells to plaid patterns showed that in area V3, as in MT and unlike in V1 and V2, many cells were sensitive to the motion of the plaid pattern, rather than to the component motion. Furthermore, a third of our sample was endstopped, and many of these cells showed orientation- or direction-selective surround effects. Our results show that there is a significant interaction between color and motion processing in area V3, and that V3 cells show many of the more complex motion properties typically observed at later stages of visual processing.
Supported by MRC G9203679N and NIH EY10021.

710.8

RECEPTIVE FIELD STRUCTURE OF V2 NEURONS IN THE PROSIMIAN PRIMATE *GALAGO CRASSICAUDATUS*. J.D. Allison* and V.A. Casagrande. Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232-2175.

Visual area 2 (V2) is the second largest visual area in the primate cortex and receives input solely from V1. There is very little data on the receptive field (RF) structure of V2 cells. A key question is how the RF structure of V2 cells compares to the RF structure of V1 cells. To answer this question we measured the response amplitude of single cells in V2 of 3 galagos using standard single-unit electrophysiological techniques with both bars and drifting sine-wave gratings. All cells were classified as simple or complex and their RF size, directionality, orientation tuning, spatial and temporal frequency tuning, and contrast sensitivity were tested. After recording, the electrode tracts were marked with lesions and reconstructed to identify the location of each cell. The results showed that all cells were complex and their RFs were located within 5° of *area centralis* with large (mean = 26 ± 5.8 deg^2 SE), elongated (mean = 4.1 x 7.5°SE) receptive fields. The majority (71%) of the cells were binocular and directionally selective (78%). The average orientation tuning was 21.5 (± 9.65 SE)°, the preferred spatial frequency averaged 0.5 cycles/deg with a mean tuning bandwidth of 2.4 (± .68 SE) octaves and a mean cutoff of 1.2 cycles/deg. The mean preferred temporal frequency was 2.76 (± 1.8 SE) Hz with a cutoff frequency of 8 Hz. The average contrast sensitivity was 20.1 (± 19.32 SE). The RF structure of V2 cells near *area centralis* is very similar to that of V1 cells in this species. V2 cells differed from V1 cells in having larger receptive fields, being mostly binocular, and having lower spatial frequency peaks and cutoffs.
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710.9

CORTICOTECTAL CELLS IN AREAS 17 AND PMLS OF THE CAT. T.G. Weyand* and A.C. Gafka, Dept. Anatomy, LSU Medical Center, New Orleans, LA 70112

Using antidromic techniques, we have recorded from corticotectal (CT) neurons in primary visual cortex (area 17, n = 21) and a lateral visual cortical area (posteromedial lateral suprasylvian cortex, PMLS, n= 13) in cats trained in oculomotor tasks. Head position was fixed, and gaze was monitored using the scleral search coil technique. The cat faced a rear projection screen onto which we could project an assortment of visual stimuli. CT cells in both areas responded well to a small spot used as the target when the spot is placed in the receptive field. None of the cells in either cortical area exhibited pre-saccadic activity. At least half the CT cells in area 17 showed post-saccadic activity associated with sweeping the retina across an illuminated field. PMLS CT cells did not show post-saccadic activity. Because CT cells in PMLS do respond to visual stimuli well in excess of saccade velocities, an oculomotor signal must actively suppress visual inputs when the cat makes eye movements. In general, area 17 CT cells in the awake cat are just like their counterparts in the paralyzed, anesthetized preparation. Most are strongly directional, orientation selective and show little evidence of length summation. PMLS cells exhibit a directional anisotropy; they respond best to stimuli moving away from gaze. PMLS CT cells exhibit this same direction anisotropy. Area 17 CT cells provide detail on the spatial attributes of the visual array to the superior colliculus. This is presumably used by the colliculus to make decisions about which target to look at next. In contrast, the large receptive fields and direction anisotropy of PMLS CT cells would be consistent with a role in supporting visually-guided locomotion (Supported by NIH Grant EY06818).

710.10

SYNCHRONIZATION OF THE SLOW (<1 Hz) OSCILLATION IN VISUAL CORTICOTHALAMIC NETWORKS. A. Nuñez*, F. Amzica and M. Steriade. Lab. of Neurophysiol., Laval University, Quebec, Canada G1K 7P4; and Dept. Morfologia, Fac. Medicina, Univ. Autonoma de Madrid, Spain.

It has been shown that a slow (<1 Hz) cortical oscillation is present in various neocortical areas as well as related thalamic nuclei (Steriade et al., J. Neurosci., 1993, 13:3252-3299). It remained however unclear whether or not this oscillatory behavior is evenly distributed within different cortical areas belonging to the same sensory modality. We performed simultaneous, multi-site, intra- and extracellular recordings from cortical visual areas 17, 18, 19 and 21 and related thalamic nuclei (mainly LG and PG) of cats under ketamine & xylazine anesthesia. The extent of synchronization as well as its spectral composition was assessed by means of correlation techniques applied to spike discharges, membrane potentials in intracellular recordings, and field potentials.

The slow (<1 Hz) oscillation occurred predominantly in areas 18, 19 and 21 (90%) and was less often seen in area 17 (58%). When it appeared in area 17, it was synchronous with the same oscillation recorded from other visual areas. In 19% of area 17 cells, they were oscillating in the delta frequency (1-4 Hz) and no synchrony with slowly oscillating neurons from other visual areas was observed. PG thalamic neurons were coherent with slowly oscillating cortical cells in 89% of cases. LG cells often (58%) reflected the cortical slow rhythm and, in those instances, were synchronized with cortical cells. In other cases (31%), LG neurons displayed delta oscillation, without relation with the slow rhythm. Light stimulation disrupted the synchrony among cortical neurons or between cortical and thalamic neurons. We suggest that the appearence of the slow oscillation in the neocortex is favored by wide-range connections between association areas, while primary receiving areas reflect a balance between the ascending clock-like, intrinsic, thalamically-generated delta oscillation and the intracortical inputs of the slow oscillation.
Supported by MRC grant MT-3689.

710.11

THALAMIC AND CORTICAL CONNECTIONS OF AREA 18 OF ALBINO CATS. Marcie Pospichal*, Andrzei Wrobel, Iwona Stepniewska and Jon Kaas, Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

The visual system of albino animals are abnormal in that many ganglion cells in the temporal retina are misrouted to the contralateral lateral geniculate nucleus (LGN). The consequences of this misrouting are only partially understood. We studied the organization of albino geniculocortical and corticocortical connections by injecting up to 6 different tracers in distinct rostrocaudal locations in area 18 (V2) of 4 animals. The results revealed a roughly normal rostrocaudal sequence of geniculate projections so that progressively more rostral injections in area 18 labeled progressively more rostral locations in the LGN. However, the zones of labeled cells were larger than those for comparable injections in normal cats, suggesting greater divergence and convergence of LGN projections. Layer A1, with abnormal retinal inputs, had discontinuous medial and lateral foci of labeled neurons from single injections, indicating topographic mismatching in projections. Cortical connections with areas 17 and 19 and suprasylvian regions were also roughly normal with more rostral injections labeling more rostral locations in these fields. However, again connections appeared less precise than normal with connections of central area 18 in particular being more widespread and overlapping than in normal cats. These abnormalities suggest that in albino cats, visuotopic organization is less precise and orderly. Supported by EY-02686 (JK), NSPB-FFS PD92037 (MP) & James S. McDonnell Foundation (AW).

710.12

Visual acuity of single neurons in area 21a of the adult cat. E. Tardif[1], A. Bergeron[1], F. Lepore[1], J.P. Guillemot*[1-2]. [1]Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal et [2]Dépt. de Kinanthropologie, Univ. du Québec à Montréal, Montréal, Québec, Canada.

The spatial selectivity of single neurons in area 21a of the adult cat was investigated using sinusoïdal gratings. Extracellular activity was recorded and analyzed in the conventional manner while the animal was paralyzed and anesthetized (halothane: 0.5% and N_2O-O_2: 70:30). Receptive fields (RFs) were located in the upper part of the visual field. Most RFs were complex type but simple and end-stopped RFs type were also found. Results showed that cells responded well to drifting sinusoïdal gratings. The spatial frequency tuning functions revealed both low-pass and band-pass characteristics. Visual acuity was lower than that found for cells in area 17 but comparable to that of other visual areas. Optimal spatial frequencies were low and the bandwidth of most cells was similar to that of striate cortex. For binocular cells, a positive relationship was found between the spatial properties derived from each eye. The contrast threshold was generally quite low under optimal parameters but some cells were found which responded only to high contrast. Cells were also tested for temporal selectivity and they were found to respond to a wide range of velocities.

710.13

MODULAR ASPECTS OF CORTICOCORTICAL CONNECTIVITY BETWEEN AREAS 17 AND 18 Joanne Matsubara* and Jamie Boyd Depts of Ophthalmology and Anatomy, University of British Columbia, Vancouver, British Columbia, Canada V5Z 3N9

We have previously shown that the cells projecting from area 17 of the cat to the posterior medial lateral suprasylvian area (PMLS) are clustered within regions that stain densely for cytochrome oxidase (CO). Here, we extend these findings to connections involving area 18.

Large, multiple injections of wheat germ agglutinin-conjugated horseradish peroxidase or cholera toxin conjugated to colloidal gold were made into areas 17, 18, or the PMLS. In each case, the aim was to saturate a large part of the visual field representation of the injected area. Labeled cells were charted in tangential sections of cortex and compared to the pattern of CO staining in alternate sections.

After tracer injections into the PMLS, labeled cells in area 18 were, as was previously found in area 17, arranged in patches that aligned with the CO blobs. CO blobs, and associated PMLS projecting patches, were slightly larger in area 18 (slightly greater than 1mm spacing) than in area 17 (slightly less than 1 mm spacing). In agreement with Ferrer et al. (1988), we found the projection from area 17 to area 18 to be discontinuous. Moreover, cells projecting from area 17 to area 18 are confined to the CO blobs. Surprisingly, labeled cells and terminals in area 18, following large injections in area 17, also have a patchy organization, which we are currently trying to relate to the pattern of CO activity. Preliminary evidence suggests that they too are confined to the blobs.

Thus, while both area 17 and 18 have similar PMLS-projecting blob and non-PMLS-projecting interblob compartments, it appears that connections between these two areas are made specifically to and from the CO blobs, with interblobs being unconnected corticocortically. These results are not easy to interpret within a strictly hierarchical framework classifying connections from 17 to 18 as feedforward, connections from 18 to 17 as feedback; rather, the similarities in connectional organization is consistent with suggestions that these two areas operate in parallel. (This work funded by MRC 5-99150)

710.14

MODULAR ORGANIZATION OF CORTICOCORTICAL INPUTS AND OUTPUTS OF AREA 19 Jamie Boyd* and Joanne Matsubara Depts of Ophthalmology and Anatomy, University of British Columbia, Vancouver, British Columbia, Canada V5Z 3N9

Our recent finding that cells in areas 17 projecting to the PMLS are confined to the CO blobs suggests that, as for primate area V1, cat area 17 may have segregated outputs to specialized extrastriate areas. In primates, CO blobs and interblobs in V1 project to thin stripes and interstripes, respectively, in V2. However, corticocortical connections between areas 17 and 18 in the cat are restricted to CO blobs (see previous abstract) begging the question of where the interblobs project.

Large, multiple injections of cholera toxin conjugated to colloidal gold (CTB-Au) were made into area 19 or the PMLS. In addition, small, focal injections of CTB-Au or unconjugated CTB were made into area 19. Labeled cells were charted in tangential sections of cortex and compared to the pattern of CO staining in alternate sections.

After large injections in area 19, labeling in areas 17 and 18 was continuous, with both blob and interblob zones labeled. Focal injections into area 19 gave rise to patches of labeled cells in areas 17 and 18 that were either confined to blobs, or confined to interblobs. In one case, after paired injections of CTB and CTB-Au, one tracer labeled blobs and the other labeled interblobs in areas 17 and 18, forming an interdigitated pattern. Interestingly, patchy, intrinsic connections in area 19 from these two injections were also partially interdigitated. These patches of local labeling in area 19 were nearly twice the size and spacing of their counterparts in areas 17 and 18. This larger spacing in area 19 was also reflected in the distribution of PMLS projecting cells in area 19, which were arranged in 0.5-1.0 mm wide bands spaced 1.5-2 mm apart and running perpendicular to the 18/19 border. We are currently studying the relationship between these output- and input-defined compartments.

These results suggest that area 19 of the cat is composed of at least two types of modules and that in cat area 19, as in primate V2, segregation of blob and interblob information is maintained, and reflected in projections to extrastriate areas. It is not clear if there is a third type of compartment in cat area 19 analogous to the division of CO stripes into thick and thin types. (This work funded by MRC 5-99150)

710.15

TWO CORTICAL VISUAL SYSTEMS IN A RODENT. C.G. Ellard* and M. Dias, Dept. of Psychology, University of Waterloo, Waterloo, Ontario, Canada.

Although it is sometimes assumed that there are two visual cortical processing streams in non-primates, there is little evidence for such a dissociation of function in rodents. We wanted to demonstrate selective effects of lesions to either dorsal or ventral posterior cortical areas on two different types of visual information processing in the Mongolian gerbil. Gerbils received either lesions of parietal cortex, temporal cortex, or sham surgery. After recovery, they were placed at random starting locations into an open field containing an object for five one-minute trials. On each trial, the total time spent in contact with the object was recorded. On the sixth trial, the object was moved to a new location in the field and a new, different object was placed in the old location. The critical measure was the amount of time spent in contact with each of the objects on the sixth trial. Normal gerbils showed decreasing contact time with the object over the first five trials. On the sixth trial, they showed elevated contact time with both the new object and the old object at a new location, demonstrating sensitivity to both object location and identity. Gerbils with parietal lesions showed dishabituation to the new object but not the new location, suggesting a spatial deficit but intact object recognition. Gerbils with temporal lesions showed no habituation to objects in the field, suggesting a deficit in object recognition. These findings constitute evidence that lesions to two different cortical areas in the gerbil produce dissociable effects on visual behaviour reminiscent of those seen in primates. (Supported by a N.S.E.R.C.C. grant to CGE).

710.16

ENHANCED PHYSIOLOGICAL DECAY OF VISUAL NEURONS IN MULTIMODAL AND NONPRIMARY VISUAL CORTICAL AREAS OF EARLY DEPRIVED CATS. U. Yinon, I. Gershon, R. Yaka and Z. Wollberg*. *Physiol. Lab., Goldschleger Eye Res. Inst., Fac. Med., Sheba Med. CTR and Dept. Zool., Fac. Life Sci., Tel-Aviv Univ., Tel-Aviv, 69978, ISRAEL.*

The modifiability of cortical cells was studied in visual primary (VI), nonprimary (SSS: PMLS, AMLS, PLLS, ALLS and medial AI) and multimodal (MS or area 7) areas. Extracellular unit recording was made in 7 cats monocularly deprived (MD) at ages of 1-11 weeks and in 31 normal (NOR) controls. They were anesthetized and paralyzed, the receptive fields (RFs) mapped and PSTHs analyzed. While similar responsiveness was found in VI

Cortical area	Total number of cells		Responsive cells, %		Binocular cells, %	
	MD	NOR	MD	NOR	MD	NOR
VI	64	429	84.4	86.5	33.3	71.1
SSS	122	398	26.2	49.7	9.4	88.9
MS	164	362	61.0	52.2	18.0	79.9

and MS areas of MD and NOR cats, a remarkable reduction was found in the SSS areas of the MDs (see table). The binocularity is consistently reduced in all areas of MDs but more vigorously in the SSS and MS. This is in accordance with our previous results suggesting reduction in selectivity of the cells' reaction and their RFs in MDs, outside VI (Soc. Neurosci. Abstr., 18:1315, 1992). There is, thus, an internal deficiency in processing visual information in areas not exclusively dedicated to vision, which is enhanced by the partial elimination of visual exposure during development.

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711.1

DIRECTIONAL RESPONSE PROPERTIES OF PURKINJE CELLS TO VESTIBULAR AND NECK INPUTS. O. Pompeiano*, P.Andre, and D.Manzoni. Dept. of Physiol., Univ. of Pisa, Pisa, Italy, I-56127.

Vestibulospinal (VS) and cervicospinal (CS) reflexes show different patterns of postural adjustements depending on the direction of head and neck displacements. This organization may require the assistance of the cerebellar anterior vermis, which integrates both the vestibular and neck inputs and influence the motor output. A prerequisite for such a proposed function is that the vermis encode the spatial coordinates of both head and neck displacements. We investigated, in decerebrate cats, the directional selectivity of the responses of vermal Purkinje (P)-cells to wobble either of the whole animal at 0.15 Hz, 10° (vestibular input) or of the body over a fixed head at 0.15 Hz, 2.5° (neck input). In both instances the direction of animal or body rotation moved at a constant speed over 360° in a clockwise (CW) or counterclockwise (CCW) direction. Bidirectional responses were represented by a vector lying midway between the maximal response directions to CW and CCW stimuli. These vectors were distributed over the entire plane of rotation for both vestibular (n=44) and neck responses (n=98), with some predominance of neck vectors in the fore-aft directions. In conclusion, the cerebellar anterior vermis provides a framework for spatial coding of vestibular and neck signals, and may intervene in the control of VS and CS reflexes during a variety of head and neck displacements.

711.2

SPATIO-TEMPORAL CODING IN THE CEREBELLAR CORTEX. V. Perciavalle*, G. Bosco and R.E. Poppele. Dept. of Physiology, University of Minnesota, Minneapolis MN 55455

The role played by parallel fibers in coincidence detection by Purkinje cells (PC) has not been investigated under natural stimulus conditions. It has been proposed that this function would depend on a synchronous activation of mossy fiber inputs similar to that of the climbing fibers. We present evidence for such activation from 208 spinocerebellar PCs recorded in anesthetized cats during rapid passive foot rotations. The stimuli evoked synchronous patterns of simple spike activity that reflected the temporal patterns recorded in the dorsal spino-cerebellar tract (DSCT) mossy fiber projection. We showed previously that the timing of DSCT post-stimulus spike density, expressed by principal components computed across cells, is generated by spinal circuits and not by stimulus parameters because the same components result from single electrical stimuli to peripheral nerves (Osborn & Poppele, J. Neurophysiol., 1989,61:447).

Three-quarters of the total variance in post-stimulus firing patterns across PCs in all parts of the spinocerebellum could be explained by four principal component waveforms which are nearly identical to those derived from DSCT activity recorded under the same experimental conditions. Further consideration of the temporal delays between DSCT and PC responses and the locations of responsive PCs relative to DSCT terminations leads to the conclusion that the synchronous patterns of post-stimulus activity evoked in cerebellar PCs results from the detection of synchronous patterns of parallel fiber activity established by spinal circuitry and transmitted via mossy fibers. (Supported by NIH Grant NS 21143.)

711.3

REGIONAL DIFFERENCES IN CEREBELLAR UNIT ACTIVITY AFTER CONDITIONING A BLINK CR TO AN ACOUSTIC CS IN CATS. C. Woody*, X.F. Wang and E. Gruen. UCLA Center for Health Sciences, MRRC, BRI, Los Angeles, CA 90024.

The % of cells responding to 70 db clicks and hisses differed by region: 83% in L V/VI, 56% in flocculus, 45% in lat. ansiform lobe, and 37% in med. ansiform lobe. After conditioning with click-CS and hiss-DS, mean activity 64-192 ms after click (n=699 units) increased relative to baseline in each region. The timing of the peak response differed from region to region. Onsets of response were typically 16-24 ms except for the med. ansiform lobe (>24 ms). (Earlier onsets were found in some units and states.) After conditioning, responses to CS were greater than to DS in the med. ansiform area, and exceeded peak responses to the CS before conditioning. The ratio of CS:DS responsive cells increased and reversed from that for adaptation and sensitization. Separation of data from L V and VI also found responses after conditioning greater to CS than DS in L VI. Discriminative initiation of the blink CR appears to depend more on motor cortex and dorsal cochlear nucleus than on cerebellum even though contributions from the cerebellum may be needed for late components of the CR to develop and be performed. (Supported by HD05958.)

711.4

SIMILAR SAGITTAL TOPOGRAPHY OF NECK AND EYE MOVEMENTS IN RABBIT CEREBELLAR VERMIS. M. Godschalk* and J. van der Burg. Dept. of Anatomy, Fac. of Medicine, Erasmus University Rotterdam, The Netherlands.

Electrical microstimulation in lobules VI and VII of monkey, cat and rabbit evokes saccadic eye movements, topographically related to the stimulation site in the vermis. In monkey and cat, the horizontal component of the saccades is directed ipsilaterally. In rabbit however, stimulation in a zone close to the midline evokes ipsilaterally directed saccades, and stimulation in a more distant zone induces saccades directed contralaterally (Godschalk et al., 1992, Soc.Neurosci.Abstr. 18:1046). To further elucidate this sagittal zonal topograpy, we studied the relation between evoked eye and neck movements in the rabbit.

Rabbits were prepared, under full anesthesia, for recording microstimulation-evoked eye and neck movements. Scleral search coils were implanted under the conjunctiva of both eyes and a similar coil was attached to a recording chamber, placed over the cerebellar vermis. After full recovery, electrode penetrations were made in the vermis while the head was relatively free-moving: the microdrive attached to the recording chamber was suspended from a rubber band, leaving the head in an upright position. The animal could freely rotate its head in the horizontal plane and shift the head for a limited extent in the horizontal plane and vertically.

On all sites where saccades were elicited, they were accompanied by neck movements. In all cases, the head made a rotatory movement in the horizontal plane and in the same direction as the accompanying eye movement; thus the sagittal zonal pattern of eye movements was preserved in the neck movements. The thresholds of the neck movements were similar to those of the elicited saccades and ranged between 8 and 60 μA. Latencies for both movement types were similar after any stimulus. We conclude that the topography of neck movements in lobules VI and VII of the cerebellar vermis, similar to saccadic eye movements, is organized in two parasagittal zones: a medial zone for ipsilaterally directed movements and a lateral zone for movements directed contralaterally.

711.5

SELECTIVE ELIMINATION OF CEREBELLAR OUTPUT IN THE GENETICALLY DYSTONIC RAT. M.S. LeDoux*, J.F. Lorden and J. Meinzen-Derr. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, Alabama 35294

The genetically dystonic(*dt*) rat, an autosomal recessive mutant, exhibits a progressive motor syndrome that resembles the generalized idiopathic dystonia seen in humans. Even with supportive measures, *dt* rats die by postnatal Day 35. A total cerebellectomy that includes the dorsal portion of the lateral vestibular nucleus (LVN) eliminates the dystonic motor syndrome of the *dt* rats, greatly improves overall motor function and prevents early death. After total cerebellectomy, *dt* rats survive into adulthood, mate and successfully rear their offspring.

Selective elimination of cerebellar output was performed on 15-day-old *dt* rats in order to determine the cerebellar components critical to the mutant's motor syndrome. Electrolytic and/or excitatory amino acid lesions of the medial cerebellar nucleus, interpositus cerebellar nucleus, lateral cerebellar nucleus and LVN were created in separate groups of *dt* rats. Rats were observed for the presence of abnormal motor signs (falls, twists, clasps, pivots) and tested on several measures of motor performance (activity, climbing, righting, homing) before surgery and again on Day 20. All nuclear lesions produced significant ($p < .05$) improvements in motor function and decreases in the frequency of abnormal motor signs. No individual nuclear structure appears entirely responsible for the *dt* rat motor syndrome.[Supported by NIH(K08 NS01593-01), Dystonia Medical Research Foundation and American Association of Neurological Surgeons]

711.6

CYTOLOGY OF THE CEREBELLAR CORTEX IN THE MEANDERTAIL MUTANT MOUSE. J.A.Heckroth*, A. Akintunde, H. Grishkat, and L.M. Eisenman Indiana University School of Medicine, THCME, Terre Haute, IN, 47809 and Jefferson Medical College, Dept. of Anatomy and Dev. Biol., Philadelphia, PA 19107.

Adult meandertail mutant mice (*mea/mea*), unaffected heterozygous mice (*mea/+*), and wild-type mice (C57Bl6) were euthanized, transcardially perfused with a buffered mixture of aldehydes, and the cerebella prepared for routine transmission electron microscopy. In its anterior region the meandertail cerebellar cortex is agranular, but retains a crude trilaminar architecture. The thin superficial layer consists of neuropil composed primarily of Purkinje cell dendritic profiles. Disarranged Purkinje cell somata with intervening neuropil form an intermediate layer. The deepest layer consists of glial and small neuronal somata, and a neuropil containing myelinated fibers, Purkinje cell dendrites, and a few mossy fiber rosettes. Scattered within the agranular cortex are microneurons, a few of which appear to be ectopic granule cells. The neuropil of the agranular cortex contains large numbers of presumed Purkinje cell dendritic spines which are wrapped in glial lamellae, and are apparently not contacted by presynaptic elements. Presumptive basket cell axons are observed to impinge upon the surface of Purkinje cell somata and dendrites, and boutons with the characteristics of climbing fibers contact some spinous appendages. The cortex of the cerebellar posterior lobe is relatively normal in appearance. This "normal" cortex, however, exhibits a high frequency of ectopic granule cells located singly, and in clusters, at the surface of and within the molecular layer. An intermediate cortical region forms a gradual transition from the normal to the agranular cortical architecture. Supported in part by NIH grant NS22093 to LME.

711.7

LOSS OF PURKINJE CELLS DOES NOT ALTER THE TOPOGRPAHY OF LOWER THORACIC-UPPER LUMBAR SPINOCEREBELLAR PROJECTIONS IN THE CEREBELLAR ANTERIOR LOBE OF *SHAKER* MUTANT RATS. T.L. Knight, N.A. Connors*, and D.L. Tolbert, Depts. of Anat., Neurobiol., and Surg. (Neurosurg.), St. Louis Univ., St Louis, MO. 63104

Lower thoracic-upper lumbar (LT-UL) spinocerebellar (SpCb) projections have a complex topography in the anterior lobe of normal rats. In adult *shaker* mutant rats, many or all of the Purkinje cells (PCs) in the anterior lobe degenerate. This study sought to determine if loss of PCs alters LT-UL SpCb topography in the anterior lobe. In *shaker* rats the distribution of WGA-HRP labeled LT-UL SpCb mossy fiber terminals were mapped in unfolded reconstructions of the anterior lobe and compared with similar reconstructions from normal rats. In *shakers*, labeled LT-UL labeled terminals were localized to clusters and sharply defined, irregularly-shaped patches surrounded by terminal free areas. The terminal patches were contained within discontinuous parasagittally oriented stripes or transversely oriented bands. The spatial distribution of LT-UL SpCb projections in *shaker* rats have numerous features of organization similar to that seen in normal rats. Based on these similarities, we conclude that PCs are not necessary for the maintenance of SpCb topography in the rat cerebellum. (Supported by NIH Grants RR07013 and NS20227).

711.8

NEW(?) PROPOSALS FOR CEREBELLAR STUDIES L.Simon*, A.Simon, J.Laczkó 1st Dept. of Anatomy, Semmelweis Univ. Medical School, 1450 Budapest, Hungary

a,The double spike generating mechanism revealed on dendritic records of Purkinje cells (Simon, 1981) suggests i, to postulate phase-related unidentical input to the (generally two) main, separated dendritic areas, which ii, due to the continuous transfolial shift of the dendritic trees (Palkovits et al. 1971), calls for a smooth, gradually changing parallel fiber input organization, rather than clearcut somatotopic maps.

b,The elementary phenomena of heterosynaptic modificating effect of climbing fiber response CFR), demonstrated on the independent dendritic spike generator patterns (Simon, 1977,1994) could fulfill both the heterosynaptic inhibition and facilitation proposed by Albus(1971) and Marr(1976), respectively.

c,The climbing fiber activation i, at sensorimotor conflict builds up the adaptive modifications, organizes and maintains task- and body/environment model (Simon, 1987)-related individual sensorimotor programs, ii, at need of changing for an other, formerly elaborated program, covering the incoming sensorimotor context, it will be loaded by inferior olivary bursts by means of fast (1-4 Sec) actualization of the dendritic input connections. Every individual PC can serve a pleanty of programs based on overlapping sensorimotor machinery.

711.9

ELECTRICAL STIMULATION OF THE INFERIOR OLIVE RESULTS IN ENHANCED ACTIVITY OF PROJECTION NEURONS IN THE CEREBELLAR NUCLEI OF THE KETAMINE ANESTHETIZED RAT.

T.J.H. Ruigrok, T.M. Teune, J. van der Burg and J. Voogd*. Dept of Anatomy, Erasmus University Rotterdam, the Netherlands.

The role of the complex spike as a 'meaningful' afferent input for the cerebellar nuclei is much debated. Here, we study the response of identified neurons in the cerebellar nuclei to graded electrical stimulation of the inferior olive.

Ketamine anesthetized male Wistar rats received stimulation electrodes in the red nucleus and inferior olive. Recordings of neurons in the contralateral cerebellar nuclei and cerebellar cortex were obtained with glass micropipettes filled with 4M NaCl. Cerebellar nuclear units were identified by their antidromic action potential triggered from the red nucleus which collided with spontaneous spikes. Purkinje cells were identified by their complex spike activity. After identification, the response pattern to graded inferior olive stimulation was recorded and analyzed.

Seventy-five cerebellar nuclear neurons were analyzed. Besides a single orthodromically induced action potential in 44 units (presumably triggered by climbing fiber collaterals; latency 4.2 $\pm$ 0.4 msec), virtually all units (73) responded with an initial pause of variable duration (mean 57 $\pm$ 38 msec). Post-stimulus time histograms showed that this inhibition phase was followed by an increase in activity in 61 units. For 36 units, the chance of firing more than doubled for at least 20 and up to 500 msec (mean 125 msec). Graded stimulation learned that, frequently, the inhibition developed before the facilitation. Purkinje cell recordings indicated that it is unlikely that the facilitatory effect upon cerebellar nuclear neurons is exclusively due to a change in simple spike firing rate.

It is concluded that olivary triggered complex spikes not only cause a phasic inhibition of cerebellar nuclear neurons but, in addition, may result in a subsequent, and potentially significant, increase of their firing rate. This effect appears to be especially prominent when large numbers of olivary neurons fire in synchrony.

711.10

INACTIVATION OF RED NUCLEUS BLOCKS BURSTING IN DEEP CEREBELLAR NUCLEUS: EVIDENCE FOR A POSITIVE FEEDBACK CIRCUIT. J. Keifer.* Dept. of Anatomy and Structural Biology, Univ. of South Dakota School of Medicine, Vermillion, SD 57069.

Microelectrode recordings in behaving animals show that burst discharges in cerebellum and red nucleus correspond to discrete limb movements. Activity in the cerebellorubral circuit is postulated to be generated by positive feedback. An anatomical basis for a recurrent circuit is provided by tract tracing studies in a variety of vertebrate species that demonstrate connections among the red nucleus, cerebellum and precerebellar nuclei. The function of a positive feedback circuit requires that the loop be closed. To further evaluate the potential role of positive feedback in producing bursts recorded in this circuit, the loop was opened by pressure microinjections of blocking agents and the effect on bursting examined.

Experiments were performed using the *in vitro* turtle brainstem-cerebellum preparation (*Chrysemys picta*). Extracellular single-unit recordings were made from the deep lateral cerebellar nucleus. Neurons were identified by antidromic activation in response to an electrical stimulus applied to the contralateral red nucleus and were readily driven to burst by a brief stimulus applied to the spinal cord. To evaluate the role of feedback in producing these bursts, cobalt (100 mM) or lidocaine (2%) mixed with fast green (2%) was microinjected (0.4 - 2.4 µl) into the red nucleus. During inactivation of the red nucleus, ascending inputs to the cerebellum from a spinal stimulus are unaffected. Ejection of these compounds into the red nucleus either blocked completely or reduced burst discharges recorded in the cerebellar nucleus. These effects were usually reversible. Location of the ejectate in the red nucleus was evaluated by histological localization of fast green. Some cells failed to show a response to the ejection. These cells could not be identified and may have been medial nucleus neurons, or ejections in these cases failed to localize in the red nucleus.

These preliminary observations provide further support for the hypothesis that burst discharge recorded in the cerebellorubral circuit is generated by a spatially distributed network that is mediated, in part, by positive feedback (Keifer & Houk, *Physiol. Rev.*, July, 1994). Supported by NIH grant R29 NS31930-01A1.

711.11

INHIBITORY SYNAPTIC RESPONSES IN TURTLE CEREBELLAR GRANULE CELLS Jens Midtgaard*, Div. Neurophysiology, Dept. Medical Physiol., Univ. of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark.

Synaptic responses were investigated in a slice preparation of the turtle (Trachemys scripta elegans) cerebellum by current clamp whole-cell recordings. IPSPs, presumably due to Golgi cell activation, were evoked by electrical stimulation in the granule cell layer and in the molecular layer. Three types of inhibitory responses were found: the classical bicuculline and picrotoxin sensitive response with reversal potential near the Cl^--equilibrium potential. This IPSP had a relatively short latency, lasted around 100 ms and strongly reduced the amplitude and duration of the mossy-fiber EPSP. In some cells a part of this IPSP persisted following the addition of bicuculline (50 µM) and picrotoxin (50 µM). Under these conditions glycine (focal application or 200 µM bath-applied) evoked a strychnine-sensitive (30 µM) response with a reversal potential close to the Cl^- equilibrium potential. The bicuculline and picrotoxin-resistant glycine response is consistent with the expression in granule cells of glycine-receptor β subunits, insensitive to picrotoxin (Pribilla et al). Following these short-lasting IPSPs, a slow IPSP developed, with a maximum after 500-1000 ms, and lasting some seconds. This IPSP had a reversal potential close to the K^+ equilibrium potential and was blocked by CGP 35348 (200-800 µM), consistent with a $GABA_B$ response. This IPSP was particularly prominent in the presence of bicuculline and picrotoxin: under these conditions mossy-fiber EPSPs displayed a marked paired-pulse depression, corresponding in timecourse to the $GABA_B$ IPSP; furthermore, the mossy-fiber paired-pulse depression was strongly reduced by CGP 35348. Thus, different IPSPs exert a strong control of the granule cell excitability on a timescale from ms to seconds.

Pribilla, I. et al. (1992). EMBO Journal, 11, (12), 4305-4311.

711.12

CONTROL OF SOMATIC SPIKING BY SYNAPTIC INPUT AND VOLTAGE-GATED DENDRITIC CURRENTS IN CEREBELLAR PURKINJE CELLS: A MODELING STUDY. D. Jaeger*, E. De Schutter^ and J.M. Bower. Div. of Biology 216-76, Caltech, Pasadena, CA 91125 and U. of Antwerp - UIA^, Belgium

The level of depolarization at the soma of a Purkinje cell is strongly dependent on the inward and outward currents distributed over the large area of its dendritic tree. Here we analyze the relative contribution of synaptic and voltage-gated currents on the spiking behavior of the soma, using a previously constructed realistic Purkinje cell model (De Schutter and Bower, J. Neurophysiol. 71: 375-419). We find that dendritic Ca^{++} currents tend to depolarize the cell in a positive feedback loop, such that a net outward synaptic current is necessary to produce physiological somatic spiking rates (0 - 100 Hz). In the absence of inhibition, bursting behavior ensues. This modeling result is supported by intracellular recordings *in vivo* showing somatic bursting behavior when inhibition is blocked with bicuculline. The control of spike timing in the model was investigated by varying inhibitory and excitatory input rates and synaptic strength. The regularity of spiking was found to be strongly dependent on the temporal structure of both inhibitory and excitatory input.

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711.13

SHORT-TERM INTERACTION BETWEEN THE COMPLEX SPIKE AND GRANULE CELL INPUTS IN A PURKINJE CELL MODEL
E. De Schutter[1]* and J.M. Bower[2]. [1]University of Antwerp - UIA, 2610 Antwerp, Belgium, [2]California Institute of Technology, Pasadena CA 91125, USA.

We used a detailed compartmental model of a Purkinje cell (J. Neurophysiol. 71:375-400, 401-419, 1994) to investigate how a climbing fiber (CF) input modifies the response to subsequent granule cell (GC) inputs. We have shown that somatic responses to small synchronous GC inputs are amplified by dendritic P calcium channels (PNAS in press); the same P channels are also activated by the complex spike (CS).

A CS fired up to 160 ms before the synchronous GC input had no effect on the amplitude of somatic EPSPs. A stimulus between 160 and 140 msec after the CF input was slightly potentiated. Between 140 msec and 90 msec there was a depression of up to 17 %, but 80 to 60 msec after the CF input there was a progressive and large potentiation (90 % at 60 msec) of the EPSP. GC inputs arriving sooner after the CF input fell into the prolonged afterhyperpolarization after the CS. Similar effects were seen for simple spike (SS) firing in response to steady asynchronous parallel fiber inputs. Between 60 and 80 ms after the CS, SS frequency increased up to 50%, between 80 and 120 ms SS frequency decreased up to 35%.

These results may explain some of the conflicting results found with *in vivo* recordings. We conclude that, even without synaptic plasticity and without changes in GC firing patterns, a CS might cause either a potentiation or a depression of SS firing, depending on which time frame after the CS is investigated.

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711.14

OPTICAL IMAGING OF CEREBELLAR CORTICAL ACTIVITY *IN VIVO* USING THE pH SENSITIVE DYE, PHENOL RED.
G.C. Chen and T.J. Ebner*. Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Neuronal activity is associated with intra- and extracellular pH changes. This raises the possibility of using pH as an indicator of neuronal activity, using optical detection methods. In this study we utilized phenol red as an optical indicator of neuronal activity. In anesthetized rats, Crus 1 and 2 of the cerebellar cortex were stained with phenol red (4 mg/ml) for approximately 1 hour. After wash out of the dye, these areas were imaged (100 ms exposure time, excitation 546 nm, emission > 590 nm) with and without surface electrical stimulation (single stimulus) using a Photometrics cooled CCD camera. The optical response in the cerebellar cortex to stimulation was determined by subtracting and averaging images without stimulation from images with stimulation. The optical response consisted of a discrete "beam" of increased signal which extended across the whole folium. The time course of the optical response peaked within 100 ms and was undetectable 200 ms after the onset of stimulation. Signal size correlated with the stimulation amplitude (100-300 μA). Field potential recordings revealed a close spatial correspondence with the optical signals. Both the electrical and optical responses were reversibly blocked with TTX. One possible origin of this optical signal is the initial, brief alkalization of the extracellular space which follows stimulus-evoked activity in cerebellum. This observation suggests that optical imaging of pH change could be a useful method for studying spatial and temporal patterns of activity in the central nervous system. Supported by NIH grant PO1 NS31318.

711.15

A NEW SILICON MICROELECTRODE ARRAY TECHNOLOGY FOR MULTICHANNEL EXTRACELLULAR RECORDING.
D.T. Kewley[a]*, D.A. Borkholder[b], C.W. Storment[b], J.M. Bower[a] and G.T.A. Kovacs[b]. [a]Computation and Neural Systems and Division of Biology, California Institute of Technology, Pasadena, CA 91125, [b]Department of Electrical Engineering, Stanford University, Stanford, CA 94305.

We have developed a new technology for the fabrication of micromachined *in vivo* extracellular microelectrode arrays. These devices ("probes") consist of thin films deposited on a single-crystal silicon substrate which is thinned to 24 μm. Their overall shape is comblike, with each shaft of the comb supporting several individual electrode sites. In a neurophysiological experiment, these shafts would be inserted into the brain of the experimental animal, allowing simultaneous recordings to be made from multiple locations both normal and parallel to the brain surface. Our efforts complement work on similar devices performed in other laboratories.

We are drawing on our combined experience as neurophysiologists and engineers to optimize the utility of this technology for the end users. For example, we are concentrating on providing a large number of electrode sites on a shaft of minimal width. We accomplish this by using two metal levels, one for high-resolution interconnects and the other for electrode sites. This allows us to position the electrodes over the interconnects, placing 4 electrode sites along the centerline of a shaft that is 24 μm wide, or 8 sites on a 40 μm shaft. Like other advanced probes, ours have iridium electrode sites, which can be used for both recording and stimulating. We are also customizing the probe geometries to meet the needs of specific experimental paradigms. For the first fabrication run, we have designed 15 different probes, including some specifically for the rat cerebellar cortex, some for the rat olfactory bulb, and some for general-purpose use. Supported by a grant from Medtronic Corporation to J.B., and an NSF NYI award to G.K.

711.16

COMPLEX SPIKE SYNCHRONY OF PURKINJE CELLS IN THE VESTIBULOCEREBELLUM OF THE RABBIT.
J.I. Simpson*, D.R. Wylie, and C.I. De Zeeuw. Department of Physiology & Biophysics, NYU Medical Center., New York, NY, 10016.

By recording from Purkinje cell pairs in the ventral nodulus of ketamine-anesthetized rabbits, we showed that neurons in the same sagittal zone and having the same complex spike (CS) response characteristics, exhibit CS synchrony, that is the tendency to fire within 2 msec (Soc. Neurosci. Abs. 19:1278). In the present study we show that CS synchrony occurs between cells in spatially separated zones but with the same class of CS responses, and between cells in the same zone but with different classes of CS responses. Neurons that exhibit CS modulation in response to optokinetic stimulation (OKS) about the vertical axis are found in 4 separate zones in each half of the cerebellum, 2 in the nodulus and 2 in the flocculus (VA zones). We recorded in the nodulus from 16 Purkinje cell pairs consisting of one cell in each of the VA zones. Six of these pairs showed CS synchrony. In addition, 3 of 14 pairs consisting of a VA cell in the nodulus, and a VA cell in the flocculus showed CS synchrony. The HA zones of the flocculus and nodulus contain contra-45° and ipsi-135° cells, both of which are modulated by OKS about a horizontal axis perpendicular to the ipsilateral anterior canal. They differ with respect to (1) ocular dominance, and (2) the origin of their CF input, which is the rostral dorsal cap and ventrolateral outgrowth, respectively. Of 7 contra-45°/ipsi-135° pairs, 5 showed CS synchrony. To summarize, CS synchrony is neither limited to Purkinje cells within a single sagittal zone nor to one class of Purkinje cells within the same zone.

711.17

MORPHOLOGICAL EVIDENCE FOR INTERZONAL INHIBITION BY GOLGI CELLS IN THE RABBIT VESTIBULOCEREBELLUM.
C.I. De Zeeuw*, D.R. Wylie, P.L. DiGiorgi, and J.I. Simpson. Dept. Physiology & Biophysics, NYU Med. Ctr., New York, NY 10016.

The complex spike (CS) and simple spike (SS) activity in individual parasagittal climbing fiber zones of the flocculus and nodulus are optimally modulated during optokinetic stimulation about approximately the same spatial axis (Kano et al.,'91). Which elements of the vestibulocerebellar cortex cause the SS activity to be spatially aligned with the CS activity is not clear, because an individual mossy fiber can have rosettes in different climbing fiber zones. To investigate whether Golgi cells might be involved in the spatial alignment, we made small injections of biocytin into the granular layer to determine the orientation of Golgi cell axons with respect to the parasagittal zones. The zones were identified using acetylcholinesterase staining of the raphes that divide the white matter into zones and/or by determining the axis of visual world rotation that produced the strongest CS modulation. The majority of our sample (n = 47) of Golgi cell axons in the flocculus and nodulus was oriented perpendicularly to the zones and crossed to neighboring zones. The basket cells showed the opposite pattern; their axons were parasagittally oriented and therefore were confined to a single zone (see also Bishop et al.,'91). We suggest that the Golgi cells may enhance the spatial alignment of the CS and SS modulation in the vestibulocerebellum by inhibiting the granule cells of neighboring zones.

711.18

ZONAL PROJECTION OF THE VENTRAL NODULUS IN THE RABBIT
D.R. Wylie*, C.I. De Zeeuw, P.L. DiGiorgi, and J.I. Simpson. Dept. of Physiology & Biophysics, NYU Medical Center, NY, NY, 10016.

The projections of Purkinje cells from zones in the ventral nodulus of pigmented rabbits were studied with the use of extracellularly injected biocytin as an anterograde tracer. The zones were physiologically identified according to the complex spike (CS) modulation of Purkinje cells in response to optokinetic stimulation (OKS). Purkinje cells in the most medial zone (zone NR) do not respond to OKS; they project to the fastigial nucleus, peri-fastigial white matter, peri-interposed white matter (plwm), and medial vestibular nucleus (MVN). In the adjacent zone (zone VA1) Purkinje cells respond best to OKS about the vertical axis; they project to the plwm and MVN. Purkinje cells in the next zone (zone HA) respond best to OKS about a horizontal axis approximately perpendicular to the ipsilateral anterior canal; they project to the plwm, dorsal group y, superior vestibular nucleus (mainly peripherally but also centrally), and MVN. In the most lateral zone, (zone VA2), Purkinje cells respond best to OKS about the vertical axis; they project to the plwm, dorsal group y, and MVN. Although all zones projected to the MVN, the projection from zone HA was largely to the caudal MVN, whereas the other zones projected to the parvicellular and caudal MVN subdivisions. A few terminal fields were found in the descending vestibular nucleus, magnocellular MVN, interposed nuclei, and ventral dendate nucleus. No terminals were found in the prepositus hypoglossi or lateral cerebellar nucleus. The majority of axons innervated more than one nucleus. Often, three or four different areas received terminals from a single Purkinje cell axon. The zonal projection pattern of the ventral nodulus is compared to that of the flocculus, which, with respect to the visual climbing fiber afferents, has similar zones.

712.1

MAXIMUM ACTIVATION DIRECTION AND VISUAL-VESTIBULAR INTERACTION OF FLOCCULUS PURKINJE CELLS IN ALERT CATS. K.D. Powell*, J.E. Killian, B.W. Peterson, J.F. Baker. Physiology, Northwestern Univ., Chicago, IL 60611.

We determined the maximum activation direction (MAD) vectors of 54 neurons in the vestibulocerebellum in the alert cat. The cat rotated sinusoidally at 0.5 Hz in conditions of total darkness and/or dim light. We characterized neurons during vestibular rotations about 3 - 12 axes. Of the 50 neurons recorded in dim light, 19 exhibited both simple and complex spikes and were classified as Purkinje cells (PC). As in our findings in decerebrate cats (Soc Neurosci 18:406), there was canal-canal convergence in alert cats. A total of 40/50 neurons (17/19 PC) had MAD vectors >20° from any single canal plane. MAD vectors of 3 neurons (1 PC) were <20° from ipsilateral horizontal canal, 5 neurons (1 PC) <20° from ipsilateral anterior canal and 2 neurons <20° from contralateral posterior canal. One PC recorded in dim light had a type III response and was >20° from any single canal plane. Other PCs had typical type I or II responses.

We recorded 11 neurons (8 PC) in total darkness, of which 8 neurons (5 PC) had MAD vectors >20° from any canal plane. The remaining 3 PCs each had MAD vectors <20° from one of the 3 ipsilateral canal planes. Of 7 neurons spatially characterized both in the dark and light conditions, 5 had MAD vectors for rotations in the dark >20° different from their MAD vectors for rotations in the light. The difference between MAD vectors for rotations in dark versus light indicates the presence of a visual signal with directional sensitivity different from that of the vestibular signal. The diversity of directional information represented by the Purkinje cells in the flocculus, both vestibular and visual, may support directional VOR adaptation. Supported by EY07342, EY06485.

712.2

INVOLVEMENT OF THE CAT CEREBELLAR INTERPOSED NUCLEUS IN THE CONTROL OF CONDITIONED AND UNCONDITIONED WITHDRAWAL REFLEXES. F.P. Kolb*, K.B. Irwin, N.K. Winters, J.R. Bloedel, V. Bracha. Barrow Neurological Institute, Phoenix, AZ, 85013.

The aim of the present study was to examine the involvement of the cat cerebellar interposed nucleus (IN) in the control of the classically conditioned and unconditioned reflexes elicited in three distinct effector systems.

In food-motivated cats, instrumentally conditioned to maintain quiet stance on small platforms, three types of classically conditioned withdrawal reflexes were established: 1) eyeblink reflex; 2) forelimb withdrawal reflex; and 3) hindlimb withdrawal reflex. Each animal was chronically implanted with a matrix of guiding cannulae used for insertion of injection needles. The effects of temporarily inactivating IN subregions were systematically examined using microinjections of muscimol (1 µl, 800 ng).

Transient inactivation of the IN affected conditioned as well as unconditioned components of the withdrawal responses in all three reflex systems. The drug injections altered both postural and phasic movement components of the limb's flexion responses. From the magnitude of the effects produced at different injection sites on the three conditioned reflexes, a topographical organization was derived. In addition, IN inactivation severely affected performance of the postural placement response in both limbs ipsilateral to the injection site and the tactile placing, hopping and magnet responses in the forelimb. The data support the view that various regions of the IN are differentially involved in the control of a variety of conditioned and unconditioned cutaneo-muscular and postural reflexes.
Supported by NIH Grants NS30013, NS 21958, Max Kade Foundation

712.3

CONTEXT-DEPENDENT MODULATION OF CEREBELLAR NUCLEAR NEURONS RELATED TO THE PERFORMANCE OF SPECIFIC MOVEMENT SEGMENTS. M.S. Milak*, V. Bracha, J.R. Bloedel. Barrow Neurological Institute, Phoenix, Arizona 85013.

This study addresses the hypothesis that the modulation of some cerebellar nuclear neurons related to the performance of a particular component of a complex movement reflects its context within the overall movement being performed. Initially cats were trained to reach for a manipulandum and move it through a template consisting of a single straight segment. Trained animals were implanted with a multiple single unit, microwire-based, recording system targeted to the anterior and posterior regions of the fastigial and dentate nuclei and the anterior and posterior interposed nuclei. After recovery, the animal was required to learn a succession of new templates, each consisting of 2 to 3 sequential, straight segments. The straight segment on which the animals were trained initially was a component of each template and was always positioned in the same location in the allocentric work space. Histograms were constructed from the activity of simultaneously-recorded units held throughout the acquisition and performance of all the templates used over one experimental day. Amplitudes of specific movement- or premovement-related unitary response components were analyzed relative to a movement segment common to all of the performed templates.

Of the 60 units recorded in seven experiments, 38% showed modulation changes dependent on the context in which the movement segment was performed. In some units these changes were related to movement components executed approximately 400 msec after the peak time of the unitary response. This finding suggests that these neurons participate in the specification and/or organization of movement sequences required for the performance of goal-directed forelimb movements. Supported by NIH grants NS30013 and NS21958.

712.4

DIFFERENTIAL CONTRIBUTIONS OF NUCLEUS INTERPOSITUS ANTERIOR AND POSTERIOR DURING REACHING TASKS IN THE MACAQUE. C.R. Mason*, R.R. Carter, J.F. Baker, J.C. Houk. Northwestern University Institute for Neuroscience, Chicago, IL 60611

Because the nucleus interpositus anterior (NIA) receives primarily cutaneous input from the rostral dorsal accessory olive while the nucleus interpositus posterior (NIP) receives primarily proprioceptive input from the medial accessory olive, we anticipated that the NIA and NIP might contribute to different aspects of motor control. A macaca fasicularis was trained in a pointing to target task, a reach to pull task and retrieval of fruit bits from Kluver board, bottles or experimenters' hands. The approximate locations of the NIA and the NIP were mapped out during a number of penetrations where single units were recorded and related to the target task. Inactivations were induced by pressure-injecting 2.0 µl muscimol (0.5µl doses four times 2.5 minutes apart)(2 mg/ml) above the NIA or NIP. Different reaching deficits were observed depending on the site of muscimol inactivation. The NIA inactivation greatly impaired the monkey's ability to shape its hand in preparation for surface contact during the pointing task or preshaping the hand prior to grasp in a reach to pull task. Once contact was made with the surface, the monkey appeared unable to use cutaneous cues to improve hand function. The monkey was able to aim its arm and place the hand in the desired spatial location for use. NIP inactivation greatly affected the monkey's ability to aim its hand to the desired location in space or maintain the position during use of the hand. The monkey did exhibit proper hand shaping for the tasks at hand. The monkey appeared unable to use proximal forelimb proprioceptive input and attempted compensation by using visual cues or distal fixation. These observations suggest that the NIA contributes to the optimal function of the hand during tasks that require coordinated use of the fingers and hand and that the NIP contributes to the overall coordinated use of the hand by coordinating the transport of the hand to where it needs to be in space (aiming the hand) and maintaining its position once there so that the hand and fingers have a stable base to work on.
Supported by N00014-90-J-1822 and 1 P50 MH48185-01.

712.5

DIFFERENTIAL TIMING OF CLIMBING FIBER DISCHARGES IN THE CEREBELLAR CORTEX DURING THE INITIATION PHASE OF THE VOLUNTARY ARM MOVEMENT IN MONKEY. J-P. Pellerin*, M-T. Parent, C. Valiquette and Y. Lamarre. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal (Qc), Canada, H3C 3J7.

Climbing fiber responses of Purkinje cells (CFR) were recorded in the vermal, intermediate and lateral regions of a monkey trained to perform rapid flexions or extensions of the elbow in response to randomly presented visual, auditory and somesthetic cues. 119 of the 314 recorded cells were time-lock activated with the movement onset in all triggering modalities. Two types of CFR were defined: 1- Pre-onset CFR: CFR frequency increasing only before the movement onset (65/119 cells) with a mean latency of -59 ms (s=18 ms) and 2- Post-onset CFR: CFR frequency increasing at/or after the onset of the movement (54/119 cells) with a mean latency of 10 ms (s=12 ms). In both cases, mean latency were statistically compared. In pre-onset CFR, the results showed a significant difference in the mean latency between the three areas investigated: Crus I and II: -81 ms (s=37 ms); Paramedian and Vermal lobules VII-VIII: -48 ms (s=24 ms) ($F(2,67) = 6.3004, P<.003$). However, there is no difference in the mean latency of the post-onset CFR in any of the cerebellar regions recorded (10 ms, s=12 ms). We suggest that pre-onset CFR are implicated in the control of the initiation of the movement because these cells are activated well before the onset of the movement. Post-onset CFR are probably more involved in feedback signal relative to the execution of the movement. Pre-onset CFR are activated earlier in the lateral cerebellum (Crus I and II) which project to the Dentate nucleus. Lateral cerebellum may be a part of a circuit regulating the initiation of the movement according to an hierarchical organisation of the sequence of activation of the olivocerebellar fibers. Supported by MRC grant of Canada.

712.6

NEURONAL CORRELATES OF DIRECTIONAL INSTRUCTION IN THE CEREBELLUM DURING PREPARATION FOR AN INTENDED MOVEMENT L.Germain,Y.Lamarre,J.Kalaska C.R.S.N. Dép. de physiologie, Univ. de Montréal

In order to assess the activity of cerebellar nuclear and cortical neurons during an instructed delay period two monkeys were trained according to the following paradigm.A 1000 hz or a 400 hz tone were used as auditory instructions for right elbow flexion or extension respectively.These auditory signals were followed by a silent immobile instructed delay period of 500 to 1500 msc duration.At the end of the preparatory period a torque pulse to the manipulandum was used as a trigger signal for movement execution.Tones and duration of delay periods were randomly mixed and set-related cells were recorded while the monkeys were performing at 92±4.4% and 85±5.9%.All neurons were recorded for approximately 100 trials and only cells which displayed a statistically significant(Student t-test p(0.01)sustained change in firing rate during the instructed delay period were included in our samples of set-related cells.Our first sample included 23 nuclear neurons and our second sample included 50 nuclear and 34 cortical neurons.Only neurons which displayed a statistically significant difference between their change in firing rate during instructed delay for flexion as compared to extension were considered as directional.The vast majority of set-related cells were directional.The mean difference in firing rate for directional nuclear set-cells were 10.88±5.29 imp/sec (n=19)and 10.91±5.64 imp/sec (n=35).In the cerebellar cortex this directional difference during the instructed delay was 14.35±15.3 imp/sec (n=22).These data are consistent with a role for cerebellar neurons in the preparation for an intended movement during an instructed delay motor task (supported by MRC and FRSQ).

712.7

SPATIAL CHARACTERISTICS OF THE RESPONSES OF CEREBELLAR PURKINJE CELLS DURING A TWO-DIMENSIONAL REACHING MOVEMENT IN MONKEYS I. SIMPLE SPIKES. Q.-G. Fu*, D. Flament, J.D. Coltz and T. J. Ebner, Depts. of Neurosurgery & Physiology, Univ. Minnesota, Minneapolis, MN 55455.

We have used a direction-distance arm reaching paradigm to study the spatial characteristics of the responses of cerebellar Purkinje cells and quantitatively analyzed the simple spike response with univariate and multivariate regression models. The paradigm involved a multijoint reaching movement in the horizontal plane from a centrally located start position to 48 targets located in 8 different directions and 6 distances. Simple spikes firing in relation to direction, distance, and target location were analyzed using regression models as previously applied to the premotor and primary motor cortex (Fu, et al, J.Neurophysiol., 70:2097-2116, 1993).

Based on a univariate model, the simple spike firing of the majority of cells (114/151,75.5%) was modulated by movement direction, distance or both parameters. Of these cells 31/114 (27.2%) showed a cosine-tuned modulation with direction and 45/114 (39.5%) cells were linearly correlated to distance, while 38/114 (33.3%) cells were modulated by both parameters. However, the majority of cells exhibited directional modulation at only one distance or distance modulation along one direction. Multivariate analysis indicated the firing of 99/128 (77.3%) cells was correlated with either direction, distance, or target location parameters (62/99, 62.7%) or some combination of these three parameters (37/99, 37.3%). The mean total R^2 for the multiple regression model was 0.34±0.15, considerably lower than the average of 0.61±0.18 obtained from the cerebral motor cortical area. Although significant correlation of simple spike discharge with kinematics occurs, still other parameters or models are needed to fully describe these cells' activity. Supported by NIH grant NS-18338.

712.8

Systematic error in multijoint coordination in cerebellar patients J Lou*#, S Massaquoi, B Marinelli#, M Hallett
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Cerebellar patients have multiple deficits in motor coordination. These deficits may share common pathophysiologic mechanisms. We studied multijoint coordination in cerebellar patients quantitatively. Two patients with olivopontocerebellar atrophy and 2 normal subjects sat in front of a chest-high table and placed the right wrist on the table 15 cm away from their chest. They reached for 3 targets in straight lines which required predominantly shoulder (direction A), synchronous elbow and shoulder (direction B), and predominantly elbow (direction C) rotation. Two infrared cameras sampled the positions of 4 diodes placed on the left and right shoulder, right elbow and right wrist at 400 Hz. The corresponding shoulder angles when elbow angles reached 1/3 of their angular trajectories were computed. At high velocities, normals deviated from straightness by making excessively synchronous elbow and shoulder rotations. In direction A, the average shoulder rotation of trials with higher velocities (AH) was 4° more than that with lower velocities (AL). In direction C, AH was 3.9° less than AL. Cerebellar patients deviated from straightness by a different systematic error. The abnormality was inadequate shoulder rotation in both direction A and C. AH was 5.3° and 7.0° less than AL in direction A and C respectively. This may be caused by inadequate torque generation at the shoulder. These findings suggest that the cerebellum may play a role in the control of force.

712.9

KINEMATIC AND EMG ANALYSIS OF WRIST MOVEMENTS IN FRIEDREICH'S ATAXIA. B.Wild, T.Klockgether, J.Dichgans (SPON:European Neuroscience Association). Department of Neurology, University of Tübingen, D 72076 Tübingen, Germany

Due to the predominant degeneration of peripheral and spinal proprioceptive neuronal pathways Friedreich's ataxia (FA) can be regarded as a model of cerebellar deafferentation. Our goal was to characterize movement abnormalities in fast goal directed wrist flexions in FA patients compared to age-matched healthy subjects and patients with chronic cerebellar degeneration.

Flexion movements (5° and 30° of amplitude and fast movements without target) were performed and measured using a manipulandum. Agonist and antagonist EMG were recorded with surface electrodes.

FA patients exhibited very slow movements with increase of reaction time, time to peak acceleration and movement duration and a decrease of peak acceleration and velocity. This was similar in cerebellar patients but significantly more pronounced in FA. In both patient groups the rise of the first agonist burst was slower and the antagonist burst delayed. In contrast to cerebellar patients there was a disruption of the triphasic pattern with diminished antagonist activity and a decrease or disappearance of the second agonist burst in FA.

We conclude that cerebellar deafferentation in FA and chronic cerebellar degeneration causes similar EMG and kinematic changes, i.e. slowing of movement initiation, accelleartion and velocity and a delay of the antagonist burst. We propose that the disruption of the triphasic pattern and the excessive movement slowing found in FA patients are suffieciently explained by peripheral deafferentation. Similar changes have been described in patients with peripheral neuropathy.

712.10

ANALYSIS OF INTERSEGMENTAL DYNAMICS IN CEREBELLAR LIMB ATAXIA

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OBJECTIVE: To determine the mechanical characteristics of multi-joint arm movements in three-dimensional space in healthy subjects and patients with cerebellar degenerative disorders.

METHODS: We studied 5 patients and 5 age and sex - matched normal subjects. Point-to-point multi - joint movements at various movement velocities were recorded using an infrared tracking system (E.L.I.T.E.) with position markers attached to the shoulder, elbow, wrist, and hand. An inverse dynamics approach modeling the upper limb as three interconnected rigid links was employed to calculate net joint torques (NET), gravitational torques (GRA), interactive motion-dependent torques (MDT), and muscle torques (MUS) acting at the shoulder and elbow joint. Effects of MDT on angular kinematics of interconnected joints were estimated.

RESULTS: When moving at high velocities, peak velocity, peak acceleration and deceleration of the hand marker, as well as angular velocity, acceleration and deceleration of shoulder and elbow joints were smaller in patients. Peak NET, MUS, and MDT torques acting at the shoulder and elbow joint were similar in both groups at slow and moderate speed, but were smaller in patients when performing high velocity movements. At all movement velocities, MDT originating in shoulder and wrist joint had a more profound effect on angular kinematics of the interconnected elbow joint in patients as compared to normals. Similarly, MDT originating at the elbow and wrist joint abnormally influenced angular acceleration of the shoulder joint in the patients.

CONLUSION: Dyscoordination of upper limb movements in cerebellar ataxia may be due to an insufficient compensation for motion-dependent interactive torques occuring during movements of a multi-segmented limb with interconnected joints. The problem may reflect a deficiency in generating appropriate levels of muscle torques at the shoulder and elbow joint.

712.11

HUMAN BRAIN FUNCTION IN BIPEDAL GAIT STUDIED WITH PET — AGING EFFECT IN CEREBELLAR FUNCTION K. Ishii, M. Senda*, H. Toyama, K.Oda, S. Ishii, K. Ishiwata and T. Sasaki, PET Center, Tokyo Metro. Inst. Gerontology, Tokyo, JAPAN.

We reported in the previous meeting on an approach to evaluate in vivo regional brain function during walk in human subjects by use of FDG-PET technique, in which the cerebellar vermis was significantly activated by a treadmill walk. The purpose of this study is to examine the aging effect on the brain function associated with bipedal gait. The subjects were twelve healthy volunteers without any neurological problems (ages 22 to 88). In each subject, two measurements were undertaken; in walking (30 minutes' treadmill walk at a steady velocity) and at rest (keeping supine rest). In both conditions, eyes were open and the brightness of the room light was controlled at the same level. After a transmission scan for attenuation correction, 150MBq of FDG was injected and the subject was instructed to keep one of the two conditions. Forty minutes after the injection, a 12- or 24-minute emission scan was performed with HEADTOME-IV. The two set of PET images from each subject were three-dimensionally registered. The whole brain activity in each study was normalized and the normalized regional activities were evaluated as the indices of regional brain function. Cerebellar vermis was markedly activated with treadmill walk in all the subjects. The mean amplitude of the reaction was 38% in the younger group (under 60, n=7, ages 31±9.4) and 27% in the elder group (over 60, n=5, ages 76±10.6). The tendency for the elderly people to lose walking activity may be partially attributed to the reduced reactivity of the brain.

713.1

NADPH-DIAPHORASE ACTIVITY IN DEVELOPING CEREBELLUM OF THE CHICK. C.R. Dermon*, A. Stamatakis (SPON: European Neuroscience Association) Dept. of Biology, Crete Univ., Iraklion 711 10, Crete, Greece.

A novel messenger nitric oxide (NO), has been suggested to play role in synaptic plasticity and to participate in shaping neuronal connections. In order to further investigate the role of NO, we studied the distribution of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) positive cells and fibers in the developing chick cerebellum, during the time that cell migration occurs and while synaptic contacts are established. From embryonic day 9 (E9) until E15 day, the most prominent feature was the strong NADPH-d activity in the undifferentiated cerebellar white matter, which contained a) NADPH-d positive ascending axons, that targeted selectively the developing folia and b) Golgi like stained neurons moving outwards from neuroepithelium to cerebellar cortex. In addition, NADPH-d labeled cells were found in cerebellar nuclei from E13 day. Cerebellar cortex exhibited weak NADPH-d reactivity until E15 day, when a) NADPH-d negative immature granule cells in external granular layer, begun massive inward migration to form internal granular layer, in which differentiated granule cells and glomeruli exhibited gradually increasing NADPH-d activity, b) molecular layer contained lightly stained basket and migrating granule cells, while diffuse NADPH-d activity increased, possibly due to the development of parallel fibers and c) Purkinje cells (PC) showed transient NADPH-d activity on their somata and apical dentrites, that peaked around E17 day and then gradually decreased, until posthatching day 1 (P1) when PC were completely devoid of NADPH-d activity, phenomenon that parallels the transient appearance of PC perisomatic processes and axo-somatic synapses. From P5 day, adult patterns of NADPH-d activity were observed in the chick cerebellum. Our data support similar pattern of NO expressing neurons in the developing avian and mammalian cerebellum and we suggest that neurons forming NO are involved in the developmental processes for the establishment of connections and normal cytoarchitecture

713.2

PURKINJE CELL HETEROGENEITY; A DIRECT COMPARISON STUDY OF TWO PARASAGITTAL BANDING PATTERNS IN THE ADULT MOUSE CEREBELLUM. A. Akintunde*(1), J. Voogd(2), T. J. H. Ruigrok(2), A. J. Haroian(3) and L. M. Eisenman(1). Depts. of Anatomy, Thomas Jefferson Univ., Philadelphia, PA 19017(1), Erasmus Univ., P.O.Box 1738, 3000 DR Rotterdam, Netherlands(2) and Hahnemann Univ. Sch. Med., Philadelphia, PA 19102(3).

Several correlational studies of the parcellation of the cerebellar cortex have been carried out in attempts to determine the "functional unit" or "cerebellar module" of the mammalian cerebellum. Purkinje cell efferents to the cerebellar nuclei have been shown to be organized into parasagittal zones as has the distribution of the zebrin II antigen. Unilateral injections of the tracer Tetramethylrhodamine ("Fluoro-Ruby") were made into the fastigial nucleus (FN) of the adult mice. Serial frontal sections of the cerebellar cortex were processed for visualization of tracer retrogradely transported from the FN injection site and for zebrin II antigenicity. Retrogradely labeled Purkinje cells were observed ipsilaterally in the posterior and anterior cerebellar vermis and in well-delineated narrow parasagittal longitudinal zones. Comparison of the banding patterns revealed the narrow longitudinal zones of retrogradely labeled Purkinje cells to be confined to a restricted portion within the boundaries of two immunonegative Purkinje cell bands. Similar observations were made in the rat (Voogd et al., Neurosci. Abstr. 17:1573, '91) NIH grant NS 16531 (LME) and NATO Grant CRG 910888 gratefully acknowledged.

713.3

CLONING OF A SELENOPROTEIN PREFERENTIALLY EXPRESSED IN THE CEREBELLUM. K. Saijoh*[a], N. Saito[b], M. J. Lee[a], M. Fujii[a], T. Kobayashi[a], K. Sumino[a], and T. Fukunaga[c]. [a]Dept. Pub. Hlth and [b]Dept. Pharmacol., Kobe Univ. Sch. Med., Kobe 650, Japan, and [c]Dept. Leg. Med. Shiga Univ. Med. Sci., Ohtu 520-21, Japan.

In order to obtain cDNA coding proteins enriched in the bovine cerebellar cortex, a subtraction library was produced. From the subtracted library, a clone of which cDNA insert displayed high homology to selenoprotein P cDNA but contained 12 rather than TGA codons and a tandem repeat of CCCCAC and CCCCAT were isolated. Thus, its primary structure was characterized as containing 12 selenocysteines as well as an 8-fold-repeat of (Pro-His). Moreover, the tandem repeat and its adjacent region included 16 histidines and 10 prolines among 28 amino acids. This selenoprotein mRNA was expressed in all the areas of the brain but most prominently in the cerebellar cortex, hippocampus, and olfactory bulb. Expression of selenoprotein P mRNA was scant in the brain. These findings suggest the possibility that this new selenoprotein, rather than selenoprotein P, is a major selenium carrier in the brain. (This work was in part supported by Grants-in-Aid of Ministry of Education, Science and Culture, Japan.)

713.4

ANATOMICAL TRACING REVEALS DIFFERENT VIBRISSA REPRESENTATIONS IN DIFFERENT CEREBELLAR LOBULES. J.J.A. Arends, H.P. Zeigler* & M.F. Jacquin. Anat. & Neurobiol., St.Louis Univ. Sch. Med., St. Louis MO 63104; Psychol., Hunter Coll., New York NY 10021; Neurology, Washington Univ. Sch. Med., St.Louis MO 63110.

Prior studies suggest that the cerebellum contains multiple representations of the body surface. It is unclear, however, whether different lobules contain representations that are biased toward particular receptor organs, and whether the latter are conveyed to the cerebellum via different mossy fiber systems. The concise and reiterated map of the facial whiskers in trigeminal brainstem subnuclei interpolaris and principalis, and their projections to the cerebellum, provide an ideal system for addressing these issues. We have previously shown that the uvula, deep lobule of Crus I, and Crus II receive the heaviest inputs, in decreasing order, from whisker-related portions of the trigeminal complex. Here, in adult rats, retrograde and anterograde tracers were used with parvalbumin immunohistochemistry to correlate single whisker representations in the trigeminal nuclei with their projections to the cerebellar cortex. We find that the distribution of labeled interpolaris cells differs markedly after tracer injections in the vermis (uvula) and the hemispheres (Crus I and II) as follows. Injections in the uvula produced a relatively homogeneous distribution of labeled cells throughout ventral interpolaris. Deep Crus I injections resulted in labeled cells mostly in ventrolateral interpolaris, where the caudalmost whiskers are represented. Crus II or paramedian lobule deposits produced a somewhat different pattern of retrogradely labeled cells that most heavily represented the ventral and rostral whiskers. Such "lobular" patterning is much less apparent in principalis. Here, mossy fibers originate exclusively in the rostral 2/3 of the nucleus, from a medial location caudally and gradually including more lateral regions rostrally. This distribution represents a bias toward a rostral whisker representation in principalis. Supported by NIH NS29885, DE07662.

713.5

THE DORSAL COLUMN NUCLEI PROJECT TO THE BASILAR PONTINE NUCLEI IN THE MONKEY. G.A. Mihailoff[1,2]* and S. Warren[1]. Departments of Anatomy[1] and Neurology[2], University of Mississippi Medical Center, Jackson, MS 39216-4505.

The basilar pontine nuclei (BPN) have long been recognized for their role in the transfer of cerebral cortical information to the cerebellum. However, recent studies in the rat indicate that the BPN receive a more diverse array of afferent projections than previously recognized. Included among these afferent systems is a projection from the dorsal column nuclei (DCN) and the spinal trigeminal nucleus. In the present studies we seek to document the existence of DCN projections to the BPN in non-human primates. In cynomolgus monkeys, multiple, unilateral injections of an orthogradely transported axonal marker were made into the DCN. In addition, in some animals, either multiple injections of an orthograde marker or an ablative lesion was made in the hand or leg region of the primary somatosensory cortex (SI). After survival periods ranging from one to twelve days, each animal was sacrificed and its brain prepared for light and electron microscopic visualization of the orthogradely transported markers. Observations from these cases indicate that the DCN in the monkey project via the medial lemniscus to territories within dorsal, medial, ventral, and lateral regions of the BPN. Some of these DCN termination sites overlap those of SI corticopontine projections. Electron microscopic studies revealed that labeled DCN boutons form asymmetrical synapses within the BPN, and further, that these spheroidal vesicle containing boutons participate in glomerular-like arrangements similar to those described in the rat. These observations suggest that in addition to the spinocerebellar systems, peripherally derived somatosensory information might be transferred to the cerebellum via mossy fibers arising in the basilar pons. Moreover, the possibility exists that peripheral and cortical somatosensory signals are integrated in some fashion within the BPN prior to transfer to the cerebellum.

Supported by NIH grant NS12644.

713.6

CONVERGENCE AND DIVERGENCE OF SPINAL AND SPINAL-CUNEATE MOSSY FIBER INPUTS TO THE RAT CEREBELLAR ANTERIOR LOBE. D.L. Tolbert* and J.M. Alisky, Depts. of Anat., Neurobiol., and Surg. (Neurosurg.) St. Louis Univ., St. Louis, MO 63104

The convergence and divergence of lower thoracic-upper lumbar (LT-UL) spinocerebellar (SpCb) and spino- and cuneocerebellar (CnCb) projections to the anterior lobe was studied in rats. Mossy fiber terminals differentially labeled with biotinylated dextran amine (BDA) and cholera toxin subunit B (CTb) were identified in the same section and quantitatively mapped in computer generated (Bioquant) unfolded reconstructions of the anterior lobe. Injections of BDA surrounded by CTb injections within a single LT-UL segment showed a higher degree of convergence than injections into rostrocaudally separated LT-UL segments. Continuing this trend, differentially labeled L_1 and L_2 SpCb terminals showed a greater degree of convergence compared to similarly labeled, but more widely separated, L_1 and $L_{3\text{-}5}$ projections. Differentially labeled SpCb and CnCb projections terminated preferentially in restricted non-overlapping areas but nonetheless showed a small degree of convergence. These findings indicate that there are varying degrees of convergence and divergence for different LT-UL SpCb inputs or for SpCb and CnCb inputs. However, terminal overlap or segregation of such inputs is never absolute. (Supported by NIH Grant NS20227).

713.7

BRANCHED NUCLEOCORTICAL PROJECTIONS TO THE INTERMEDIATE CEREBELLUM IN THE CAT. L. PROVINI [2], W. MARCOTTI [1], S. MORARA [1], A. ROSINA [1*, 1] Ist. Neurosci. Bioimm., C.N.R. and [2] Ist. Fisiol. Gen. Chim. Biol. Univ. Milano, Milano, Italy.

Cerebellar nucleocortical projections have been reported to be topographically organized, in relation to both the olivocorticonuclear compartments and the somatotopic arrangement of the olivocerebellar input. This study was aimed to address two related questions, i.e. I) whether or not somatotopically homologous areas of the anterior lobe (PIAL) and paramedian lobule (PML) - in C1, C2 and C3 compartments - differ in the extent and topography of their nucleocortical input; and II) whether or not there are nuclear neurons that connect by way of branched collaterals these two areas, as it was previously found for the olivocerebellar system. With this aim, one of the fluorescent retrograde tracers Fast blue (FB) and Diamidino yellow (DY) was pressure injected into the forelimb-related folia (FL) of the intermediate compartments of either PIAL or PML.

The analysis of single- and double-labeling in the inferior olive complex (IO) allowed to verify that the FB and DY injected areas were centered into the FL areas, and only those experiments in which the two IO labeled populations were in register, were selected for the present study. The results show that the FL-PIAL and FL-PML areas in C1, C3 and C2 cortical zones are projected upon by neurons located in the anterior (NIA) and posterior (NIP) interposed nuclei, thus roughly reciprocating the corticonuclear organization. Within these nuclei, the two populations of labeled neurons overlap and show a topographic relation to the cortical somatotopy. In the areas of overlap, double-labeled neurons are found. A minor non reciprocal projection stemming from the fastigial nucleus, and a contralateral projection from the interposed nuclei were also observed.

These data are taken to indicate that the corticopetal fibers from the interposed nuclei to C1, C2, C3 compartments mainly operate as a closed loop feed-back system. The non-reciprocal and contralateral nucleocortical fibers may be seen as a system which allows communication among different compartments.

713.8

VISUAL INPUT TO THE CEREBELLUM OF RATS. B. Mercier, E. Jenkinson, I. Kralj-Hans, C. Swales and M. Glickstein*. Dept. of Antomy, University College London, Gower Street, London WC1E 6BT.

To trace visual pathways to the cerebellum we injected wheatgerm agglutinin (WGA-HRP) into the visual cortex or the superior colliculus of rats and plotted terminal distribution of fibres in the pontine nuclei and nucleus reticularis tegmenti pontis (NRTP). In parallel experiments we injected WGA-HRP into cerebellar lobules VII or IX of the vermis or the petrosal lobule, and plotted retrogradely filled cells in the pons. The visual cortex projects principally to a dorsolateral region in the rostral half of the pontine nuclei. Although collicular projection overlap somewhat with those from the visual cortex, they extend far more caudally. Unlike the visual cortex, the superior colliculus also projects to NRTP. We compared the location of retrogradely labelled cells following cerebellar injection with the results of studies of orthograde projections from cortex and colliculus. The petrosal lobule receive its input from the same region of the pontine nuclei which receives fibers from the primary visual cortex. The region of pontine nuclei whose cells project to lobule IX receives inputs from both visual cortex and superior colliculus. Unlike the petrosal lobule and lobule IX, lobule VII receives a major input from NRTP. The results suggest that there are two relatively independent pathways by which visual information can reach the cerebellar cortex. One originates in the primary visual cortex and projects by way of the dorsolateral pons to the petrosal lobule and lobule IX. Another originates in the superior colliculus and projects by way of NRTP to lobule VII. The pattern of organization is similar to that seen in parallel experiments with monkeys.

713.9

ORGANIZATION OF THE OLIVOCEREBELLAR PROJECTION IN THE *MEANDER TAIL* MUTANT MOUSE. J. A. Napieralski, H.L. Grishkat and L. M. Eisenman*. Department of Anatomy/Developmental Biology, Thomas Jefferson University, Philadelphia, PA 19107.

There is a considerable amount of evidence which demonstrates that the histologically uniform cerebellum is actually divided into anatomical, biochemical and functional zones. One of the parasagittally organized afferent systems which has been particularly well characterized in several species is the olivocerebellar system. Although the mechanism underlying the organization of this system is unknown, useful information has been obtained through the analysis of the projection in neurologically mutant mice with cerebellar defects. This study presents the results of the analysis of the olivocerebellar projection in the cerebellum of the *meander tail* (*mea*) mutant mouse. The cerebellum of the *mea/mea* is characterized by an apparently normal cytoarchitecture posteriorly with an abrupt transition to an abnormal anterior region characterized by abnormal foliation, agranularity and Purkinje cell (PC) ectopia. Injections of either wheatgerm agglutinin-horseradish peroxidase or tetramethyl rhodamine into the inferior olive of *mea/mea* mice demonstrated that the parasagittal organization of the olivocerebellar system is preserved in both the "normal" posterior and abnormal anterior regions of the cerebellum. These results suggest that neither the agranularity of the cortex nor the abnormal location of the PC, the target cells of the olivocerebellar fibers, disrupts the normal parasagittal organization of these fibers in the cortex. NIH Grant NS22093 (to LME) gratefully acknowledged.

713.10

ANTEROGRADE LABELLING OF SPINOCEREBELLAR FIBERS IN FETAL MOUSE CEREBELLUM. H. L. Grishkat* and L.M. Eisenman. Department of Anatomy/Developmental Biology, Thomas Jefferson University, Philadelphia, PA 19107.

A large amount of information has been collected on the adult organization of the spinocerebellar projection in several different species. However, studies on the development of this projection are few and have been limited to select species such as the opossum, chick, cat and the early postnatal rat. An analysis using anterograde tracing techniques on the spinocerebellar projection in the fetal mouse (E13-E15) is presented. Biocytin crystals were applied to the cut end of the cervical spinal cord of embryonic mice. A tissue slab containing the brain stem, cerebellum and cervical spinal cord was maintained *In vitro* for 23 hrs and then fixed, sectioned and processed for biocytin revelation. Only a few labelled fibers were found in the lateral most portions of the rostral cerebellum at E13. At E14 labelled fibers were limited to rostrolateral portions of the cerebellum. At E15 labelled fibers were evident coursing to the rostromedial areas of the cerebellum. These fibers were just ventral to the migrating EGL and parallel to the pial surface, although a few fibers could be seen coursing more deeply within the cellular layer. Some fibers were seen to cross the midline. No labelling was found in the posterior cerebellum at this age. These studies indicate that spinocerebellar fibers have penetrated the cerebellar anlage significantly prior to the differentiation and migration of their target, the granule cell. Supported by NIH grant NS22093 to LME.

713.11

THE PREFRONTAL CORTEX IS THE TARGET OF OUTPUT FROM THE BASAL GANGLIA AND CEREBELLUM. F.A. Middleton*[1] and P.L. Strick[2]. [2]Research Service, VA Medical Center and Departments of [2]Neurosurgery and [1,2]Physiology, SUNY Health Science Center, Syracuse, NY 13210.

There have been a number of recent suggestions that output from the cerebellum and basal ganglia influences regions of the prefrontal cortex involved in language and higher cognitive function. To begin to examine this issue we injected the McIntyre-B strain of herpes simplex virus type 1 (HSV1) into the dorsolateral prefrontal cortex (Walker's area 46) of cebus monkeys (*Cebus apella*, n=3). This strain of HSV1 is transported transneuronally in the retrograde direction. All injection sites were confined to the principal sulcus and virus did not spread to adjacent cortical regions, such as the frontal eye field (FEF) in area 8. Five days after virus inoculation, many labeled neurons were found in both the dentate nucleus (DN) of the cerebellum and the internal segment of the globus pallidus (GPi). The size and shape of labeled neurons in DN and GPi were typical of neurons in these structures that project to the thalamus. Labeled DN neurons were located in the most ventral portion of DN and were concentrated rostro-caudally in the middle third of the nucleus. This location is clearly distinct from that of DN neurons labeled by retrograde transneuronal transport from the primary motor cortex (M1), ventral premotor area (PMv), or FEF. Labeled GPi neurons were located in the most dorsomedial region of both the inner and outer portions of GPi and were concentrated rostro-caudally in the middle-third of the nucleus. This location is clearly different from that of GPi neurons labeled by transneuronal transport from M1, PMv, or the supplementary motor area. These findings provide an anatomical substrate for the participation of cerebellar and basal ganglia output in higher cognitive function. Support: VA Medical Research Service and USPHS 2957 & 24328 (PLS).

714.1

CORTICOTROPIN RELEASING FACTOR (CRF) AXONS AND BINDING SITES IN THE REELER CEREBELLUM. J. S. King, G. A. Bishop, and P.C. Madtes, Jr.* Dept. Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

It is our working hypothesis that CRF functions in early development as a growth regulating factor. We previously reported in the developing opossum cerebellum that (1) CRF is present in early arriving axons and growth cones and (2) the initial CRF binding sites are expressed over Purkinje cells subsequent to their migration (King & Madtes, '93 Neurosci. Absts. 19:1215). In this study we used an antibody to CRF and ^{125}I labeled CRF to describe the distribution of CRF labeled axons and CRF binding sites in the cerebellum of the mutant mouse, reeler. In the reeler cerebellum, most Purkinje cells fail to migrate to the typical monolayer and are malpositioned in either the internal granule cell layer or in three paired deep cellular masses. CRF immunoreactive axons terminate in their adult phenotypes as climbing fibers and mossy fibers, but only in the reduced molecular and granule cell layers. Punctate immunolabeling is present within the lateral and intermediate deep cellular masses, but absent in the medial cellular mass. CRF binding sites are present over Purkinje cells that have migrated to form the typical monolayer, and those that reach the internal granule layer. In contrast, CRF binding sites are absent over Purkinje cells that fail to reach the diminutive cortex and are positioned in the deep cellular masses. Taken together, these findings are compatible with our developmental data that indicate CRF receptor binding is expressed over Purkinje cells only after they reach the cortical surface, and supports our working hypothesis. (Supported by NS-08798).

714.2

PEPTIDE LOCALIZATION IN THE MOUSE INFERIOR OLIVE. K.V. Gregg, J.S. King* and G.A. Bishop. Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Recent studies from our laboratory have defined the roles of various neuropeptides in modulating circuits in the cerebellar cortex (Prog. in Neurobiol., 39:475, 1993). A major afferent system to the cerebellum is derived from neurons in the inferior olivary complex (IOC). Modulation of this afferent system also will alter the pattern of activity in cerebellar circuits. The intent of the present study is to determine the distribution of several neuropeptides within the IOC of the adult C57BL/6J mouse using the PAP technique. These peptides are known to modulate cerebellar activity and include cholecystokinin (CCK), enkephalin (ENK), calcitonin gene-related peptide (CGRP), substance P (Sub P) and corticotropin releasing factor (CRF). Kooy's terminology is used to describe olivary subdivisions (Folia Neurobiol. 10:205, 1917). Each of these peptides, with the exception of Sub P, labeled small ($\leq 1\mu m$) varicosities in all nuclei of the olivary complex. In addition, CGRP and CRF immunostaining were present in larger profiles ($\geq 1\mu m$) that were restricted in their distribution. The larger CGRP varicosities were localized primarily in the lateral pole of the dorsal accessory olive (DAO), the dorsal cap of Kooy, and the dorsomedial extension of the ventral lamella of the principal olive (POv). Large CRF varicosities were exclusively found in the dorsal cap of Kooy. Substance P immunolabeling was confined to the dorsal cap of Kooy, the POv, the dorsomedial extension of the POv, and portions of the medial accessory olive (MAO). CRF was the only peptide that labeled olivary cell bodies. The overlap in the distribution of these peptides suggests that they may be colocalized with each other as well as with excitatory or inhibitory amino acids known to be present in afferents to the IOC. These findings serve as baseline data for future physiological studies designed to address the functional role of peptides in olivary circuitry. (Supported by NS08798).

714.3

PHYSIOLOGICAL EFFECTS OF CALCITONIN GENE RELATED PEPTIDE IN CEREBELLAR CIRCUITRY. G.A. Bishop*. Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Calcitonin gene related peptide (CGRP), composed of 37 amino acids, has been localized within specific populations of mossy fibers in the cat's cerebellar cortex (J.Comp.Neurol. 322:201-212, 1992). In the present study the physiological effects of CGRP on spontaneous, amino acid induced, and synaptically mediated activity were analyzed. In addition, interactions between CGRP and serotonin (5HT), another modulator of cerebellar circuitry, also were recorded. CGRP suppresses spontaneous and amino acid induced activity. However, the peptide has a more potent effect in blocking aspartate and quisqualate mediated excitation as compared to that elicited by glutamate. CGRP decreases or eliminates synaptic activity elicited by stimulation of the inferior cerebellar peduncle. 5HT also suppresses neuronal firing in the cerebellum. When CGRP and 5HT are applied together, the individual suppressive effects are potentiated. However, the potentiation is most effective if 5HT is applied prior to the application of CGRP. The results suggest that the release of amino acids from climbing and mossy fibers may not always result in excitation of their target neurons. Rather the response of the postsynaptic cell is dependent on its chemical microenvironment at a particular point in time. This chemical microenvironment may be different for each cell, thus each may have a unique response that is determined by interactions between peptides, monoamines and classic excitatory amino acids. These complex interactions between peptides, excitatory amino acids and monoamines provide evidence for functional plasticity in cerebellar circuitry. (Supported by NS18028).

714.4

COLLATERALS OF OLIVOCEREBELLAR PATHWAY SYNAPSE ONLY ONTO NON-GABAERGIC NEURONS OF THE DEEP CEREBELLAR NUCLEI. ★T. Borsello, ★F. Rossi, •J. van der Want and ★P. Strata*. ★Dpt. Anat. Human Physiol. Univ. of Torino, 10125 Torino, Italy and •Dpt. of Morphology, Netherlands Ophtalm. Res. Inst., 1100AC Amsterdam, The Netherlands.

Collaterals of the olivocerebellar fibres to the cerebellar nuclei have been assumed to be important to set a background excitation to allow Purkinje cells to sculpture their inhibitory action. The pattern of nuclear cell discharge to the motoneurons is important to shape movements. The discovery that the cerebellar nuclei contain cells which project back to the inferior olive and that are inhibitory (GABAergic) in nature leads to reexamine the problem as to the functional significance of these collaterals to the nuclear cells. Therefore, the question is whether such collaterals project to all types of nuclear cells or whether they are specific to a cell type.

Phaseoulus vulgaris leucoagglutinin (PHA-L) was injected into the inferior olive of adult albino rats, in order to identify at E.M. level collaterals to the cerebellar nuclei. GABAergic cells were identified by immunoreactivity with postembedding colloidal gold particles. Choleratoxin (CTb) was also injected into the inferior olive to label anterogradely the collaterals to the nuclei and retrogradely the nucleo-olivary projections.

Collaterals of inferior olive cells to the intracerebellar nuclei were present exclusively on non-GABAergic deep cerebellar neurons. In addition, CTb labelled retrogradely only deep nuclear cells which are GABAergic, whereas orthodromic labelling was confined to terminals ending on non-GABAergic neurons.

The lack of collaterals of the olivocerebellar pathway to the GABAergic neurons which project back to the inferior olive suggests the olivo-cerebellar-olivary loop to be a closed loop circuit which contributes to motor performance with a different mechanism from the open loop circuit where the deep nuclei neurons project towards the motoneurons.

714.5

17β-ESTRADIOL ALTERS SENSORY PROCESSING IN WHISKER RESPONSIVE CELLS OF PRINICPAL TRIGEMINAL (PrV) AND ROSTRAL DORSAL ACCESSORY OLIVARY (rDAO) NUCLEI OF THE RAT M.C. Kennedy[1]*, S.S. Smith[1], M.A.L. Nicolelis[2] and J.K. Chapin[2]. Depts. of Anatomy[1] and Physiology[2], Inst. of Neuroscience, MCP-Hahnemann University, Broad and Vine, Philadelphia, PA 19102-1192.

The estrous cycle of the rat is associated not only with changes in reproductive function, but also with facilitation of an entire array of sensorimotor parameters, including lowered sensory detection threshold, increased whisker barrelfield size and more precise limb coordination. Previous studies from this lab have demonstrated that systemic injection of 17B- estradiol enhances synchronized rhythmic discharge from the rDAO during a variable speed treadmill paradigm. In the present study the effects of this hormone (100 ng in oil injected i.p.) were tested on sensory reponses of simultaneously recorded neurons within the rDAO and PrV. For this study female rats were chronically implanted with separate bundles of 8 microwires (50 μ dia stainless steel, NB Labs) lowered independently into the whisker projection areas of the above structures. Up to 47 individual units were recorded using software from Spectrum Scientific on a Motorola computer. The whiskers were stimulated using a motor-driven wooden probe brushed in three different planes (rostral-to- caudal, dorsal-to-ventral, ventral-to- dorsal). Post-stimulus histograms were then constructed before and 30-90 min after administration of the steroid. Administration of 17β- estradiol enhanced the signal-to-noise ratio of sensory responses to brushing in the rostral-to-caudal (7/12 rDAO; 9/11 PrV cells) and ventral-to-dorsal (14/26 rDAO; 6/9 PrV cells) direction both by enhancing the sensory response (by ~20%) and by decreasing background discharge (by 30-50%) of those neurons demonstrating an excitatory response. In contrast, only 4/24 (rDAO) and 1/11 (PrV) responses of neurons to brushing in the dorsal-to-ventral direction were enhanced by hormone treatment; the remainder were either primarily depressed (13/24 rDAO; 4/11 PrV cells) or not changed (7/24 rDAO; 6/11 PrV cells) by 20 min post-steroid. Thus, these results suggest that the reproductive hormone estradiol alters sensory processing in whisker responsive cells of both trigeminal and rDAO neurons. (Supported by USAF Gr AFOSR#93-0136 to SSS and NS26722 to JKC.)

714.6

LOCAL APPLICATION OF ESTRADIOL FACILITATES SYNCHRONIZED RHYTHMIC OLIVARY ACTIVITY. Sheryl S. Smith*. Dept. of Anatomy, Inst. of Neuroscience, Hahnemann-MCP University, Phila., PA 19102-1192.

Ongoing studies in this laboratory have demonstrated that synchronized olivary oscillations are modulated by the hormone (estrous) cycle of the rat. Limb response to changes in treadmill speed is most accurate and timely on estrus, following endogenous increases in estradiol, relative to diestrus (lower hormone levels). One potential strategy by which estrous hormones might facilitate rapid limb movements is via an action on the synchronization of rhythmic olivary activity. Synchronized oscillations of this structure are thought to facilitate rapid, alternating movements of the limbs (Llinas, 1991). Previous studies from this lab (Smith, 1991, 1992) have demonstrated that synchronized rhythmic olivary activity recorded during treadmill locomotion is facilitated on the night of behavioral estrus, when limb coordination is improved, or following systemic injection of estradiol and progesterone at physiological doses. For the present study, the effect of local administration of estradiol was tested for its effect on synchronized olivary activity. This study was conducted in diestrous female rats chronically implanted with arrays of microwires (50 μ dia. stainless steel, teflon- coated) to record from as many as 23 neurons simultaneously within the rDAO during treadmill locomotion. The wires encircled a 23 g. cannula implanted 1 μM above the wire tips. Local infusion of drugs was then accomplished using fused silica capillary tubing (144 μ dia.). Local administration of 600 nM 17β-estradiol (E_2, in 1 μl 0.02% ETOH/saline) triggered synchronized olivary activity with a 3 Hz oscillation during treadmill locomotion using a variable acceleration paradigm by 15 min post-steroid. Local administration of vehicle had no effect on rhythmic olivary activity. Local administration of the NMDA receptor antagonist MK-801 (20 μM) 5 min prior to administration of the steroid prevented the synchronizing effect of E_2 administration. These results suggest that E_2 can increase synchronized olivary oscillations by acting withing the vicinity of the rDAO, and that this effect is dependent upon functional NMDA receptors. These modulatory effects of E_2 may underlie its reported facilitating action on rapid movements of the limbs. (Supp. by USAF Grant# 93- 1-0136)

714.7

ULTRASTRUCTURE OF MUSCARINIC AND NICOTINIC RECEPTOR PROTEIN IN THE RAT, RABBIT, AND MONKEY CEREBELLUM. A.R. Caffé, R. K. Hawkins and J.Voogd. Dept. Anatomy, Erasmus University Rotterdam, The Netherlands. **(SPON: European Neuroscience Association).**

The cerebellum is innervated by cholinergic mossy fibres from the medial vestibular and prepositus hypoglossi nuclei, and thin varicose fibres of which the origin is still uncertain. Furthermore, cholinergic agents administered within the cerebellum evoke behavioural modifications. Physiological studies, however, have yielded conflicting results about the identity of the cholinoceptive elements. To characterise cerebellar cholinoceptive profiles at the ultrastructural level, antibodies against cholinergic receptor proteins have been used. Muscarinic receptor protein immunoreactivity has been found in astrocytes. In the granular layer some Golgi cells, mossy fibre terminals, and granule cell bodies also express this staining. Some Purkinje cell somata display cytoplasmic labelling but most of these perikarya receive labelled axon terminals. In the molecular layer prominent muscarinic receptor immunolabeling has been observed in some Basket cells, Bergmann glia and parallel fibres. Nicotinic receptor protein is differently expressed. Some granule and Golgi cells, but no astrocytes or mossy fibre terminals, express immunoreactivity. Most Purkinje cells and their dendrites show strong staining. In the molecular layer parallel fibre varicosities, but no Bergmann glia fibres, express nicotinic receptor protein. Species differences exist in topography of both muscarinic and nicotinic stained cerebellar profiles. The results indicate that muscarinic and nicotinic receptors are synthesised by multiple and distinct cerebellar cells. Combined with the species differences this might explain the present inconsistent data on the identity of cerebellar cholinoceptive profiles.

714.8

GLUTAMATE RECEPTOR SUBUNITS AT THE GIANT MOSSY FIBER-UNIPOLAR BRUSH CELL SYNAPSE. E. Mugnaini*, and D. Jaarsma. Lab. of Neuromorphology, Univ. Connecticut, Storrs, CT 06269-4145, USA

The unipolar brush cell (UBC), a previously neglected small neuron of the cerebellar granular layer, is densely concentrated in the vestibulo-cerebellum and is characterized by its single brush-like dendritic arbor arising from a short stalk (Mugnaini and Floris, J. Comp. Neurol., 339: 174-180, 1994). The UBC is innervated by one or two mossy fibers (mf) via an extraordinarily extensive synaptic contact (Mugnaini et al., Synapse, 16: 284-311, 1994). White matter activation results in NMDA and non-NMDA glutamate receptor-mediated EPSCs in the UBC (Rossi et al.,this meeting). Here we used previously characterized antibodies specific for AMPA, kainate and NMDA receptor subunits to immunohistochemically identify the receptor/s associated with the mf-UBC synapse. Frozen and Vibratome sections of rat and cat cerebella, perfused with buffered aldehydes, were incubated with polyclonal antisera to GluR1, GluR2/3, GluR4, GluR6/7, KA-2, NMDA-R1 and NMDA-R2A/B (kindly donated by R. Wenthold), diluted 1:50 to 1:200, and with monoclonal antibodies (mab) recognizing GluR5/6/7 (Clone 4F5, Pharmingen) and NMDA-R1 (clone 54.1)subunits, diluted 1:500 to 1:2000. The PAP or the ABC method were employed using DAB as the chromogen. UBCs were strongly immunostained with the GluR2/3 and GluR5/6/7 (4F5) antibodies. Weak staining was observed with antibodies against the NMDA-R1 subunits. Antisera against the GluR1, GluR4, GluR6,7, KA-2 and NMDA-R2A/B subunits did not substantially immunoreact with the UBCs. At the electron-microscopic level, GluR2/3 and GluR5/6/7 immunoreaction products were found throughout the UBC cytoplasm. The mab to G5/6/7 stained the post-synaptic densities at the mf-UBC contact more densely than the polyclonal Glu2/3 antiserum. Thus we were able to clearly identify both AMPA and kainate glutamate receptor subunits at the mf-UBC synapse. Supported by USA PHS grant NS 09904.

714.9

SPATIOTEMPORAL STUDY OF FOS ACTIVITY IN THE INFERIOR OLIVE AND CEREBELLUM FOLLOWING SYSTEMIC HARMALINE. D.W. Saxon* and A.J. Beitz. Department of Vet.PathoBiology, University of Minnesota, St Paul, MN 55108

The tremorgenic alkaloid, harmaline when given systemically produces repetitive synchronous activity in neurons of the inferior olive (IO) and presumably release of excitatory amino acid neurotransmitter at the olivo-Purkinje cell synapse. Following subcutaneous injection (25 mg/kg in saline) and survival times between 15 minutes and 24 hours, rats were perfused with paraformaldehyde, sectioned and reacted for FOS protein immunocytochemistry. The results indicate that as early as 15 min following harmaline there are detectable levels of FOS in caudal subdivisions of IO (i.e., IOA, B & C) and that by 30 min most other subdivisions of IO (i.e., IOD, IOM, IODM,& IOPr) show FOS activity. Activity in the IO peaked by 2 hours with regard to numbers and intensity of positive FOS nuclei and had returned to control levels by 18 hours post-harmaline. Medial subdivisions of IO were found to contain FOS activity for protracted times after lateral IO subdivisions returned to control levels. The cerebellar cortex showed marked increase in FOS activity by 1 hour post-harmaline. At 1 hour the granule cell layer throughout the cerebellum and the Purkinje cell (PC) layer of the vermis and paravermis showed FOS nuclear staining. A subset of PC in the vermis and paravermis were found to display FOS activity at 1 hour and all later times tested. FOS positive nuclei in the molecular layer (ML) were numerous in the vermis but sparse in the lateral hemispheres. Labeling in the ML peaked by 2 hours and gradually returned to control levels by 12 hours post-harmaline. The deep cerebellar nuclei also showed spatial and temporal changes in FOS activity. A morphologically diverse group of neuronal nuclei were evident in the fastigial nucleus by 30 min, but changed to a single morphological group of small nuclei by 18 hours. The interposed and lateral cerebellar nuclei displayed intense FOS activity by 2 hours and returned to control levels by 24 hours post-harmaline. Taken together these results suggest that there are distinct spatial and temporal changes in the cortex, IO and deep cerebellar nuclei in response to harmaline activation of the IO. Supported by Grant # NS31318 and DC01086.

714.10

ETHANOL SUPPRESSION OF GLUTAMATE-INDUCED ENHANCEMENT OF CEREBELLAR PURKINJE CELL DISCHARGE IN RAT. R. Grigorian*, G. Zernova, A. Khorkov. Sechenov Institute, St. Petersburg, Russia.

It is commonly accepted that both ethanol (ET) and glutamate (GLU) have powerful actions on the activity of cerebellar Purkinje cells (PC). The effects of intravenous injections of 1.0 g/kg of ET on glutamate-induced enhancement of cerebellar PC discharge was studied. The mean change in PC discharge frequency produced by microiontophoretic application of GLU across a range of current intensities (30, 80, 120 and 180 nA) was evaluated before and after the injection of small doses of ET. The circulating level of ET was determined by gas chromotography. Before application of ET, the mean frequency of PC discharge activated by mossy fibers was 26.56 $\pm$ 0.33 imp/s, the standard deviation was 2.6%, and the time to return to baseline after GLU was 600-1600 ms, depending upon the current intensity used for GLU injection. In contrast, after ET injection, the mean frequency of PC discharge in response to GLU was always less than before ET, across all current intensities employed. The standard deviation of PC discharge frequency after ET injection was reduced to 0.6%, one-fourth as variable as before ET. Further, the restoring time of PC discharge frequency in response to GLU was as much as 1.5-2.0 times faster after ET injection. These results demonstrate that ET suppresses the GLU-induced enhancement of cerebellar PC discharge frequency.

714.11

ALTERATION OF CERBELLAR GLUTAMATE RECEPTOR SUBUNITS FOLLOWING INFERIOR OLIVE AND PONTOCEREBELLAR TRACT LESIONS. J. J. Sanderson, R. H. Price, Jr.* and A J. Beitz. Department of Veterinary PathoBiology, University of Minnesota, St. Paul MN 55108

The major cerebellar afferent pathways, climbing and mossy fibers, are reported to use an excitatory amino acid as their neurotransmitter. Chemical lesions of the inferior olive and mechanical lesions of the pontocerebellar mossy fiber tract were made in an effort to elucidate the receptor subtypes responsible for mediating cerebellar afferent neurotransmission . Intraperitoneal (IP) injections of 50 to 60 mg/kg 3-acetyl pyridine (3-AP) were used to chemically lesion the inferior olivary nucleus of young (150 to 250 g), male SD rats. The right middle cerebellar peduncle (MCP) was lesioned with a short, horizontal sweep of a 28 g needle inserted through the occipital skull plate. *In situ* hybridization histochemistry was used to monitor postsynaptic changes in the levels of mRNA for subunits of the NMDA (NR1), AMPA (GluR1) and metabotropic (mGluR1) subtypes of glutamate receptors. In addition, immunocytochemistry with specific antisera for the NMDA and AMPA receptor subunits NR1, GluR1, GluR2/3, and GluR4 was used to detect differences between control, 3-AP-lesioned and MCP-lesioned rats. Preliminary results suggest that GluR2/3 immunostaining in subpopulations of Purkinje cells oriented along parasagittal zones becomes more intense in the 3-AP-lesioned animals. In non-lesioned controls, all Purkinje cells appear to be uniformly stained. These results suggest a selective glutamate receptor subunit upregulation after climbing fiber destruction. Supported by NS31318.

714.12

EVOKED RESPONSE MODULATION IN INTRACELLULARLY RECORDED CEREBELLAR PURKINJE CELLS BY SEROTONIN. L.J. Larson-Prior* and R.M. Bushey. Dept. of Neuroscience and Anatomy, Penn State College of Medicine, M.S. Hershey Medical Center, Hershey PA 17033.

Distinct differences in the extent and distribution of serotonergic input to the cerebellar cortex have been reported for several mammalian species based upon immunocytochemical data. In addition, the physiologic responses of extracellularly recorded cerebellar Purkinje cells to exogenously applied seotonin have been reported to be variable; eliciting both increased and decreased spontaneous firing rates. Recently, the $5HT_{1A}$ receptor has been implicated in the reduction of Purkinje cell activity (Darrow *et al., Eur. J. Pharmac.* 175:145, 1990; Kerr and Bishop, *Brain Res.* 591:253, 1992). Utilizing an isolated cerebellar preparation from the brain of the freshwater diving turtle which maintains the three-dimensional architecture of this brain region (Larson-Prior *et al., J. Neurophysiol.* 63:293, 1990), we have tested the effects of exogenously applied serotonin on synaptic responses of intracellularly recorded cerebellar Purkinje cells.

Consistent with activation of the $5HT_{1A}$ receptor subtype, recorded Purkinje cells were hyperpolarized with application of bath applied serotonin (5-10 mM) and showed a consistent decrease in membrane resistance. Firing rate, as assessed by injection of depolarizing current, was slowed following application of serotonin. Evoked responses were obtained by electrical stimulation of either the cerebellar peduncle or the cerebellar granule cell layer. In both cases, unitary EPSPs were reduced by bath application of serotonin. Trains of stimuli (5 stimuli, 50 Hz) produced a late, slow EPSP which was also attenuated with the application of serotonin. Supported by NS 30759.

715.1

IDENTIFICATION OF PASSIVE AND REFLEX COMPONENTS OF HUMAN DYNAMIC ANKLE STIFFNESS. R.E. Kearney[1]*, R.B. Stein[2] and L. Parmeswaran[1], [1]Dept. of Biomed. Eng, McGill University, Montréal, Canada, H3G 1Y6 and [2]Div. of Neurosci., University of Alberta, Edmonton Canada T6G 2S2

The role of stretch reflexes in the control of movement and posture remains unclear since it is difficult to distinguish the relative contributions of passive and reflex mechanisms to the torques generated in response to a joint perturbation. System identification techniques, using random perturbations, have yielded good descriptions of overall joint stiffness and reflex EMG dynamics but have had little success in characterizing the reflex contribution to the stiffness. Recent evidence suggests that the reflex response is strongly nonlinear and that on-going movements inhibit reflex action in proportion to their average velocity. This motivated us to re-examine human ankle stiffness using experimental and analytic methods designed to overcome these problems. We have used position perturbations which were designed to provide a wide-bandwidth position input with low average velocity. Possible nonlinearities in stiffness dynamics were accounted for through the use of a parallel-cascade system technique for non-linear system identification. Experiments using these new methods consistently identify two distinct components in the overall stiffness dynamics. The first component is position-dependent, acts at short-latency (< 40 ms) and display high-pass dynamics; it seems certain to reflect the passive contribution to stiffness. The second component is velocity-dependent, acts at longer latency (> 40ms) and has dynamics which are described by a static non-linearity followed by low-pass dynamics; this component likely represents the *reflex contribution* to ankle stiffness. The reflex contribution to stiffness was most significant at low frequencies (below 10 Hz); its size relative to the passive contribution varied strongly with the parameters of the perturbation as well as joint position and level of activation. Thus, the role of stretch reflexes in motor control is likely to be very context sensitive. (Supported by the Medical Research Council of Canada)

715.2

NONLINEAR SHORT-LATENCY REFLEXES PREVENT INSTABILITY IN ELBOW POSITION. R.B. Stein*[1], I.W. Hunter[2], S.R. Lafontaine[2] and L.A. Jones[3], Div. of Neuroscience[1], Univ. of Alberta, Edmonton, Canada T6G 2S2, Dept. of Biomed. Eng.[2] and School of Phys. and Occ. Therapy[3], McGill Univ., Montreal Canada.

A motor and digital control system have been developed which allow rapid stretches to be applied to the human elbow joint. The forearm is returned to the initial position and the properties of the control system can be varied before the reflex contraction. Thus, short latency reflex responses can be cleanly separated from intrinsic muscle properties under a wide variety of loading conditions. The reflex force varies linearly with the velocity of stretch over nearly two orders of magnitude. The reflex force also varies linearly with the tonic level of force over the entire range of forces studied (0 - 100 N). This contrasts sharply with, for example, the human ankle joint which shows a very limited linear range. As the control system is made more compliant (less stiff) reflex shortening increases dramatically and becomes more prolonged, whereas the reflex force becomes somewhat smaller and shorter. With compliant loads the reflex shortening is on average equal to the stretch that generated it. Simulations the results show that the dependence of reflex shortening and force on the stiffness of the load is mainly determined by the mechanics of the limb and muscles. The gain of the reflex is as high as it can be without causing instability. The presence of a rectification nonlinearity (e.g., lengthening the muscle produces a reflex, but shortening the muscle does not) is mainly responsible for preserving the stability of the elbow system.

Supported by MRC and NCE of Canada.

715.3

MOTOR UNIT ACTIVITY DURING SPASTICITY. B. H. Ross, and C. K. Thomas*. The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL 33136.

Spasticity is a common phenomenon following human spinal cord injury. Most studies to date have examined changes in reflex pathways in individuals who experience spasticity but during the absence of actual spasms. These data therefore provide important clues about the baseline condition from which spasticity stems. However, they provide little information about the processes which occur during spasms per se. The aim of the present study was to examine the patterns of motor unit activity during induced spasms. Seven individuals with chronic (>1 yr post injury) spinal cord injury between C3-C6 induced spasms by either gentle touch to the side of the knee or weak upper body movements. The evoked electromyogram (EMG) was recorded unilaterally from the surface of each of the triceps surae muscles and intramuscularly from the medial gastrocnemius muscle. None of the triceps surae muscles were under voluntary control. However, during spasms, there were usually short bursts of EMG in all three muscles. Occasionally, periods of rhythmic clonic activity or sustained activation of these and many other muscles were induced. During the brief and sustained spasms, motor units responded: 1) at slow tonic rates (< 10 Hz), 2) with repetitive doublets, and/or 3) with an increase then decrease in rate as the strength of the spasm rose and fell. The first two patterns of motor unit activity seem unique to involuntary contractions whereas the third pattern is typical of motor unit behavior during voluntary contractions. During clonus, motor unit activity also rose and fell rhythmically with the repetitive rise and fall in surface EMG. Peak rates for different motor units occurred in phase. These data suggest that the asynchronous versus synchronous activity of different motor units may explain the nature of the spasm (brief burst versus clonic form).

715.4

AFFERENT CONDITIONING PRODUCES DIFFERENT EFFECTS ON FLEXION AND H REFLEXES IN MAN. J. Liu and C.W.Y. Hui-Chan*. School of Physical and Occupational Therapy, McGill University, Montréal, Québec, Canada

The objective of this study was to determine whether human flexion reflex (FR) and H-reflex (HR) were modified in a similar or different manner by repeated daily transcutaneous electrical nerve stimulation (TENS).

Twenty young healthy subjects were randomly assigned to a TENS or a placebo group, with 10 in each group for the FR test and 4 in each group for the HR test. Ten daily 60 min TENS or placebo stimulation was applied to the lumbro-sacral region over a two-week period. The FR was elicited by electrically stimulating the sole of subject's right foot and recorded electromyographically from the tibialis anterior (TA) and biceps femoris (BF) muscles. The HR was elicited by electrically stimulating the right posterior tibial nerve in the popliteal fossa and recorded electromyographically from the soleus muscle. ANOVA and planned comparison tests were used to compare the data obtained on Day_1 and Day_{10} of the treatment period and between the two groups.

For the TA FR area, the pre-stimulation control value obtained on Day_1 was significantly decreased to 68.6% on Day_{10} in the TENS ($p<0.01$), but not placebo group (92.5%; $p>0.05$). For the BF FR area, the pre-stimulation control value was depressed by both TENS and placebo stimulation to 63.8% ($p<0.01$) and 84.8% ($p<0.05$) respectively on Day_{10}, with TENS producing greater inhibitory effects than placebo stimulation ($p<0.01$). For the HR, no significant effect was found on the pre-stimulation control value on Day_{10} either in the TENS (= 96.7% of Day_1 value; $p>0.05$) or placebo group (= 107.3%; $p>0.05$), or between the two groups.

Our results indicated that repeated daily TENS applications over a two-week period produced significant inhibitory influence on the FR but not the HR. Such a differential effects produced by the *same* afferent conditioning are consistent with the FR and HR being mediated respectively by a *poly*synaptic and predominantly *mono*synaptic reflex pathway. *(This study was financed by the Fonds de la Recherche en Santé du Québec)*

715.5

Interaction of Flexion- (FR) and Crossed Extension Reflexes (CER) with Tilt-Evoked Responses (TER) in Standing Humans. N. Paquet * and C.W.Y. Hui-Chan, School of Phys. and Occ. Therapy, McGill University, Montreal, Canada, H3G 1Y5.

With the use of ischemia, ankle and foot somatosensory inputs were found to contribute to the early component of the TER in lower limb muscles (*Bussel et al., Prog. Clin. Neurophysiol., 8:310-322, 1980*). The purpose of this study was to examine whether FR afferent inputs would modify the lower limb TER in a functionnally meaningful manner.

Thirteen young healthy subjects were blindfolded and stood fixed to a tilting apparatus with straps and a neck collar. They underwent sudden forward head-body tilts of 15° with an axis of rotation co-linear with the ankle joint. The mean head acceleration was 0.7g, as measured with a linear accelerometer mounted on a dental bite. FR and CER were elicited with electrical stimulation (ES) of the right tibial nerve behind the medial malleolus during standing (ES alone, n=10 trials) and tilting (tilt+ES, n=20 trials), when the background contraction of the ipsilateral tibilalis anterior (TA) muscle was maintained at 10-20% of maximum voluntary contraction. The ES was delivered as a train of 1 ms pulses at 200 Hz during 30 ms at a mean of 3.3 x sensory threshold. TERs with tilt alone (n=10 trials) were also elicited for comparison purpose. EMG responses were recorded in the TA, soleus (SO), biceps femoris (BF) and vastus lateralis (VL) muscles bilaterally.

The FR area in the ipsilateral TA was increased ($p<0.05$) during tilt in the majority (n=6/7) of subjects whose TA FR area was significantly modified by tilt, while it was decreased in only 1 subject. The remaining 6 subjects showed no effect of tilt on the ipsilateral TA FR area (n=3), or less than 30% of FR occurrence (n=3). Moreover, more subjects showed a response in the contralateral TA during tilt+ES (n=6) than during ES (n=2) or tilt alone (n=1). Finally, a majority of respondents had a tendency for a larger EMG area in the ipsilateral VL (n=8/10 respondents), and contralateral SO and/or VL (n=5/6) during tilt+ES than ES alone.

These results indicate that whole head-and-body tilt tended to increase the excitability of lower limb flexor (including FR) and extensor (including CER) muscles activated by the flexion reflex afferents. The resultant co-contraction of ankle muscles and increased knee extensor activation is consistent with the more difficult task of maintaining a stance against perturbation. *This project was financed by Parkinson's Foundation of Canada and a MRC studentship for N.P.*

715.6

H-REFLEX CHANGES INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION DURING VOLUNTARY MOVEMENT. A.M. Sherwood*, W.B. McKay and M. R. Dimitrijević; Restorative Neurology, Baylor College of Medicine, Houston, TX 77030-3498

Voluntary dorsiflexion movement modified early and later responses to transcranial magnetic stimulation (TMS) with marked, phase-dependent changes in amplitude and latency. To explore movement-related modification of segmental excitability, we evaluated the effects of TMS on H-reflexes over conditioning to test (C-T) intervals ranging from -10 to 100 mS while 5 healthy subjects lay supine during relaxation and during voluntary ankle movement. Half-maximum amplitude H-reflexes were conditioned by TMS at 20% above EMG threshold in the soleus (typically 50-60% of the maximum TMS output). During relaxation there was a slight overall facilitation, but no dependence on C-T interval. In plantar flexion, there was a dependence on C-T interval ($p < 0.01$), with an enhancement of H at 0 mS compared to 30-100 mS ($p < 0.01$). During dorsiflexion movement, there was an early decrease followed by *increased* excitability at a 60 mS C-T interval compared to control reflexes obtained during movement alone (260% increase, $p < 0.01$), and compared to TMS-conditioned responses at -10 to 40 mS ($p < 0.01$). These findings support the polysynaptic excitation of soleus motoneurones acting as antagonist during voluntary dorsiflexion movement hypothesized to be responsible for generation of the late MEP responses.

715.7

THE EFFECTS OF LOW INTENSITY CUTANEOUS STIMULATION ON THE H REFLEX MODULATION DURING STATIC AND DYNAMIC CYCLING MOVEMENTS. C. Labrecque and M. Bélanger*. Dép. de Kinanthropologie, Univ. du Québec à Montréal, Montréal, Québec, Canada, H3C 3P8.

Numerous studies have shown modulations of the cutaneous reflex during rhythmic movements such as walking and cycling. Similarly, the H reflex has also been shown to be modulated and depressed during such movements. However, in real life situations, the nervous systems must consider these two sources of interacting feedback during the ongoing movement. The main objective of this study was to examine the effects of cutaneous stimulation on muscular inputs during stationary positions and active cycling movements. The cutaneous stimulation consisted of a 10 ms train of 5-1 ms pulses, at 2 times the sensory threshold, and applied to the dorsal surface of the foot. The H reflex stimulation consisted of a 1 ms pulse applied to the tibial nerve and it followed the cutaneous stimulation by 50 ms. The intensity of the H reflex stimulation was adjusted throughout the experiment and trials which had a M-wave between 10-20% of the maximal M-wave were selected for analysis. Four muscles ipsilateral to the stimulation (Tibialis Anterior, Soleus, Biceps Femoris and Vastus Lateralis) and 4 phases of cycling (0°=top pedal position, 90°,180°and 270°) were examined for both the static and dynamic conditions (cycling rate of 1 Hz and workload of 1 kpm). During the static condition, the soleus H reflex varied in amplitude depending on the phase, the largest one being at 90° and the lowest at 0°. The low intensity cutaneous stimulation had a tendency to increase the H reflex response particularly at the 270° position. During the active cycling, the H reflex was also modulated, the greatest response being at 90° while the lowest amplitude was observed at 0° and 180°. The H reflex continued to be modulated in a similar fashion following the cutaneous stimulation, however, the responses tended to be slighlty inhibited. (Supported by PAFACC, UQAM)

715.8

SPASTICITY IN THE CONSCIOUS RAT? QUANTITATIVE EXAMINATION OF STRETCH AND VELOCITY SENSITIVE REFLEX COMPONENTS. WJ Thoman & JS Taylor*. Dept. Neurosci., U of FL Gainesville.

Although the rat has often been used as a model of spinal cord injury (SCI), no study has yet identified spasticity, defined as enhanced stretch activity and velocity-sensitive (VS) reflex components.[1] Here we present preliminary data of a SCI model using the conditioned rat, with recording of long-latency triceps surae stretch reflex activity following subtotal lesions at the upper lumbar level. Background forces were closely matched before and after SCI[2] to obtain more direct measures of test reflex activity. Injury-induced stretch reflex deficits were similar to those obtained previously from the conscious cat[3], with increases in both dynamic and static torque amplitude and no change in either the VS or dynamic index components. However, and in contrast with the cat, hyper-reflexia was not observed in the first week post lesion, but developed to a maximum over the first month[4]. We conclude that with appropriate reflex testing conditions rats can also be defined as spastic following selective spinal lesions[3], but that increased VS of long-latency stretch reflexes as a hallmark of SCI should be re-examined using other testing procedures[4]. Supported by Grant NS27511 and IDSTF.

(1)Lance,JW (1980) Symposium synopsis. In: Feldman,RG et al. (eds) Spasticity: Disordered Motor Control. pp485-494. (2)Katz,RT & Rymer,WZ (1989) Arch.Phys.Med.Rehabil. 70:144-155. (3)Taylor,JS, Vierck,CJ,Jr., Munson,JB, (1993) Development of an animal model for the study of spinal spasticity. In: Thilman,A et al. (eds) Spasticity: Mechanisms and Management. pp204-221. (4)Thompson,FJ, Reier,PJ, Lucas,CC, Parmer,R (1992) J.Neurophys. 68(5):1473-1486.

715.9

PARALLELS BETWEEN CHANGES IN EXCITABILITY OF THE H REFLEX AND RECOVERY OF MOTOR FUNCTION IN CATS AFTER CUTTING AFFERENT AND EFFERENT CONNECTIONS OF MOTOR CORTEX. **J.A. McMillan*, M. Sinyaya, A. Shelyakin, and T. Tobias, Montana State Univ., Bozeman, MT, 59717 and Pavlov Inst. Physiol. and Inst. Exp. Med., St. Petersburg, Russia**

Earlier work has demonstrated compensatory plasticity of sensory projections to the cortex in cats following unilateral isolation of the cerebral cortex from its afferent and efferent connections with the spinal cord. (Sinyaya et al., 1991, Sechenov Physiol. J. of Russia, 77(8):24-31). The goal of the present study was to examine changes in excitability of the H reflex in cats after brain damage and to determine if such changes correlate with reported changes in assymetry of reflex function in children undergoing functional biofeedback therapy for cerebral palsy (Bogdanov et al., 1991, Sechenov Physiol. J. of Russia, 77(10):24-28.

The H reflex was recorded from medial gastrocnemius in intact cats and in cats in which the corona radiata supplying left motor cortex was cut while leaving the cortex itself intact (cf. Sinyaya et al. above). Threshold for the reflex was lower in the right leg immediately after the lesion. Presynaptic inhibition of the reflex, monitored by comparing amplitude of control and test reflexes evoked by paired stimuli 100 msec apart, was less pronounced following the lesion. These changes were less evident 1-3 months after the lesion. We propose that the long-term changes in excitability of the H reflex reflect a compensatory process which affects motor as well as sensory function following brain damage. (Supported by NSF EPSCoR RII-8921978 and NIH 5S06GM08218-09).

715.10

IDENTIFYING THE INTERNEURONS INVOLVED IN THE REFLEX RESPONSE TO ELECTRICAL STIMULATION OF THE INTERNAL BRANCH OF THE FELINE SUPERIOR LARYNGEAL NERVE. Y. Tanaka, C.L. Ludlow*, and W.S. Selbie. VSS, NIDCD, NIH, Bethesda, MD 20892.

Electrical stimulation of the internal branch of the superior laryngeal nerve (ISLN) produces a bilateral adductor response in the feline larynx. The purpose of this study was to identify interneurons in the medulla oblongata that comprise this reflex pathway using the retrograde tracer cholera toxin B to label the nucleus ambiguus (NA) and the stimulus induced expression of the proto-oncogene, c-fos. Cholera toxin B was injected into the thyroarytenoid, lateral cricoarytenoid, cricothyroid and the posterior cricoarytenoid muscle to identify the efferent neurons in the (NA.) After 72 hours, the cats were anesthetized with alpha chloralose and the right ISLN was placed in a bipolar cuff electrode. Following 4 hours rest the ISLN was stimulated at 0.5 Hz at supra maximal levels for 20 minutes immediately before euthanasia. Serial 50 µm transverse frozen sections were made through the medulla oblongata. Odd numbered sections were processed for immunohistochemistry by means of anti-c-fos protein. Even numbered sections were incubated with anti-cholera toxin B. As expected, cholera toxin B labeled neurons and dendrites in the NA and terminals in the NTS. C-fos-like immunoreactive neurons were identified bilaterally in the NTS from the level of the most rostral portion of the dorsal motor nucleus of the vagus to the most caudal portion of the inferior olivary nucleus (IO), and in the NA from the rostral end of the hypoglossal nucleus to the caudal end of the IO. Bilateral c-fos like reactions were also observed in the reticular formation around the NA. Neurons expressing c-fos that lay outside the anatomically defined NA and NTS are candidates as interneurons.

715.11

PREPULSE FACILITATION OF THE ACOUSTIC STARTLE REFLEX IN THE RAT: STIMULUS PARAMETERS AND DRUG EFFECTS. M.K. Taylor, J.R. Ison* & S.B. Schwarzkopf, Depts. of Psychology and Psychiatry, University of Rochester, Rochester, NY, 14627.

Noise prepulses (PP) presented in a masking noise (N) just prior to a startle stimulus can either facilitate (PPF) or inhibit (PPI) the acoustic startle reflex (ASR). There are many studies of the psychophysical and pharmacological characteristics of PPI, but little is known about PPF. Here we report that a 20 msec PP ending with the startle pulse facilitates the startle in inverse linear proportion to its signal to noise ratio (PP/N). Accordingly, our weakest PP/N (a +3 dB ratio) approximately doubled the ASR to a 115 dB stimulus, whereas ratios greater than +9 dB resulted in increasing amounts of PPI. The PPF for a consistant PP/N ratio (a +3dB difference) increased with noise levels from 30 to 40 dB, but then remained constant for higher intensities (tested to 73/70 dB). As PP lead time increased, PPF was enhanced up to 20 ms and changed to PPI beyond 60 - 80 ms. Both effects increased with experience. Diazepam enhanced PPF (revealing that PPF is different from other forms of ASR potentiation) but reduced PPI. In contrast, preliminary data show that PPF is reduced by apomorphine. Because these drugs affect PPF as well as PPI, their effects may not be restricted to inhibitory processes, as is generally thought. These drug effects on PPF and PPI may result from their disruption of attentional processes involved in stimulus discrimination and reflex control; and that PPF is altered at lead times as short as 5 ms indicates that these mechanisms have a peripheral site of action.

(Supported by PHS grants, AG09524 and MH40381)

715.12

SYNAPSES IN ROSTROLATERAL MIDBRAIN MEDIATE "FEAR" POTENTIATION OF ACOUSTIC STARTLE AND ELECTRICALLY EVOKED STARTLE. J.S. Yeomans* and P.W. Frankland, Dept. Psychology, University of Toronto, Toronto Ont. M5S 1A1.

Electrical stimulation of the caudal ventral amygdalofugal (VAF) pathway from the amygdala to the brain stem evokes startle-like responses, or at lower currents enhances acoustic startle. Electrolytic lesions of these sites block fear potentiation of acoustic startle. Collision tests show that startle-like responses are due to short axons from the amygdala to the diencephalon-midbrain border, which synaptically activate long axons from the rostral midbrain to the medulla (Yeomans & Pollard 1993). The short axons that link the amygdala with the midbrain have moderate conduction velocities of about 10 m/s and 0.4-0.8 ms refractory periods, while the long axons that link the midbrain and medulla have fast conduction velocities of about 60 m/s and 0.2-0.5 ms refractory periods. The functional synapses were located in a small region of the rostrolateral midbrain in two ways: 1) The transition zone from axonal to transsynaptic collision for electrically evoked startle was located by placing a series of electrodes near the midbrain-diencephalon border and testing for collision with medulla and diencephalon sites; 2) Bilateral ibotenate lesions (3 ug) of this same zone, dorsal to the substantia nigra, blocked fear potentiation of acoustic startle.

715.13

LESIONS OF THE CENTRAL GRAY BLOCK SENSITIZATION AND FEAR POTENTIATION OF THE ACOUSTIC STARTLE RESPONSE IN RATS. M. Fendt, M. Koch and H.-U. Schnitzler. (SPON: European Neuroscience Association). Tierphysiologie, Universität Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany.

The acoustic startle response (ASR) can be enhanced by conditioned or unconditioned fear. It has been shown that the central nucleus of the amygdala (cA) and its efferent pathway to the caudal pontine reticular nucleus (PnC), an essential part of the primary startle circuit, is important for the enhancement of the ASR. It was unclear, however, whether these modulations were directly mediated by the amygdaloreticular pathway or whether there exists a relay nucleus within this pathway. We tested the hypothesis that the midbrain central gray (CG) is important for the effects of fear on the ASR. Neuroanatomical tracing studies described a descending projection from the cA to that part of the CG where a descending projection to the PnC takes its origin. We lesioned this part of the CG with the neurotoxin quinolinic acid and then measured the effects of conditioned and unconditioned fear on the ASR. Lesions of the dorsal and lateral parts of the CG totally blocked the sensitization of the ASR by footshocks (unconditioned fear) and blocked the enhancement of the ASR by conditioned fear without affecting the ASR amplitude in the absence of the conditioned or unconditioned stimuli. This finding suggests a crucial role of the CG for the enhancement of the ASR by conditioned and unconditioned fear. The pathway from the cA via the CG to the PnC represents one part of a complex circuitry mediating the effects of conditioned and unconditioned fear to the ASR.

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715.14

STRIATED MUSCLE ACTIVITY DURING THE MICTURITION REFLEX IN THE RAT AND MICTURITION PATTERNS IN A SEXUAL CONTEXT. J. Manzo*, A. Esquivel, M.I. Vázquez, Y. Cruz and P. Pacheco. INE, Univ. Veracruzana, Xalapa, Ver; CIF, Univ. Autón. Tlax., Tlaxcala, Tlax; Inst. de Invest. Biomédicas, UNAM, México, D.F. México.

In rats, EMG activity of the external urethral sphincter (EUS) has been recorded during voiding, that is, during the high frequency oscillation phase (HFO) of the micturition reflex. Here we investigated if the EUS is the only striated muscle activated during HFO. The cystometrogram technique was combined with simultaneous EMG recordings of several muscles in the pelvic region. Results indicated that in addition to the EUS, the bulbospongiosus, levator ani, and pubococcygeus muscles show phasic EMG activity during HFO. The EMG frequency of all these muscles was 8 Hz, similar to the frequency of HFO. The duration of HFO and phasic EMG was also similar (6.5±0.25s). HFO phase and EMGs were reliably correlated with voiding.

Also we explored the existence of a behavioral pattern occurring during micturition in rats. An arena with two compartments (30x30 cm each) separated by a mesh was used. A receptive female was placed in one compartment and a sexually experienced male in the other. 40 such pairs were tested four times. Only males urinate consistently (62.5%) in this condition. Urine was expulsed in a burst pattern, and placed in a line parallel to the dividing mesh at a mean distance of 12.65cm. These results are correlated with the data obtained from a group of n=10 males and n=10 females in which residual urine was measured after stress-induced micturition. It was showed that males retain significatively more urine than females ($F(1,19)=15.63$ $p<0.05$).

It is suggested that the somatic motor control of micturition in the rat is complex and serves not only elimination *per se*, but also other behavioral patterns associated with micturition and possibly having a communicatory function. SEP (PP) and CONACYT and PADEP-UNAM (JM)

715.15

MUSCULAR REFLEXES ELICITED BY GENITAL STIMULATION IN THE FEMALE RABBIT. M. Martínez-Gómez*, M. Carro, P Ordinola, H. Distel, R. Hudson and P. Pacheco. Centro de Investigaciones Fisiologicas-UAT, Mexico; Inst. Med. Psychol., Univ. Munich, Germany; Instituto de Investigaciones Biomedicas-UNAM, Mexico.

The present findings represent part of a larger study investigating the contribution of pelvic innervation to reproductive procceses in the rabbit. In adult chinchilla -breed females, reflex EMGs of the Musculi constrictores vulvae and vestibuli, ischiocavernosus, bulboespongiosus, obturatorius internus, pubococcygeus, and rectus abdominis, were recorded in response to stimulation of the clitoral sheath, vagina, cervix, perineal, and perianal skin, and the flanks. Strong EMG activity was elicited by clitoral stimulation in all muscles except the rectus abdominis, and to a slightly lesser degree by stimulating the first and second thirds of the vagina using a glass rod. Both kinds of stimulation resulted in prolonged afterdischarges- up to 50 sec for the pubococcygeus and the obturatorius muscles. Stimulating the cervix produced a response only in the rectus abdominis with an afterdischarge of about 18 sec. Brushing or pressing the clitoral sheath, perineal or perianal skin resulted in EMG activity only in the pubococcygeus and, possibly, the constrictor vestibuli. Stimulating the flanks failed to elicited any response.

In summary: Clitoral and vaginal stimulation provoked strong EMG activity in almost all the muscles studied which was blocked by cervical stimulation. Withdrawal of cervical stimulation resulted in the reapparence of EMG activity which, however, was somewhat attenuated if clitoral stimulation was avoided.

SPINAL CORD AND BRAINSTEM: PATTERN GENERATION

716.1

SPINAL CORD CONTROL OF BILATERAL HINDLIMB COORDINATION IN THE TURTLE. Field E*, Stein PSG; Biology Department and Movement Science Program, Washington University, St. Louis MO 63130.

The spinal turtle (*Trachemys scripta elegans)* produces three different forms of scratching behavior in location-specific responses to gentle mechanical stimulation of the lateral body surface (J. Neurophysiol. 53:1501, 1985). In such behaviors, activity is rhythmic with the force against the stimulated site generated by the limb ipsilateral to the stimulus.

Bilateral stimulation applied simultaneously to mirror-image locations, one on the left and one on right lateral body surface, elicits bilateral scratch behaviors. The most usual kinematic characteristic observed, in response to bilateral stimulation of rostral/rostral or pocket/pocket receptive field sites, is an out-of-phase relationship between the left and the right hip angles. The distal limb elements maintain their form-specific kinematic and electromyographic (EMG) intralimb relationships. When bilaterally asymmetric (i.e., rostral/pocket) sites are stimulated, the hip angles maintain an out-of-phase relationship while the distal limb segments perform disparate movements consistent with the site of stimulation on that side. The animal thereby produces, simultaneously, each of two different forms of scratching behavior, while maintaining an out-of-phase relationship between hip angles.

The pattern of hindlimb coordination observed during bilateral scratch in spinal turtles is similar to that of swimming behaviors in intact turtles. In forward swimming, the hindlimb movements are out-of-phase with each other. In the performance of turning behaviors, the intact turtle maintains the out-of-phase relationship between hip angles while the limb movements and the intralimb EMG relationships differ between the right and left sides. One limb displays forward swim kinematics; the other displays backward swim kinematics. The results in both intact and spinal turtles are consistent with the hypothesis that neural signals, related to control of the hips, play a major role in left/right interlimb coordination. Supported by NIH Grant NS 30786 to PSGS.

716.2

BILATERAL MOTOR RHYTHMS DURING FICTIVE ROSTRAL SCRATCHING IN THE TURTLE. Paul S.G. Stein*[1], John C. Victor[1], Edelle C. Field[1], and Scott N. Currie[2], [1]Dept. Biology and Movement Science Program, Washington Univ., St. Louis, MO 63130, and [2]Dept. Neuroscience, Univ. of California, Riverside, CA 92521.

Fictive rostral scratching is produced in a spinal, immobilized turtle by gentle mechanical stimulation of the ipsilateral (ipsi) midbody shell bridge (J. Neurophysiol. 53: 1517, 1985). Most previous work has focused on ipsi motor output. This output includes bursts of hip flexor (VP-HP) motor activity that rhythmically alternate with bursts of hip extensor (HR-KF) motor activity. Knee extensor (FT-KE) motor activity occurs during the latter portion of each burst of hip flexor activity.

Berkowitz and Stein (J. Neurosci. 14: in press, 1994) demonstrated rhythmic bursts of contralateral (contra) hip flexor activity that alternate with bursts of ipsi hip flexor activity during fictive rostral scratching.

We add to these previous findings. Stimulation of the ipsi shell bridge also produces a fictive motor pattern of contra hip extensor bursts that alternate with contra hip flexor bursts. Contra hip extensor activity occurs during the early part of ipsi hip flexor activity. Contra hip flexor activity begins during the latter part of the ipsi hip flexor activity and continues during ipsi hip extensor activity.

Simultaneous bilateral stimulation of mirror-image locations, one in the left rostral scratch receptive field and the other in the right rostral scratch receptive field, elicits bilateral fictive rostral scratching motor patterns. At the onset of stimulation, there is in-phase activation of left and right hip flexor nerves. After the first cycle of response and for the remainder of the response, the activity of each left motor pool alternates with the activity of its mirror-image motor pool on the right.

The patterns of activity seen during bilaterally activated fictive rostral scratching are similar to the patterns observed during bilaterally activated actual rostral scratching in spinal turtles as well as during actual forward swimming in intact turtles (Field and Stein, this volume). Thus, bilaterally activated fictive rostral scratching is an excellent model system to examine spinal mechanisms for the coordination of left and right rhythmic motor behavior. Supported by NIH Grant NS30786 to PSGS.

716.3

DEPRESSION OF MONOSYNAPTIC GROUP II AND OTHER FIELD POTENTIALS DURING MLR-EVOKED FICTIVE LOCOMOTION SUGGESTS A REDUCTION OF TRANSMISSION IN SENSORY AFFERENT PATHWAYS. M-C.Perreault*, I.Jiménez[1], S.J.Shefchyk and D.McCrea. Dept. Physiol., Univ. of Manitoba, Winnipeg, Canada, R3E 0W3, and CIEA-IPN[1], Mexico.

The possibility that transmission from sensory afferents might be affected during MLR-evoked fictive locomotion was examined in decerebrate cats. Monosynaptic field potentials were evoked by stimulation of extensor (Q, GS), flexor (Sart, PBSt) and cutaneous nerves and recorded in lumbar and sacral spinal segments. During MLR-evoked fictive locomotion monosynaptic components of group II field potentials were reduced up to 70%. In those trials where locomotion appeared several seconds after the onset of MLR stimulation, group II field potentials were maximally depressed only when the locomotor pattern was fully developed. Group I and cutaneous field potentials were also depressed but to a lesser degree. All monosynaptic field potentials were tonically depressed with the onset of fictive locomotion and for several seconds after its cessation. A smaller phasic depression during extension was often superimposed on the tonic depression. We suggest that both descending pathways and spinal locomotor networks contribute to the depression of transmission from group II, group I and cutaneous afferents during MLR-evoked fictive locomotion. Although the mechanisms have not yet been determined, presynaptic inhibition of the terminals of segmental afferents is likely. *Supported by the MRC and the Rick Hansen Legacy Fund.*

716.4

PROLONGED MODIFICATION OF THE MECHANICS OF THE STEP CYCLE BY SINGLE AND REPETITIVE MECHANICAL STIMULI IN CHRONIC SPINAL CATS. K. Nakada, J.A. Hodgson, R. de Leon, R.R. Roy and V.R. Edgerton*. Physiological Science Dept and BRI, UCLA, LA, CA 90024

After step training, the hindlimbs of the spinal cat transected at T12-T13 can regain the ability for full weight-bearing locomotion on a treadmill. When an obstacle is placed in front of the foot during the swing phase of a step cycle, the limb can be lifted above the obstacle after contact and the swing phase completed. In this study, a plexiglass rod instrumented with a strain gauge was introduced during the swing phase to perturb the hindlimb step cycle. The swing phase of the first step following contact of the foot with the obstacle was elevated relative to pre-contact steps. With repetitive obstruction, the step height increased in succeeding steps thus minimizing contact with the obstacle. In some instances, contact with the rod led to complete avoidance in the subsequent step. Impact force records demonstrated that the stimulus strength necessary to elicit the response gradually decreased with successive perturbations. Several steps after the perturbation was removed, the step height usually returned to pre-contact levels. When many consecutive steps were disrupted, the step height returned to pre-contact levels more slowly and sometimes remained slightly elevated. Similar kinematic modifications were elicited when air puffs were used to displace the hairs on the dorsal surface of the paw during the middle of the swing phase. Thus, it appears that the spinal cord, in the absence of supraspinal input, can "learn" of the presence and absence of an obstacle and modify the hindlimb kinematics in a manner that improves the probability of continuing successful locomotion. (Supported by NIH Grant NS16333)

716.5

THE INFLUENCE OF N-TYPE CALCIUM CHANNEL BLOCKERS ON THE RESPIRATORY NETWORK OF ADULT CATS. J.M. Ramirez*, O. Pierrefiche, S.W. Schwarzacher, F. Filloux, J.M. McIntosh, B.M. Olivera and D.W. Richter. Departments of Physiology and Anatomy, University of Göttingen, D-37073 Göttingen, FRG, Departments of Biology, Neurology and Psychiatry, University of Utah, Salt Lake City. USA.

The N-type calcium channel regulates the release of certain neurotransmitters and is specifically blocked by the ω-conotoxin GVIA. We used this ω-conotoxin to investigate the functional role of the medullary Pre-Bötzinger complex in the generation of respiratory rhythmic activity in adult mammals. Lesion experiments have demonstrated that the Pre-Bötzinger Complex is critical for rhythm generation in neonatal rats. In adult animals it has been demonstrated that all types of respiratory neurons are present within this medullary area but their importance for rhythm generation remains uncertain. In this study we pressure injected ω-conotoxin GVIA into the Pre-Bötzinger Complex of adult cats. Male and female cats were anaesthetized (Pentobarbital 40mg/kg i.p.), paralyzed, vagotomized and artificially ventilated. Respiratory activity was monitored from ipsi- and contralateral phrenic nerves while simultaneously recording intracellulary respiratory neurons. When injected into the Pre-Bötzinger Complex, ω-conotoxin GVIA had profound effects on respiratory activity. In this limited area blockade of the N-type calcium channel decreased the amplitude and the slope of phrenic nerve activity and increased significantly the cycle length. Prolonged injection induced central apnea. Although ω-conotoxin GVIA was injected into the Pre-Bötzinger Complex of one side, the induced respiratory effects were bilateral. All effects lead to a decrease of synaptically induced drive potentials to late-inspiratory, post-inspiratory and expiratory neurons. We conclude that ω-conotoxin GVIA blocks tonic excitatory drive to a specific area in the respiratory network, the Pre-Bötzinger Complex. The data also demonstrate that in adult cats the Pre-Bötzinger Complex is essential for respiratory rhythm generation.

716.6

ANTIDROMIC ACTIVITY OF DORSAL ROOT FILAMENTS DURING TREADMILL LOCOMOTION IN THALAMIC CATS. I. Beloozerova and S. Rossignol*. Cntr Res. Neuro. Sci., Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Previous work has shown that the proximal stump of cut dorsal roots (DR) are cyclically depolarized twice per cycle during **fictive locomotion** in paralysed thalamic cats, with a greatest maximum during the activity of flexors and a second maximum during activity in the extensors. Single DR units discharging antidromically (AD) and rhythmically are often recorded in these cut rootlets. Since DR depolarizations can also be readily evoked by activation of peripheral afferents such as stimulation of the foot pads, we investigated whether the natural peripheral inputs during **real locomotion** could also play a role in the AD firing of DR units. Nine cats were decerebrated under Halothane anesthesia. Locomotion was either spontaneous or triggered by MLR stimulation. Units were recorded from the proximal ends of the cut rootlets with Ag/AgCl electrodes in an oil pool. Representative EMGs of ipsilateral limb were recorded and the movements videotaped. The Table shows the distribution and characteristics of 73 modulated units out of a total of 92 recorded units. Their peak discharge may occur during swing or stance; they may start (ON) or end (OFF) their firing in stance or swing or be entirely confined within one phase (ON & OFF). It is concluded that not only central mechanisms but also peripheral events, such as foot contact during stance, may exert important presynaptic influences as revealed by the large proportion of units firing during stance. (Supported by MRC and FCAR).

	swing	stance	total
PEAK	39	34	73
ON & OFF	15	7	22
ON	27	24	51
OFF	17	12	29

716.7

FDL AND SOLEOUS MOTONEURON ACTIVITY DURING SCRATCHING. S.H. Dueñas* and L. Castillo. CUCS, Universidad de Guadalajara and C.B., Universidad de Aguascalientes, México

This study analyzed weather the normal patterns of scratching activity of FDL and Sol motoneurons prevail during fictive scratching (FS). In four normal cats, six hindlimb muscles were chronically implanted with patch electrodes for EMG recordings. In thalamic and later on, spinal cats, several hindlimb nerves were used for ENG recordings. The changes in resting potential (RP) in four FDL and five Sol motoneurons were also analyzed. In normal scratching cats, FDL-EMG exhibited tonic or phasic or intermingled activity, in contrast, Sol-EMG remain silent almost the entire episode of scratching. Occasionally occurred phasic Sol-EMG activity. In thalamic cats, FDL-ENG exhibited tonic, or phasic or intermingled activity. In 36% of the episodes of scratching occurred a sustained decrease in RP of FDL motoneurons. In 64% of the episodes, sustained RP decrease was followed by phasic changes. At the onsets of the phasic bursts of activity occurred high frequency firing (doublets). Sol-ENG exhibited absence of motoneuron activity. Four out five Sol motoneurons were hyperpolarized during the episodes of fictive scratching. In spinal cats, FDL motoneurons only exhibit phasic activity but, without doublets. Sol motoneurons were phasically activated. Spinal scratch generator produces phasic activity in FDL and Sol motoneurons, and supraspinal nuclei elicited sustained facilitation and inhibition in FDL and Sol motoneurons respectively.

716.8

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CAT SPINAL NEURONS ACTIVATED DURING FICTIVE LOCOMOTION. P.A. Carr*, B. Noga, A. Huang, X. Dai, D.M. Nance and L.M. Jordan. Depts. of Physiology and Pathology, University of Manitoba, Winnipeg, MB, CANADA, R3E 0W3.

Anti-c-fos immunohistochemistry or intracellular injection of a 3000 MW tetramethylrhodamine-dextran conjugate (TMR-D) was used to localize spinal neurons activated during fictive locomotion in paralysed, decerebrate cats. The neurochemical characteristics of c-fos-immunoreactive or TMR-D labelled cells were subsequently investigated using a variety of cytochemical markers. Locomotor-related spinal neurons were injected with TMR-D and tissue containing the intracellularly-labelled cells subsequently processed using anti-choline acetyltransferase (ChAT)-, aspartate- or c-fos-immunohistochemistry. Subpopulations of TMR-D labelled lamina VII neurons were seen to contain either ChAT- or aspartate-immunoreactivity. Simultaneous double labelling for c-fos and ChAT, aspartate, calcitonin gene-related peptide (CGRP) or NADPH-diaphorase was carried out on non-TMR-D labelled spinal cord tissue obtained from animals that displayed at least six hours of robust, MLR stimulus-evoked fictive locomotion. The majority of c-fos-immunoreactive cells were located in lamina VII and VIII of the lumbar spinal cord. Subpopulations of c-fos-immunoreactive neurons were seen to contain either NADPH-diaphorase reaction product, ChAT-immunoreactivity (-IR), aspartate-IR or to have close contacts with CGRP-immunoreactive fibres. These preliminary results reveal certain neurochemical characteristics of subpopulations of locomotor-related cat spinal neurons. They show that a subpopulation of c-fos-immunoreactive cells are first-order interneurons and some may utilize acetylcholine, aspartate and/or nitric oxide as neurotransmitters/modulators. Sponsored by MRC Canada, Human Frontier Science Program and the Health Sciences Centre Foundation.

716.9

LOCOMOTOR-RELATED PRESYNAPTIC MODULATION IN DORSAL CELL AXONS IN THE LAMPREY. A. El Manira*, J. Tegnér and S. Grillner. Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Using the lamprey spinal cord *in vitro* preparation, we investigated the presynaptic control in the glutamatergic skin sensory neurons (dorsal cells) during fictive locomotion. The membrane potential of dorsal cells showed phasic depolarizations that occurred in phase with the ipsilateral ventral root burst. Negative current injections increased the amplitude of the depolarizations indicating that the depolarization is chemically mediated. In cells where no depolarization could be recorded, simultaneous intracellular recordings from their axons showed large depolarizations suggesting that the input synapses underlying these depolarizations are located on the axons. These depolarizations were insensitive to specific $GABA_A$ (bicuculline) or $GABA_B$ (phaclofen and saclofen) antagonists. On the other hand, GABA receptors are present on dorsal cells. GABA, muscimol and baclofen induced depolarizations in the dorsal cells which persisted after blockade of synaptic transmission with tetrodotoxin. Specific GABA antagonists had no effect on the amplitude of the induced depolarizations, suggesting that the GABAergic effects on dorsal cells are not mediated by conventional $GABA_A$ and $GABA_B$ receptors. Small bipolar neurons containing GABA-ir and NPY-ir have close apposition on dorsal cell axons. Our evidence show that the synaptic transmission from sensory cells is phasically modulated by the neuronal network generating locomotion, as previously shown for network interneurons (Alford and Grillner, J. Neurosci. 1991).

716.10

TTX-RESISTANT, NMDA RECEPTOR-MEDIATED MEMBRANE POTENTIAL OSCILLATIONS IN SPINAL NEURONS OF *XENOPUS LAEVIS* LARVAE ARE 5HT-DEPENDENT. J. F. S-Wedderburn & K. T. Sillar*, School of Biological and Medical Sciences, University of St. Andrews, St. Andrews, Fife, KY16 8LB, Scotland.

The spinal network controlling swimming in *Xenopus laevis* embryos (stage 37/38) is one of the best understood locomotor central pattern generators (CPG's) in vertebrates (see Roberts, 1990, *Sci. Prog. Oxf.* **74**, 31-35.). Unlike more adult systems, however, the activity is stereotyped and inflexible, with motorneurons normally firing only once in each cycle. During a brief period of post-embryonic development (24hrs), the progressive rostro-caudal invasion of the spinal cord by axons of developing serotonergic brainstem interneurons is implicated in the ontogeny of a more adult-like locomotor pattern, involving bursts of motorneuronal discharge in each cycle. Here we have begun to examine the cellular mechanisms that might underlie the developmental modulation of an embryonic motor circuit.

In the lamprey, spinal motorneurons express intrinsic NMDA receptor-mediated membrane potential oscillations which may play a role in swimming rhythm generation (Wallén & Grillner, 1987, *J. Neurosci.*, **7**, 2747-2755). In *Xenopus* tadpoles (stages 37/38-42), in the presence of 1μM TTX, the bath application of 100μM NMDA depolarises motorneurons by about 20mV, but unlike the lamprey, does not lead to oscillations in membrane potential. However, the addition of 2-5μM 5HT induces sustained oscillations, whose amplitude (but not frequency) is voltage-dependent. Our results suggest that 5HT acts via receptors on spinal neurons to modulate the activity of the NMDA ionophore, thereby enabling expression of intrinsic membrane potential oscillations.

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716.11

EARLY APPEARANCE OF THE SYNCHRONIZED OSCILLATORY DISCHARGE OF THE CRANIAL NERVES IN THE IN VITRO ISOLATED BRAINSTEM OF NEWBORN CATS. F. Kato, R. T. Kado*, M.-P. Morin-Surun and M. Denavit-Saubié, Institut A. Fessard, CNRS, 91198, Gif-sur-Yvette, France.

To clarify the mechanism for the generation of high-frequency oscillatory discharges (HFO) correlated among the inspiratory nerves, we have applied the in vitro preparation of perfused isolated brainstem developed by Morin-Surun et al. (1992) to the newborn cats. One to 15 days-old cats were decapitated under pentobarbital anesthesia and the vascular system was perfused caudo-rostrally from the basilar artery with Krebs solution kept at 28°C. The efferent discharges of facial (VII), vagus (X) glossopharyngeal (IX) and hypoglossal (XII) nerves were recorded simultaneously. By the spectral analyses, the following results were obtained: i) In the power spectra of the inspiratory nerve discharges, a clear peak with a modal frequency of 30.1±1.7 Hz (mean±sd, n=19) was consistently observed. ii) In the coherence spectra for any pair of these nerves recorded, a peak with high values of coherence (0.91±0.09, n=9) was consistently observed, demonstrating the synchronization of this oscillatory discharge between nerves. iii) A rise in the temperature of Krebs solution of the superfusion chamber significantly augmented the modal frequency of the oscillation. iv) Perfusion with a high CO_2 Krebs solution accelerated the respiratory rhythm but did not give any significant influence on the oscillation frequency. v) This synchronized oscillatory activity was observed unchangedly in X, IX and XII even after ponto-medullary transection. Based on these observations, we conclude that this synchronized oscillatory activity in the isolated brainstem of the newborn cats is identical to the HFO in mature animals and that the HFO could be generated in the medulla without any connection between other neural structures at least as early as ~12 hours after birth.

716.12

FAST OSCILLATORY ACTIVITY IN MESENCEPHALIC TRIGEMINAL NEURONS. C. Pedroarena, I. Pose, J. Yamuy, F.R. Morales*, and M.H. Chase. Depto. de Fisiología, Facultad de Medicina, Montevideo, Uruguay, and Dept. of Physiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

In contrast to all other muscle afferents, primary afferent fibers that innervate the jaw musculature have their cell bodies located within the central nervous system, in the mesencephalic trigeminal nucleus (MES-V). During experiments designed to study neurons within the reticular formation we found a remarkable oscillatory activity of the membrane potential of MES-V neurons, which is the object of the present report.

Intracellular recordings from MES-V neurons were obtained in the standard tissue slice preparation from adult rats; neurons were labeled by intracellular biocytin injection. The membrane potential of MES-V cells exhibited epochs of sinusoidal-like oscillatory activity within the frequency range of 80 to 125 Hz; its amplitude ranged between 1 and 5 mV. Large oscillatory waves often triggered bursts of action potentials. The oscillatory activity was not observed at resting membrane potentials levels more negative than -65mV, but it could be evoked by the injection of depolarizing currents. Tetrodotoxin abolished the oscillatory activity.

This is the first report of an oscillatory activity in the cell bodies of primary afferents. Consequently, we can only speculate on their functional significance. It is possible that this high frequency activity plays a role in the transmission of information from the jaw musculature to central neural structures. Because this activity originates in cell bodies, it is also possible that it may be synaptically modulated during different behaviors. Supported by USPHS Grant NS 09999.

SPINAL CORD AND BRAINSTEM: NEONATES

717.1

INTRACELLULAR PATCH ELECTRODE RECORDINGS FROM NEURONS IN THE ISOLATED SPINAL CORD FROM NEONATAL RATS.

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The use of patch electrodes for intracellular recordings has several advantages compared to the use of traditional microelectrodes, particularly a better signal to noise ratio and the possibility for recording from small cells. We wanted to utilize these advantages in an intact spinal cord preparation, which has the benefit of intact large neurons and functional neuronal networks.

Neonatal rats (0-3 days old) were decapitated and the spinal cords dissected out with the preparation in ice-cold extracellular medium containing 0 mM Ca^{++}. For the experiments, the cord was submerged in extracellular medium at room temperature, containing 2 mM Ca^{++}. Pia mater could not be penetrated by the patch electrodes. Therefore, either a shallow cut was made from the ventral side immediately medial to the ventral root, or a hemisected preparation was used. The patch pipette, with positive pressure, could then be lowered 100 to 500 μm into the spinal cord, and GΩ seal formed by slight suction. This approaches gave a high (>50%) success rate for stable (20 to 60 minutes) whole cell recordings, often with access resistance around 5 to 10 MΩ.

Recordings from a variety of cell types, visualized with biocytin, were made in the intermediate and ventral areas of the cord. Typical resting membrane potentials were -45 to -55 mV, spikes were 2 to 3 ms wide with 70 to 90 mV amplitude. Cells could be recorded from during fictive locomotor activity, and the good signal to noise ratio made spontaneous and stimulated potentials from individual synapses clearly detectable.

717.2

NON INACTIVATING CHLORIDE AND POTASSIUM CHANNELS OF INSPIRATORY NEURONS IN THE IN VITRO BRAINSTEM-SPINAL CORD PREPARATION OF NEWBORN RAT. T.D. Jacquin, J. Champagnat, M. Denavit-Saubié*. Laboratoire de biologie fonctionnelle du neurone, Institut Alfred Fessard, CNRS, 91198 Gif sur Yvette Cx, France

Single channel activity was studied in inspiratory neurons identified by their pattern of firing related to phrenic motor output and located in the ventrolateral region (VLR) of theisolated in vitro brainstem-spinal cord preparation of a newborn rat. In outside-out recording under physiological condition, three channel types were identified by reversal potential,single-channel conductance, voltage sensitivity, pattern ofactivity and tetraethylammonium sensitivity. Two non inactivating potassium channels of 38 and 70 pS were found. Both channels showed voltage dependency, becoming more active at moderate depolarized potentials. The 70 pS channel showed tetraethylammonium sensitivity at 0.1-0.4 mM. Also, a chloride channel of 25 pS was seen. These channel types may be involved in the control of the resting membrane potential and bursting activity of the inspiratory neurons and contribute to their functions in the mammalian brainstem.

717.3

L-DOPA AND QUIPAZINE ELICIT AIR-STEPPING IN NEONATAL RATS WITH SPINAL CORD TRANSECTIONS. D.J. Stehouwer*, M.L. McEwen and C. Van Hartesveldt. Dept. Psychology, Univ. Florida, Gainesville, FL 32611-2065

Decerebrate neonatal rats respond to systemic injections of L-DOPA with coordinated locomotor activity on the ground, in water, or suspended in air (air-stepping). However, L-DOPA fails to elicit stepping in the hindlimbs of rats with mid-thoracic transections of the spinal cord, suggesting that the critical locus of action of L-DOPA is in the brainstem. Alternatively, it is possible that L-DOPA acts on spinal circuits, but that locomotor activation requires input from an additional descending system that originates in the brainstem. In the present study we examined the possibility that L-DOPA can elicit air-stepping in the hindlimbs of spinally-transected neonatal rats in the presence of the serotonergic receptor agonist, quipazine. Intact neonatal rats and those that had received complete mid-thoracic (T5) transections of the spinal cord were injected subcutaneously with L-DOPA (100mg/kg), quipazine (1.6 mg/kg), L-DOPA and quipazine in combination, or the vehicle solution. Results showed that neither L-DOPA nor quipazine alone elicited locomotor activity in the hindlimbs of rats with spinal cord transections. However, the combination of the two drugs elicited well-coordinated stepping in both the forelimbs and the hindlimbs, although the forelimbs and hindlimbs stepped independently of each other. In comparison to stepping of intact rats given L-DOPA alone, the forelimbs of spinal rats receiving the drug combination stepped faster, and the hindlimbs slower. The rate and topography of stepping were more variable in intact rats given the L-DOPA/quipazine combination than in intact rats receiving L-DOPA alone or in the hindlimbs of spinally-transected rats given the drug combination. These results show that quipazine and L-DOPA must both act on spinal targets to produce locomotion.

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717.4

LOCOMOTOR RESETTING PATTERN FOLLOWING MUSCLE AFFERENT STIMULATION IN AN ISOLATED SPINAL CORD-HINDLIMB PREPARATION OF THE NEWBORN RAT. M. Iizuka[1], O. Kiehn[2], O. Kjærulff[2] and N. Kudo[1]*. [1]Dept. Physiol., Inst. Basic Medi. Sci., Univ. of Tsukuba, Tsukuba 305, Japan and [2]Dept. Neurophysiol., Univ. of Copenhagen, Denmark.

Effects of muscle afferents stimulation on the central pattern generator (CPG) for locomotion were examined using an isolated spinal cord-hindlimb preparation of newborn rats (P0-6). Locomotor activities were evoked by bath application of a combination of NMDA and 5-HT and recorded from flexor and extensor muscles. The locomotor rhythm was reset by a train of stimuli (20Hz, 10-20 pulses) to the quadriceps nerve (Qn). At P0-1, resetting was characterized by the generation of a new flexor burst when stimuli were applied during flexor phase in all of the 8 preparations tested. This resetting pattern was observed at all stimulation intensities (1.8-50 times threshold, T). At P2-6, however, different resetting patterns were observed depending on the stimulus intensity. Below 3xT Qn stimulation delivered during flexor phase truncated the flexor burst and reset the rhythm by initiating a new extensor burst in 4 of 6 cases at P2-3 and in 5 of 5 at P4-6. Higher intensity stimuli (>5xT) initiated a new flexor phase in all of the preparations tested. These results suggest that the pathway conveying suppressive effects from low threshold muscle afferents to the flexor CPG develops later than the pathway mediating excitatory effects from muscle afferents.

717.5

LOCALIZATION OF THE CENTRAL PATTERN GENERATOR FOR HINDLIMB LOCOMOTION IN THE NEONATAL RAT *IN VITRO*. A LESION STUDY. O. Kjaerulff and O. Kiehn (SPON: Euopean Neuroscience Association). Sect. of Neurophysiology, Dept. of Medical Physiology, Univ. of Copenhagen, Copenhagen 2200, Denmark.

Our previous studies with sulforhodamine 101 as a marker of neuronal activity have shown that laminae VII and X in the lower thoracic and lumbar regions of the spinal cord may be of possible importance for the generation of locomotion in the neonatal rat (Kjaerulff, Barajon and Kiehn, *J. Physiol.*, in press, 1994). To test this conclusion we have monitored the rhythm-generating ability in surgically isolated subregions of thoracolumbar spinal cord (Th12-L6).

Rhythmic activity (RA) recorded from the ventral roots was induced with a combination of 5-HT and NMDA in spinal cords prepared from rats 0-2 days of age. RA was seen in isolated preparations consisting of segments Th12-L1 or L1-L3, while in preparations consisting of L4-L6 or L5-L6 either no RA or only slow, low-amplitude RA was found.

Preparations consisting of all segements from Th12 to L6 were sectioned in various planes. A midsagittal section did not prevent RA. Similarly, RA - alternating between the two sides - persisted after removal of the dorsal half of the spinal cord with a horizontal section. In contrast, preparations left from a paramedian section and comprising the lateral motoneuron pool, the lateral intermediate area and the dorsal horn showed no RA.

We infer that the neuronal networks important for spinal hindlimb locomotor activity predominate medially and ventrally in the thoracolumbar transition zone.

717.6

DURING LOCOMOTOR-LIKE ACTIVITY *in vitro*, NEONATAL RAT INTERMEDIATE LAMINAE SPINAL CORD NEURONS DISPLAY DRIVE POTENTIALS. Jason N. MacLean and David S. K. Magnuson*. Spinal Cord Research Centre, U. of Manitoba, Winnipeg, MB, Canada.

Studies using activity dependent[1] and retrograde labelling[2] suggest that neurons in lamina X and medial lamina VII of the lumbar spinal cord may participate in hindlimb locomotor activity. An *in vitro* spinal cord preparation which involved a midsagittal lumbar hemicordectomy while keeping the thoracic cord intact, was used to examine these neurons. This reduced preparation exhibits drug-induced locomotor-like activity and provides unobstructed access to the lumbar intermediate grey matter. Locomotor-like activity recorded in ventral roots L3 and L5 was elicited by superfusion of the cord with N-methyl-D-aspartate (NMDA) and serotonin. During this activity, neurons in the medial grey matter were recorded from and labelled intracellularly. Thirteen of 15 cells displayed locomotor-like drive potentials (LDPs) up to 7mV peak to peak. These LDPs were either in or out of phase with the recorded ventral root rhythmic activity. The LDPs appeared to result from the summation of post-synaptic potentials. Dorsal root stimulation resulted in excitatory post-synaptic potentials (latency 0.2 - 3.6msec longer than monosynaptic VR latency) in 10 of these neurons. Five neurons received excitatory and 1 received inhibitory synaptic input from ventrolateral funiculus stimulation. Labelled neurons were located lateral and slightly ventral to the central canal, and had dorsoventrally oriented processes. These observations lend support to the hypothesis that neurons in this region of the lumbar spinal cord participate in the production of locomotor output.

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[1](Kjaerulff, J Physiol 1994 in press) [2](Hoover, Somato Motor Res, 1992).

717.7

LARGE AMPLITUDE EXCITATORY SYNAPTIC CURRENTS MEDIATE STRYCHNINE-DEPENDENT SYNCHRONOUS MOTOR RHYTHMS IN THE NEONATAL RAT SPINAL CORD. K.C. Cowley*, S. Hochman, L.M. Jordan, and B.J. Schmidt. Depts. Medicine and Physiology, University of Manitoba, Winnipeg, Canada, R3E 0W3.

Application of the glycine receptor antagonist strychnine to the bilaterally intact *in vitro* rat spinal cord transforms locomotor-like and other alternating patterns of hindlimb motor activity into synchronous rhythms (*Harder & Schmidt, Soc. for Neurosci. abstr. 1992*). We have now applied whole-cell recording methods to examine intracellular events in lumbar motoneurons and interneurons during strychnine-induced synchronization. NMDA, serotonin and acetylcholine were used alone or in combination to induce baseline rhythmic activity. Following strychnine (1 μM), large amplitude rhythmic excitatory synaptic actions, in-phase with synchronous rhythmic ventral root activity, were recorded in all neurons examined. Rhythmic EPSCs (E_{rev}= 0 mV) as large as 200 pA resulted in synaptic drive potentials measuring over 50 mV in some neurons. An interneuron which previously received exclusively inhibitory synaptic input (E_{rev}= -50 mV) in response to dorsal root stimulation and during acetylcholine (10 μM)-induced rhythmic activity, displayed only rhythmic EPSCs following strychnine. The maximal EPSC amplitude in this cell occurred at -40 mV suggesting involvement of NMDA receptor activation. In one interneuron rhythmic synaptic activity was not observed, despite the induction of phasic ventral root activity by serotonin (10 μM) and NMDA (2.5 μM); however, the cell developed rhythmic 10 mV depolarizing potentials after addition of strychnine. Thus blockade of glycine receptors may result in the phasic recruitment of neurons not previously associated with the production of motor rhythm. Finally, although rhythmic inhibitory synaptic currents (E_{rev}= -50 mV) re-appeared in interneurons following removal of strychnine from the bath, ventral root activity remained synchronized. *(Supported by HSCF and MRC)*

717.8

NMDA RECEPTOR-MEDIATED VOLTAGE OSCILLATIONS IN MAMMALIAN MOTONEURONS. S. Hochman*, L.M. Jordan, and B.J. Schmidt. Depts. of Medicine and Physiology, University of Manitoba, Winnipeg, Canada, R3E 0W3.

Whole-cell current clamp recordings were obtained from motoneurons in the bilaterally intact *in vitro* neonatal rat spinal cord in order to examine the effects of NMDA receptor activation. In one motoneuron, bath application of serotonin (30 μM) and NMDLA (10 μM) produced a progressive membrane depolarization accompanied by repetitive firing (6 Hz). Re-adjustment of the membrane potential to -60 mV, via intracellular current injection, resulted in the emergence of prolonged post-spike after-depolarizations (ADPs) measuring 30 mV as well as an associated slowing of firing frequency (2 Hz). Following hyperpolarization of the membrane to -85 mV, Na^+ spikes and ADPs were no longer present but rhythmic 10-15 mV slow-rising membrane potential oscillations (1-2 Hz) persisted. After blocking Na^+ spikes and synaptic transmission with TTX (1 μM), 10-15 mV oscillations and occasional superimposed faster-rising high amplitude (30 mV) potentials remained. In another motoneuron, NMDLA (20 μM) in the presence of TTX induced sustained rhythmic membrane oscillations between -55 and -25 mV (2 Hz). Hyperpolarization of the membrane to -60 mV had no effect on frequency but the oscillations were reduced in amplitude (10-15 mV). These voltage oscillations lasted 4 minutes after which the cell remained depolarized at -25 mV. Repolarization of the membrane to -60 mV and injection of a current ramp waveform revealed step changes motoneuron membrane potential compatible with the presence of a bistable property. In other motoneurons the frequency of NMDA-induced, TTX-resistant membrane potential oscillations was also insensitive to adjustments in membrane voltage between -90 and -40 mV. However, the frequency of oscillation was sensitive to the concentration of NMDA in the bath. These observations suggest that mammalian hindlimb motoneurons are capable of endogenous oscillatory behavior in the presence of NMDA receptor activation. *(Supported by HSCF and MRC Canada)*

717.9

VOLTAGE-DEPENDENT OSCILLATIONS OF MOTONEURON MEMBRANE POTENTIAL DURING RHYTHMIC MOTOR ACTIVITY IN THE *IN VITRO* NEONATAL RAT SPINAL CORD. B.J. Schmidt*, S. Hochman and L.M. Jordan. Depts. of Medicine and Physiology, University of Manitoba, Winnipeg, Canada, R3E 0W3.

The role of voltage-dependent properties in the generation of the rhythmic depolarizations in motoneurons during neurochemically-induced activity was examined using whole-cell recording methods. QX-314 (5 mM) was included in the pipette to block Na^+ currents. Serotonin and NMDLA alone or in combination were used to induce activity. In voltage clamp mode, phasic inward currents (e.g. 50 pA at V_h = -80 mV) coincided with rhythmic ventral root discharge. In current clamp mode, NMDLA (12 μM) induced fast rhythmic motor activity (4-6 Hz) associated with 5-10 mV voltage oscillations at hyperpolarized membrane potentials (e.g. -80 mV) and larger amplitude oscillations (20 mV) at resting potential (-60 mV). In one motoneuron, slowly depolarizing current ramp injections (15s) demonstrated a voltage-dependent 9 mV step increase (from 6 to 15 mV) in the amplitude of the NMDA (5 μM)-induced 1.8 Hz membrane oscillations. In another motoneuron, slow frequency (0.4 Hz) 5 mV oscillations with superimposed long-duration EPSPs (40-100 ms) were observed during rhythmic ventral root discharge induced by serotonin (60 μM) and NMDLA (20 μM). Short-duration current ramps (250 ms) were delivered between -60 and -20 mV during the depolarized phase of the slow oscillations. An abrupt increase of superimposed EPSP amplitude, from 4 mV at -60 mV to 10mV at ramp voltages of -40 mV, was observed.

These observations suggest that membrane depolarization can enhance the amplitude of excitatory events underlying motor rhythms and may reflect the voltage-dependent removal of Mg^{2+} blockade at NMDA receptor-activated synapses. *(Supported by HSCF and MRC Canada).*

CIRCUITRY AND PATTERN GENERATION V

718.1

EVIDENCE FOR FUNCTIONAL PARTITIONING WITHIN THE MASTICATORY PATTERN GENERATOR NETWORK. K.-G. Westberg, P. Clavelou, C. Valiquette, S. Lepage, J.P. Lund and H.H. Jasper*. Centre de Recherches en Sciences Neurologiques et Fac. Méd. Dent., Université de Montréal, Montréal, Québec, H3C 3J7, Canada.

During mastication, jaw muscle activity is adapted to the size and the texture of food. It has already been shown that last-order interneurones of subnucleus oralis-γ of the spinal trigeminal tract (NVspo) form part of the pattern generating circuits. The present study was performed to determine how these neurones behave during different forms of mastication.

Rabbits were anaesthetized with urethane. Different jaw movement patterns were elicited by repetitive electrical stimulation of four sites within the masticatory areas of the right and left hemispheres of the cerebral cortex. The animals were paralyzed and the fictive patterns of rhythmic activity were recorded in the trigeminal motor nucleus. The firing properties of trigeminal interneurones were analyzed during mastication induced from each cortical stimulation site. Changes in their responsiveness to low-threshold peripheral afferents were quantified by measuring the latency and frequency of axon potentials caused by stimulation of the inferior alveolar nerve.

Eighty-five neurones were recorded within NVspo-γ and 56 were rhythmically active during fictive movements. Thirty % of these showed rhythmic bursts only during one pattern. The other 70 % altered both their burst duration and frequency when the movement program changed. It was also observed that the response to the afferent input was specific to the type of motor pattern.

Our results suggests that last-order NVspo-γ interneurones form functional subpopulations to produce different patterns. In addition, the gain of reflex connections is specifically modulated according to the pattern of movement.

Supported by a Group Grant from the Canadian MRC, the Swedish MRC (K94-10133; K93-10529) and Glaxo-France.

718.2

ACTIVITY OF SPINDLE AFFERENTS RECORDED AT THE LEVEL OF TRIGEMINAL SUBNUCLEUS ORALIS OF RABBIT DURING FICTIVE MASTICATION. P. Clavelou, K.-G. Westberg, S. Lepage, C. Valiquette and J.P. Lund*. Centre de Recherches en Sciences Neurologiques et Fac. Méd. Dent., Université de Montréal, Montréal, Québec, H3C 3J7, Canada.

Muscle spindle afferents from the jaw closing muscles have their cell bodies in the mesencephalic trigeminal nucleus (NVmes). Axon collaterals are sent to the trigeminal motor nucleus (NVmt), its border areas, and to the medial part of the oral nucleus of the spinal trigeminal tract (NVspo). We report here on the firing properties of slowly adapting massceteric spindle afferents recorded near the NVspo during fictive mastication in urethane-anaesthetized rabbits. Afferents were identified by their response to stretching of the jaw and to probing the muscle, and by short latency (<1ms) action potentials evoked by single pulse or repetitive (>300Hz) electrical stimulation of the motor nerve. Repetitive stimulation of the sensory motor cortex was used to evoke fictive movements that were monitored by recording the rhythmical activity in NVmt. The results show that the tonic firing of these afferents to a constant stretch is phasically inhibited during the opening phase, while an increase in frequency occured during the closing phase of the cycle. Blocking of peripheral inputs with anaesthetic injected into the muscle or around the nerve supports the concept of a central origin for the observed modulation.

Kolta et al. (J. Neurophysiol. 1990, 64:1067-1076) recorded from the same type of spindle afferents at the level of NVmes. In agreement with us, they observed a phasic inhibition of all units during the opening phase of the chewing cycle, but only 2 of 33 fired bursts during closure. We conclude that the centrally-induced blocking of orthodromic invasion during the opening phase affects cell body, stem and central axons, while the burst of activity during the closing phase are probably generated at terminals of the central axonal branches.

Supported by a Group Grant from the Canadian MRC, the Swedish MRC (K94-10133; K93-10529) and Glaxo-France.

718.3

THE THALAMIC VPL IN RATS PROMOTES BEHAVIORAL RECOVERY AFTER SOMATOSENSORY LESIONS. J. Wells[1], S. M. Henry[1,2], and J. M. Held[*2]. Department of Anatomy and Neurobiology[1] and Department of Physical Therapy[2], University of Vermont, Burlington, VT 05405.

The transfer of information through VPL was found to be critical for the successful completion of a motor task and for the normal trajectory of the hindlimb. Bilateral VPL lesions resulted in a significant increase in the time to traverse a narrow bar for a reward and a significant modification of the swing phase of the gait cycle. The rats gradually recovered the rapid traverse of the bar, but the deficit in the swing cycle did not recover within 46 days. The cerebellar, basal ganglia and other motor areas did not serve as alternatives to the VPL, at least during the first 2 weeks for the locomotor task or the first 46 days for the hindlimb movement pattern. After unilateral lesions of only the gracile nucleus, there was no deficit in the task at the behavioral level, but recovery occurred in the movement pattern of the hindlimb over 3 weeks. VPL itself may promote the recovery in two ways. Structural recovery of the number of synapses begins at about the time that the movement pattern returns to normal. In addition, when there is a decrease in the somatosensory input to VPL, there may be a widespread disinhibition in VPL that would unmask normally ineffective synapses which may act as alternative inputs. Supported by the Foundation for Physical Therapy.

718.4

HIGH FREQUENCY OSCILLATIONS IN MEMBRANE POTENTIALS (MP) OF MEDULLARY INSPIRATORY (I) AND EXPIRATORY (E) NEURONS. W.-X. Huang*, M.I. Cohen, Q.P. Yu and Q. He. Dept. of Physiol., Albert Einstein Col. Med., Bronx, NY 10461.

In 32 decerebrate, unanesthetized and paralyzed cats, we studied the characteristics of high frequency oscillations (HFOs) in MPs of medullary I and E cells. Simultaneous recordings were taken from bilateral phrenic (Phr) and recurrent laryngeal (RL) nerves and from cells (0 - 1.5 mm caudal to obex, 3.2 - 4.2 mm lateral). HFOs during I were found in MPs of 24 E and 15 I cells. Fourteen of these were identified as RL cells (7 I, 7 E) by a sharp peak in the spike-RL nerve discharge cross-correlogram. The HFO in MP during I, superimposed on depolarization in I cells or hyperpolarization in E cells, developed gradually after I onset, reached maximum amplitude around mid-I, then gradually decreased. The MP-Phr coherence peaks had a frequency range of 39.1 - 66.4 Hz (mean 55.6 ± 8.3 SD Hz), with a mean coherence value of 0.55 ± 0.18 (range 0.38 - 0.80). The MP-Phr cross-correlogram lags (peak MP depolarization to Phr) were: a) for E cells, mean of 5.6 ± 1.8 ms (range 0 to 0.46 of an HFO cycle); and b) for I cells, 6.5 ± 4.7 ms (range -0.06 to 0.93 of an HFO cycle). The results confirm that there are widespread short-term network interactions among I cells as well as between I and E cells; and the dispersion of lags suggests the existence of distributed connections between cells of the generating networks. (Supported by N.I.H. Grant HL-27300.)

719.1

NEUROMUSCULAR PARTITIONING OF THE HUMAN BICEPS BRACHII LONG HEAD. M.H. Mann, S.L. Wolf, R.L. Segal*, Div. Phys. Ther., Dept. of Rehab. Med., Emory Univ. Sch. of Med., Atlanta, GA 30322.

Previous studies (ter Haar Romeny et al., 1984; Segal 1992) have suggested three functionally discrete longitudinally oriented partitions within the biceps brachii long head (BLH). A recent pilot study from our lab (see English et al., 1993) suggested the organization of BLH may be different than proposed by ter Haar Romeny et al.,(1984).

In an attempt to clarify the organization of BLH, fine-wire electrodes were inserted into 3 strips of BLH running from medial to lateral in 6 subjects as determined from dissections. The activation order of motor units was determined for 3 tasks: elbow flexion, forearm supination, and combined flexion and supination. In addition, recordings were made during elbow extension, forearm pronation, and shoulder extension, flexion and abduction. Spike-triggered averages (STA) between motor units from the different recording sites were used to determine the level of correlated activity. During both elbow flexion and forearm supination medial motor units were usually recruited first while motor units from the lateral recording site were usually recruited last. Motor units from the central recording site were recruited more easily than the lateral site, especially when elbow flexion and supination were combined. However, motor units from the lateral recording site were most easily recruited during shoulder movements. Activity of motor units from the lateral site were not correlated with the motor units from the other sites, but motor units from the medial and central recording sites showed some correlated activity. This study supports partitioning of BLH, but possibly not as previously proposed. Future studies should study the activity of BLH during shoulder movements in more detail.

719.2

NONHOMOGENEOUS AND TASK DEPENDENT ACTIVATION OF THE FIRST DORSAL INTEROSSEOUS MUSCLE. D.H. Laidlaw†, G.H. Yue†, A.L. Alexander‡, A.F. Gmitro‡, E.C. Unger‡, and R.M. Enoka†*. †Dept. Biomedical Engineering, Cleveland Clinic Foundation, Cleveland, OH 44195, and ‡Dept. Radiology, University of Arizona, Tucson, AZ 85724

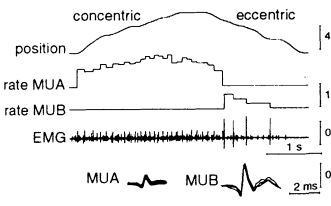
Spin-spin relaxation time (T2) measured from the magnetic resonance image of skeletal muscle varies linearly with activation intensity of the muscle. T2 was used to investigate functional compartmentalization of the first dorsal interosseous (FDI) muscle of four healthy human subjects. Spin-echo images of two cross sections from the proximal half of the muscle were analyzed to determine regional differences in activation immediately following flexion and abduction exercises of the left index finger. The exercise was performed against an elastic load that was 20% of the maximum work capacity. Each cross section of the muscle was divided into eight regions about the major axes of the muscle. In each cross section, the signal intensity at four echo times (25, 50, 75, 100 ms) was measured and T2 relaxation time was calculated. Only areas showing homogeneous signals (i.e., no apparent high connective tissue or fat content) were measured. Percent change of T2 from initial resting measurements was used to quantify relative activation. The two major findings were: (1) during abduction, the dorsomedial portion was more active (10.8% increase) than the ventrolateral portion (3.7%); and (2) during flexion, the middorsal and ventrolateral portions were more active (11.0% and 12.6% increases respectively) than the other portions (4-9%). These findings suggest that the relative activation of the FDI muscle is nonhomogeneous and varies with the task performed.

Supported by NIH grants AG 09000 and NS 20544 to RME.

719.3

MOTOR UNIT FIRING DURING SHORTENING AND LENGTHENING CONTRACTIONS. J.N. Howell*,A.J. Fuglevand, M.L. Walsh and B. Bigland-Ritchie. J.B. Pierce Laboratory, 290 Congress, New Haven, CT 06519.

In order to examine motor unit firing patterns during movement, fine wire (25μ) electrodes were inserted into the first dorsal interosseus (FDI). Unit activity was recorded as the FDI shortened and lengthened under constant loads and was compared to activity during isometric ramps. Single units, identified by wave shape analysis, could be followed reproducibly over many cycles of movement and in repeated isometric contractions. In most cases the first units to be recruited during shortening (concentric) were the last to be derecruited during lengthening (eccentric). An exception is shown, in which unit A turned off as B was recruited. B had a higher torque threshold during isometric activation than A. Unit B activity was simultaneously detected by a second electrode pair (not shown), making it unlikely that the appearance of B in the trace shown represented an artifact of electrode movement rather than unit recruitment.



719.4

ADAPTATION OF MOTOR UNIT DISCHARGE RATE AND VARIABILITY DURING SUSTAINED MAXIMUM VOLUNTARY CONTRACTIONS. A.J. Fuglevand*, V.G. Macefield, J.N. Howell and B. Bigland-Ritchie. John B. Pierce Laboratory, 290 Congress Ave., New Haven, CT 06519.

Although the average discharge rate of motor unit populations is known to decline during a sustained maximum voluntary contraction (MVC) (Bigland-Ritchie et al., *J. Physiol.* 340: 335, 1983), little is known about how individual motor units adapt during this fatigue task. To address this issue, motor unit action potential trains were recorded with intramuscular tungsten microelectrodes during an MVC (60 - 120 s) of the extensor hallicis longus muscle in 27 experiments on 7 human subjects. The analyses were restricted to discharge trains in which at least 100 spikes were recorded. Each train was divided into segments having equal number of spikes but with no less than 50 spikes/segment. Mean discharge rate and the coefficient of variation in interspike intervals (cvISI) were determined for each segment. Adaptation was estimated for each unit from the segment values as the average change in discharge rate (Δrate/10 s) or in variability (ΔcvISI/10 s) that would occur for each 10 s of activity. 15 trains with a mean (± SD) duration of 10.8 ± 4.8 s were included in the analyses. The discharge rate of all units but one recorded within the initial 30 s of the MVC decreased with time from an initial mean rate of 19.5 ± 8.0 imp/s (mean Δrate = -4.4 imp/s/10 s). Units monitored after 30 s showed little adaptation (mean Δrate = 0.7 imp/s/10 s). Discharge variability (initial cvISI = 0.2 ± 0.07) altered little over time for trains recorded within and after the initial 30 s of the MVC (mean ΔcvISI/10 s = -0.03 and -0.04, respectively). These findings imply that discharge-rate adaptation is confined to the early stages of sustained activity and that discharge variability remains stable. Supported by USPHS NS 14576 & HL 30062.

719.5

ELECTROPHYSIOLOGICAL PROPERTIES OF DISSOCIATED PARAPODIAL MUSCLE FIBERS FROM *APLYSIA BRASILIANA*. Paul J. Laurienti* and James E. Blankenship. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77555-0843.

The marine mollusc *A. brasiliana* swims by rhythmic flapping of its bilaterally symmetric wing-like parapodia. Motor neurons (MNs) and serotonergic modulatory neurons (POP cells) which innervate peripheral parapodial muscle are located in each pedal ganglion. POP cells and exogenous serotonin (5-HT) have been shown to modulate the activity of the cholinergic neuromuscular junction. The modulatory effect of 5-HT may be mediated through either pre- or postsynaptic mechanisms. We have recently demonstrated that 5-HT acts, at least in part, through a presynaptic mechanism. However, the possibility of postsynaptic modulation has not been investigated. To test the direct effect of 5-HT on parapodial muscle fibers, we have developed a dissociated muscle preparation in order to characterize the properties of these cells. We have successfully dissociated viable muscle fibers and have performed current clamp experiments using standard intracellular recordings. The resting membrane potential (RPM) of the dissociated fibers is approximately -70 mV, which is consistent with reports of the RMP in muscles in the intact parapodium. Muscle fibers are able to contract when depolarized by injecting intracellular current or in response to high K^+. I-V curves from single fibers are non-linear, showing a decrease in cell resistance with depolarization and an increase in resistance with hyperpolarization. Preliminary experiments investigating the effect of exogenously applied acetylcholine (ACh) has revealed a Cl^--dependant hyperpolarization; other components of the ACh response are currently under investigation. Future experiments are planned to analyze the effect of 5-HT on both the intrinsic and ACh-induced currents in these muscle cells.

719.6

THIXOTROPIC BEHAVIOR OF THE PLANTARFLEXOR MUSCLES IN NORMAL INDIVIDUALS. Lamontagne A., Malouin F., Richards CL.*, Tardif D. Physiotherapy Dept., Faculty of Medicine, Laval University and Neurobiology Research Center, Hôpital de l' Enfant-Jésus, Quebec City, Canada.

The primary aim of this study was to develop a method of assessing the thixotropic behavior of normal muscles with torque measures. Stiffness variations that are dependent on the previous history of muscle contraction or length (thixotropy) are thought to reflect intrinsic muscle fibers properties. We thus compared the effect of brief (30 s) lengthening and shortening periods of the plantarflexors on the resistive torque obtained during passive dorsiflexion (DF) of the ankle. In seven healthy persons (23 to 40 yrs), passive ankle movements (n=6) ranging from -23° to -8° of DF were imposed at 20°/s with an isokinetic dynamometer (Kin-Com). Passive DFs were made before (pre-test) and after (post-test) a 30 s pause in a lengthened (-8°) position (condition 1) or a shortened (-23°) position (condition 2) of the plantarflexors. The foot was maintained in the footplate between pre-tests and post-tests. Surface electrodes on the tibialis anterior and soleus muscles monitored unwanted muscle activations during the tests. The last five trials of each test were averaged and the differences in the area (dA) under the torque-angle curves (-18° to -10°) between the pre-test and post-test were computed for each condition. In condition 1, the resistive torque was decreased in all 7 subjects (dA=1.94 N.m.deg $^{-1}$ ±1.11) while in condition 2, we observed only a slight decrease for 6 subjects (dA=0.18 N.m.deg $^{-1}$ ±0.12), indicating that resistive torque responses are sensitive to the recent history of muscle lengthening. Wilcoxon signed rank tests computed between the dA for condition 1 and 2 indicated that the lenghtened position induced a larger effect (p=0.01) on torque than the shortened position. These results indicate 1) that normal plantarflexor muscles exhibit a thixotropic behavior that can be detected by measuring resistive torque to passive movement and 2) that this method may be applicable to the evaluation of changes in intrinsic properties of spastic muscle. (Supported by MRC of Canada).

719.7

SPROUTED MOTOR UNITS SHOW TYPE-SPECIFIC RESPONSES TO CHRONIC ENLARGEMENT FOLLOWING PARTIAL DENERVATION OF RAT LATERAL GASTROCNEMIUS. K.L. Seburn and P.F. Gardiner*. Physical Activity Sciences, Univ. of Montréal, Montréal, Que., Canada H3C 3J7.

Motor units (MUs), enlarged subsequent to partial denervation may, with extended periods, diminish in their capacity for force production. We studied this issue by comparing normal lateral gastrocnemius (LG) MUs, isolated via splitting of the L5 ventral root, with MUs which had been sprouted (L4 section), for either 30 or 90 days. Compared to control, MU proportions were unchanged and overall mean MU tetanic forces doubled at both 30 and 90 days. This increased tetanic force varied according to MU type: mean forces for S, FR and FF MUs were 330%, 195%, and 267% respectively, of controls. After 90 days FR MU force was further increased (25%), S was unchanged and FF decreased (26%), compared to 30 days. Twitch/tetanic ratios also responded differently according to MU type. Our results indicate that after the initial sprouting response is complete, MU reorganization continues based on size and/or activity level. Supported by NSERC Canada.

719.8

PROPAGATION OF CALCIUM IONS IN CULTURED COLONIC SMOOTH MUSCLE CELLS. S.H. YOUNG*, H.S. ENNES, AND E. MAYER. Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

Smooth muscle cells are isolated by collagenase digestion from rabbit distal colonic circular or longitudinal smooth muscle, and then suspended in medium with high glucose (12 mM), non-essential amino acids, and 10% fetal bovine serum, and plated onto coverslips. After 5 or more days in culture, cells are loaded with the calcium indicator FURA-2 (5uM, 1 Hr, 37°C), and cell calcium concentrations are measured with an imaging system (Attofluor Instruments).

Intracellular calcium increases in response to bath application of bethanecol (10-5 M), or caffeine (10 mM). Increases are usually apparent first in the subplasmalemmal space, followed by spread to other regions of the cell. The response is usually symmetric around the cell, and no oscillations of calcium are seen. In contrast, single cells mechanically stimulated with a smooth glass pipette show strong response local to the stimulus site, followed by propagated waves of calcium away from the stimulus site. In cultures of high cell density, where several cells are in contact, these calcium waves propagate into neighboring cells.

719.9

A DYNAMIC, 3-D, 7 DEGREE-OF-FREEDOM UPPER EXTREMITY MODEL. A. Kakavand, G.T. Yamaguchi, A.B. Schwartz*. Barrow Neurological Inst., 350 W. Thomas Rd., Phoenix, AZ 85013 and Arizona State University, Tempe, AZ 85287-6006

A 7 degree-of-freedom (DOF) dynamic model of the rhesus monkey upper extremity has been developed for use in trajectory planning studies. Dynamic equations of motion were derived using Kane's method and coded on a computer-graphic workstation. Included are 36 musculotendon forces to actuate the 7 DOF. Musculotendon origin and insertion points were obtained by dissection and measurement to +/- 0.1 mm in Cartesian space via an Optotrak digitizing probe and transformed to bone centered reference frames. Via points describing each musculotendon pathway were also computed where necessary to prevent the paths from penetrating bone surfaces during joint rotation, using the graphical display program SIMM.

The model can be used to explore the neuromuscular strategies employed by the monkey to select joint angles when (i) holding the finger at a specified point in free space, or (ii) creating a trajectory in free space. When the finger position (or trajectory) is well within the physiological workspace, an infinite number of possible arm configurations will deliver the same endpoint position (trajectory). Optimal control theory was therefore used to compute minimum cost solutions to the kinematic redundancy problem. Cost functions include minimizing gravitational potential energy (C_1) and the sum of muscle stresses squared (C_2), which relates to metabolic energy expenditure.

A pseudoinverse method was used to compute musculotendon stresses with minimum positional (trajectory) error. Preliminary results for the positional study indicate that cost function C_2 predicts the preferred shoulder angles, elbow flexion angle, and wrist flexion/extension angle, but cost function C_1 does not perform as well. Supported by the NIH (NS 26375).

BRAIN METABOLISM AND BLOOD FLOW: DISEASE MECHANISMS

720.1

INDOMETHACIN PRETREATMENT ABOLISHES ATTENUATION BY N^{G}-NITRO-L-ARGININE (L-NNA) OF CORTICAL SPREADING DEPRESSION (CSD)-INDUCED CEREBRAL VASODILATION. D. Busija*, D. Colonna, and W. Meng. Dept. of Physiology & Pharmacology, Bowman Gray Sch. of Med., Winston-Salem, NC 27157-1083.

The role of nitric oxide in cerebrovascular dilation during CSD is controversial. The purpose of this study was to examine effects of inhibition of nitric oxide synthase on cerebrovascular dilation during CSD. Since indomethacin pretreatment potentiates cerebrovascular dilation during CSD (*Am. J. Physiol.*, 261: R828, 1991), we speculated that nitric oxide might play a larger role under these conditions. Urethane-anesthetized rabbits were equipped with a closed cranial window and pial arteriolar diameter was examined via intravital microscopy. Baseline arteriolar diameters ranged from 80-100 μm. We examined arteriolar responses to CSD in the absence of other drugs, and after intravenous administration of L-NNA (15 mg/kg) and/or indomethacin (10 mg/kg). CSD was induced by microinjection of 5% KCl. L-NNA reduced nitric oxide synthase activity by 90% in cerebral cortex ($n=12$ for controls and $n=24$ for L-NNA-treated) and reduced pial arteriolar dilation in response to 10^{-5}M acetylcholine from $30\pm10\%$ to $9\pm3\%$ ($n=3$). In the absence of indomethacin, L-NNA reduced peak cerebral arteriolar diameter from $56\pm6\%$ to $23\pm3\%$ during CSD ($p<0.05$; $n=8$). In other animals, indomethacin pretreatment increased arteriolar dilation from $41\pm5\%$ to $76\pm6\%$ during CSD ($p<0.05$; $n=5$). However, administration of L-NNA in the presence of indomethacin failed to reduce arteriolar dilation. In conclusion, following administration of indomethacin, inhibition of nitric oxide production by L-NNA fails to attenuate CSD-induced arteriolar dilation. Supported by HL 30260 and HL 46558.

720.2

MECHANISMS FOR PRESERVED CEREBROVASCULAR AUTOREGULATION DURING HYPERTENSION IN RATS AFTER SINOAORTIC DENERVATION (SAD). W.T. Talman*, D. Nitschke-Dragon. Dept. of Neurology, VAMC and Univ. of Iowa, Iowa City, IA 52242.

We have shown that cerebral blood flow (CBF) and cerebral vascular resistance (CVR) autoregulate at higher levels of arterial blood pressure (AP) after SAD in rat. In this study we sought to assess potential mechanisms for this phenomenon. CBF was monitored by laser flowmetry in intact rats in whom AP was increased by phenylephrine (PE), vasopressin, or angiotensin. AP was increased by PE in additional rats after: 1) SAD; 2) SAD plus bilateral removal of superior cervical ganglia; 3) SAD plus bilateral ligation and transection of renal vessels and nerves (isolation); or 4) SAD plus sympathectomy and renal isolation. Body temperature, arterial pCO_2, and rate of rise of AP were controlled. CBF and CVR broke through autoregulation when mean AP's were raised to 155 ± 4 mmHg by PE in intact rats but did not breakthrough at even higher levels of AP in SAD rats with intact (185 ± 2 mmHg) or interrupted (181 ± 5 mmHg) sympathetic innervation of cerebral vessels. Breakthrough was seen in rats in which AP was increased with vasopressin but not in those treated with angiotensin, which blocked baroreflex-mediated bradycardia that occurred in intact rats in response to increased AP. Nonetheless, breakthrough did not occur in SAD rats even when increases in plasma renin activity were prevented by isolation of the kidneys before SAD. This study suggests that the arterial baroreflex may participate in breakthrough of autoregulation at hypertensive levels of AP independent of its effects on sympathetic innervation of cerebral vessels or on plasma angiotensin levels. We speculate that breakthrough of cerebral autoregulation in response to hypertension may result from release of an active vasodilator. Support: VA merit review and career award, AHA grant in aid, and NIH HL32205 and HL14388.

720.3

THROMBIN INDUCES BRAIN EDEMA AND CONSTRICTION IN CEREBRAL MICROVESSELS: POSSIBLE ROLE IN INTRACEREBRAL HEMORRHAGE. K.R. Lee, A.L. Betz, O. Sagher*, R.F. Keep, G.L. Heath, and J.T. Hoff Section of Neurosurgery, University of Michigan, Ann Arbor, MI 48109

Thrombin is a naturally occurring protein involved in the clotting cascade. In addition to its role in thrombogenesis, it is thought to have vasoactive and inflammatory effects. While under normal conditions the level of thrombin in the brain is undetectable, during intracerebral hemorrhage (ICH) large quantities of prothrombin are converted to thrombin, exposing the brain to thrombin. The present series of studies investigated the possibility that the intracerebral injection of thrombin induces local brain edema and microvascular constriction.

Rats received a 10 µl stereotaxic injection of either saline, thrombin (1, 10, or 100 units), or thrombin (10 units) **and** a thrombin inhibitor (hirudin, 10 units; or Nα-(2-naphthalenesulfonylglycyl)-4-amadino-DL-phenylalaninepiperidide [αNAPAP], 0.2mM). Local brain water increased in rats injected with 10 or 100 units of thrombin (4.0% or 5.8% respectively; $p<0.01$ vs. sham animals). Animals injected with thrombin **and** hirudin had less water accumulation (2.0%; $p<0.05$ vs. thrombin **alone**). Similar results were obtained with the thrombin inhibitor, αNAPAP.

In order to investigate the possibility that thrombin may induce microvascular constriction, a second series of experiments was performed in which in vitro brain slices were obtained from rats and examined with computerized videomicroscopy. Microvessels (30-70µm in diameter) within the slices were monitored while the slices were superfused with thrombin (1 unit/µl in artificial cerebrospinal fluid) or vehicle (0.8% albumin solution) for 30 minutes. Thrombin induced a 35.4% microvascular constriction after 30 minutes, whereas treatment with vehicle resulted in a 2.9% constriction ($p<0.005$). These studies suggest that thrombin induces brain edema, and that this effect may be due in part to the microvascular effects of thrombin. Brain edema/toxicity seen in ICH may be mediated by the local effects of thrombin. The results also suggest that the neurosurgical use of thrombin to augment hemostasis may be accompanied by local edema and vasoconstriction.

720.4

HEMOGLOBIN AND BLOOD OXYGEN METABOLISM DURING ACUTE CYANIDE TOXICITY OF MINIATURE SWINE. E.U. Maduh*, E.W. Nealley, and S.I. Baskin. Biochem. Pharmacol. Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425

Monitoring changes in blood hemoglobin (Hb) and the related oxygen (O_2) parameters provides a means for evaluating treatment progress in acute cyanide (CN) poisoning. In the present study, serial measurements/ calculations of O_2, vol. %, Hb and its derivatives were obtained before and at time points over a period of 60 min after i.v. injection of NaCN (2.5 or 4.2 mg/kg) in the male miniature pig. Animals were anesthetized by isoflurane inhalation and cannulated for arterial blood collections. Whole blood was analyzed with a hemoximeter, for O_2, carboxyhemoglobin (COHb), oxyhemoglobin (HbO_2), and methemoglobin (metHb). NaCN injection significantly decreased metHb (Gram% ± SE) from 1.5 ± 0.17 to 1.05 ± 0.15 at the 10 min time point whereas COHb concentrations increased (0.46 ±0.2 to 1.15 ± 0.35) over the same period after the 2.5 mg/kg dose was administered. The 4.2 mg/kg dose decreased metHb (1.68 ± 0.26 to 0.80 ± 0.11) and increased COHb, 0.5 ± 0.05 to 1.1 ± 0.4 at the same time point. With either dose, the decrease (metHb) or increase (COHb), continued in the subsequent 20 min reaching a plateau between the 30 and 60 min time points. Levels of O_2 and HbO_2 tended to increase following NaCN exposure but did not attain statistical significance. Previously, pretreatment with 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7) a protein kinase C (PKC) inhibitor, was shown to blunt the loss of brain metabolic energy elicited by NaCN in vivo (*Maduh et al., FASEB J*, 8:A647, 1994), suggesting a possible role of PKC in CN toxicity. Results of the present study further support the PKC possibility as H7 (1 mg/kg) pretreatment administered 30 min before NaCN at the 4.2 mg/kg dose, also diminished but did not eliminate the changes in the arterial blood Hb and O_2 parameters analyzed. Since changes in arterial blood Hb factor very importantly in brain viability and metabolic status, it is concluded that monitoring these parameters may be useful in assessing the effectiveness of prophylactic treatment to counter cyanide effects in the intact organism.

720.5

CEREBROVASCULAR RESPONSES TO HYPERCAPNIA AND HYPERTENSION AFTER SUBARACHNOID HEMORRHAGE IN RATS. S.Yamamoto*, S.Nishizawa, and K.Uemura. Dept. of Neurosurg. Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan, 431-31.

We sought to determine whether chronic cerebral vasospasm following subarachnoid hemorrhage (SAH) would modify cerebrovascular responses. To do this we: (a) analyzed the effect of SAH upon vessel calibers on cerebral angiogram 48 hours later; (b) investigated cerebral blood flow (CBF) reactivities to increase in pCO_2 and mean arterial blood pressure (MABP). SAH was produced by intracisternal injection of 0.45 ml of autologous blood or saline (sham) in anesthetized Sprague-Dawley rats. Angiography was performed by injection of contrast material via carotid artery in control (n=15), SAH (n=6) and sham (n=6) groups. CBF was measured by laser-Doppler flowmetry over the parietal cortex 48 hours after SAH in sham (n=8) and SAH (n=6) animals. Graded hypertension was induced with phenylephrine. SAH reduced, by over 50%, the mean vessel caliber of internal carotid, anterior cerebral and middle cerebral arteries significantly ($p<0.05$, ANOVA). In other rats, hypercapnia (36 to 54 mmHg) increased CBF to a similar degree (sham 2.61, SAH 2.11 %/mmHg) in both groups. SAH did not abolish CBF autoregulation but shifted the least squares curve toward higher MABP ($p<0.01$, ANOVA with covariates). SAH also increased cerebrovascular resistance calculated by MABP/CBF at any levels of MABP. We conclude chronic vasospasm following SAH changed the cerebrovascular responses to hypertension but not to hypercapnia.

720.6

THREE-DIMENSIONAL IMAGE ANALYSIS OF LOCAL METABOLISM - BLOOD FLOW UNCOUPLING IN PHOTOTHROMBOTIC MCA OCCLUSION IN RATS. T. Back, M.D. Ginsberg*, W. Zhao, J.Y. Loor, I. Valdés, O.F. Alonso, B.D. Watson, and R. Busto. Cerebral Vasc. Disease Research Center, Univ. of Miami School of Medicine, Miami, FL, 33101.

We applied 3D autoradiography to characterize the relationship between local cerebral glucose utilization (lCMRglu, studied by ^{14}C-2-deoxyglucose) and blood flow (lCBF, studied by ^{14}C-iodoantipyrine) at 1.5 h following permanent distal middle cerebral artery (MCA) occlusion coupled with 1 h contralateral and permanent ipsilateral carotid occlusions in halothane-anesthetized, physiologically monitored normothermic Sprague-Dawley rats. The MCA was occluded by laser-driven photochemical thrombosis. Brains were coronally sectioned subserially and aligned by disparity analysis to yield average 3D data sets for both lCMRgl and lCBF (n=7 each). Computer division produced a 3D ratio image depicting the average metabolism/flow couple. lCMRgl images showed remarkable maintenance of metabolism despite the fact that a volume of 109 mm^3 exhibited penumbral flow values of 0.15-0.30 ml/g/min. Metabolic suppression (CMRgl < 50% of control) was present in a volume of only 60 mm^3 and corresponded to the most ischemic cortical zone (CBF < 0.15 ml/g/min). The ratio image revealed marked uncoupling (ratio values > 100% + 4xSD) in 152 mm^3 of the ipsilateral hemisphere. Thus, CMR>CBF uncoupling, even at low CBF, is a prominent feature of this stroke model.

720.7

CYTOCHROME OXIDASE ENZYME ACTIVITY IS UP-REGULATED IN RAT NUCLEUS ACCUMBENS AND DOWN-REGULATED IN MEDIAL PREFRONTAL CORTEX DURING WITHDRAWAL FROM CHRONIC COCAINE. **J.R. Walker* and K.A. Sevarino. Division of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.**

Cytochrome c oxidase (CO) subunit I mRNA was previously found to be up-regulated by chronic cocaine treatment (15 mg/kg, i.p., twice daily for 14 days) in rat nucleus accumbens (NAc) (Walker and Sevarino, Soc. Neurosci. Abs. 19, 1858). CO enzyme activity, measured spectrophotometrically, was examined in multiple brain regions, and was found to be regulated in the medial prefrontal cortex (mPFC), as well as in the NAc, each with a unique time dependence during withdrawal from chronic cocaine. The peak increase in enzyme activity in the NAc occurred 18 hours after the last cocaine injection, at a time when the peak decrease in CO activity was observed in the mPFC. CO activity was not regulated in any other brain region studied. CO activity up-regulation was not observed in the NAc 18 hours after acute cocaine, chronic desipramine, chronic fluoxetine, chronic lidocaine, or chronic morphine treatments. We suggest that CO enzyme activity may serve as a biochemical marker for cocaine withdrawal.

720.8

Reversibility of Hyperammonemic Encephalopathy

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Liver failure or shunting of blood past the liver leads to hyperammonemia and encephalopathy. Metabolic changes associated with this condition include decreased brain glucose consumption, increased neutral amino acid transport into the brain, and an abnormal amino acid spectrum in plasma and brain. The question is open whether these changes, especially those that occur in brain, are completely reversible after a sustained period of liver deficiency. We developed an animal model whereby portacaval shunting, which produces pronounced liver atrophy, could be reversed by restoration of blood flow to the liver. Three experimental groups were studied: 1) sham operated rats, 2) portacaval shunted rats and, 3) portacaval shunted rats with full blood flow restored to the liver for one week. Three weeks after the first operation (shunt or sham) we measured the plasma and brain amino acid spectrum, the liver-to-body weight ratio, brain glucose consumption, phenylalanine transport across the blood-brain barrier and the circadian rhythm of activity. Shunted animals showed the characteristic metabolic and kinetic abnormalities and a disruption of the normal circadian rhythm. Shunted rats that had restored normal liver blood flow regained the normal liver size, and were indistinguishable from the control group in every respect.

Supported by NIH grant NS 16389.

720.9

INCREASE IN CORTICAL INTERSTITIAL PURINE METABOLITES AND DECREASE IN CEREBRAL BLOOD FLOW FOLLOWING ASPHYXIA IN NEWBORN PIGS: IMPLICATIONS FOR SUPEROXIDE FREE RADICAL FORMATION. E.R. Gonzales, C.O. Park, A.R. Shah, R.G. Dacey*, T.S. Park, J.M. Gidday. Department of Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63010

Previous studies showing increases in cortical SOD-inhibitable NBT reduction and increases in cortical lipid peroxidation products following asphyxia in piglets indicate that superoxide anion is generated in response to asphyxia. To test the hypothesis that superoxide free radical production may derive, in part, from xanthine oxidase activity, we implanted cortical microdialysis probes in isoflurane anesthetized newborn piglets (n=7) and measured purine metabolites at 20 min intervals over an 80 min post-asphyxic reventilation period. Cortical cerebral blood flow (CBF) and pial arteriolar diameter were measured concomitantly by laser doppler flowmetry and cranial window videomicroscopy, respectively. Significant increases in hypoxanthine, xanthine, and uric acid concentrations were measured throughout the 80 min reventilation period; peak increases of 167±49 % (p=.002), 61±13 % (p=.001) and 1906±377 % (p=.042), respectively, occurred at 40 min post-asphyxia. Significant reductions in CBF and arteriolar diameter occurred at 40-80 min post-asphyxia; peak reductions of 23±5 % (p=.001) and 39±13 % (p=.033), respectively, occurred at 80 min post-asphyxia. These findings are indicative of significant xanthine oxidase activity in piglet cortex, and suggest that purine degradation may contribute importantly to free radical formation following asphyxia, which may, in turn, play a role in post-asphyxic hypoperfusion and neuronal injury.
(Supported by NIH RO1 NS21045).

720.10

EPIFLUORESCENCE VIDEOMICROSCOPY REVEALS INCREASED LEUKOCYTE ADHERENCE IN PIGLET PIAL VENULES DURING REPERFUSION FOLLOWING GLOBAL CEREBRAL ISCHEMIA. T.S. Park*, E.R. Gonzales, C.O. Park, A.R. Shah, J.M. Gidday. Department of Neurological Surgery, Washington University, St. Louis, MO 63010

We developed an *in vivo* model of cerebral ischemia-reperfusion wherein on-line videoimages of fluorescently-labelled leukocytes flowing within pial venules of anesthetized newborn pigs can be obtained. A closed plexiglass cranial window was implanted over the parietal cortex, and pial venules were imaged with an epifluorescence microscope (Olympus BHMJ) outfitted with a 100 watt mercury lamp, rhodamine filter cube (535/35 nm excitation; 565 dichroic; 610/75 nm emmision), 10x immersion lens (Olympus; 0.4NA), and newvicon tube camera with gain/contrast enhancement capabilities (Hamamatsu C2400). Final image magnification captured to Super VHS videotape was 470x. Rhodamine 6G was administered as a 0.5 mg/kg i.v. loading dose and a 0.5 mg/kg/h i.v. maintenance dose in saline to selectively label circulating leukocytes. Under baseline conditions in 6 pigs, few labelled leukocytes were observed adhering to the endothelium of 75-150 μm diameter venules. However, following 10 min of global cerebral ischemia induced by reversible subclavian and brachiocephalic occlusion, a dramatic increase in rolling and adherent leukocytes was observed at 30, 60, and 90 min of reperfusion. At these times, cortical blood flow, measured by both laser doppler flowmetry and hydrogen clearance, was decreased by 25-40 % ($p<0.05$). These findings demonstrate the feasibility of continuous *in vivo* monitoring of leukocyte adherence in cerebral venules, and suggest that reperfusion-induced adherence to venular endothelium may contribute to hypoperfusion following global cerebral ischemia. (NIH RO1 NS21045)

LEARNING AND MEMORY: PHARMACOLOGY—OTHERS II

721.1

NITRIC OXIDE FORMATION IS ESSENTIAL FOR ADAPTIVE MODIFICATION OF THE VESTIBULO-OCULAR REFLEX, A MODEL FOR NEUROPLASTIC CHANGES IN THE GOLDFISH. Jun Li[1]*, Sheryl S. Smith[1], and James G. McElligott[2]. [1]Dept. of Anatomy, Inst. of Neuroscience, MCP- Hahnemann University, Phila., PA 19102; [2]Dept of Pharmacology, Temple University School of Medicine, Phila., PA 19140.

The gaseous second messenger nitric oxide (NO), implicated in neuroplasticity using in vitro models (Schuman and Madison, 1991), was tested for its role in adaptive modification of the vestibulo-ocular reflex (VOR) in the goldfish, an in vivo model for neuroplastic changes. Two potential mechanisms for NO release in the cerebellum, activation of climbing fiber afferents (Southam and Garthwaite, 1991) and activation of non-NMDA excitatory amino acid receptors (Garthwaite et al, 1989), also occur during adaptation of the VOR. This reflex functions to maintain a stable visual image on the retina during head movement. Adaptive increases in VOR gain were produced by sinusoidal rotation of the head in the horizontal plane 180° out- of-phase with a visual stimulus. NO synthase (NOS) was first localized to goldfish cerebellum, as identified by NADPH- diaphorase staining. For the VOR study, NO production was prevented by L- N^{G}- monomethyl-arginine (L-NMMA), a specific blocker of NOS, injected bilaterally (12.5μg/0.5μl/side) into the vestibulo-cerebellum. Treatment with L-NMMA, but not D-NMMA (the inactive isomer) significantly inhibited the robust adaptive gain increase ($P<0.01$) normally observed in vehicle- injected controls trained over a 3-hr period, but did not alter the basal, pre-adapted VOR gain. L-arginine, the substrate of NOS, injected concomitantly with L- NMMA, prevented the observed L-NMMA-induced inhibition of VOR adaptation. The inhibitory effect of L-NMMA application on VOR gain adaptation was not due to a vasoactive action as assessed by laser Doppler blood flow measurements. Thus, these results suggest a role for NO, within the cerebellum, in adaptive modification of the VOR, a learned response. (Supported by NIH#DC01094 to JGM and USAF Gr AFOSR#93-0136 to SSS.)

721.2

A selective neuronal nitric oxide synthase inhibitor has amnestic effects in the chick

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Nitric oxide (NO) is a putative retrograde messenger that is supposed to play a role in synaptic plastic processes and in learning and memory formation. Previous studies show that inhibitors of NO synthases cause amnestic effects in chicks or rats. However, NO also is a blood vessel relaxing factor which is produced by endothelial cells. It is not certain if the amnestic effects might be the result of blood vessel constriction rather than inhibition of synaptic transmission.

7-nitro indazole (7-NI) is an NO synthase inhibitor that is selective for the neuronal isoform[1]. We tested this inhibitor in a one-trial passive avoidance task in day-old chicks, in which the birds peck at and subsequently avoid a bitter tasting bead, displaying a typical disgust reaction. Chicks injected with 50mg/kg 7-NI i.p.1 h before training and tested 30 min or 24 h after training showed amnesia for the training and the birds pecked the test bead (75% avoidance of test group compared to 30% of control group with received saline injections). Injection 5 min after training and testing 2 h after training did not produce amnesia.

This result supports the theory that NO acts as a neuronal transmitter which is of importance in processes of learning and memory formation.

1: Moore et al., Br. J. Pharmacol., 108 (1993) 296-297

721.3

MICRODIALYSIS WITH NITRIC OXIDE IN THE SEPTUM IMPROVES SOCIAL MEMORY OF RATS. T.Horn*, R.Landgraf+ and M.Engelmann+. The Scripps Research Institute, La Jolla, CA, U.S.A., +Max Planck Institute for Psychiatry, Munich, Germany.

Nitric oxide (NO) synthase immunoreactivity in limbic areas of the rat brain including the septum implicates an involvement of NO in learning and memory. The present study tested whether NO in the septum of adult male adult rats is potent to modulate their juvenile recognition capacities. Animals which received artificial cerebrospinal fluid (aCSF) pregassed with a mixture of 5% NO in nitrogen via microdialysis into the septum showed a significantly improved juvenile recognition ability ($p<0.01$, ANOVA, n=13) compared to control sessions in which the same animal received aCSF pregassed with 100% nitrogen. To quantitate the applied amount of NO, chemiluminescence detection was used to analyze the *in-vitro* release from the microdialysis probes showing that approximately 40 pmol NO per min were released from the probes into the surrounding medium. Blockade of endogenous NO-synthesis by intraperitoneal administration of L-NAME (36mg/kg b.wt.), however, failed to affect juvenile recognition in another group of rats (n=13). These results indicate, that neuronal substrate of olfactory memory located in the septum is sensitive to NO. However, the fact that blockade of endogenous NO synthesis did not affect the behavioral performance of rats suggests that other mechanisms may substitute for endogenous NO. Supported by the DAAD.

721.4

INHIBITION OF NITRIC OXIDE SYNTHASE AND LONG-TERM HABITUATION TO NOVELTY IN THE NAPLES HIGH AND LOW-EXCITABILITY RAT LINES. M.P.Pellicano, F.Siciliano and A.G.Sadile, (SPON: European Brain and Behaviour Society). Lab. Neurophysiol. Behav. & Neural Networks, Dept. Human Physiol. "F.Bottazzi", SUN, Naples, Italy.

The role of nitric oxide (NO) as a neuromodulator in the CNS was assessed by a behavioral study in a genetic model of hippocampus-dependent trait by inhibition of the NO-synthase (NOS) enzyme. Adult male rats of the Naples High (NHE) and Low-Excitability (NLE) lines, and of random-bred controls (NRB) were tested for 10 min in a Làt-maze during which two theta-related activity components such as walking and rearings, and an emotionality index such as defecation score were measured. Immediately upon completion of the test, they were given an intraperitoneal injection of the NOS inhibitor N^{G}-nitro-L-arginine (L-NOARG) 1-10 mg/Kg or vehicle. Retention testing took place 7 days later. The results indicate that in NRB-rats 10 mg/Kg impaired long-term habituation (LTH) of the vertical component with prevailing emotional meaning, whereas both 1 and 10 mg/Kg inhibited LTH of the defecation score, i.e. emotional habituation. Conversely, both doses exerted no effect on LTH of the horizontal component in the NLE, but had an impairing effect in the NHE. In addition, while 1 mg/Kg had no effect on LTH of the vertical component, 10 mg/Kg exerted a small improvement in both strains. The disruptive effect of L-NOARG on long-term habituation of the vertical component in random-bred rats supports the hypothesized role NO may play in the coupling of spatial and emotional information. In addition, the genotype-dependent differential effect of NOS inhibition in NLE and NHE lines, compared to NRB-rats, strengthens their usefulness as a genetic model for altered spatial and non spatial processing and storage in the mammalian brain. **(Supported by CNR 93.00408.CT04, and MURST 40% grants).**

721.5

CHRONIC ADMINISTRATION OF L-NAME IN DRINKING WATER ALTERS WORKING MEMORY IN RATS. B.L. Cobb*[1], K.L. Ryan[2], M.R. Frei[2], V. Guel-Gomez[2] and G.A.Mickley[1], [1]Radiofrequency Radiation Division, Armstrong Laboratory, Brooks AFB, Tx, 78234; [2]Depart. of Biology, Trinity University, Tx 78212

In order to examine the role of nitric oxide (NO) in the maintenance of working memory of rats, the effects of chronic administration (in drinking water) of the NO synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME), on this behavior was examined with a simple test of remembering recently explored objects. Unlike other working memory tasks that require a subject to perform for a reward such as food or water or to avoid shock, our task measured spontaneous exploration of novel and familiar objects and has been described as a "pure" working memory task (Ennaceur, 1988). Normal subjects spend significantly more time in contact with new environmental components and less time with familiar objects. A subject that extensively reexplores a stimulus with which it has previous experience is presumed to exhibit some memory loss associated with the object. Memory changes were evaluated by measuring the relative time subjects explored familiar vs. new stimulus objects. Rats (n=15) that chronically drank for 14 days, a dose of ≈100 mg/kg/day L-NAME, spent significantly less time exploring a novel object than rats (n=13) that drank only tap water ($p<.05$). This effect of L-NAME was significantly attenuated by concurrent administration of L-Arginine (≈5g/kg/day). Total object exploration was not affected by our drug treatments, suggesting that our object discrimination task is not activity dependent. These data are consistent with the hypothesis that NO is required for some forms of working memory.

721.6

REGULATION OF SPONTANEOUS ALTERNATION IN THE RAT BY GLUCOSE: A ROLE FOR GLYCOLYSIS. M.R. Stefani, M.E. Ragozzino, P.K. Thompson, K.Hellems, R.C. Lennartz and P.E. Gold*. Dept. of Psychology, University of Virginia, Charlottesville, VA 22903.

D-glucose administered peripherally has previously been shown to enhance memory on a variety of tasks and to attenuate the effects of certain memory-impairing drugs such as the opioid agonist morphine. The present studies show that morphine (3.95 nmols) injected centrally into the rat medial septum 30 minutes prior to behavioral testing produced memory deficits as measured in spontaneous alternation and inhibitory avoidance tasks. Glucose (18.3 nmols) administered concurrently with the morphine reversed the amnestic effects of morphine. To test whether the glucose effects were due to metabolites of glucose, pyruvate (18.3 nmols), the end product of the glycolytic metabolism of glucose, was injected intraseptally together with morphine. Pyruvate reversed the spontaneous alternation performance deficits induced by morphine administered alone. Furthermore, blockade of the glycolytic pathway with intraseptally administered iodoacetic acid (0.5 nmols), an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, produced deficits in spontaneous alternation which were reversed by concurrent intraseptal injections of pyruvate (25 nmols), but not glucose (10 nmols). These results suggest that the memory-enhancing properties of D-glucose may be mediated by glucose metabolism. Glucose may act via the citric acid cycle to increase the availability of ATP and/or neurotransmitter precursors. [Supported by NSF (BNS 90-12239), NIA (AG07648) and ONR (N0001489-J-1216)]

721.7

INVOLVEMENT OF CYCLIC AMP IN THE ACOUSTIC STARTLE RESPONSE AT THE LEVEL OF THE NUCLEUS RETICULARIS PONTIS CAUDALIS. T.C.M. de Lima & M. Davis* Dept. of Pharmacology, Univ. Fed. Santa Catarina, Fpolis, SC, Brazil & *Dept. of Psychiatry, Yale Univ. Sch. Med., New Haven, CT.

The acoustic startle reflex has a well-described neural circuitry in which the nucleus reticularis pontis caudalis (RPC) plays an essential role. Nevertheless, the pharmacology of this reflex is not fully known. Local infusion into the RPC of excitatory amino acid (EAAs) antagonists suggests that glutamate may actually mediate startle at this level. EAA release at other synapses has been shown to be enhanced by increases of the second messenger cAMP. Previous studies have demonstrated the involvement of the amygdala in the modulation of the startle response, perhaps via a cAMP-dependent process at the RPC. As an initial test of this hypothesis, the present experiments evaluated whether a cAMP analog, 8-bromo-cAMP (8BR), or a phosphodiesterase inhibitor, Rolipram, would increase startle when infused directly into the RPC.

Rats implanted with cannulae in the RPC (AP= -12.0, ML= ±1.5, DV= -9.6, at an angle of 78^0) were tested, one week after surgery, with 20 startle stimuli (115 dB bursts of noise, 50 msec duration, at a 30 sec interval) as a pre-drug baseline, and then infused successively with artificial cerebral spinal fluid (ACSF) and increasing doses of 8BR (0.125, 0.25, 0.5, 0.75 and 1.0 µg). Immediately after each infusion they were retested with 40 startle stimuli. Other groups of animals were tested as above, but received repeated infusions either of ACSF alone or of 8BR (0.25 µg). 8BR produced a dose-dependent increase in startle amplitude. Repeated infusions of vehicle or 0.25 µg of 8BR did not reliably affect startle amplitude. Another group of animals infused with Rolipram 10 µg/ µl showed a marked increase in startle amplitude (108.1 %). These results suggest that the cAMP second messenger system can modulate startle amplitude at the level of the RPC. To characterize more precisely the involvement of cAMP in startle modulation, we are currently examining the effect of other compounds known to alter cAMP activity. (Post-doctoral fellowship CNPq-Brazil # 201260/93-0).

721.8

EFFECTS OF ADENOSINE A_1 AND A_2 AGONISTS AND ANTAGONISTS ON CONFLICT LEARNING AND MEMORY IN THE MOUSE. M.E. Judge*. Pharmaceuticals Division, Novo Nordisk A/S, DK-2760 Måløv, DENMARK.

Adenosine is an inhibitory neuromodulator, exerting both activation-dependant and tonic effects on central neurotransmission, mediated in part by A_1 and A_2 receptors. Since drugs influencing these receptors have been shown to affect memory in several animal models, the effects of agonists and antagonists of these receptors were evaluated in a conflict memory test in mice. Thirsty mice were trained 30 min after an i.p. injection. They were given 5 min to drink for 5 sec. Side effects at high doses inhibited drinking, but not the next phase, delivery of mild shocks until drinking ceased. Memory was tested the next day. Anterograde amnesia was produced by treatment with the A_1/A_2 antagonist aminophylline, the A_2 antagonist DMPX, and the A_1 agonist R-PIA at doses below those suppressing responding during training. In contrast, the A_1 antagonist CPT and the A_2 agonist CGS 21680 only impaired memory at doses which also impaired responding during training. None of these compounds affected memory processes when injected after training or before testing. These results indicate that the formation of an associative memory can be impaired by either activation of A_1 receptors or blockade of A_2 receptors.

721.9

EFFECTS OF APAMIN ON TIME-DEPENDENT CHANGES OF REGIONAL BRAIN METABOLISM DURING MEMORY PROCESSING IN MICE. W Belcadi-Abbassi, B. Bontempi and C. Destrade.* Lab. des Neurosciences Comportementales URA CNRS 339, Univ. Bordeaux I, 33405 Talence France.

We previously showed that apamin, a blocker of calcium-activated potassium channels, facilitated memory processes when injected at non-convulsant doses, immediately after partial acquisition of a bar-pressing task in a Skinner-box (MESSIER et al, 1991, Brain Research, 551, 322-). Using the same learning paradigm we also analysed the post-test kinetics of regional mapping of 14C 2-Deoxyglucose (2-DG) uptake (SIF et al, 1991, Behav. Neural Biol. 56, 43-). The results showed a U-shaped curve of 2-DG labelling intensity in hippocampal and cortical areas as a function of the post-learning period (increase in labelling 15min and 3hr after training; decrease at the 1hr time interval).

In an attempt to characterise the effects of the post-training injection of apamin (APA) on the post-test kinetics of 2-DG uptake, APA (0.2 mg/Kg i.p.) or saline (SAL) was injected either before or at various time intervals (0min, 1hr, or 3hr) after training. 5min later, 2-DG was injected into the jugular vein. 40min later brains were processed for autoradiography. Quantitative densitometric comparisons of the autoradiographs of APA and SAL animals show that the post-training injection of APA, 1) significantly alters the 2-DG labelling patterns in the medial septum, hippocampal formation, subiculum, mammilllary bodies, entorhinal, cingulate and pyriform cortices, which exhibit the highest density of central APA receptors, and 2) accelerates the temporal evolution of the 2-DG patterns. The diminution in 2-DG labelling which normally occurs at 1h 45min post-training in controls appears significantly earlier (45min) in APA treated animals. This phenomenon could be a consequence of an early APA-induced increase in neuronal activity which results in an acceleration of the brain mechanisms implicated in memory processing. Supported by CNRS and DRET grants.

721.10

THE TYROSINE PHOSPHORYLATION OF A SET OF PROTEINS IN THE RAT GUSTATORY CORTEX, THAT IS MODULATED BY GUSTATORY EXPERIENCE, IS ALSO MODULATED BY *IN VIVO* ADMINISTRATION OF CARBAMYLCHOLINE. K. Rosenblum, S. Hazvi, D. E. Berman, C. Naor and Y. Dudai*. Weizmann Institute of Science, Rehovot, Israel.

We have previously reported that gustatory experience alters tyrosine phosphorylation in the rat insular cortex (Rosenblum et al., Soc. Neurosci. Abst. 18: 743, and *submitted).* Thus, conditioned taste aversion (CTA) training, and to a lesser extent exposure to a novel taste (saccharin or NaCl), increased tyrosine phosphorylation of several polypeptides, the major ones being of MW 100, 115 and 180kD. The effect was specific to the insular cortex, peaked within minutes and vanished within hours. The 100 and 180kD proteins are synaptic membrane glycoproteins. We have set out to identify neurotransmitters that mediate the effect in *vivo* and hence might molecularly encode sensory input in the cortex. Toward that end, we have microinjected transmitter agonists and antagonists into the insular cortex and quantified their effect on tyrosine phosphorylation by immunoblotting the cortical tissue with anti-phosphotyrosine antibodies. We found that carbamylcholine increased tyrosine phosphorylation of the same proteins that were affected by the gustatory experience. The effect was dose dependent at the range of 1-100 mM carbamylcholine and time dependent, peaking at ca. 1 hr. Choline chloride had no effect, while atropine abolished the effect of carbamylcholine. In parallel experiments we found that injection of scopolamine into the insular cortex prior to the exposure to saccharin in CTA training blocked CTA memory, thus corroborating the role of acetylcholine in mediating sensory input to the cortex in CTA training. (Supported by the Whitehall Foundation.)

721.11

MICE RECEIVING CHRONIC TREATMENT WITH ERYTHROPOIETIN SHOW INCREASED HEMATOCRIT AND IMPROVED PERFORMANCE IN A SWIM MAZE. *J. Hengemihle, O. Abugo, J. Rifkind, E. Spangler, D. Danon, and D. Ingram*, Lab. of Cellular and Molecular Biology, Gerontol. Res. Ctr., Natl. Inst. Aging, NIH, Baltimore, MD 21224*

Erythropoietin is a glycoprotein produced endogenously in the kidney, which stimulates red blood cell production. We evaluated the effects of chronic treatment with recombinant human erythropoietin (Epogen®:Epo) on the performance of 8-mo old male C57BL/6J mice in a complex spatial task, the Morris water maze. Mice were treated with either Epo (1.5 units injected s.c. every other day) or vehicle (PBS also injected s.c. every other day). Results indicated that the treatment had no effect on maze performance after 9 wks, but after 20 wks the Epo-treated mice learned the task significantly faster than controls over 4 days of training as measured by mean distance (cm) to reach the goal platform. The results suggested that the improved performance in mice resulted from an increase in hematocrit which was not achieved until after 20 weeks of Epo treatment. Evaluation of several hematological parameters indicated that the only significant effect of Epo treatment was to raise hematocrit values at 20 wks. This rise was found to be significantly correlated with swim maze performance.

721.12

EFFECT OF AIT-082 ON ETHANOL-INDUCED AMNESIA AND BEHAVIOR. R.F. Ritzmann*, I. Prisecaru, C.L. Melchior, F. DeLeon-Jones and A.J. Glasky. Olive View UCLA Medical Center, Sylmar, CA 91342 and Advanced ImmunoTherapeutics, Tustin, CA 92680

AIT-082, a derivative of the purine hypoxanthine, has been shown to improve memory in the win-shift test of working memory. This test consists of placing a mouse in a T-maze with a reward in both goal boxes; the mouse is allowed to choose one of the goal boxes. On the next trial, if the mouse remembers which box it entered on the previous trial, it will enter the other box. By increasing the delay between trials, the length of time a mouse can remember can be determined. In C57BL/6 mice, the longest delay that these mice can remember is 120 seconds. Mice receiving AIT-082 (30 mg/kg) were able to remember at delays over 180 seconds. Ethanol (0.5 mg/kg), 10 minutes prior to testing, at 120 second delay, reduced the correct responses to 65%, without altering the time to leave the start box or the time to traverse the maze, indicating that ability to perform was not impaired. AIT-082, given 20 minutes prior to ethanol, blocked the amnestic effect of ethanol.

A behavioral analysis of the effects of AIT-082 was performed at doses from 0.005-60 mg/kg. The tests included: locomotor activity, exploratory activity, plus maze test of anxiety, hot plate analgesia and roto-rod test of motor coordination. The only significant effects of AIT-082 were improved performance on the roto-rod test at doses from 0.05 to 60 mg/kg and a decrease in locomotor activity only at the highest dose (60 mg/kg). The decrease in locomotor activity along with the lack of any effect on exploratory activity may be an increase in the rate of habituation, which would be consistent with the effects of AIT-082 on memory in other tests. Supported by grants from NIAAA and NIA (AG09911)

721.13

ETHANOL IMPAIRS PLACE LEARNING BUT NOT RESPONSE LEARNING IN THE RAT. W.T. Kendrick; J.L. Vandergriff; D.B. Matthews; P.J. Best; and P.E. Simson*. Lab. of Neuropsychopharmacology, Dept. of Psychology, Miami University, Oxford, OH 45056.

Ethanol blocks long-term potentiation *in vitro* (Sinclair and Lo, 1986) and inhibits NMDA-evoked activity of hippocampal neurons both *in vitro* (Lovinger *et al.*, 1989) and *in vivo* (Simson *et al.*, 1993). In behavioral studies, acutely administered ethanol impairs the use of spatial memory (Matthews *et al.*, RSA Meeting, 1994), and chronically administered ethanol impairs learning of a spatial task (Arendt *et al.*, 1988) that has been shown to be sensitive to hippocampal damage (Olton and Papas, 1979). More recently, Matthews *et al.* (RSA Meeting, 1994) report that ethanol, but not diazepam or MK-801, markedly alters the spatial properties of hippocampal neurons recorded electrophysiologically in awake behaving rats. In contrast to these results, however, Devenport *et al.* (1989) conclude that ethanol is virtually without influence on spatial localization.

In the present study, we investigated the effect of ethanol on spatial and non-spatial tasks that Matthews and Best (1993) demonstrated to be differentially sensitive to fimbria/fornix lesions. Results show that animals injected with ethanol prior to each training session learned significantly slower than saline-injected animals on the spatial task ($p < .01$). In contrast, ethanol-injected and control animals performed similarly on the non-spatial task ($p > .1$). These results suggest that ethanol produces an impairment in spatial learning similar to that produced by fimbria/fornix lesions.

721.14

ACUTE ETHANOL IMPAIRS SPATIAL MEMORY BUT NOT NON-SPATIAL MEMORY IN RATS: COMPARISONS WITH DIAZEPAM AND MK-801. P.J. Best*, D.B. Matthews, J.L. Vandergriff, W.T. Kendrick & P.E. Simson. Cognitive Neurosci. Lab. Dept. Psych. Miami Univ. Oxford, OH 45056.

Ethanol blocks LTP in hippocampal slices (Sinclair & Lo, 1986) and inhibits NMDA-evoked activity of hippocampal neurons in vivo (Simson et al., 1993). Chronic ethanol also impairs learning a spatial task (Arendt et al., 1988) that has been shown to be sensitive to hippocampal damage (Olton & Papas, 1979). In addition, Matthews et al. (see accompanying abstract) report that ethanol alters the spatial properties of hippocampal neurons.

We investigated the effects of acute ethanol on two tasks requiring spatial memory and one requiring non-spatial memory. Ethanol significantly reduced the ability to use spatial memory but not non-spatial memory. Also, MK-801 (only at doses that produce ataxia) but not diazepam impaired spatial memory. Neither drug affected non-spatial memory.

Therefore, ethanol's contribution to auto accidents, may not be limited to impairment of judgment and reflexes but may include diminished cognitive capacities necessary for the use of spatial memory.

721.15

HIPPOCAMPUS: POSSIBLE SITE OF AMELIORATION OF EYEBLINK CLASSICAL CONDITIONING BY NEFIRACETAM D.S. Woodruff-Pak* & Y.-T. Li. Lab. of Cognitive Neuroscience, Phila. Geriatric Cntr, Phila., PA & Temple Univ., Phila., PA 19122.

We demonstrated that Nefiracetam (NEF) ameliorates eyeblink classical conditioning (EBCC) in older rabbits in the 750 msec delay paradigm. The present study is an attempt to identify the brain site of action. If NEF affects EBCC via the hippocampus, injection of NEF in rabbits with intact hippocampus should ameliorate EBCC whereas injection in hippocampectomized rabbits should not. Five groups of older rabbits were run in 750 msec delay EBCC: 1) hippocampectomy, 10 mg/kg NEF (N=8); 2) partial cortical removal, 10 mg/kg NEF (N=9); 3) sham surgery, 10 mg/kg NEF (N=9); 4) no surgery, 10 mg/kg NEF (N=8); 5) no surgery, vehicle (N=8). Among groups treated with NEF, trials to learning criterion (T/C) was significantly greater in hippocampectomized rabbits (1,038) than in rabbits with intact hippocampus (cortical = 738; sham surgery = 445; no surgery = 473). Rabbits with vehicle had significantly more T/C than those with NEF (845 vs. 445 and 473). Analyses of percentage of conditioned responses supported the results with T/C. Hippocampectomized rabbits with NEF learned more slowly than cortical surgery and controls with intact hippocampus. NEF may act to ameliorate EBCC in older rabbits via the hippocampus. *Supported by Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.*

721.16

BENEFICIAL EFFECTS OF PRE-084, A SELECTIVE σ LIGAND, ON PHARMACOLOGICAL MODELS OF LEARNING IMPAIRMENT IN MICE. T. Maurice*[1], T.-P. Su[2], D.W. Parish[3] and A. Privat[1].

[1] INSERM U 336, ENSCM, 8 rue de l'Ecole Normale, 34053 Montpellier cedex 1, France; [2] NIDA-Addiction Research Center, NIH, POB 5180, Baltimore, MD 21224; [3] Bio-CAD Corporation, 545 Oakmead Parkway, Sunnyvale, CA 94086.

We investigated the effects of PRE-084, a selective sigma ligand derived from phencyclidine, on several pharmacological models of learning impairments in young adult mice. Tests included spontaneous alternation in a Y-maze, for immediate spatial working memory, and the step-down type passive avoidance, elevated (+)-maze, and place learning in a water maze, for long-term memory. (1) PRE-084, at doses about 1 mg/kg sc, significantly attenuated the impairment of alternation, decrease in step-down latency, and increase in transfert latency induced by MK-801 (0.2 mg/kg ip). These effects were antagonized by the simultaneous administration of BMY-14802 (10 mg/kg ip), and suppressed in mice chronically treated with haloperidol (4 mg/kg/day sc for 7 days). (2) At the same dose range, PRE-084 significantly attenuated the impairments induced by mecamylamine (10 mg/kg ip), but not by scopolamine (1 mg/kg ip). (3) PRE-084, at 0.3 - 1 mg/kg, also prevented the impairment of alternation, decrease in step-down latency, and impairment of place learning induced by the calcium antagonist nimodipine (0.3 mg/kg ip). These results confirm previous studies showing that sigma ligands may modulate the learning processes, mediated via the NMDA receptor or the nicotinic cholinoceptor activation, and the resulting calcium mobilization. Thus, PRE-084, by acting selectively at sigma sites, may be useful in improving the cognitive impairments due to aging or Alzheimer's disease.

721.17

THE NOVEL COGNITION ENHANCING AGENT S 12024–2 FACILITATES OBJECT AND SOCIAL RECOGNITION MEMORY AFTER ACUTE OR CHRONIC TREATMENT IN RATS. W. Raaijmakers, J. Prickaerts, A. Schoffelmeer*[1], and J. Jolles. Dept of Psychiatry and Neuropsychology, Univ. of Limburg, Maastricht, NL-6200 MD, and [1]Dept of Pharmacol., Univ of Amsterdam, The Netherlands.

Preliminary experiments suggested that S 12024–2, a novel compound [Servier], might enhance memory. We used two different tests to establish whether S 12024–2 facilitates recognition memory. Twenty adult male Wistar rats were tested in the object exploration test [Ennaceur & Delacour, Behav Brain Res, 1988]. In the first trial (3 min) exploration times were scored for two identical objects. After 24 h the rats did no longer discriminate between an identical, familiar object and a novel object. When they had received S 12024–2 (3.4 mg/kg ip) immediately after the first trial, they discriminated between the two objects indicating that the treatment had facilitated recognition memory. This effect of S 12024–2 was replicated. Subsequently, two groups were formed that received either distilled water (n=8) or a solution of S 12024–2 (16 mg/kg/day; n=12) to drink. After 10 days the S 12024–2-treated, but not the control, rats showed significant recognition of the familiar object. This effect was replicated after 17 days of treatment.

Another group of 20 rats was used for the social memory test [Dantzer et al., Psychopharmacol., 1987]. A similar design was used: all animals were tested after post-training ip injection of saline or 3.4 mg/kg S 12024–2. After 24 hours a decrease in exploration of a juvenile rat was found only when treated with S 12024–2. This effect was replicated. In the chronic treatment condition S 12024–2 facilitated social recognition after 6, 10 and 12 days of treatment. From these results it can be firmly concluded that S 12024–2 is effective as a cognition enhancing agent with respect to recognition memory.

(Supported by I.R.I.S., Courbevoie, France.)

721.18

ACUTE ADMINISTRATION OF ESTRADIOL ENHANCES SPATIAL MEMORY IN INTACT MALE RATS. G.M. Alexander, M.G. Packard, W.L. Nores, & R.D. Olson* Dept. Psychology, Univ. New Orleans, N.O.,LA 70148.

We examined the effects of acute post-training injections of Estradiol-Hydroxypropyl-B-Cyclodextrin Inclusion Complex on memory of intact male rats trained in a single-platform spatial water maze task. Unlike traditional forms of sex hormone administration, injection of estradiol inclusion complex results in a rapid rise of hormone in blood plasma, similar to that induced by normal hormone release.

Rats received a single 8 trial (30 second inter-trial-interval) training session with a submerged escape platform located in the same quadrant of the maze on all trials. Following trial 8, rats received a post-training subcutaneous injection of estradiol (0.25, 0.5, or 0.75 mg/kg) or saline. On a retention test session 24 hours later, latency to mount the escape platform was used as a measure of memory. The retention test escape latencies of the animals given 0.75 mg/kg of estradiol were significantly lower than those of saline treated rats, indicating an enhancement of spatial memory. Estradiol at doses of 0.25 and 0.5 mg/kg had no effect on retention test latencies. The findings indicate that acute post-training administration of estradiol can enhance spatial memory in male rats.

721.19

WHY DOESN'T DRUG DISCRIMINATION TRAINING LOWER THE DOSAGE AT WHICH DRUG STATES CONTROL RESPONSE OCCURRENCE. D.A. Overton*, G.D. Stanwood, S.R. Pragada, B. Patel, N.M. Bormann and H. Chai. Psychiatry Dept., Temple Medical School, Philadelphia, PA 19140.

Two studies were conducted to determine the lowest dosage of phenobarbital that could (1) maintain discriminative control in a drug discrimination (DD) task, and (2) produce appreciable state dependent learning (SDL). Both studies progressed through successive cycles of training in a 2-lever operant task with a different dosage used in each cycle. Both used Long Evans male rats and injection of phenobarbital (D) or saline (N). The SDL study used the following procedure: (1) Train for 5 sessions while D (or N) to press lever 1; (2) Give one D and one N test session (either lever reinforced); (3) Train for 5 sessions to lever 2 while N (or D); (4) Give two D and two N test sessions; (5) Untrain the rats by reinforcing responses on the drug-inappropriate lever. SDL was noted after D-->N transitions with doses as low as 3 mg/kg. The DD study used conventional D vs N training with FR10 vs extinction schedules of reinforcement on the correct and incorrect levers. Dosage during successive cycles of 18 sessions was selected by a dosage titration paradigm to determine the lowest dosage that could support above-criterion discriminative control. The threshold regularly-discriminated dosage was 3 mg/kg and extrapolation from the accuracy vs. dosage plots indicated an absolute threshold dosage of about 2 mg/kg. Hence SDL generalization failures occur with dosages almost as low as the lowest dosage that can be discriminated after prolonged DD training. The results suggest that "drug cues" may not be processed via mechanisms that allow the rats to "pay attention" to drug effects.

BIOLOGICAL RHYTHMS: LIGHT

722.1

CIRCADIAN CLOCK REGULATES ROD AND CONE INPUT TO GOLDFISH CONE HORIZONTAL CELLS. S.C. Mangel* and Y. Wang. Dept. of Ophthalmology, University of Alabama School of Medicine, Birmingham, AL.

To determine whether a circadian clock regulates the effects of dark adaptation in the retina, goldfish L-type cone horizontal cells (HCs) were studied. Fish were maintained in constant darkness. Surgery was performed under dim red or infrared light. Retinas were superfused in darkness for 90 min ("prolonged darkness"), following which a HC was impaled without the aid of any light flashes. Following prolonged darkness during subjective day, light responses were fast and response amplitudes averaged 17 mV to the brightest stimuli. Spectral sensitivity was similar to that of red (625 nm) cones. During subjective night, light responses were very slow, response amplitudes averaged 5 mV to the brightest stimuli and response duration was 5-6 times longer than stimulus duration. Spectral sensitivity was similar to that of rod HCs. Reversing the light-dark cycle for 10 days reversed the rhythm of dark adaptation. These results indicate that a circadian clock regulates HC light responses such that following dark adaptation, red cone input predominates during the subjective day and rod input predominates during the subjective night.

722.2

LIGHTING CONDITION AS AN ENVIRONMENTAL VARIABLE AFFECTING ETHANOL SELF-SELECTION IN LABORATORY RATS. F. L. W. Goodwin and Z. Amit* Ctr. Stud. Behav. Neurobiology, Concordia Univ., Montreal, Quebec, Canada H3G 1M8.

Wistar rats were exposed to two lighting conditions, constant light or a standard 12/12 hr light/dark cycle. The animals were acclimatized to lighting conditions for 2 wks prior to ethanol (EtOH) acquisition with water and food available ad lib. EtOH was presented in increasing concentrations from 2% (v/v; 95% EtOH with water) to 10% on alternate days in free choice with water. Immediately following the acquisition phase, a maintenance period was initiated which began with everyday presentations of 10% EtOH solution in free choice with water. After 10 days maintenance, lighting conditions were switched such that rats in constant light were exposed to 12/12 light/dark cycle and vice versa. EtOH and water intake were recorded for an additional 10 days. Rats exposed to constant light conditions during EtOH acquisition and maintenance consumed significantly less EtOH during the maintenance period than rats exposed to normal lighting conditions. There were no differences between the two groups in water consumption reflecting some specificity for EtOH intake. When lighting conditions were switched, rats previously exposed to constant light increased their water consumption. In addition, EtOH intake between the two groups was no longer different. Although fluid consumption patterns differed among the groups, both demonstrated undifferentiated weight gain throughout. The present study showed that the environmental variable of lighting can exert a selective influence on EtOH self-selection in rats.

722.3

THE HAMSTER POSTERIOR LIMITANS NUCLEUS (PLi) RECEIVES AFFERENTS FROM RETINA AND INTERGENICULATE LEAFLET (IGL). J. Blanchard* and L.P. Morin. Dept. Psychiatry, Stony Brook Univ., NY 11794.

Hamster NPY- or ENK-IR neurons in the IGL project to the suprachiasmatic nucleus (SCN) by a geniculo-hypothalamic tract (GHT). Cells also project to the contralateral IGL, but few contain NPY- or ENK-IR. A third major IGL-efferent path is now identifiable.

NPY- and ENK-IR fibers extend dorsally from the IGL into the superior thalamic radiation. A thin, vertically oriented terminal field is evident in the PLi along the lateral border of the anterior pretectal nucleus, extending ventrally and laterally along the medial geniculate nucleus. Retrograde studies (cholera toxin β-IR) show IGL cells projecting to the dorsal thalamic and pretectal nuclei. Anterograde transport of Phaseolus vulgaris leuco-agglutinin reveals a strong ipsi- and a weaker contra-lateral, projection to the PLi from the IGL. Anterograde transport of cholera toxin from the eye indicates that the dense NPY-/ENK-IR plexus in the PLi also receives retinal input. The results suggest that the circadian system communicates with an area integrating visual, acoustic and somasthetic stimuli.

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722.4

EFFECTS OF LESIONS OF THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS ON PHOTOPERIODIC PARAMETERS. C.C. Purvis*, M.J. Duncan. Dept. of Anatomy & Neurobiology, Univ. of Kentucky Med. Center, Lexington, KY 40536-0084.

This study of Siberian hamsters, investigated whether lesions of the paraventricular thalamic nucleus (PVT), which contains melatonin binding sites, affect pineal-dependent short photoperiod (SD)-induced responses: testicular regression, loss of body weight, winter molt, and torpor. Adult male hamsters received stereotaxic injections of ibotenic acid (LES, N=11) or saline (CON, N=10) into the anterior PVT. One week later, animals were transferred to SD (10L:14D); after 12 weeks, they were transferred to a cold room (7°C) and were observed daily for the display of torpor. After 60 days, the animals were perfused transcardially with fixative to histologically verify the location of the lesion. Although PVT lesions did not affect molting, they inhibited the expression of the other three photoperiodic responses. Testes weight (mg) was greater in the LES than in the CON hamsters (LES: 139±43 vs CON: 42±3, $p<0.05$); body weight (g) was also higher (LES: 42.1±1.8 vs CON: 32.2±1.9, $p<0.005$). A reduction was seen in the incidence of torpor (LES: 19.3±2.6 vs CON: 43.3±7.0, $p<.005$). The results also showed that the incidence of torpor was correlated with body weight ($p<0.001$). Current studies are further investigating the role of midline thalamic nuclei and body weight in the expression of photoperiodic responses.

722.5

PHASE SHIFTS TO LIGHT AT CT18 ARE INCREASED BY PRETREATMENT WITH ANTISERUM TO NEUROPEPTIDE Y. S.M.Biello, N.Mrosovsky and M.Ralph*. Depts. of Zoology and Psychology, University of Toronto, Toronto, Ont., Canada M5S 2L8

Previous work has indicated that antiserum to neuropeptide Y (aNPY) blocks phase shifts to certain non photic stimuli at CT 4. To insure this effect was specific to non photic phase shifts, and not a general interference with the ability of the clock to phase shift, we administered aNPY just prior to a light pulse at CT 18. Hamsters housed in constant darkness (DD) were implanted with a guide cannula aimed at the suprachiasmatic nucleus. Animals were injected with 200nl of aNPY or 200nl of normal serum (NS) at CT 18 under a dim red safe light. They were then either exposed to light (approximately 200 lux) for 15 minutes or returned to DD. Hamsters shifted significantly more to the light pulse when pretreated with aNPY (mean NS + light = 1.18 minutes; mean aNPY + light = 1.92 minutes; $p < 0.01$ on both two-tailed paired t test and Wilcoxon test). Therefore, antiserum to NPY did not block photic advances and in fact potentiated them. This indicates that the low levels of NPY found in the SCN at this time may be involved in photic phase shifting.

722.6

INTRAVENTRICULAR APPLICATION OF C-FOS and JUN B ANTISENSE-OLIGONUCLEOTIDES BLOCKS LIGHT-INDUCED PHASE SHIFTS OF THE MAMMALIAN CIRCADIAN SYSTEM F. Wollnik*[1], F. Gillardon[2], R. Bravo[3], M. Zimmermann[2], W. Brysch[4], K.H. Schlingensiepen[4], T. Herdegen[2]. [1] University of Konstanz, Germany, [2] University of Heidelberg, Germany, [3] Bristol-Myers Squibb Institute, Princeton, USA, [4] Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

Light-induced phase shifts of the mammalian circadian system result in the expression of c-Jun, JunB, JunD, c-Fos, FosB and Krox-24 transcription factor gene proteins in the ventrolateral subdivision of the suprachiasmatic nucleus (SCN) lasting up to 8 hrs. In order to assess the role of these transcription factors in the control of the circadian system we blocked the expression of c-Fos and JunB proteins by intraventricular application of antisense phosphorothioate oligodeoxynucleotides (ASO; 4mM in 2 μl).

While a light pulse (300 lux, 1 hr) at circadian time (CT) 15 induced a phase shift (-125±15 min) of the circadian rhythm, application of ASO 6 hours before the light pulse blocked the phase shift (-10±16 min). Application of nonsense-(control)-ASO (NSO; 4mM in 2 μl) did not prevent the effect of the light pulse, resulting in a phase shift (-127±20 min) not significantly different from the one induced by light alone. These results demonstrate that transcription factors of the *jun* and *fos* gene families are an essential part of the cellular signal transduction pathway mediating light-induced phase shifts of the circadian pacemaker.

722.7

EFFECTS OF PROTEIN SYNTHESIS INHIBITION ON PRESUMPTIVE OSCILLATOR PROTEINS AND CIRCADIAN PHASE OF THE OCULAR CIRCADIAN PACEMAKER OF BULLA GOULDIANA, Michael H. Roberts* and Nancy A. Krucher, Dept. of Biology, Clarkson University, Potsdam, NY, 13699-5806

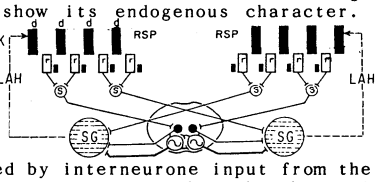
The eye of the marine snail, Bulla gouldiana contains a circadian pacemaker. In previous work we have proposed that members of two protein families, some of whom regulate the eukaryotic cell division cycle, are involved in circadian rhythm generation. Using Western blotting techniques we have identified a protein (p40) possibly related to the cell division kinase, p34cdc2, in the B. gouldiana eye. We have also identified a presumptive 66kDa homolog (p66) of the regulatory protein cyclin.

In the current work we have characterized the phase dependent effects of the protein synthesis inhibitor cycloheximide (CHX 10mM) on both the phase of the circadian rhythm and the levels of p40 and p66. CHX had no effect on the rhythm when given from CT 2-6, while phase delays appeared at CT 6-10 (1.6 ± 0.75 hrs) and increased gradually to 3.0 ± 1.17 hrs from CT 22-2. In preliminary studies, p40 levels are increased by CHX during the day and are decreased at night. In contrast, CHX increases p66 when given from CT 18-22 and decreases p66 at other phases. The effects of CHX on circadian phase as well as on the level of these proteins are consistent with our hypothesis that p40 and p66 may be involved in circadian rhythm generation.

722.8

AFFERENT COMPONENTS IN THE PROTOCEREBRAL (pc) CIRCADIAN SYSTEM OF CRAYFISH. B. Barrera-Mera* and E. Barrera-Calva. Depto de Fisiología Fac. Medicina UNAM. A. Postal 70-250 México 04510 D.F.

The pc system of crayfish (Fig 1) has been studied in isolated preparations "in vivo". Under This conditions a robust circadian (c) rhythm of sustaining response neurons (s), retina (r) and distal (d) retinal shielding pigments (RSP) show its endogenous character. While this activity can occur spontaneously, presumably, in the normal animal, the pc c pacemaker (pm) is modulated by interneurone input from the rest of the nervous system, as shown by the variety of elements in the optic tract. In the pc c system we found that only the c response of r, s and the jittery fibers remain unchanged. They aparently are the afferent input of both left and right pc c pm, which are expressed throughout the synchronous release of neurohemal neurosecretions as light-adapting hormone (LAH) from the sinus gland (sg).



722.9

LYMNAEA STAGNALIS LACKS CIRCADIAN PERIODICITY IN THE SPONTANEOUS ACTIVITY OF ITS OPTIC NERVE. P.M. Casey and T. J. Mueller*. Department of Biology, Harvey Mudd College, Claremont, CA 91711.

The circadian clock of the gastropods *Aplysia* (Jacklett, 1969), *Bulla* (Block & Wallace, 1982), *Bursatella* (Block & Robert, 1981), and *Navanax* (Eskin & Harcombe, 1977) has been found to be located in the eye. Recordings made in complete darkness from each of these organisms' optic nerves showed a marked increase in the rate of spontaneous firing during their preferred activity period.

Like these other gastropods, the freshwater snail *Lymnaea* displays a locomotive circadian rhythm (Tobin & Mueller, SNS 1993). However, recordings made from its optic nerve with a suction electrode showed no evidence of circadian periodicity in over twenty individuals tested.

This is an unusual finding since it differs from the classic *Aplysia* circadian model. However, there is evidence from *Aplysia* (Lickey & Wozniak, 1979) which indicates the presence of secondary circadian clocks outside of the eye. Since *Lymnaea's* visual system appears to behave differently from *Aplysia* and *Bulla*, and its circadian behavior is weaker, it is possible that *Lymnaea's* circadian system may be based on the use of these other clocks.

722.10

CHANGES ON THE CIRCADIAN LOCOMOTOR ACTIVITY IN CRAYFISH PROCAMBARUS UPON RED LIGHT STIMULUS. V Inclán-Rubio*, R. Valladares-Bejar and A. Merlín-Pérez. Departamento de Fisiología. Facultad de Medicina, UNAM. AP. 70250, CP. 04510, México, D.F. México. The crayfish Procambarus has a circadian rhythm in locomotor activity that shows a free running period (t) in darkness (23.4h) and lightness (26.2h) constant conditions, a shifting phase response to white light pulses and t modifications by photoperiodic changes. In other circadian rhythms, the application of red light promotes important changes in acrophase and amplitude. The present study pursues to know the effect evoked by red light (623 nm) on the locomotor activity. We used adult Procambarus bouvieri. Each crayfish was placed within an automatic monitor locomotor activity, and exposed to (LD:12:12) cycle, with white, red or white and red light illumination, during at least 24 days. The temperature was alwaysthe same (19°C). The results obtained by radiogram analysis of the animals activity show: 1) A clear 24h period in LD cycle. 2) An important reduction in total amplitude when red light is present in the photoperiod. 3) The red light effect disappears between the 4th or 5th day when the amplitude of locomotor activity increases again. 4) No difference in the acrophase value between white and red light stimulus was noted.

BIOLOGICAL RHYTHMS: MODELS

723.1

USE OF LED TECHNOLOGY IN PROVIDING CONTROLLED LIGHTING FOR ANIMAL RESEARCH. M. Drews, I-H Tang, T. Tandon, and C.A. Fuller*. Section of Neurobiology, Physiology & Behavior, University of California, Davis, California 95616-8519.

The light environment is critical for animal research, including both basic husbandry and experimentation. In order to study how animals respond to different aspects of light, we utilized the characteristics of light emitting diodes (LED). The LED technology offers several advantages, including: 1) they are solid state devices, 2) they provide narrow spectral band output near their peak wavelength, 3) they are available in several discrete wavelengths, 4) they demonstrate negligible shift in spectral distribution at different intensities, 5) they have a high degree of stability and longevity, 6) they produce virtually no heat, 7) they are small and have minimal power requirements, and 8) their output intensity can be controlled linearly and dynamically. We have developed an autonomous computer based controller to provide variable profile light cycles, including: 1) rate of light intensity change, 2) range of minimal and maximal intensity, and 3) duration. The output intensity profile can be monitored in real time. We have examined the photic entrainment of hamsters in a simulated natural light-dark cycle using this device. This device can also be applied to study the photic entrainment thresholds of a defined spectrum. We have also studied the entrainment behavior of a small primate, the squirrel monkey, using the narrow spectral qualities of the LED light source. (Supported by NASA Grant NAG2-792.)

723.2

PHOTIC ENTRAINMENT BY SIMULATED NATURAL LIGHT-DARK CYCLES. I-H Tang, D.M. Murakami*, C.A. Fuller. Section of Neurobiology, Physiology & Behavior, University of California, Davis, California 95616-8519.

This study compared the entrainment characteristics of simulated natural light-dark (LD) cycles and square wave LD cycles (14:10) in golden hamsters. The simulated natural LD cycle was composed of a 4 hr linearly ramped dawn-mimicking period, a 6 hr full light intensity day period and a 4 hr linearly ramped dusk-mimicking period. Animals were implanted with biotelemetry transmitters to record body temperature and activity at 10 min intervals via microcomputer. The phase angle at which animals entrained was determined by onset times of the animals' ambulatory activity. Animals under simulated natural LD cycles exhibited advanced phases relative to square wave LD cycles. An additional study was performed to examine the role of bilateral vs. unilateral vision on the entrainment responses. Animals that received unilateral optic nerve transection exhibited a further advance of activity onset. In all conditions, there was a stable phase angle between body temperature and ambulatory activity under simulated natural LD cycles, suggesting anticipatory behavior. Preliminary data also demonstrated that animals with a unilateral optic nerve transection showed different c-Fos expression compared to intact animals in response to identical night time light pulses.

723.3

A MONTE CARLO TECHNIQUE FOR CHARACTERIZING EPOCHS OF ELECTROCORTICAL ACTIVITY IN THE FETAL SHEEP. S. R. Robinson[1]*, W. P. Smotherman[1], C. H. Wong[1], S. S. Robertson[2], and P. W. Nathanielsz[3]. [1]Laboratory of Perinatal Neuroethology, Dept. Psychology, Binghamton Univ., Binghamton, NY 13902; [2]Dept. Human Development and Family Studies, Cornell Univ.; [3]Laboratory of Pregnancy and Newborn Research, College of Veterinary Medicine, Cornell Univ., Ithaca NY 14853.

Electrocortical activity (ECoG) recorded from fetal sheep is a measure of sleep-wake state that differentiates into epochs of low voltage and high voltage late in gestation (term = 145 days). Five fetal sheep were surgically prepared at 113-114 days of gestation with an array of chronic instrumentation, including stainless steel electrodes overlying the dura of the parietal lobes to measure fetal electrocortical activity. ECoG signals were sampled at 32 Hz, amplified, full-wave rectified, averaged over 1-s intervals, and recorded continuously over days 121-133. Brief epochs of low- and high-voltage ECoG were distinguished on day 121, with durations that persisted significantly longer than predicted by Monte Carlo reordering of ECoG time series. Average ECoG voltage increased with age, and duration of low- and high-voltage ECoG epochs also increased, with the most rapid development evident about days 129-131. The developmental increase in duration appeared to be due to the selective elimination of brief (< 5-s) transitions in ECoG activity that interrupted epochs of longer duration. These results suggest an objective, quantitative strategy for characterizing epochs of low- and high-voltage electrocortical activity that may be relevant for understanding the prenatal ontogeny of behavioral states.

This research was supported by a subcontract with Cornell University to WPS and SRR under NIH Grant HD 28014.

723.4

EVIDENCE THAT MAMMALIAN CIRCADIAN RHYTHM IS COMPOSED OF ULTRADIAN SUBCYCLES; SIMILARITIES BETWEEN CALCIUM OSCILLATION IN SUPRACHIASMATIC NUCLEUS CELLS TO THE MAMMALIAN CIRCADIAN PACEMAKER. Shin Yamazaki*[1], Shin-Ichi T. Inouye[2] and Yoichiro Kuroda.[1]

[1]*Department of Molecular and Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu-shi, Tokyo 183, Japan;* [2]*Laboratory of Integrative Brain Function, Mitsubishi Kasei Institute of Life Sciences, 11 Minami-Ooya, Machida-shi, Tokyo 194, Japan.*

A central question in the elucidation of the mechanism of circadian rhythm generation in the suprachiasmatic nucleus (SCN) is whether individual cells have an intrinsic capacity to generate circadian rhythms or the rhythmicity requires the interaction of cells. Like cortical neurons in culture (Kuroda et al. Neurosci. Lett. 135, 255, 1992), dissociated cultures of rat SCN show spontaneous oscillations of intra-cellular Ca^{2+} with 5-8 second period (ultradian rhythm). Replacement of H_2O by deuterium oxide (D_2O) in the culture medium lengthened the period of this oscillation, while tetrodotoxin did not inhibit the deuterium oxide sensitive Ca^{2+} oscillation. Since these characteristics resemble those of the mammalian circadian pacemaker, the ultradian Ca^{2+} rhythm may be involved in the circadian rhythm or have a similar rhythm generation mechanism. The gap junction inhibitor, halothane, stopped this oscillation. Gap junctions may be necessary in order to generate rhythmic oscillations.

723.5

THETA EEG GENERATOR IN RAT NEOCORTICAL BRAIN SLICES. H. S. Lukatch* and M. B. MacIver. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

The present study describes a novel neocortical EEG theta generator in rat brain slices. Theta EEG frequencies (7.31 +/- .94; n=19) were evoked and maintained *in vitro* by mimicking ascending cholinergic and GABAergic tone with carbachol (100 μM) and bicuculline (10 μM). Mapping studies revealed that this theta activity was limited to a strip of medial cortex running rostral caudally (partially overlapping cortical areas Oc2MM, RSA, Fr1, and Fr2). The presence of a theta generator intrinsic to cortical areas Oc2MM and RSA was confirmed by isolating mini-slices of these areas and inducing theta EEG activity in these mini-slices. Two electrode differential recordings were used to determine that theta generating cells were localized to layer 5. This theta activity was blocked by the muscarinic receptor antagonist atropine sulfate (0.5 μM), similar to *in vivo* cholinergically driven theta. Preliminary loose patch single unit recordings revealed two cell types which exhibited different firing patterns in relation to the theta oscillations. One cell type fired only at the beginning of each theta burst and remained silent during the theta train. The other cell type was quiet at the beginning of each theta train, and attained its highest rate of discharge during periods of sustained theta activity.
Supported by USAF OSR/SCEEE Graduate Fellowship.

723.6

PREDICTABILITY OF BIOLOGICAL RHYTHMS? K.-D. Kniffki*, C. Braun, A. Klusch and P. Tran-Gia‡ Physiologisches Institut and Institut für Informatik, Universität Würzburg, Röntgenring 9 and am Hubland‡, D-97070 Würzburg.

Temporal fluctuations which cannot be explained as consequences of statistically independent random events are found in a variety of physical and biological systems. The fluctuations of these systems can be characterized by the power spectrum density $S(f)$ decaying as f^{-b} at low frequencies with an exponent $0.5 \leq b \leq 1.5$. We present a new approach to describe the individual biological rhythms of humans using data from a colleague who has kept daily records for four years of his state of general well-being applying a fifty-point magnitude category rating scale. This time series $\{R(t_i)\}$ was described as a point process by introducing discriminating rating levels r and s for the occurrence of $R(t_i) \geq r$ ('ups') and $R(t_i) \leq s$ ('downs'). $\{R(t_i)\}$ after introducing a certain r or s is described as $y(t) = \sum \delta(t - t_i)$, whereby $\delta(t - t_i)$ represents Dirac's delta function, and $t_{i,j}$ the time of a particular value $R(t_i) \geq r$ or $R(t_j) \leq s$. Applying counting statistics (Kniffki et al.,Fractals 1:380,1993) to evaluate $S(f)$ two scaling regions were found: first scaling region $1d \leq \Delta t \leq 15d$ for the 'ups' and 'downs' where $S(f) \propto f^{-b}$ with $b \approx 0.7$; second scaling region $\Delta t \geq 15d$ exhibiting an almost random behavior with $b \approx 0.00$.

We speculate that the basic mechanisms of the neuronal/humoral systems responsible for biological rhythms are expressions of a 'self-organized critical state' as introduced by Bak, Tang and Wiesenfeld (Phys.Rev.Lett.59:381,1987) for physical systems. Because of the first scaling region and based on one's own monitored biological rhythm it should be possible to predict future episodes ($\Delta t \leq 15d$) with a certain probability by using methods of nonlinear time series analysis or modified feed-forward neural networks learning with backpropagation algorithm.

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STRESS: NEUROCHEMISTRY AND BEHAVIOR II

724.1

NEONATAL ISOLATION ENHANCES LTP AND RESPONSES TO AMPHETAMINE CHALLENGE IN JUVENILE RATS. J. Hoffman, A. Arabshahi, R.J. Austin-LaFrance, J.D. Bronzino and P. Kehoe*. Neuroscience Program, Trinity College, Hartford, CT 06106.

Using a within-litter design, pups were isolated from mother and littermates for 1 hr. per day over PN days 2-9. At 30 days of age, the response of the dentate granule cell population to tetanization of the perforant pathway was examined in previously isolated and non-isolated littermates. Measures of population spike amplitude (PSA) and population EPSP slope obtained at several times following tetanization were used to assess the magnitude and duration of LTP obtained from each group. Preliminary results indicate that the degree of enhancement to both EPSP slope and PSA measures obtained from neonatally isolated animals was markedly greater than that of controls across the 24 hr. recording period. In a separate group of littermates, striatal dopamine response to amphetamine (7.5mg/kg) challenge was assessed on PN day 27 by *in vivo* microdialysis. In comaparison to controls, previously isolated animals showed significantly higher dopamine levels over the first 1hr. following amphetamine administration. Behaviorally, these animals demonstrated a significantly enhanced amphetamine hyperactivity. In summary, neonatal isolation significantly enhances LTP and amphetamine-induced behavioral and dopaminergic responsivity. The results suggest that the impact of neonatal isolation on response properties of both the hippocampus and striatum endures into the juvenile period of development and can be revealed by both electrophysiological and pharmacological challenges administered well after cessation of the stress paradigm.

724.2

EFFECT OF CHRONIC STRESS AND ANTIDEPRESSANT TREATMENT ON PHOSPHORYLATION OF MICROTUBULE-ASSOCIATED PROTEIN IN RAT BRAIN

Y. Sasuga, T. Higuchi*, M. Asakura, S. Miyamoto, J. Imafuku, K. Osada, T. Tsukamoto, Y. Gamo and A. Aoba. Dept. of Neuropsychiatry, St. Marianna Univ. Sch. of Med., Kawasaki, 216 Japan

We examined the effect of long-term treatment with antidepressant drugs on the tubulin polymerization in rat cerebral cortex, and also compared effects of acute and chronic stress (sigle and variable) on tubulin assembly. The tubulin was prepared in buffer containing protein phosphatase inhibitors, calyculin A and sodium orthovanadate. The polymerization of microtubule was monitored in turbidity at 345 nm by a spectrophotometer. Treatment with desipramine, mianserin or citalopram for 7 days decreased the initial rate of tubulin assembly while a single injection of desipramine increased the rate. However, the tubulin preparation without phosphatase inhibitors nullified the inhibiton of tubulin assembly following long-term desipramine treatment, suggesting that the inhibition of tubulin assembly is attributed to the phosphorylated microtubule-associated proteins (MAPs; MAP2 and/or tau factor). On the other hand, A single restraint stress increased the assembly rate, and the increase in the rate was recovered after chronic restraint stress for 7 days. In contrast, after chronic variable stress (cold-swimming, restraint, tail-pinch, shaking) for 7 days the increase in the rate was not recovered. It is thus proposed that the inhibition of tubulin assembly by the phosphorylation of MAPs may be an adaptive change to a novel stressor and this mechanism might be enhanced by long-term antidepressant treatment.

724.3

EFFECTS OF NEONATAL ISOLATION ON BEHAVIOR FOLLOWING AMPHETAMINE. C. Arons*[1] P. Kehoe[2] and W. Shoemaker[1]. [1]Dept. of Psychiatry, UCONN Health Center, Farmington, CT 06030, [2]Dept. of Psychology, Trinity College, Hartford, CT 06106.

As part of a study on the ontogeny of stress-induced behavioral sensitization we examined the proximal effects of neonatal isolation on locomotor activity and vocalizations following administration of amphetamine. One half of each litter was marked weighed and subjected to a daily one hour isolation from postnatal days (PND) 2-9. The other half of the litter was marked weighed and immediately returned to the nest and dam. Additional litters served as non-handled controls. Pups from these litters remained with their dams undisturbed through PND 9. On PND 10 all pups were tested. Pups from each group were randomly divided into one of three challenge groups, saline 0.5 mg/kg amphetamine (AMPH) and 2.0 mg/kg AMPH. Activity measures were recorded for a five minute period beginning 15 minutes after drug administration. Ultrasonic vocalizations were recorded during this time with a bat detector. Females that were isolated and received 0.5 mg/kg AMPH showed higher levels of activity than the two control groups, significantly so in the case of patterned movements. These effects of isolation were not apparent in females tested with saline or 2.0mg/kg AMPH. Males that were isolated and tested with 0.5 mg/kg AMPH showed an opposite trend, they had lower activity scores than control males. Both doses of AMPH reduced vocalizations compared to saline. Among saline animals there was a clear effect of handling on the pattern of vocalizations over the five minute test period. There was a slight but insignificant trend for isolated animals to vocalize less than non-isolates, particularly with 0.5 mg/kg AMPH. These results suggest that neonatal isolation alters behavior following the administration of a low dose of amphetamine, at least when pups are tested 24 hour after the final isolation period. In addition, females seem to be effected to a greater degree than males, with regards to activity measures.

724.4

Stimulation of macrophages reduces cholesterol levels in stressed and unstressed rats. **F. X. Brennan*, M. Fleshner, L. R Watkins, & S. F. Maier. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.**

Stress can increase levels of cholesterol in plasma. Cholesterol is cleared from plasma primarily by high affinity cell-surface receptors. However, there is a secondary mechanism involving macrophages, a phagocytic immune cell. Thus, the effect of macrophage stimulation was examined as a potential mechanism for attenuating stress-induced increases in cholesterol. Rats (12 per group) were exposed to three sessions of inescapable tailshock (100 1.6 mA shocks, one session per day for three successive days), or remained in their home cage. Half of each group were injected with zymosan (30 mg/kg, s.c.), a drug known to stimulate macrophage function *in vivo*. Animals received one zymosan injection for each of the three days prior to, as well as 15 min prior to each stress session. Control animals received vehicle injections. Results indicated that both stressed and control animals receiving zymosan had lower total plasma cholesterol levels than their saline-treated controls. Zymosan also blocked stress-induced decreases in spleen weight, and exacerbated stress-induced decreases in thymus weight. These data suggest that macrophage suppression is not the primary mechanism for stress-induced increases in cholesterol. However, stimulation of macrophages may prove to be be a prophylactic manipulation in attenuating the cholesterol increases seen after stressor exposure.
NIH-MH45045

724.5

STRAIN-SPECIFIC DEFICITS OF MORRIS WATER-MAZE PERFORMANCE: EFFECTS OF NEONATAL HANDLING. M.D. Zaharia[1], N. Shanks[2], M. J. Meaney[3], and H. Anisman[1]*. [1]Dept. of Psychology, Carleton Univ., Ottawa, Ontario, K1S 5B6, [2]Clinical Immunopathology, Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA. 15213, [3]Douglas Hosp. Res. Ctr., Montreal, Que.

BALB/cByJ mice are highly reactive to stressors relative to other strains (e.g., C57BL/6ByJ), exhibiting hyper-corticosterone secretion in response to stressors, as well as particularly marked brain catecholamine alterations. Behaviorally, BALB/cByJ mice exhibit a wide variety of behavioral disturbances after stressors that are more pronounced than in other strains. Interestingly, BALB/cByJ mice fail to acquire a spatial learning response in a Morris water-maze paradigm. In view of the particularly marked stress reactivity of the BALB/cByJ mouse, it was of interest to determine if the spatial learning deficit in this strain could be modified by neonatal stimulation, just as such a manipulation has been found to dampen stress responses in mature animals. We observed that the performance deficit, which is not evident when visual intramaze cues are present, is apparent as early as 30 days of age. Moreover, using a cross fostering paradigm it was determined that the deficits were not related to maternal factors. If pups were handled (i.e., separated from the Dam for brief periods over the course of the initial 21 days postpartum) the behavioral deficits were prevented. The data were related to central neurochemical and endocrine changes induced by stressors and the modification of such effects by neonatal handling.

724.6

SPECIFIC CHANGES IN AMPA RECEPTORS ASSOCIATED WITH BEHAVIORAL STRESS. S. Standley, J.J. Kim, G. I. Wong, M. Baudry*, and R. F. Thompson. Neurosciences Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520.

Behavioral stress has effects on LTP and learning and memory. Autoradiographic studies of ligand binding to the AMPA/GluR receptor have also shown that the binding characteristics of the receptors are rapidly altered in the hippocampus and other brain structures following stress. While these studies reported a general increase in ^{3}H-AMPA binding in stressed animals, the nature (Bmax, or Kd), and type (high affinity or low affinity) of receptor change have not been reported in detail. To evaluate the nature of the changes in AMPA receptors, we performed equilibrium saturation kinetic studies with ^{3}H-AMPA on synaptic fractions (P2) prepared from telencephalon obtained from both acutely stressed (60 tail shocks: 1 mA, 30-90 s interval) and control Long Evans rats. As previously reported, saturation kinetic data were better fitted to a two site than a one site model, indicating the existence of a small population of high affinity sites (Kd $\cong$ 20 nM) and a large number of low affinity sites (Kd $\cong$ 600 nM). Stressed animals exhibited a significant increase in the maximal number of low affinity binding sites without significant changes in high affinity sites. As the low affinity receptors have been postulated to be synaptic receptors, whereas the high affinity receptors are enriched in nonsynaptic subcellular fractions, the results suggest that stress might alter some steps involved in receptor insertion or internalization. The results might also account for the impairment of LTP associated with stress, inasmuch as LTP shares the same cellular mechanisms of expression via AMPA receptor regulation. Supported by grants from NRSA 1F32MN10521-01 BNR to JJK, NSF 91103787 to MB, and NSF BNS-8718300 & NIH (NIA) AG05142 to RFT.

724.7

BEHAVIORAL STRESS ENHANCES LTD IN RAT HIPPOCAMPUS. J. J. Kim*[1], M. R. Foy[2] and R. F. Thompson[1], [1]Neurosciences Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520 and [2]Psychology Dept., Loyola Marymount Univ., Los Angeles, CA 90045-2669.

Uncontrollable and unpredictable stress is known to impair certain learning tasks, and also to impair hippocampal long-term potentiation (LTP). In the present study, we examined the effect of stress on another form of hippocampal synaptic plasticity, long-term depression (LTD). Hippocampal slices were prepared from adult Long-Evans male rats immediately following exposure to behavioral stress (60 tailshocks: 1 mA, 1-s, 30-90 s apart; animals immobilized). Field EPSP recordings (amplitude and slope) from dendrites in area CA1 following Schaffer/commissural stimulation (900 pulses at 1 Hz) indicate that LTD was significantly greater in stressed animals than that recorded from control animals. These results argue that an additional form of hippocampal plasticity can be modified by behavioral stress.

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724.8

RECURRENT BRAIN POLYAMINE RESPONSE AFTER REPEATED STRESS AND ITS ENHANCEMENT AFTER AN ADDITIONAL DELAYED STRESS EPISODE. V.H. Gilad* and G.M. Gilad. Faculty. of Med., Technion-Israel Inst. of Technology., Haifa, Israel.

Rapid changes in brain polyamine (PA) metabolism, termed the PA response, are generally induced with a magnitude proportional to the stressor intensity. The effects of repeated stress episodes on the PA response are unknown. Therefore, we examined effects of repeated restraint, a relatively mild stressor, on ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAM-DC), two key PA synthesizing enzymes. Rats were subjected to 2 h restraint once daily for 5 days, and enzyme activities assayed in the hippocampus 6 h after the beginning of each stress episode. We found that ODC activity was increased repeatedly after each stress episode to about 130% of control. In contrast, SAM-DC activity was reduced after the first episode (86% of control), but remained unchanged thereafter. Additional stress application, 7 days after the last (5th) episode, resulted in a much larger ODC increase (180% of control), without a change in SAM-DC activity. We conclude that: 1) even mild stress can induce a characteristic PA response in the brain; 2) the ODC response is induced after each episode of stress, and 3) additional stress episode applied days after cessation of the initial stressful stimuli, results in a much greater increase in ODC activity. The study implicates an overactive PA response in the (mal)adaptive response to stressful events.

724.9

CHANGES IN BOMBESIN (BN) LEVELS AND ITS RECEPTORS IN THE RAT BRAIN FOLLOWING ACUTE IMMOBILIZATION STRESS P. Kent[1,*], Z. Merali[1,2], and H. Anisman[3], [1]Psychol & [2]Pharmacol, Univ of Ottawa, [3]Carleton University, Ottawa, Ontario, Canada.

The neurochemical mechanisms underlying the coincident activation of the pituitary-adrenal axis and the sympathetic nervous system in response to stress remain unclear. Central injection of the neuropeptide bombesin (BN), potently releases epinephrine from the adrenal medulla and adrenocorticotropic hormone (ACTH) from the pituitary, and elicits behaviors typically associated with increased emotionality and arousal. The objective of the current study was to determine whether stress is associated with changes in the endogenous levels of BN-like peptides and/or their receptors. Male Sprague-Dawley rats were subjected to acute immobilization stress for 0 (control), 30 min or 120 min. Plasma ACTH levels increased in response to stress, peaking at 30 min. BN-like immunoreactivity increased significantly at the hypothalamus and medulla, within 30 min, and declined to control levels by 120 min. The peptide levels in several other regions, including the hippocampus, striatum, midbrain, pituitary, and pons, failed to change significantly. Autoradiographic analysis of BN/GRP receptor densities for 8 regions examined are listed in ascending order; hippocampus, nucleus tractus solitarius (NTS), paraventricular (PVN), arcuate (Arc) and periventricular hypothalamic nuclei, paraventricular thalamic nucleus, central amygdala and nucleus accumbens. Significant increases in binding were found after 30 and 120 min of stress in the NTS, and after 120 min in the PVN and Arc. These data indicate that BN-like peptides may serve to mediate and/or integrate responses to stress.

724.10

THE PROTECTIVE EFFECTS OF STRESS CONTROL MAY BE MEDIATED BY INCREASED BRAIN LEVELS OF BENZODIAZEPINE RECEPTOR AGONISTS. R.C. Drugan*, A.S. Basile, J.H. Ha and R.J. Ferland. Department of Psychology, Brown University, Providence, RI 02912 and Laboratory of Neuroscience, NIDDK, NIH, Bethesda, MD 20982.

Control over stress protects against many of the deleterious effects of stress exposure, but the endogenous mediators responsible for these prophylactic effects have remained elusive. Using behavioral pharmacology, in vitro radioligand binding and neurochemical analyses, we demonstrate that exposure to escapable stress results in brain and behavior changes reminiscent of benzodiazepine administration. The stress control group shows significant protection against picrotoxinin-induced seizures, reductions in [35s] t-butylbicyclophosphorothionate binding at or near the chloride channel and a 3-fold increase of benzodiazepine-like agonists in brain comparison to both yoked-inescapable shock and non-shock controls. These observations suggest that active coping behavior leads to the release of endogenous benzodiazepine-like agonists that protect the organism from stress pathology. Research supported by PHS grant MH 45475 to RCD.

724.11

ALCOHOL AVAILABILITY AND AGGRESSION LEVEL AFFECT BEHAVIOR IN THE OPEN FIELD AND PLUS MAZE, AND 5-HT RECEPTORS IN TRIAD-HOUSED RATS. L.A. POHORECKY*, X. HUANG, S. LARSON, C. QUINN, D. BENJAMIN. Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

Individually- and triad-housed male Long Evans rats were tested in the open field and elevated plus maze. Subjects were 18 triads and 14 single caged rats, half of which had a choice of drinking a 6% solution of ethanol (ET) or water. Dominance status was determined by monitoring behavior during the initial 30 minutes of colony formation. Initially, the number of attacks by the Alpha (α) on the Beta (ß) and Gamma (τ) was observed daily to classify triads by aggression level. Body weight loss 1 day after group housing was similar for ß and τ but minimal for α, and singles gained. Less weight gain was observed in high aggression (HT) than in middle aggression (MT) or low aggression triads (LT). Weight gain of α was not affected by aggression level. Only the ß were affected by ET availability, with lower weight gain overall. Headpokes were affected by level of aggression; in LT headpokes were less frequent, and of shortest total duration, but in MT, α showed the lowest duration and frequency. In the LT, ET increased headpoke duration and frequency. In triads with ET available, α had significantly lower headpoke frequency. In the MT, ET decreased grooming, but had no effect in LT. In triads with ET available, the MT showed significantly less grooming than MT or LT. In the ß, availability of ET increased center time significantly. The availability of ET produced anxiolytic effects in singly housed, but not in triad-housed rats. 5-HT_{2A} receptor binding was significantly affected by aggression level; whereas HT showed rank-related increases above control levels in ß and τ, LT showed rank-related decreases in ß and τ. Effects on 5-HT_{2C} binding were also observed. These results demonstrate that aggression levels vary between triads and interact with rank and ET to produce complex effects on 5-HT receptor binding and behavior (Supported by NIAAA 05306).

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: OTHER II

725.1

TEMPORAL REGULATION OF PREPROENKEPHALIN-A (PPE-A) mRNA EXPRESSION BY ESTROGEN IN THE POSTERIOR DORSAL MEDIAL AMYGDALA (MeApd) OF THE FEMALE RAT. C. B. Eckersell*, C. A. Priest and P. E Micevych. Dept. Anatomy and Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

Gonadal steroids modulate reproductive behavior through their effects on intercellular signaling in the limbic-hypothalamic neural circuit. Within this circuit, the up-regulation of PPE-A mRNA (encoding met-enkephalin) by estrogen, in the ventromedial nucleus of the hypothalamus, has been correlated with reproductive behavior in the female rat. The MeApd also is an integral part of the limbic-hypothalamic neural circuit that plays an important role in opioid regulation of female reproductive behavior. To examine the temporal effects of acute estrogen exposure on PPE-A mRNA levels in the MeApd, adult ovariectomized Long-Evans rats were injected with 50 μg estradiol benzoate (EB), perfused at one of nine successive time points from 0 to 72 hours after injection and used for quantitative *in situ* hybridization histochemistry of PPE-A mRNA. PPE-A mRNA was localized to neurons within the MeApd using an ^{35}S-labelled, single-stranded, cRNA probe complementary to the entire coding sequence of the PPE-A mRNA (gift from Drs. Yoshikawa and Sabol, NIH, Bethesda, MD). EB treatment induced a biphasic increase in the number of PPE-A mRNA expressing cells in the MeApd with maximal levels occurring at 1 and 24 hours post treatment. These peaks were interrupted by a dramatic decrease in the number of PPE-A mRNA expressing cells at 4 hours after EB injection. By 72 hours post-EB, PPE-A expressing cell numbers returned to basal levels. The biphasic increase in PPE-A mRNA expressing cell numbers with acute estrogen treatment supports the idea that there are two temporal and perhaps mechanistic regulatory events for PPE-A transcription within the MeApd. Supported by NS 21220.

725.2

PROGESTERONE ENHANCES AN ESTRADIOL-INDUCED INCREASE OF FOS-IMMUNOREACTIVITY IN LOCALIZED REGIONS OF FEMALE RAT FOREBRAIN. A.P. Auger and J.D. Blaustein.* Neurosci. & Behav. Program, Univ. of Massachusetts, Amherst, MA 01003.

In female rats, the onset of reproductive behavior depends on the sequential presence of estradiol followed by progesterone. Although treatment with high doses of estradiol has been shown to increase immunostaining for the Fos protein, an immediate early gene product that is expressed upon cellular activation, another report conflicts with this finding. However, the previous reports agree that subsequent treatment with progesterone has no apparent effect on Fos expression. In order to resolve this discrepancy and investigate possible effects of progesterone, we used Fos-immunocytochemistry combined with computer-aided image analysis. In experiment one, we found that treatment with 5 µg of estradiol increased Fos-immunoreactivity (Fos-IR) within the medial preoptic area and the dorsal medial hypothalamus. Subsequent treatment with 500 µg of progesterone one hour before perfusion increased the intensity of the immunostaining within the medial preoptic area and the dorsal medial hypothalamus, although it had no significant effect on Fos-IR cell number. In experiment two, a lower concentration of Fos antiserum was used in order to diminish the immunostaining sensitivity to a level in which no increase of Fos-IR cell number was observed after treatment with estradiol. Under these immunocytochemical conditions, subsequent treatment with progesterone increased the number of Fos-IR cells in the medial preoptic area, the dorsal medial hypothalamus and the steroid receptor-rich area lateral to the ventromedial hypothalamus. Thus, treatment with behaviorally-effective doses of both estradiol and progesterone induces Fos expression in localized regions of female rat brain.
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725.3

PROGESTERONE DECREASES EXTRACELLULAR SEROTONIN IN THE VENTROMEDIAL HYPOTHALAMUS AND MIDBRAIN CENTRAL GREY IN ESTROGEN-PRIMED OVARIECTOMIZED RATS. C.J. Farmer, T.R. Isakson and K.J. Renner*. Dept. Biomedical Sciences, Southwest Missouri State Univ., Springfield MO 65804.

Progesterone (P) effects on extracellular (EC) 5HT were monitored in the ventromedial hypothalamus (VMH), midbrain central grey (MCG) or medial preoptic area (mPOA) using microdialysis. Rats primed with estradiol benzoate (5 μg) were anesthetized with chloral hydrate (400 mg/kg, i.p.). Samples were collected at 20 min intervals. Once a stable 5HT baseline was obtained, rats were injected (s.c.) with 0.5 mg P or vehicle (V). In the mPOA, EC 5HT levels were not affected by P. Significant decreases in EC 5HT levels were present in the MCG and VMH 40 and 60 min after P, respectively. In the VMH, EC 5HT decreased to 57 $\pm$ 5.9 % of pretreatment values 100 min after P. 5HT levels in the MCG decreased to 56 $\pm$ 5.1 % of pretreatment values 80 min after P. The decreases in EC 5HT in the VMH and MCG persisted during the remainder of the sampling period. 5HT baseline levels were stable in the respective V-treated groups. These results demonstrate that P-influenced decreases in 5HT release in the MCG and VMH occur in vivo and support previous studies which suggest that decreases in 5HT activity in the VMH and MCG contribute to the facilitation of female receptivity. (supported by IBN-9309451)

725.4

SUBCELLULAR LOCALIZATION AND KINETIC PROPERTIES OF AROMATASE IN THE RAT BRAIN. C.E. Roselli* Department of Physiology, Oregon Health Sciences University, Portland, OR. 97201.

The conversion of testosterone (T) to estradiol is catalyzed by cytochrome P450 aromatase. *In situ* aromatization is required for the full expression of the effects of T in the brain. This study examined the subcellular distribution and reaction kinetics of aromatase in the adult rat brain. Preoptic area (POA), hypothalamus (HYP) and amygdala (AMYG) were homogenized in isotonic sucrose buffered with potassium phosphate. Homogenates were fractionated by a sequential series of centrifugations to obtain: 800 x g pellet (nuclei); 11,000 x g pellet (mitochondria and synaptosomes); 100,000 x g pellet (microsomes); and 100,000 x g supernatant (cytosol). Aromatase activity (AA) was measured using a previously validated ^3H_2O assay. Marker enzymes were measured to identify organelles in the different subcellular fractions. We found that AA in all 3 tissues was enriched 8-14 fold in microsomes, but not in other cell subfractions. The addition of either a NADPH-generating system or 1 mM NADPH to the reaction mixture stimulated AA in all subfractions, whereas NADH was only minimally effective. Affinity constants were equivalent in all subfractions ($\sim$10 nM) suggesting that only one form of the enzyme is present in the rat brain. One week after castration, AA was significantly reduced in all cell subfractions of POA and in the homogenate and microsomes of HYP. Castration did not significantly alter AA in any subfraction of AMYG. To more critically evaluate its subcellular localization, AA was measured in purified synaptosomes. AA was not enriched in these preparations suggesting that aromatase is not substantially associated with nerve terminals in rat brain.

725.5

HYPOTHALAMIC δ OPIOID RECEPTORS IN MALE JAPANESE QUAIL (*Coturnix c. japonica*). P. Deviche*[(1)], C.C. Gulledge[(1)], N. Thompson[(2)], and M.A. Ottinger[(2)] [(1)]Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775; [(2)]Dept. Poult. Sci., Univ. Maryland, College Pk, MD 20742.

Central opioids regulate mammalian reproductive behavior and physiology, but little information on this is available for nonmammalian species. In Japanese quail (*Coturnix c. japonica*), administration of the preferential δ receptor agonist enkephalin (ENK) dose-relatedly decreases the *in vitro* hypothalamic release of gonadotropin-releasing hormone (GnRH), and hypothalamic regions of the avian brain (dark-eyed junco, *Junco hyemalis*; pigeon, *Columba livia*) contain δ receptors. The present study used quantitative *in vitro* autoradiography to measure specific pCl-DPDPE binding site densities in hypothalamic (paraventricular n.; medial preoptic area) and extrahypothalamic (medial and lateral septum; neostriatum) regions of sexually active and inactive young, middle-aged, and old male quail. High densities of δ opioid receptors were found in hypothalamic regions, suggesting a local action of endogenous opioid peptides. Receptor densities did not differ as a function of sexual activity level or age. These receptors are localized in areas important in GnRH regulation and are likely to be involved in ENK modulation of GnRH release. Supported by NSF Award BNS-9121258 to P.D. and USDA # 88-37242-2913 and 92-37203-7742 to M.A.O.

725.6

EVOLUTION OF THE GNRH FAMILY OF PEPTIDES INFERRED FROM CDNA SEQUENCES. R.C. Francis* and R.D. Fernald, Department of Psychology, Stanford University, Stanford, CA.

The decapeptide GnRH is an important regulator of vertebrate reproductive function and has been highly conserved during the course of vertebrate evolution. Most, and perhaps all, vertebrates express at least two forms of GnRH. Chicken-II GnrH (cGnRH-II) is the most ubiquitous form, and has been identified in every major vertebrate taxon. Previously it was thought that eutherian mammals do not express cGnRH-II but the gene encoding this form has now been cloned and sequenced in members of this taxon as well. While it has potent gonadotropin releasing action *in vitro*, cGnRH-IIir neurons are generally confined to midbrain regions, and with the possible exception of elasmobranchs, are not thought to function in gonadotropin release *in vivo*. In this study we used a variety of evolutionary distance measures and tree-building techniques to analyze amino acid and cDNA sequences, both to explore the evolutionary history of this family of peptides and their functional divergence across vertebrates. Our results suggest that there are two distinct GnRH lineages, which we call GnRH-I and GnRH-II, which resulted from a single gene duplication event early in the course of vertebrate evolution. The GnRH-I lineage includes mammal GnRH (mGnRH), chicken-I GnRH (cGnRH-I) and salmon GnRH (sGnRH) and all other forms of placodal origin. The GnRH-II lineage includes cGnRH-II and other forms expressed in the midbrain. All forms of GnRH exhibit, at both the cDNA and amino acid levels, a high degree of sequence identity in the GnRH region, somewhat less so in the signal sequence, and markedly less so in the associated peptide coding region. All GnRH-I forms have a single associated peptide (GAP), while GnRH-II forms have two associated peptides. The low degree of conservation of these associated peptides implies that whatever function they serve, if any, requires little in the way of sequence specificity.

725.7

GALANIN - IS IT A REPRODUCTIVELY RELEVANT PEPTIDE IN THE TELEOST FISH, *HAPLOCHROMIS BURTONI*. T. Bushnik* and R.D. Fernald, Department of Psychology, Stanford University, Stanford, CA.

Galanin, a 29 amino acid peptide present in both the gut and the brain, has been called a hypothalamic-hypophysiotropic hormone because it can effect the release of pituitary hormones such as growth hormone, prolactin, and gonadotropins. Galanin and its mRNA can be co-localized in gonadotropin releasing hormone (GnRH) cells in the hypothalamus/preoptic area (POA) although it is labile. We decided to investigate the role of galanin in regulating GnRH release in the teleost fish, *H. burtoni*. Males of this species exhibit size changes in the POA GnRH cell population that are correlated with changes in social status and reproductive maturity. These changes in cell size are plastic; that is, up-grading or down-grading the social status of an adult *H. burtoni* male results in a corresponding increase or decrease in the size of the GnRH cells in the POA. We addressed three questions: 1) is there galanin-like immunoreactivity (GAL-LI) present in the POA of *H. burtoni* males?, 2) do these GAL-LI cells project to the pituitary?, and 3) do these GAL-LI exhibit a correlation between cell size and social/reproductive status similar to that which has been reported for GnRH cells in the POA? *H. burtoni* males were observed behaviorally for 4 weeks to establish social status, then sacrificed, and crystals of fast DiI were applied to the transected pituitary stalks of the paraformaldehyde fixed brains. After 35-38 days, the brains were sectioned on a vibratome and immunostained for galanin using a fluorescein label. It was observed that 1) GAL-LI cells were present in the POA of all males, 2) the majority of these GAL-LI cells did not contain fast DiI suggesting that only a very small minority of these cells may project to the pituitary, and 3) the GAL-LI cells showed a tendancy towards a correlation between social status and cell size with socially dominant males having larger GAL-LI cells although the size differential was small.

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725.8

THE POSSIBLE ROLE OF NPY Y1 RECEPTORS IN THE CONTROL OF LORDOSIS. Jan Thornton* and Laurie Holcomb. Neuroscience Program and Dept Biology, Oberlin College, Oberlin OH 44074.

Previously we have shown that NPY plays a facilitatory role in the control of lordosis. These studies examined if NPY acts at Y1 receptors to affect lordosis. Adult guinea pigs were ovx and implanted with a cannula into the lateral ventricle. In Exp 1, females were injected with 20ug Estradiol Benzoate (EB) followed 40h later by .5mg Progesterone (P). Females which displayed lordosis after P were injected ICV with either 0.5 or 5 ug of the NPY Y1 agonist leu31, pro34 NPY, or were used as controls. The 5 ug dose of the Y1 agonist significantly increased lordosis responding. In Exp 2, ovx cannulated females were injected with 20ug EB and tested for lordosis 40h later. They were then injected ICV with NPY Y1 agonist (5ug) or were used as controls. The Y1 agonist did not facilitate lordosis in these females. These data suggest that 1) NPY acts at Y1 receptors to facilitate lordosis and 2) NPY may affect some progesterone mediated component of lordosis since only EB+P but not EB induced lordosis was facilitated.

725.9

AQUEOUS EXTRACT ACTION OF RUTA CHALEPENSIS ON SPERM MOTILITY. E. Gijón *, L. Cartas, M. Lorenzana-Jímenez and X. García. Dept. of Physiol. and Dept. of Pharmacol. Sch. Med. UNAM México D. F. 04510. México.

Muccitelli and Ferguson (1) point out flaws in the experimental desing of a recent study by Kupitz and Atlas (2), where the experimental condition do not guarantee that the applied concentration of each compound was present at the membrane surface of the mature egg, and that a more likely target for the compounds is the sperm if any of the applied compounds interfered with sperm activation or motility. This prompted us to tried aqueous extract of Ruta chalepensis on rat sperm motility, as it has been suggested that Ruta chalepensis extract has an anticonceptive action, and it is used in traditional medicine as vaginal wash before sexual intercourse. Exposing sperm recently obtained from rat testicle to Ruta chalepensis extract, we found under light microscope: a reduction in motility, decomposition of tail motility, until complete immovilization of spermatozoa without determining the involment of ion channels. This Ruta chalepensis effect may play a role in its postulated anticonceptive action. 1. Science 263:98, 1994; 2. Science 261:484, 1993.

726.1

REGULATION OF GLUTAMATE RECEPTOR BINDING FOLLOWING CHRONIC NEUROLEPTIC TREATMENT. Ian Creese*, F.I. Tarazi and W.I. Florijn. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Glutamate receptors may play an important role in the pathophysiology of schizophrenia and the motor side effects of chronic neuroleptic drug treatment. We examined changes in the binding of three glutamate receptor subtypes using ^{3}H-MK801 (NMDA-R antagonist), ^{3}H-CNQX (AMPA-R antagonist) and ^{3}H-Kainic acid (kainate-R agonist) *in vitro* receptor autoradiography following one month's treatment of rats with SCH23390 (SCH) (0.5 mg/kg/day), clozapine (CLZ) (25 mg/kg/day), haloperidol (HAL) (1.5 mg/kg/day), or raclopride (RAC) (10 mg/kg/day). Chronic SCH treatment elevated ^{3}H-MK801 binding in the hippocampal formation with significant increases in CA_2 and dentate gyrus. This result suggests a specific role for dopamine D_1 receptors in the regulation of hippocampal NMDA receptor function. In contrast, chronic HAL or RAC treatment did not alter ^{3}H-MK801 binding in any of the brain regions analyzed. However, chronic CLZ treatment caused a significant decrease in ^{3}H-MK801 binding in caudate putamen (medial: -34%, lateral: -22%) without affecting the hippocampal formation, nucleus accumbens or the cortex. This might explain why it produces minimal extrapyramidal side effects. Results for AMPA and kainate receptors will also be reported.

Supported by MH44211 and Sigma Xi grant (F.I.T.)

726.2

REGULATION OF DOPAMINE RECEPTOR BINDING FOLLOWING CHRONIC NEUROLEPTIC TREATMENT. F.I. Tarazi*, W.I. Florijn and Ian Creese. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

We examined changes in dopamine receptor binding in various brain regions following one month's treatment of rats with SCH23390 (0.5 mg/kg/day), haloperidol (HAL) (1.5 mg/kg/day), raclopride (RAC) (10 mg/kg/day) or clozapine (CLZ) (25 mg/kg/day) using *in vitro* receptor autoradiography. Chronic SCH23390 treatment significantly increased ^{3}H-SCH23390 binding in nucleus accumbens (NA) [+55%] and caudate putamen (CP)[+28%]. ^{3}H-7OH-DPAT binding to dopamine D_3 receptors was not changed by any of the neuroleptic drugs. In HAL-treated rats ^{3}H-YM-09151-2 and ^{3}H-spiperone binding was increased in CP [+50% and +45%] and in NA [+89% and 45%]. RAC treatment also elevated ^{3}H-YM-09151-2 and ^{3}H-spiperone binding in CP [+20% and +28%] and in NA [+28% and +25%]. ^{3}H-raclopride binding was only increased in the lateral CP [+17%] of HAL-treated rats. Since ^{3}H-YM-09151-2 and ^{3}H-spiperone have similar affinities for D_2 ,D_3 and D_4 receptors but ^{3}H-raclopride has low affinity for D_4 receptors, these results suggest that D_4 receptors are majorally upregulated by the HAL treatment. Thus, ^{3}H-YM-09151-2 and ^{3}H-spiperone binding in the presence of a D_2 and D_3 receptor saturating concentration of raclopride (300 nM) to quantify selectively putative D_4 receptors was significantly increased in both the CP and NA (+70%) of HAL-treated rats. Interestingly, the binding of both radioligands in the presence of 300 nM raclopride was significantly increased in CP (+26%) but not in NA of CLZ-treated rats.

Supported by MH44211 and Sigma Xi grant (F.I.T.)

726.3

AKATHISIA-LIKE RESPONSE TO HALOPERIDOL IN RATS TRAINED TO PERFORM A SUSTAINED ATTENTION TASK. B.J. Brockel* and S.C. Fowler. Univ. of Mississippi, University, MS 38677.

Rats were trained to remain immobile with their heads in observation tunnels where brief (0.12-s) visual stimuli were presented. The rats learned to react to 1 of 3 visual stimuli by executing a nose-poke response within 3 s of stimulus presentation. Exits from the tunnel were penalized by a 7.5-s time out, a contingency that made the rats refrain from locomotion for most of the 900-s session. Forty animals were equally divided into 5 separate groups with each group receiving its own dose (0.0 to 0.12 mg/kg) of haloperidol (Hal) daily for 23 days. Hal decreased reinforcers and dose-dependently increased proportion of errors of omission, suggesting that Hal not only reduces responding but also produces attention deficits. Number of head exits from the observation tunnel was dose-dependently increased by Hal. This effect showed gradual tolerance across the chronic dosing. Head exits in this paradigm may serve as an animal model of akathisia. Supported by MH43429.

726.4

HALOPERIDOL-INDUCED MONOAMINE DEPLETION: TYROSINE HYDROXYLASE-NEGATIVE SUBSETS OF DOPAMINE NEURONS ARE AFFECTED IN GASTROPOD CNS

D. Sakharov*, E. Voronezhskaya, L. Nezlin[1], K. Elekes[2], M. Baker, R. Croll[3]
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Long-term treatment of leeches with haloperidol (HAL) was shown to result in depletion of specific subsets of putative dopamine (DA) neurons (NeuroReport 1994, 5:667). With the aim of further investigating what appears to be a novel action of HAL, the present study was performed on adult and juvenile specimens of the pond snail *Lymnaea stagnalis* kept for up to 20 days in 0.5-2.0 µM Hal solutions. HPLC-EC analysis of the CNS revealed a concentration-dependent DA depletion which reached 20-50% over the first 4-8 days and thereafter remained stable. (A comparable DA depletion was observed in the land snail *Achatina fulica* as a result of daily HAL injection, 1 µmol/kg, for 4 days.) In *L. stagnalis* a transient, dose-dependent, serotonin depletion was also observed which, by day 12, reached its 60 to 80% maximum followed by a complete (for 0.5 µM HAL) or partial recovery by day 20 of treatment. Glyoxylate-induced fluorescence was found depressed or undetectable in some, but not all, identifiable clusters of DA neurons in the CNS of animals treated with HAL for 3-4 days or more. A peculiar feature of HAL-sensitive DA neurons was that they were non-reactive to antibodies raised against tyrosine hydroxylase, whereas HAL-resistent DA neurons showed such reactivity. Thus the previous and present reports suggest that the depleting action of low doses of chronic HAL observed in specific subsets of DA neurons may be a phylogenetically conserved characteristic which, if generalized to vertebrates, may account for therapeutic effects of this and related drugs.

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726.5

NITRIC OXIDE SYNTHESIS INHIBITION ATTENUATES HALOPERIDOL-INDUCED SUPERSENSITIVITY. C.M. Pudiak*, & M.A. Bozarth. *Department of Psychology, University at Buffalo, Buffalo, NY 14260-4110.*

Previous work has shown that nitric oxide is involved in sensitization to the locomotor-stimulating effect of repeated cocaine injections (Pudiak & Bozarth, *Life Sci.* 53: 1517-1524, 1993.). Increased responsiveness to stimulants can also be produced by chronic dopamine-receptor blockade. This study examined the effectiveness of nitric oxide synthesis inhibition in blocking the supersensitivity following chronic neuroleptic treatment (i.e., dopamine-receptor blockade). Nω-nitro-L-arginine (L-NAME) was used to inhibit nitric oxide synthesis, while haloperidol was used to produce supersensitivity by chronically blocking dopamine receptors.

Male, Long-Evans rats were tested in locomotor activity chambers for 30 min following an injection of normal saline (1 ml/kg, i.p.). On the next day, they were retested following an injection of cocaine hydrochloride (10 mg/kg, i.p.). All rats then received daily injections of haloperidol (0.2 mg/kg, i.p.) for 14 days. Half of the rats were pretreated with normal saline (1 ml/kg, i.p.) and half were pretreated with L-NAME (30 mg/kg, i.p.) 30-min before their haloperidol injections. Seventy-two hours after their last injections, all rats received an injection of cocaine (10 mg/kg, i.p.) and were tested for 30 min in locomotor activity boxes.

Rats treated with saline plus haloperidol showed greater stimulation of locomotor activity than during their first cocaine test, while rats treated with L-NAME plus haloperidol showed no change between the two tests. This finding suggests that inhibition of nitric oxide synthesis attenuated the haloperidol-induced supersensitivity to cocaine. Similar mechanisms may be involved in neuroleptic-induced supersensitivity and stimulant-induced sensitization.

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726.6

EFFECTS OF ANTIPSYCHOTICS ON THE PENTYLENETETRAZOLE (PTZ) SEIZURE THRESHOLD RELATIVE TO *IN VIVO* POTENCY MAY BE PREDICTIVE OF CLINICAL SEIZURE LIABILITY. S.C. McInerney, D.C. Hoffman, H. Donovan, L.J. Cornfield, R.J. Meade, J.V. Cassella* and D.W. Gallager. Neurogen Corp. Branford, CT 06405.

Antipsychotic drugs are known to cause a variety of neuropsychiatric side effects including the precipitation of seizures. However, as yet there is no animal model which is predictive of seizure liability. In the present studies, several clinically used antipsychotic drugs were evaluated for their ability to affect the onset of seizures induced by IV infusion of the chemical convulsant PTZ in rats. When administered IV prior to threshold determination, all of the reference antipsychotics reduced the PTZ seizure threshold at some dose when tested across a wide range of doses. In order to determine a behaviorally relevant dose range, the potency of each antipsychotic agent was assessed for its ability to antagonize *d*-amphetamine-induced hyperactivity, one measure of *in vivo* potency. The minimum efficacious dose (MED) for decreasing the PTZ seizure threshold was determined and compared to the ED50 (dose calculated to produce 50% reduction in hyperactivity) for antagonizing amphetamine-induced hyperactivity by the ratio of MED/ED50. In our studies, clozapine produced the lowest ratio of MED/ED50 of 0.67, with proconvulsant activity occurring well within the behaviorally relevant dose range. Haloperidol, trifluoperazine and chlorpromazine produced the highest ratios (25, 16.7 and 14.7, respectively). The ratio of proconvulsant activity to *in vivo* potency for these and additional drugs was found to correlate with available reports of clinical seizure incidence. This ratio may therefore serve as a predictor for proconvulsant liability among antipsychotic agents.

726.7

EFFECTS OF SOCIAL ISOLATION ON LOCOMOTOR ACTIVITY AND PREPULSE INHIBITION IN WISTAR RATS AND INTERACTIONS WITH ANTIPSYCHOTICS. T.C. McCloskey*, H.J. Ketteler, G.M. Fadayel, C.J. Schmidt and J.H. Kehne, Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215.

Social isolation of postweaning rats results in a syndrome of behaviors and a pattern of biochemical alterations that are, at least in part, indicative of enhanced dopaminergic sensitivity. Given the postulated role of dopaminergic overactivity in schizophrenia, and the need to develop new, non-pharmacological therapeutic models, the present study began investigating the behavioral effects of social isolation in rats, and interactions with antipsychotics. Male Wistar rats were housed either singly ("isolated" condition) or in groups of 4 ("grouped" condition) beginning at 25 days of age. When tested 5 weeks later, isolated rats placed in a novel test environment showed a significant locomotor hyperactivity relative to grouped rats. This hyperactivity was particularly notable during the first 10 min of testing when initial exploratory levels of activity are high. Isolated rats also tended to show disrupted prepulse inhibition of acoustic startle using either an auditory prepulse (weak white noise burst) or a visual prepulse (lightflash). Dose-response studies indicate that the locomotor hyperactivity can be reduced by the DA antagonist haloperidol at doses that did not significantly reduce baseline levels of activity. Preliminary findings of increased dopamine concentrations in the striatum and n. accumbens are also consistent with enhanced dopaminergic activity in the isolates. These data support the conclusion that the locomotor hyperactivity produced by social isolation is attributable, at least in part, to dopamine hyperactivity.

726.8

A TEMPORAL SHIFT IN THE LOCOMOTOR RESPONSE TO APOMORPHINE MAY PREDICT EPS LIABILITY OF ANTIPSYCHOTICS. M.J. Majchrzak*, V. Guanowsky, P.A. Seymour, & S. H. Zorn, Dept. of Neuroscience, Central Research Division, Pfizer Inc., Groton, CT 06340

Apomorphine is a dopamine receptor agonist that produces hyperactivity and stereotypy in rats. In order to examine the effects of known typical and atypical antipsychotics on the pattern of the behavioral response to apomorphine, locomotor activity was measured in habituated rats using photocell chambers. Stereotypy was measured in separate experiments by an observer blind to treatment. A dose of 1.78 mg/kg, s.c., initially produces stereotypy without locomotor stimulation, followed by a period of hyperactivity that begins 75-90 min later and lasts approximately 30 minutes. Pretreatment with the typical antipsychotics haloperidol, raclopride and fluphenazine produce a "temporal shift" in the apomorphine locomotor response. This response is characterized by a dose dependent leftward shift in the time course of the hyperactivity without attenuation of its magnitude. The temporal shift observed with typical antipsychotics is not due to an attenuation of stereotypy, since doses that temporally shift locomotor activity are not sufficient to antagonize stereotypy. The atypical antipsychotic clozapine, produces a dose dependent antagonism of the apomorphine locomotor response without a temporal shift or antagonism of stereotypy. The D1 antagonist SCH-23390, and the mixed D2/5HT-2 antagonist risperidone, inhibited the hyperactivity with no temporal shift. They did, however, both antagonize stereotypy. Since agents that produce temporal shifts are known to produce EPS clinically, and, those that do not produce temporal shifts have reduced EPS liability, it is hypothesized that the temporal shift may be predictive of antipsychotic efficacy associated with EPS liability. This behavioral assay may be useful for the identification of antipsychotics with a reduced propensity for EPS.

726.9

S 17828 AND S 14956, NOVEL BENZIZOXAZOLES DISPLAYING ANTIPSYCHOTIC PROPERTIES AND A LOW SIDE-EFFECT PROFILE IN ANIMAL MODELS: A COMPARISON TO RISPERIDONE, J.-L. Peglion, V. Audinot, M. Brocco, K. Bervoets*, C. Daquet, A. Newman-Tancredi, H. Canton and M.J. Millan, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, France.

In addition to dopamine receptors, both serotoninergic and adrenergic receptors are implicated in the pathogenesis and treatment of schizophrenia. Risperidone (RIS) is of interest in view of its high affinity at 5-$HT_{2A}/\alpha_1/\alpha_2$ vs D_2 receptors (pKis = 9.2/8.9/8.3 vs 8.3) as compared to haloperidol (7.1/7.9/5.9 vs 8.7), and, also, as it elicits a less pronounced extrapyramidal (EPS) syndrome in man at antipsychotic doses. In this study, we show that the novel benzizoxazoles, S 17828 and S 14956, also manifest high affinity at 5-$HT_{2A}/\alpha_1/\alpha_2$ vs D_2 receptors; pKis were 8.5/8.2/8.2 vs 8.7 and 8.5/9.1/8.2 vs 8.5, respectively. Interestingly, the affinity of S 17828 for cloned, human D_3 receptors was high (8.8) as compared to S 14956 (7.7) and RIS (8.0). Further, S 17828, S 14956 and RIS all showed low affinity at M_1 sites (< 6.0) and S 17828 (7.0) and S 14956 (7.1) showed only low affinity at H_1 sites as compared to RIS (8.9). *Ex vivo*, in (male) rats, S 17828 and S 14956 mimicked RIS in showing high occupancy of 5-HT_{2A} and α_1 vs D_2 sites: % occupation at 10.0 mg/kg, p.o.; S 17828 (71/33 vs 39), S 14956 (59/75 vs 13) and RIS (78/48 vs 18). RIS elicited catalepsy in rats only at doses (ED_{50} = 2.1 mg/kg, p.o.) higher than those inhibiting amphetamine-induced locomotion in rats (ID_{50} = 0.5 mg/kg, p.o.) and apomorphine-induced climbing in (male) mice (ID_{50} = 0.02 mg/kg, p.o.); such dose-differences were likewise marked for S 17828 (1.7 vs 0.04 and 0.02) and S 14956 (16.7 vs 1.1 and 0.6). In a model reflecting putative hypotensive properties in vivo, RIS potently elicited ptosis in rats (ED_{50} = 0.1 mg/kg, p.o.) whereas S 17828 (5.3) and S 14956 (1.3) were only weakly active. In conclusion, these rodent models suggest that S 17828 and S 14956 may be effective antipsychotics with a low side-effect profile.

726.10

A PARAMETRIC CHARACTERIZATION OF THE LATENT INHIBITION-CONDITIONED EMOTIONAL RESPONSE (LI-CER) MODEL OF SCHIZOPHRENIA IN RATS. R. Schreiber, S. Monneyron and M.J. Millan*, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, France.

Disruption of LI, that is, the inability to ignore irrelevant stimuli, has been proposed as a model of the attentional-cognitive deficits of schizophrenia. In the present study, we characterized this model by use of a three-step paradigm. After establishment of a stable baseline of licking behaviour, (male) rats were first pre-exposed (PE) or not pre-exposed (NPE) to a series of tones. One day later, during a conditioning session, they were exposed to two tone-shock pairings and 24h later, on the test-day, allowed to make 100 licks until the tone commenced. The time to complete licks 90-100 (t_1) and licks 100-110 (t_2) was measured and the suppression ratio (SR) was defined as: t_1/t_1+t_2. It was found that weight (200 vs. 300 g), pretest-criterion (completion of either 600 or 1000 licks within 10 min), duration of pre-exposure (40 tones/40 min vs. 40 tones/15 min) and the duration of shock (0.8 mA/1s vs. 0.8 mA/3s) were not critical for the establishment of LI. An inverted U-shaped shock-SR curve was obtained with maximal effects emerging at 0.5 mA (SR ± SEM: 0.09 ± 0.02 and 0.28 ± 0.03, for NPE and PE, respectively, $P < 0.05$). D,l-Amphetamine (2 x 1.5 mg/kg, i.p.) disrupted LI when injected 15 min before PE (40 tones) and conditioning using a *24h delay* between sessions but failed to disrupt LI when PE and conditioning were performed on the *same* day. Under the former conditions, haloperidol, 2 x 0.1 and 2 x 0.16 mg/kg, i.p., evoked LI in rats pre-exposed to 10 tones. It is concluded that, in the LI-CER model, the intensity of the unconditioned stimulus is decisive for the induction of LI and that, based on the results obtained with d,l-amphetamine and haloperidol, this paradigm is an appropriate model for the characterization of antipsychotic drugs.

726.11

STRUCTURAL ANALOGUES OF THE ANTIPSYCHOTIC SERTINDOLE. STUDIES ON THE FIRING OF DOPAMINE NEURONES IN A10 AND A9 RAT BRAIN AREAS AFTER CHRONIC ADMINISTRATION. J.Perregaard*, T. Skarsfeldt, K.Andersen, K.P.Bøgesø, K.Frederiksen, Research & Development, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark.

The atypical antipsychotic sertindole, 1-[2-[4-[5-chloro-1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-piperidinyl]ethyl]-2-imidazolidinone, has high affinity for serotonin 5-HT_{2A} (IC_{50}=0.39 nM), 5-HT_{2C} (IC_{50}=1.1 nM), and dopamine D_2 (IC_{50}= 4.1 nM) receptors and for α_1 adrenoceptors (IC_{50}=3.4 nM). *In vivo* sertindole has a long lasting antiserotonergic effect in the rat (inhibition of quipazine-induced head twitches; ED_{50}=0.039 µmol/kg, 24 hours after po administration). Despite high affinity *in vitro* for dopamine D_2 receptors sertindole exerts no cataleptogenic effects. Within the group of close structural analogues of sertindole similar neurochemical binding profiles were found. Such analogues include variation in the 5-substituent of the indole,[1a] moving the 5-chlorine atom to the 6-position,[1b] isosteric interchange of the indole N-1 and C-3 atoms,[1c] and the corresponding (1*R*,3*S*)-*trans*-1-piperazino-3-phenylindan derivative. Sertindole potently and selectively decreases the number of firing dopamine neurones in A10 versus A9 in the rat after 3 weeks of treatment.[2] The receptor binding profiles will be presented and discussed in relation to the effects on A10/A9 dopamine neurones after chronic administration with the sertindole analogues. A partial and selective inhibition of A10 dopamine neurones was found for some compounds. Results for the antipsychotics haloperidol, clozapine, and remoxipride will be included for comparison.

1a) Perregaard et al., *J.Med.Chem.* **1992**, *35*, 1092. 1b) *ibid.* **1992**, *35*, 4813.
1c) Andersen et al., *ibid.* **1992**, *35*, 4823.
2) Skarsfeldt and Perregaard, *Eur.J.Pharmacol.*, **1990**, *182*, 613.

726.12

PHARMACOLOGICAL PROFILE OF SM-13496, A NOVEL ANTIPSYCHOTIC AGENT WITH MINIMAL EXTRAPYRAMIDAL AND CNS DEPRESSIVE SIDE EFFECTS. Y. Ohno*, K. Ishida, T. Ishibashi and M. Nakamura. Res. Cent., Sumitomo Pharmaceuticals Co., Ltd., Konohana-ku, Osaka 554, Japan.

SM-13496 is a newly discovered antipsychotic that preferentially acts on dopamine D2 and 5-HT2 receptors. SM-13496 showed high to moderate affinities for D2 (Ki=14.4 nM) and 5-HT_2 (Ki=26.8 nM) receptors, but had negligible affinities for D_1, 5-HT1, 5-HT3, α_1, β, muscarinic, $GABA_A$, benzodiazepine, σ, or glutamate receptors. Oral administration of SM-13496 blocked dopaminergic behaviors (e.g., methamphetamine-induced hyperactivity in rats and apomorphine-induced climbing behavior in mice) and selectively suppressed the conditioned avoidance response in rats (ED_{50}=1.7-4.9 mg/kg). SM-13496 also inhibited 5-HT2 receptor-mediated behaviors (e.g., tryptamine-induced clonic seizure and *p*-chloroamphetamine-induced hyperthermia in rats, ED_{50}=3.0-5.6 mg/kg) and had anticonflict activity in the Vogel's conflict test (MED=10 mg/kg). Despite its potent D2 blocking activities, SM-13496 showed only negligible actions in inducing extrapyramidal side effects (i.e., catalepsy and bradykinesia, ED_{50} >1000 mg/kg), potentiation of anesthesia (ED_{50} > 1000 mg/kg), muscle relaxation (ED_{50} >1000 mg/kg) and inhibition or potentiation of convulsion (ED_{50} >1000 mg/kg). In conclusion, SM-13496 is a novel atypical antipsychotic with minimal extrapyramidal and CNS depressive side effects.

726.13

5-HT2 RECEPTOR BLOCKADE BY SM-9018, A NOVEL 5-HT2 AND D2 ANTAGONIST, COUNTERACTS ITS ACTIONS AT STRIATAL DOPAMINE D2 RECEPTORS. T. Ishibashi, Y. Ohno, K. Ishida, K. Ikeda, T. Tatsuno* and M. Nakamura. Res. Cent., Sumitomo Pharmaceuticals Co., Ltd., Konohana-ku, Osaka 554, Japan.

SM-9018 is a potential atypical antipsychotic that has potent 5-HT_2 and D_2 blocking activities. To elucidate the role of its 5-HT2 blocking activity in the striatum, we studied the effects of SM-9018 (5-HT2/D2 antagonist), haloperidol (D2 antagonist) and 5-HT2 antagonists on the induction of extrapyramidal side effects (i.e., bradykinesia), *ex vivo* c-fos mRNA expression in the striatum and *in vitro* acetylcholine (ACh) release from striatal slices. Antipsychotics (e.g., SM-9018 and haloperidol) dose-dependently induced bradykinesia in the pole-descending behavior of mice with relative potencies consistent with those for catalepsy induction. SM-9018 was about 70 times weaker than haloperidol in inducing bradykinesia and had a 13 times higher therapeutic index (anti-apomorphine /bradykinesia ratio). 5-HT2 antagonists (e.g., ritanserin) had no effects by themselves, but significantly reduced the haloperidol-induced bradykinesia. Oral administration of haloperidol markedly enhanced c-fos mRNA expression in the rat striatum (about 7 fold at 30 mg/kg) whereas SM-9018 showed only a slight increase (about 1.8 fold) at doses of up to 30 mg/kg. 5-HT2 antagonists failed to affect c-fos mRNA expression by themselves, but markedly attenuated the haloperidol-induced c-fos expression. Finally, SM-9018 was about 10 times weaker than haloperidol in enhancing the *in vitro* [^{3}H]ACh release upon 3Hz electrical stimulation while it was as potent as haloperidol in binding to D_2 receptors and in antagonizing the inhibitory effects of exogenous quinpirole (a D_2 agonist) on [^{3}H]ACh release. Simultaneous application of the 5-HT2 antagonist significantly reduced the haloperidol-induced enhancement of [^{3}H]ACh release. Our findings suggest that the 5-HT2 blocking activity of SM-9018 counteracts its antagonistic actions at the striatal D_2 receptors, which seems to contribute to its low incidence of extrapyramidal side effects.

726.14

STUDY OF THE STRUCTURE-METABOLISM RELATIONSHIPS OF ANALOGUES OF CI-1007 (PD 143188), A DOPAMINE AUTORECEPTOR AGONIST AND POTENTIAL ANTIPSYCHOTIC AGENT.

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We have recently described CI-1007 (PD 143188) as a dopamine (DA) receptor agonist and potential antipsychotic agent. As part of studies directed at understanding the pharmacokinetics and metabolism of CI-1007, we have identified three phenyl-hydroxylated metabolites of CI-1007. One of these metabolites shows pharmacological activity similar to CI-1007 and could contribute to the activity of CI-1007 *in vivo*. Fluorinated analogues of CI-1007 were synthesized to block metabolism. One compound retained DA autoreceptor agonist properties and had a longer half-life in isolated rat hepatocytes than CI-1007. However its *in vivo* half-life was short and it showed no improvement over CI-1007 in oral activity in the monkey conditioned avoidance test. A 3-pyridyl analogue of CI-1007 was more potent *in vitro* but this did not translate into superior oral activity in the monkey. The half-life of this compound was extremely short in rat hepatocytes but the corresponding 4-methyl-3-pyridyl analogue was more stable. This compound showed improved activity versus the 3-pyridyl analogue in the monkey but none of these compounds had an overall profile as optimal as CI-1007. We have learned that simple blockade of metabolism does not necessarily improve oral activity of compounds.

726.15

COMPARATIVE INTRINSIC ACTIVITIES OF DOPAMINE D2 AGONISTS.

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Intrinsic activity may be an important determinant of the clinical antipsychotic profile of partial dopamine (DA) D2 agonists. In order to determine the utility of preclinical tests for estimating intrinsic activity we measured the maximal effects of a series of partial DA agonists in models commonly used to characterize dopamine D2 agonists in vivo and compared the results to the maximal inhibition of forskolin-stimulated cyclic AMP accumulation in GH4C1 cells expressing the human D2 receptor in vitro (cAMP). In vivo tests included substantia nigra dopamine neuron firing (DNF), gamma-butyrolactone-stimulated brain dopamine synthesis (GBL) and intrastriatal microdialysis (ISMD) in rats. Drugs were tested at 1 μM in cAMP, a level verified as supramaximal for selected agonists. Dose-effect curves were determined for DNF (i.v.) and high single doses were used for GBL (i.p.) and ISMD (i.p. or s.c.). Relative intrinsic activity in cAMP was: apomorphine > B-HT920 ≈ EMD38362 > CI-1007 = terguride ≈ (-)-3-PPP > SDZ 912 > haloperidol. Maximal effects in DNF and GBL revealed a similar order of intrinsic activity. However, maximal effects in DNF and GBL suggested: apomorphine = B-HT920 and CI-1007 > (-)-3-PPP. While apomorphine and B-HT 920 produced large decreases in striatal DA overflow in ISMD, SDZ 912, terguride, (-)-3-PPP and haloperidol caused increased DA overflow. The close agreement with results from the cAMP assay suggest that the GBL and DNF tests may be useful for estimation of DA agonist intrinsic activity, while the ISMD test does not discriminate between weak partial agonists.

726.16

ANTIPANIC-LIKE ACTIVITY OF S 21357-1 IN MICE

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S 21357-1 (Trihydrochloride of 3-{-2-[-4-(-4-fluorobenzoyl)-piperidino]-ethyl}-6-{4-[4-(2-methoxyphenyl)1-piperazinyl]-1-nbutyl}benzothiazolin-2-one) binds *in vitro* with high to moderate affinity to the $5HT_{2A}$ (Ki=2nM), $5HT_{1A}$ (Ki=7 nM), $5HT_{1D}$ (Ki=32 nM), $5HT_{1B}$ (Ki=35 nM) and $5HT_{2C}$ (Ki=240 nM) receptors and with low affinity (Ki>1000 nM) to the $5HT_3$, α and β-adrenergic, D_1, D_2, sigma and opioid receptors.

S 21357-1 was evaluated in the anxiety/defense test battery which is able to measure escape and defensive reactions of a mouse after exposure to a predator (i.e. a rat). The animals were tested in the oval runway in two situations: after exposure to the predator (a stress mimicking generalized anxiety disorders) and in the presence of the predator reproducing a panic attack. In the first situation, S 21357-1 was able to prevent the increase of the jump escapes induced by the stress and the stress-induced decrease of wall rears at the doses of 0.125, 0.5 and 2 mg/kg i.p.; in addition, S 21357-1 prevented the increase of the wall climbs only at the lowest dose. In the second situation, S 21357-1 also reduced the escape behaviors: the number of avoidances was reduced at the doses of 0.125 and 0.5 mg/kg i.p. (respectively -29%, p<0.01 and -19%, p<0.05) as well as the avoidance distances (94% for the doses of 0.125, 0.5 and 2 mg/kg IP, p<0.01). During the forced contact, S 21357-1 significantly decreased the frequency of biting towards the rat at 0.125, 0.5 and 2 mg/kg i.p. (respectively -38%, -31%, -24%, p<0.01). S 21357-1 showed anxiolytic-like activity in the 2-compartment exploratory model in mice: it dose-dependently increased the number of transitions in the oral doses of 0.25, 1 and 4 mg/kg (respectively +195%, p<0.02, +438%, p<0.01 and +590%, p<0.005) and the time spent in the light compartment (respectively +141%, p<0.05, +300%, p<0.02 and +265%, p<0.01) without affecting locomotor activity or rearing.

These results suggest the interest of S 21357 as an antipanic-like and anxiolytic-like agent with minimal side effects.

PSYCHOTHERAPEUTIC DRUGS: OTHER

727.1

THE DELAYED ANTICONFLICT EFFECT OF MK-801 IN RATS: SIGMA VERSUS NMDA (PCP SITE) RECEPTOR MECHANISMS. **Z.C Xie* and R.L. Commissaris, Dept. of Pharmaceutical Sciences, Wayne State Univ., Detroit, MI 48202 U.S.A**

The present studies examined the receptor mechanism for the delayed anticonflict (i.e., anxiolytic-like) effect produced by dizocilpine (MK-801) in Conditioned Suppression of Drinking (CSD) conflict paradigm. Two questions were addressed: (1) do other agents which reduce NMDA neurotransmission mimic the delayed anticonflict effect? and (2) what treatment(s) can block the MK-801 effect? In Experiment 1, female rats were tested in the CSD conflict task using a multiple within-day test procedure (3 conflict sessions/day; 5 minutes/session). A thorough (0-60 hours in 6-hour increments) time course for the effects of MK-801 and a variety of agents which reduce NMDA neurotransmission was determined. As expected, MK-801 (0.1-0.4 mg/kg, IP) exerted a delayed anticonflict effect with a latency to onset of approximately 12 hours and a maximal effect at approximately 18-24 hours; no other NMDA antagonists exerted a delayed anticonflict effect, although CPP and AP-7 did produce modest anticonflict effects at a short (i.e., 40 minutes) pretreatment interval. In Experiment 2, the delayed anticonflict effect of 0.2 mg/kg MK-801 was selectively antagonized by the sigma site antagonist rimcazole (10 mg/kg, IP); in contrast, the PCP site antagonist metaphit (0.2 mg, ICV), the opiate antagonist naltrexone (1.0 mg/kg, IP) and the benzodiazepine antagonist Ro15-1788 (0.5 mg/kg, IP) did not antagonize MK-801. Together, these data indicate that the delayed anticonflict effect of NMDA antagonist MK-801 may due to its actions at sigma, rather than NMDA, receptors. **(Supported in part by MH 48171 to RLC).**

727.2

CENTRAL INFUSION OF MK-801 DISRUPTS PREPULSE INHIBITION OF THE STARTLE RESPONSE. V.P. Bakshi* and M.A. Geyer. Dept. of Neuroscience, UCSD, La Jolla, CA 92093.

Intense auditory or tactile stimuli elicit an involuntary startle response. Presentation of a weak stimulus just prior to the primary stimulus attenuates the magnitude of the startle response. This phenomenon of "prepulse inhibition" (PPI) is thought to reflect sensorimotor gating mechanisms. Because deficits in sensorimotor gating are characteristic of several disorders including schizophrenia, it is of interest to determine the neural substrates underlying PPI. Systemic administrations of dizocilpine (MK-801) and other non-competitive NMDA antagonists such as phencyclidine markedly disrupt PPI in rats. In humans, administration of non-competitive NMDA antagonists can result in psychosis that closely resembles schizophrenic symptomatology. Relatively little work has focused on the effects on sensorimotor gating of central administration of these compounds. The purpose of the present investigation was to determine the effects on PPI of intracerebral microinfusions of MK-801. Male Sprague-Dawley rats received intracerebroventricular infusions of MK-801 (0, 10 or 30 μg/ 5 μl) in a counterbalanced order over three test days. Five min after drug administration, animals were tested in startle chambers. The test session consisted of presentations (in a counterbalanced order) of a 120 dB "pulse" (the primary startling stimulus) either alone or immediately preceded by non-startling "prepulses" that were 3, 6, or 12 dB above the background noise (65 dB). MK-801 markedly reduced PPI and increased startle amplitude following intraventricular administration. This result suggests that the PPI-disruptive effects of MK-801 are mediated by CNS receptors. Studies to identify the specific structures subserving this effect are in progress.

727.3

EFFECT OF CARBAMAZEPINE ON PREGNENOLONE SYNTHESIS IN C6 GLIOMA CELLS AND RAT BRAIN

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Although carbamazepine (CBZ) has proven effective in the treatment of both neurological and psychiatric disorders, its mechanisms of action remain unclear. We have begun to explore the hypothesis that CBZ may affect the brain levels of pregnenolone, a neurosteroid known to modulate $GABA_A$ receptor in CNS. In the present study, cultured C6 glial cells were incubated with CBZ at concentrations of 1 to 100 μM for 0 to 120 min. After incubation, samples were homogenized and extracted with ethyl acetate. Pregnenolone was separated by HPLC, and quantitated using a radioimmunoassay method. The pregnenolone content increased after incubation with CBZ for 30 min. with the maximal effect being apparent after 60 min. The synthesis of pregnenolone was stimulated by CBZ in a concentration-dependent manner with the maximal effect at 50 μM. In rat brain slices, CBZ also produced a dose- and time-dependent increase in pregnenolone content. These results provide evidence that CBZ can increase steroid synthesis in rat brain tissue at concentrations used therapeutically in humans. This finding may provide novel insights into the mechanisms underlying one or more clinical effects of CBZ.

727.4

DESENSITIZATION AFTER A SINGLE DOSE OF THE 5-HT_{1A} RECEPTOR AGONIST FLESINOXAN UNDER BASAL AND DEFENSIVE-BURYING CONDITIONS.

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A single injection of a 5-HT_{1A} receptor agonist has been shown to attenuate 8-OH-DPAT-induced corticosterone secretion in the rat (Kelder and Ross, N.-S. Arch. Pharmacol. (1992) 346: 121). In order to assess whether this also implies behavioral desensitization, we studied the effects of a single pretreatment with flesinoxan under basal conditions and in the shock-prod defensive-burying paradigm. Male rats received an anxiolytic dose of flesinoxan (3 mg kg^{-1} s.c.), which suppresses defensive burying, or vehicle on day 1. Plasma corticosterone and glucose concentrations were determined on day 2 or day 8, 60 min after a second flesinoxan or vehicle treatment. On day 2 only, drug-induced increases in corticosterone and glucose under basal conditions were found to be diminished after flesinoxan pretreatment. No effect of pretreatment on lower lip retraction was observed. In the defensive-burying condition, flesinoxan pretreatment on the previous day also attenuated plasma corticosterone and glucose elevations. It is suggested that desensitization related to hormonal effects of 5-HT_{1A} receptor agonists involves a mechanism distinct from that underlying other symptoms of 5-HT_{1A} receptor activation.

727.5

ANTAGONISM OF AMPHETAMINE-INDUCED BEHAVIORAL CHANGES IN SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES BY LOW DOSE APOMORPHINE.

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Low doses of the dopamine (DA) agonist apomorphine (APO) have been shown to lessen psychotic behavior in schizophrenic patients. This activity appears to be related to action on the pre-synaptic DA autoreceptor resulting in decreased DA release. Recently, there has been interest in developing agents with this property as antipsychotic candidates. In this study, we tested the effect of low dose APO on amphetamine (AMPH)-induced behavioral changes in monkeys that appear to have face validity or correlative validity to human psychotic behavior. The effect of APO was compared to the atypical antipsychotic clozapine (CLOZ). The subjects were 4 females of a stable social colony of 5 adult stumptail macaques (Macaca arctoides). The study followed a cross-over design with 2 monkeys receiving drug per treatment day. Following observation of normal behavior (baseline), d-AMPH, 1.0 mg/kg, was given i.m. 15 min before observation for 2 days. Then, either APO, 0.1 mg/kg, was administered i.m. 15 min or CLOZ, 5 mg/kg, n.g. 2.5 hr before observation for 2 consecutive mornings and in the afternoon of day 1. AMPH was given as before. On each day, a "blind" observer quantitated and recorded the behavior of each animal using a checklist of over 40 social and solitary behaviors. Alone, AMPH significantly increased submissive gestures, checking (visual scanning), induced stereotypy, and eliminated initiated social grooming. APO antagonized AMPH-induced submissiveness, stereotypy, and restored social grooming, whereas, CLOZ blocked AMPH-induced submissiveness and checking. Neither APO or CLOZ induced movement abnormalities, but CLOZ-treated monkeys had a significant increase in resting. The results of this study suggest that this paradigm may be valuable in screening DA partial agonists as potential antipsychotic agents.

727.6

In Vivo Electrophysiological Effects of ABT-418: A Novel Cholinergic Channel Activator (ChCA).

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ABT-418[(S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole] has *in vitro* nicotinic-cholinergic functional and receptor binding properties (Arneric, et. al., *J Pharmacol Exp Ther*, in press), that, in conjunction with with animal behavior studies (Decker, et. al., *J Pharmacol Exp Ther*, in press), suggests a more selective and less toxic action compared to nicotine. Nicotine itself desynchronizes neocortical EEG while also supressing paroxysmal spike wave discharges. This study evaluated the effects of ABT-418 on these neocortical EEG pararmeters that are affected by nicotinic acetylcholine (nAChR) receptor activation.

Six and 12 month Wistar rats were surgically implanted with recording electrodes over the frontal and parietal cortices. ABT-418 (0.62, 1.9, & 6.2 μmol/kg, i.p.) did not produce any significant change in FFT-analyzed EEG activity in unanesthetized 6 month rats. In contrast, all tested doses of (-)-nicotine (0.19, 0.62, 1.9 μmol/kg, i.p.) significantly ($p<0.05$) lowered 1-13 Hz total power values. ABT-418 (1.9 & 6.2 μmol/kg, i.p.) significantly lowered the incidence of spontaneous 6-10 Hz neocortical spike wave discharges in awake 12 month rats. This effect was inhibited by the cholinergic channel blocker mecamylamine (5.0 μmol/kg, i.p.) indicating that ABT-418 attenuation of spike wave activity is mediated by nAChRs.

The relative lack of ABT-418-induced neocortical desynchronization distinguishes this compound from nicotine. This study suggests that the attenuation of spike wave discharges and the reported behavioral effects of ABT-418 are not a simple consequence of the neocortical activation.

727.7

SEROTONERGIC OVERACTIVATION, LIKE DOPAMINERGIC OVERACTIVATION OR GLUTAMATE UNDERACTIVATION, DISRUPTS CROSS-MODAL SENSORY GATING AS MEASURED BY AUDITORY AND VISUAL PREPULSE INHIBITION IN RATS.

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Dopamine (DA) and/or glutamate (GLU) imbalances are generally thought to contribute to schizophrenic symptomatology. Increasing evidence suggests a possible role for serotonin (5-HT) as well. Prepulse inhibition (PPI), a measure of sensory gating, is disrupted in schizophrenics, and by psychotomimetics in rats. In the present study, cross-modal PPI of acoustic startle in Wistar rats was produced by using either an auditory stimulus (sound) or a visual stimulus (light) as the prepulse. DA overactivity induced by d-amphetamine or apomorphine, or GLU underactivity produced by the cation channel blockers phencyclidine (PCP) or MK-801, disrupted auditory and visual PPI. MDL 100,453, a competitive NMDA antagonist, decreased baseline without markedly disrupting PPI. The 5-HT releaser fenfluramine (5.0 mg/kg) disrupted both auditory and visual PPI, and these effects were attenuated by the 5-HT uptake blocker MDL 28,618A, or by central 5-HT depletion produced by the 5-HT neurotoxin *p*-chloroamphetamine (PCA; 10 mg/kg) injected two weeks prior to testing. These findings indicate that excessive 5-HT overactivation, like DA overactivation or GLU underactivity, can disrupt auditory or visual sensory gating processes. The findings with PCA also suggest the utility of PPI as a tool to evaluate functional deficits accompanying CNS 5-HT neurotoxicity.

727.8

USE OF NON-PERMEABLE MICROCARRIER BEADS IN ALKALI METAL ION TRANSPORT NMR STUDIES OF PERFUSED NEUROBLASTOMA CELLS.

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In Nuclear Magnetic Resonance (NMR) studies of cells in suspension, it is possible to distinguish between the chemical shifts of the intracellular and extracellular ^{7}Li, ^{23}Na, ^{39}K and ^{89}Rb NMR resonances by the use of anionic shift reagents (SR) in the extracellular medium. In NMR studies of cells anchored on microcarrier beads, which are permeable to alkali metal ions and/or SR, the intracellular resonance may overlap that of the internal bead volume.

Cell-free suspensions of four types of beads which differed in size, ion permeability, matrix composition, magnetic susceptibility and charge distribution were used in this study. Cytodex 1 and CultiSpher-G beads in buffered solutions gave rise to two separate ^{7}Li and ^{23}Na resonances, while only one ^{7}Li and ^{23}Na resonance was observed in suspensions containing Biosilon or glass beads. Hence the physical basis for the discrimination between the alkali metal NMR resonances in cell-free suspensions of Cytodex 1 and CultiSpher-G is the cation and anion permeabilities of these materials. ^{7}Li NMR transport experiments of human neuroblastoma cells anchored on impermeable Biosilon beads will be presented. The relevance of these studies to understanding the mechanisms of lithium action in the treatment of manic depression will be discussed.

727.9

HYDROFLUORIC ACID ENHANCES RECOVERY FROM ISOFLURANE ANESTHESIA IN MICE: BEHAVIORAL AND PHYSICOCHEMICAL MEASURES. T.P. Jerussi*, R.A. Del Vecchio, J. Bandekar, C.G. Huang and M.J. Benvenga. Dept. of Biological Research, Ohmeda, PPD, Murray Hill, NJ 07974.

When isoflurane (I) with trace amounts of hydrofluoric acid (HF) was tested for anesthesia in mice, the recovery appeared markedly faster than that of I alone. Therefore, a systematic investigation of the mixture was initiated. Mice were exposed to I or I containing 0.006-0.052% HF. LC_{50}s, EC_{50}s for loss of righting, and recoveries, the time for the return of righting, were determined for each concentration. Differences between I and I + HF, and the soda lime stability of I + HF were analyzed by FT-IR. I and HF interactions were also investigated by theoretical (*ab initio* quantum chemical) calculations using a model system of HF-dimethyl ether.

All of the EC_{50}s were significantly different from I alone whereas the LC_{50}s did not change. Moreover, recoveries of mice exposed to I + 0.023-0.130% HF were significantly faster than those of I. The main differences between the FT-IR spectra of I and I + 0.023% HF were shifts in peak positions and band-broadenings. The FT-IR spectra of the liquid and vapor phases of I + 0.023% HF exposed to soda lime were identical to that of an untreated sample. Quantum chemical calculations indicated that a strong (8.85 kcal mole^{-1}) hydrogen bond was formed between the oxygen of dimethyl ether and the H of HF.

The addition of small amounts of HF to I creates an I·HF adduct that has distinct physicochemical properties and produces a different pharmacological profile than I, resulting in greater potencies and faster recoveries from anesthesia.

727.10

PHARMACOLOGICAL STUDIES OF YM-35992 - A NOVEL SELECTIVE SEROTONIN RE-UPTAKE INHIBITOR WITH 5-HT_2 ANTAGONISTIC ACTIVITY - S. Yatsugi, H. Takeuchi, K. Nakato, H. Hattori, K. Koshiya, M. Fujii, E. Wanibuchi* and T. Yamaguchi. Yamanouchi Institute for Drug Discovery Research, 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

YM-35992 ((-)-(S)-2-[[(7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride) possesses affinities for not only serotonin (5-HT) uptake sites (Ki=21 nM) but also 5-HT_2 receptors (Ki=100 nM), and has a relatively weak affinity for α_1 receptors (Ki=247 nM). YM-35992 induced symptoms of central serotonergic activation in mice when coadministered with l-5-HTP (90 mg/kg, iv), the 5-HT precursor. The ED_{50} values for the induction of these symptoms including tremors, head-twitches and hindlimb abductions were 6.3, 7.2 and 14.1 mg/kg ip, respectively. On the other hand, trazodone, which also has affinities for 5-HT uptake sites (Ki= 142 nM) and 5-HT_2 receptors (Ki=38 nM), did not show any interactions with l-5-HTP even at a dose of 60 mg/kg ip. YM-35992 and trazodone significantly reduced the number of head-twitches in mice induced by DOI (2.5 mg/kg ip), a 5-HT_2 agonist, with the ED_{50} values of 7.1 and 7.8 mg/kg ip, respectively. YM-35992 (10 mg/kg ip) significantly reduced the immobility time in the mouse tail suspension test, but trazodone conversely prolonged the immobility time in a dose-dependent manner. Interestingly, both YM-35992 and trazodone ameliorated the learning impairment in olfactory bulbectomized rats after an acute administration, but citalopram did not. In this model, most antidepressants are known to ameliorate the learning impairment only after chronic but not acute administration. These results suggest that YM-35992 would be a novel type antidepressant with more potent effects than trazodone and earlier onset of action than conventional selective serotonin re-uptake inhibitors.

727.11

BIOCHEMICAL STUDIES OF YM-35992 - A NOVEL ANTIDEPRESSANT. T. Yamaguchi*, S.Yatsugi, H.Takeuchi, M.Fujii. Yamanouchi Institute for Drug Discovery, 21 Miyukigaoka, Japan 305.

YM-35992, (-)-(S)-2-[[(7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride, showed biochemical characteristics of potent selective serotonin re-uptake inhibitor. In the ^{3}H-citaroplam binding study, YM-35992 showed the same high affinity as fluoxetine(Ki=21nM). 5HT selective uptake inhibition was also observed in the study using synaptosome preparation(IC50=140nM). YM-35992 did not inhibit both dopamine re-uptake and norepinephrine re-uptake(IC50>10μM and =6.7μM respectively). These results show YM-35992 is a potent and selective 5HT re-uptake inhibitor as compared with amitriptyline and trazodone. However citaropram and fluoxetine have no affinity against 5HT$_2$ receptor, YM-35992 showed the almost same affinity as trazodone in ^{3}H-ketanserin binding study(Ki=186nM). In another receptor binding studies, a relatively weak affinity for only α_1 receptor was observed. YM-35992 was also found to inhibit potently the aggregation of platelets(IC50=2.15μM), the results revealed an antagonistic activity of this compound for 5HT$_2$ receptor.

In contrast to the first generation of antidepressant(ex.tricyclic), several selective serotonin re-uptake inhibitors(SSRI) show relatively weak side-effects and fast onset of effectiveness. Up-regulation of 5HT$_2$ receptor is observed in the brain of patients with depression, and also clonic treatment of antidepressant down-regulate this receptor. Such down-regulation of 5HT$_2$ receptor possibly contribute to the clinical effectiveness. Thus YM-35992, SSRI with 5HT$_2$ antagonistic activity, might show the potent therapeutic action as a novel antidepressant in comparison with conventional SSRIs.

DEGENERATIVE DISEASE: ALZHEIMER'S—MODELS, ASSESSMENT, AND TREATMENT

728.1

DEATH OF SEPTAL CHOLINERGIC NEURONS PRODUCED BY CHRONIC EXPOSURE TO GLUTAMATE IS PREVENTED BY MK-801: ROLE OF NGF AND NITRIC OXIDE. Y. Agid* and P. P. Michel. INSERM U289, Hôpital de la Salpêtrière, 47, bld de l'hôpital, 75013 Paris, France.

To study the sequence of neurodegenerative events which could be associated with cholinergic cell death in Alzheimer's disease, rat septal cholinergic neurons in culture were exposed to chronic excitotoxic stress by glutamate. Counts of ChAT immunopositive cells and measurement of ChAT activity revealed that concentrations of glutamate on the order of 70μM killed 50% of cholinergic neurons after 24 h. of treatment. Neurotoxic effects were not aimed at cholinergic neurons specifically since other populations of cells present in these cultures were also affected. The non competitive NMDA receptor/channel antagonist, MK-801 (10μM), abolished acute neuronal swelling and rescued from late degeneration both cholinergic and non cholinergic cells when concentrations of glutamate did not exceed 500μM. The same degree of neuroprotection was afforded by the polyamine antagonist, Ifenprodil (20μM). On the other hand, the kainate/quisqualate receptor antagonist, CNQX lacked protective effects. NGF used in standard culture conditions to enhance expression of the cholinergic phenotype was shown not to influence excitotoxic processes. It should be noted that the presence of the NO synthase enzyme in most of the cholinergic neurons did not confer to these cells a particular resistance to glutamate intoxication. Furthermore despite the fact that the NO generating agent, sodium nitroprusside, was capable of mimicking some of the effects of glutamate, the lack of protection of various NO synthase inhibitors (L-NAME, L-NOARG; 30-1000μM) against glutamate-induced septal cell death suggests that NO does not participate to the toxic process as an intercellular messenger.

728.2

LACK OF EFFECT OF UNILATERAL NEUROTOXIC LESIONS OF THE BASAL FOREBRAIN (BF) CHOLINERGIC SYSTEM ON CEREBRAL GLUCOSE UTILIZATION (CMRglc): A POSITRON EMISSION TOMOGRAPHY (PET) STUDY IN BABOONS. C. Chavoix*[1], C. Le Mestric[1], P. Rioux[1], C. Fallet-Bianco[1], P. Allain[1,2], A.R Young[1], J. Epelbaum[3], F. Mézenge[1], E.T. MacKenzie[4] and J.C. Baron[1]. INSERM U320[1], CEA DSV-DRIPP[2], CNRS URA1829[4], Centre Cycéron, BP5229, 14074 Caen Cédex, and INSERM U159[3], 2Ter rue d'Alésia, 75014 Paris, France.

Loss of BF cholinergic neurons and associative neocortex hypometabolism are two presumably inter-related features of Alzheimer's disease (AD). Unilateral lesions to the nucleus basalis of Meynert (nbM) in baboons were reported to induce a marked but transient neocortical, preferentially ipsilateral, hypometabolism (Kiyosawa et al., Brain, 1989; 112:435); however, the lesions were electrolytic and there was no control group. To re-evaluate this issue, we studied with PET the effects of ibotenic acid lesions of the entire BF cholinergic system on CMRglc in 4 baboons and 3 sham-operated controls. In each baboon, magnetic resonance imaging (MRI) was used to 1) pre-operatively locate the target sites (n=17), 2) post-operatively confirm damage to the BF area, 3) determine internal cerebral landmarks for PET positioning, and 4) identify brain structures for CMRglc measurements. High-resolution coronal PET coregistered with MRI was performed in baboons under phencyclidine-N2O, once before and 1-4 times after surgery (at days -7, 5, 12, 35, and 70; sacrifice was at day 75). CMRglc and choline acetyltransferase (ChAT) activity were measured in neocortical areas and hippocampus. Lesion accuracy was confirmed by post-mortem histology (e.g., 25-100% neuronal loss in the nbM) and significant reduction in ChAT activity (e.g., ~ 60% decrease in frontal cortex). Nevertheless, there was no statistically consistent metabolic effects of BF lesions as compared to sham-animals, and no positive correlation between CMRglc and ChAT activity values in both groups for any time post-surgery. These results suggest that cholinergic deafferentation does not contribute to the brain hypometabolism observed in AD.

728.3

Animal model of wandering in Alzheimer's disease: Neural changes in proximal and distal sites following colchicine lesions of the dorsal hippocampal formation. R.J. Donahue* and J.P. Ryan. Neurobehavioral Research Lab, Psychology Department, SUNY Plattsburgh, Plattsburgh, New York 12901.

The neuropathology and neurobehavioral effects of wandering in Alzheimer's disease (AD) have been virtually unexplored despite the problematic nature of the behavior. Ryan and colleagues (1990, 1993) have investigated the behavior in an animal model using intrahippocampal injections of the neurotoxin colchicine. Marked behavioral deficits have been observed implicating not only the hippocampus but associated regions of the hippocampal system. The present study investigated changes in the hippocampus and related structures following dorsal hippocampal formation lesions. Twenty-four Long Evans hooded rats received either bilateral injections of one of three colchicine doses (10 μg, 15, μg, 25 μg) or artificial CSF into the dentate gyrus and/or CA1 regions. Ten days following surgery the animals were tested in the T-maze for spontaneous alternating behavior. Neural changes were detected using two immunocytochemistry procedures for Glia Fibrial Acidic Protein (GFAP) and for dopamine. Results indicate marked changes in the hippocampal formation as well as related brain regions supporting the hypothesis that behavioral alterations in the animal model of wandering in AD are the consequence of damage to the hippocampal system.

728.4

Colchicine lesions of the dorsal hippocampal formation induce psychomotor impairments and perseveration. J.P. Ryan*, Z. Israelian, and D. Wagoner. Neurobehavioral Research Lab, Psychology Department, SUNY Plattsburgh, Plattsburgh, New York 12901.

The animal model of wandering in Alzheimer's disease (AD) developed by Ryan and colleagues (1990, 1993) has identified two behavioral components which may contribute to wandering in patients with AD: spatial memory impairments and psychomotor dysfunction. The present study elaborated on the psychomotor aspects and further investigated contributions of attention, activity level and perseveration in the animal model. Forty-four Long-Evans hooded rats received either bilateral injections of one of three colchicine doses (10 μg, 15 μg, 25 μg) or artificial CSF into the dorsal hippocampal formation. Ten days following surgery the animals were behaviorally tested in the activity chamber for attention and exploration followed by four consecutive days of T-maze testing for spontaneous alternating behavior. Increased activity, decreased attention and perseveration were indicated in rats with marked cell loss in the dorsal dentate gyrus and CA1. The study supports the hypothesis that deficits in spatial memory characterized in the animal model of wandering in AD are exacerbated by perseverative behavior and hyperactivity.

728.5

PREHENSION IN ELDERLY AND DAT SUBJECTS. V. Diggles-Buckles*, JC Morris, J Duchek, L Hunt, J Zieleskiewicz. Alzheimer's Disease Research Center, Washington Univ. Sch. of Med., St. Louis, MO 63110

Dementia of the Alzheimer type (DAT) is associated with reduced functional abilities, e.g. driving. The effect of impaired motor control mechanisms for these functional abilities have been underemphasized relative to cognitive loss. To explore basic kinematic and coordination differences in elderly controls and DAT subjects, we examined phases of simple prehension. Prehension was chosen for comparison as a pure motor function to minimize skill, past experience, and cognitive load. DAT subjects were divided into 2 groups according to dementia severity: very mild (n=5) and mild (n=5). Controls and DAT subjects were asked to grasp a cylinder (5 cm wide) 20 cm from starting hand position, lift and place it on a target 20 cm from the cylinder's original location when they heard a tone. Subjects were instructed to "move as quickly and accurately as possible," e.g. the cylinder must be on the target (7 cm wide) and still standing after release. **Results**: DAT subjects were slower in reaction time and movement time (based on switches) but used larger peak velocities, accelerations and decelerations to achieve the task. The coordination of finger preshaping and limb transport to the cylinder was more tightly coupled temporally in controls than in the mildly demented subjects. There are motor changes associated with DAT that go beyond simple slowing to include disruption of coordination. Supported by NIA AG10145

728.6

CORPUS CALLOSUM PATHOLOGY IN ALZHEIMER SUBJECTS: DISRUPTION IN INTERHEMISPHERIC TRANSFER. Y. Lakmache[1]; F. Lepore[1]*; S. Gauthier[2] and M. Lassonde[1]. [1] Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal et [2] Centre McGill pour Etudes sur le Vieillissement, Montreal, Qc., Canada.

The main feature of Alzheimer's disease (AD) concerns the neuropathological and physiopathological changes in the associative areas of the cortex. Recent studies have demonstrated that besides the grey matter, the white matter of the centrum semiovale, which includes the minor and major forceps of the corpus callosum (CC), is also severely affected in AD. This might be in part a result of the degeneration of the pyramidal cells within these areas which furnish most of the commissural fibers. Fibers of the CC link the two hemispheres following a rostro-caudal topographical distribution. Thus, the motor fibers of the CC are mainly found in the genu, the somesthetic fibers occupy the trunk, the auditory fibers cross in the isthmus and the visual fibers project through the splenium. The aim of this study was to evaluate interhemispheric transfer in AD subjects and hence to assess the integrity of the CC. Several tasks, including tactile denomination, tactile and visual discrimination, simple reaction time to lateralized flashes of light or to puffs of air, bimanual coordination, etc., which attempted to test interhemispheric transfer within these systems were administered to 10 AD and 10 matched controls. Results showed that inter- but not intrahemispheric performance was significantly impaired in AD relative to controls. This finding is interpreted as showing that AD affects not only the well established memory and cognitive functions but also interhemispheric integration.

728.7

THE CASE FOR DIAGNOSTIC DICHOTOMY BETWEEN EARLY- AND LATE-ONSET ALZHEIMER'S DISEASE. A. Carta, D. Bravi, M. Calvani, S. NíBhuachalla, M. Raskind*. Scientific Affairs Dept., Sigma-Tau Pharmaceuticals, Gaithersburg, MD 20878. *Alzheimer's Disease Research Center, Univ. of Washington, Seattle, WA 98108.

The practice of diagnosing Alzheimer's disease (AD) without classification as to age of onset is under increasing challenge. Recent reports of genetic dissimilarity between early- and late-onset AD patients provide qualitative support for previously noted differences between the two groups. These observations span clinical, neurobiological, and neurohistological parameters. Even the classic pathology of AD has yielded to observations that the substantive histological similarities, once used to unify early- and late-onset patients, segregate the groups in terms of quantity and distribution. To assess the validity of an age-based dichotomy, published data supporting and refuting the argument were reviewed. Conclusive claims cannot be made from a review of small patient cohorts; yet, a substantial amount of data differentiate early- and late-onset AD. Among them, evidence for differences in disease course, language, cholinergic deficits, and metabolic function are strongly supportive of heterogeneity in AD identified by age of disease onset. These observations are reviewed in light of the recent genetic data, and their implications for continuing AD research are explored.

728.8

ASSOCIATION BETWEEN MOTION SENSITIVITY DEFICITS AND DIFFERENTIAL LUMINANCE SENSITIVITY IN SENILE DEMENTIA OF THE ALZHEIMER'S TYPE. A. Bellefeuille[1], G. L. Trick[1,3], P. Morris[2], M. Wolf[2] and M. Ptito[1]*. [1]University of Montreal, Montreal, H3C 3J7, Canada, [2]Washington University, St Louis, MO 63110 and [3]Henry Ford Hospital, Detroit, MI 48202.

The visual abnormalities associated with senile dementia of the Alzheimer's type (SDAT) include both motion sensitivity deficits and reductions in differential luminance sensitivity (DLS). The principal objective of this study was to determine the relationship between these deficits in patients with early to mild SDAT. Random dot kinematograms (RDKs) were used to assess global motion sensitivity in 16 patients with diagnosed SDAT and 19 visually and cognitively normal control subjects of similar age. Motion sensitivity testing was conducted twice within a month on each participant and the results of the two tests were averaged. DLS was evaluated with automated static perimetry (Humphrey, 30-2). Only participants with reliable visual fields (by manufacturer's criteria) were included. The global sensitivity, indices of visual field sensitivity (i.e., mean deviation [MD], pattern standard deviation [PSD], and corrected PSD [CPSD]) were used to quantify differential luminance sensitivity. Motion sensitivity was reduced significantly in SDAT patients compared to the controls ($p < 0.05$). DLS was depressed significantly (i.e., significant visual field defects were detected) in 11/16 SDAT patients. MD, an index of generalized reduction in DLS, was significantly correlated with motion thresholds ($r = -0.37$, $p<0.05$). PSD and CPSD, more representative of focal reduction of DLS, were not significantly correlated with motion thresholds ($p>0.10$). These results support previous findings of significant motion and differential luminance sensitivity deficits in patients with SDAT. In addition, the correlation between motion and luminance sensitivity suggests a link between these two deficits. (Supported in part by NIH grant AG05681).

728.9

REDUCTION IN AREA 17 BLOOD FLOW TO HIGH BUT NOT LOW FREQUENCY FLASHING LIGHTS IN DEMENTIA OF THE ALZHEIMER TYPE (DAT).M.J. Mentis, J. Stoll*, C. Grady, P. Pietrini, W. Hong, D. Mangot, G.E. Alexander, J.Ma, Maisog, J.W. VanMeter, M. Kurkjian, U. Freo, S.I. Rapoport, M.B. Schapiro. Lab. Neurosci., Natl. Inst. on Aging, NIH, Bethesda, MD 20892.

To determine the functional integrity of area 17 in DAT across a parametric range of passive visual stimuli we compared regional cerebral blood flow (rCBF) in 8 patients with DAT (75±6yr, Mini Mental State 21±4) and 12 healthy controls (63±13yr) using positron emission tomography (PET), [15-O] water and arterial catheterizaton for rCBF calculation. The visual stimulus was administered by goggles which flashed red lights alternately into each eye. All subjects had 6 scans, one at each frequency (0, 2, 4, 8, 16, 32 Hz) in randomized order. The healthy controls showed a linear rCBF increase from 0 through 16 Hz and then a decline at 32Hz which confirmed earlier reports of linear increase with maximal rCBF at ± 8Hz (simultaneous flashing into both eyes). In area 17, compared to the healthy controls, the DAT group had a) significantly lower rCBF values for all frequencies (p=0.001), b) maximum rCBF response at 8 not 16 Hz, and c) similar percentage rCBF increases at 2, 4, and 8 Hz but significantly lower increases at 16 and 32 Hz (p=0.001). This suggests that in DAT patients the neural circuits responsible for generating the area 17 response to flashing lights are normal for low but fail at high flash frequencies, perhaps a differential failure of magno and parvocellular systems.

728.10

RELATIONSHIP BETWEEN NEUROIMAGING AND PATHOLOGY IN ALZHEIMER'S DISEASE.
N.Nakano[1], R.Fukatsu[1], M.Fujii[1], J.Miyazawa[1], S.Murakami[1], K.Utsumi[1], S.Hayashi[1], T.Saito[1]*, K.Tsuzuki[2], and N.Takahata[1] Dep. of Neuropsychiatry[1], and Microbacteriology[2], Sapporo Med. Univ., Sapporo, Japan 060.

Neuroimaging is very useful in establishing the clinical diagnosis, and has been widely available in clinical neuropsychiatry. But little is known about the significance of alterations in neuroimaging. In this paper, we present comparative study between alterations in neuroimagings and neuropathological findings in a case of Alzheimer's disease (AD).
case: 64 yro female, began to show memory disturbance, followed by difficulties in house keeping at 59 yro. showed disorientation and apraxia, language disability, and Bálint's syndrome. was gradually deteriorated.
Neuroimagings: Longitudinally examinations of 123I-IMP SPECT revealed that asymmetry of hypoactive regions (lt. side dominant) was observed in parietal and temporal lobes at early stage and expanded into surrounding areas in advanced stages. Asymmetrical atrophy was evident in the same regions.
Neuropathology: AD was confirmed pathologically. 16 cortical regions in each hemisphere, which were matched to ROI in SPECT, were selected for further quantitative analysis. Neuronal cell count (NCC) and ß/A4 deposited area (BDA) were estimated using computer-assisted image processing. NCC reduced in number remarkably in lt. parietal, temporal, and frontal lobes. BDA in lt. hemisphere was significantly larger than that in rt. side.
Neuroimagings, especially hypoactive lesions in SPECT show good correlation with NCC and BDA. And functional decline in regions revealing hypoactive in SPECT could precede pathological changes in AD.

728.11

A META-ANALYSIS EVALUATION OF CLINICAL TRIALS IN ALZHEIMER'S PATIENTS TREATED WITH ACETYL-L-CARNITINE. D. Bravi*, G. Koch†, M. Calvani, A. Bacchieri, P. Pola, S. NíBhuachalla, A. Carta. Scientific Affairs Dept., Sigma-Tau Pharmaceuticals, Gaithersburg, MD 20878. †Univ. of North Carolina, Chapel Hill, NC 27599-7400.

Alzheimer's disease (AD) is divided according to DSM-III-R into presenile (onset before age 65) and senile groups. There are many genetic, neurobiological, and clinical data indicating that presenile AD patients may differ from their senile counterparts. Thus, different therapeutical approaches may be considered for early- and late-onset AD patients. Several clinical trials with acetyl-L-carnitine (ALCAR) have been conducted in AD patients in recent years. It seemed appropriate to look retrospectively at the effect of ALCAR in presenile AD patients. Eight placebo-controlled, double-blind, clinical studies with ALCAR in patients diagnosed with AD were pooled in a comprehensive meta-analysis. For each study, cognitive and functional outcome measures for efficacy were evaluated. By using standardized ranks for treatment comparison on cognitive tests, ALCAR-treated early-onset patients tended toward slower progression of symptoms ($p<0.1$) than placebo-treated subjects, whereas using logrank scores the effect of ALCAR achieved significance ($p<0.05$). Functional tests showed a treatment effect favoring ALCAR by using standardized ranks ($p<0.006$). These data suggest that ALCAR may affect the clinical progression of presenile AD.

728.12

LIPOPHILIC VIP ANALOGUES: NOVEL DRUGS FOR TREATMENT OF NEURODEGENERATIVE DISEASES
I. Gozes*, A. Bardea, A. Reshef, R. Zamostiano, S. Zhukovsky, S. Rubinraut, M. Fridkin and D.E. Brenneman. Sackler Med. Sch., Tel Aviv Univ.; Weizmann Inst., Israel; SDMP, NICHD, Bethesda, MD.

Vasoactive intestinal peptide (VIP) has been shown to be involved in promotion of neuronal survival as well as in acquisition of learning and memory. We now demonstrate that a superactive lipophilic VIP agonist inhibited neuronal cell death in an Alzheimer's model in vitro and ameliorated cognitive functions in an in vivo Alzheimer's paradigm. Thus, addition of 25uM of a fragment of the beta-amyloid peptide to rat cerebral cortical cells in vitro resulted in 70% cell death which was attenuated by co-treatment with stearyl-Nle^{17}-VIP. Furthermore, in an in vivo Alzheimer's model of rats treated with the cholinergic blocker AF64A, which induced an impairment in spatial learning, stearyl-Nle^{17}-VIP injected i.c.v or inhaled intranasally alleviated the impairment characteristic of Alzheimer's disease. These data supports neuropeptide intervention as a therapeutic strategy for neurodegenerative diseases.

We thank Dr. H. Leder for the initial sample of AF64A. Supported by Fujimoto Corp. Japan.

DEGENERATIVE DISEASE: PARKINSON'S—HUMAN STUDIES

729.1

USE OF ADVANCED INFORMATION FOR PROGRAMMING A PREHENSION TASK IN PARKINSON'S DISEASE. P. Weiss*, G.E. Stelmach, A. Chaiken and C. Waterman Motor Control Laboratory, Arizona State University, Tempe, AZ 85 287 -0404, USA

The influence of precuing on the generation and modification of motor programs in young and elderly control subjects and 9 PD patients was examined by giving advanced information about the target size before a prehension movement: Subjects were instructed to prepare the prehension movement for the first shown (precued) target and then actually grasp the second shown target, which was of either the same or a different size. While under the high probabilty condition (valid size precue), the preparation of a adequate motor program for the grasp was feasable, the motor program had to be restructured in the low probability condition (invalid size precue).

3D movement trajectories were recorded by an Optotrak®-system at 100 Hz. The kinematics of the wrist movement (transport time (TT), peak velocity (PV) and peak acceleration (PA) amplitudes) were used for the analysis of the reaching component, while the maximum grip aperture (MGA) between thumb and index was utilized for analysing the manipulation component.

Changes in target size led to a combined modification of the manipulation as well as the reaching component in both, the controls and in the patient group, indicating a preserved global motor programming in PD. But for the PD patients motor coordination was impaired indicated by the later occurrence of MGA during the reach. In addition, PD patients failed to use a safety grip margin in comparison to the elderly controls. An invalid target size precue forced the subjects to restructure their motor program reflected by longer reaction times (RT). While controls showed no additional modifications of the reach, PD patients failed to completely restructure the motor program in the prolonged RT under the invalid precue condition revealed by an additional changed reaching component.

PD patients are generally able to adjust their prehension program to the task demands, however, they are disadvantaged in high demanding task because of their reduced rate in restructuring a motor program.

729.2

WHEN ASKED TO SWING THEIR ARMS, PERSONS WITH PARKINSON'S DISEASE WALK BETTER WITH FASTER, LARGER STEPS. WHY?
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Persons with Parkinson's disease (PD) typically walk with small, shuffling steps and little or no arm swing. We hypothesized that persons with PD could improve the temporal and spatial aspects of their gait patterns if asked to walk in specific ways. Five community-dwelling subjects with PD (age 71.8 +/- 4.8 years; 10.2 +/- 6.4 years since diagnosis) and 5 age- and gender-matched subjects without PD (age 70.8 +/- 3.9 years) participated. Subjects were videotaped while walking across a 23' path under various instructional cues. Trials of natural walking were interspersed between four randomized cued conditions: walk while deliberately swinging their arms, counting aloud, taking large steps, or taking fast steps. When asked to swing their arms, 4 of 5 subjects with PD did so. This markedly increased the size of steps and speed of walking. Conversely, when asked to walk with larger steps, arm swing increased substantially (2/5). When asked to walk fast, arm swing increased (2/5). All control subjects when: 1) asked to swing their arms, did so with concomitant increases in speed and in step size; 2) taking large or fast steps, increased arm swing excursion; 3) taking large steps increased the speed of walking; and 4) fast walking increased step size. For both PD and control subjects, counting aloud resulted in inconsistent patterns of change in speed and arm swing. Two theoretical models of motor control, reafferent allied reflexes and dynamical systems, can account for these findings. Variability between subjects with PD and level of disability may further explain these findings. Training with self-instructional cues may be a potential intervention technique to improve walking patterns in persons with PD.

729.3

IMPAIRMENTS IN PARALLEL PROCESSING OF MOTOR RESPONSES DURING SWITCHING TASKS IN ELDERLY SUBJECTS AND PARKINSON'S DISEASE (PD) PATIENTS M Plotnik[1,3], R Inzelberg[1,2], E Schechtman[1], T Flash[1] & A Korczyn[2,3] 1. Dept. of Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel. 2. Dept. of Neurology, Tel - Aviv Sourasky Medical Center. 3. Dept. of Physiology and Pharmacology, Tel - Aviv University, Sackler school of Medicine, Israel.

The objective of the present study is to examine the switching abilities of PD patients and elderly subjects using the double-step target displacement paradigm. The subjects were instructed to move their hands toward a visual target presented on the surface of a horizontal table. This target could remain lit (a control trial) or, unexpectedly change after an inter stimulus interval - ISI, within the reaction time - RT (a switching trial). In the switching trials two types of hand trajectories were observed: double segment movements in which the hand moved first toward the first target and then toward the second target, and movements that were initially directed toward the second target. The second response was more prevalent in PD patients than in control subjects. In both groups, almost no movements had intermediate values of initial movement direction (IMD) in between the first and second target locations. Both control subjects and patients, had prolonged RT to the second stimulus (RT2) compared to the RT to the first stimulus - RT1 ($p < 0.01$). The ratio RT2/RT1 was larger in PD ($p < 0.05$). The values of RT2 obtained in the switching trails were highly compatible with the values expected according to the single channel theory for preparing motor responses for two consecutive stimuli (Welford Q J Exp Psychol 1959; 11: 193-210).

Analyzed with reference to the modification time (definition: D = RT - ISI), the IMDs in the switching trials were directed toward the first target for small values of D, and toward the second target for large values of D. Hence, when grouping together all switching trials, the IMDs were correlated with the values of D (Spearman, $P < 0.01$). However this pattern was not observed when the two classes of responses were separately analyzed . Our results suggest that unlike in neurologically healthy young adults (Van Sonderen et al. Exp Brain Res 1988; 71: 139-146), in elderly subjects and PD patients a gradual shift in the direction of the movement to be initiated does not occur within the preparatory period of the movement. Instead, beyond a certain threshold of D, a sharp change in the IMD is observed. Taken together, our observations can be interpreted as expressing impairments in the abilities of elderly subjects and more prominently PD patients to parallelly prepare two motor responses in the arm trajectory modification task.

729.4

RECRUITMENT OF MOTOR UNITS IN ANTAGONIST MUSCLES IN PARKINSON'S DISEASE. D.S. Glendinning*, E.B. Montgomery Jr., R.M. Enoka. Depts. of Exercise and Sport Sciences and Neurology, Univ. Arizona, Tucson, AZ 85721, and Dept. of Biomedical Engineering, Cleveland Clinic Foundation, Cleveland, OH 44195.

Several authors have reported that the surface electromyogram (SEMG) is diminished during attempted fast movements in persons with Parkinsons disease (PD). We examined MU recruitment in PD to determine if abnormalities could explain the reduced SEMG. Ten persons with PD (40-78 yrs), ten older (51-77 yrs), and ten younger (21-39 yrs) healthy persons volunteered for the study. We measured muscle activity and forces associated with contractions of the first dorsal interosseous (FDI) muscle of the left hand. We also used nerve stimulation to evoke a maximal twitch response in the FDI. Both forces and SEMG signals during maximal voluntary contractions were reduced in the PD group. However, the evoked maximal twitch and M-wave showed no reduction. In all three groups, MU recruitment followed the size principle such that progressively larger MUs, as measured with spike-triggered averaging, were sequentially recruited as the isometric force increased. Threshold derecruitment forces for single MUs were higher than recruitment forces, but a greater discrepancy between these forces was found in the PD group. For all tasks, antagonist activity was increased in the PD and elderly subjects, but was rarely present in the young group. These results suggest that the diminished SEMG signal is not caused by abnormal MU recruitment order in PD, but may be related to inadequate motor neuron activation, increased reciprocal inhibition from coactive muscles, or premature derecruitment of motor units.

Supported by NIH grants AG09000 and NS09145

729.5

POSTURAL ADJUSTMENTS DURING WRIST AND ELBOW MOVEMENTS IN PARKINSON'S DISEASE . A.A. Aruin, M.L. Latash*, I. Neyman, J.J. Nicholas, M.B. Shapiro. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

Patients with Parkinson's disease, age-matched controls, and young control subjects performed fast, discrete elbow or wrist flexion or extension movements in a sagittal plane under the instruction to move one of the joints "as fast as possible". Relative stability in the other, postural joint was comparable in the patients and in both control groups. Typically, EMG patterns in muscle pairs acting at both joints displayed a commonly observed tri-phasic pattern. During both elbow and wrist movements, in both flexion and extension, the elbow flexor and the wrist flexor demonstrated similar, synchronized EMG patterns, while the elbow extensor and the wrist extensor also showed similar patterns of activation. A cross-correlation analysis of the EMGs confirmed virtually simultaneous bursts in the wrist and elbow flexors and in the wrist and elbow extensors. In all three groups, there were no signs of anticipatory activation of postural muscles in about 90% of movements. The analysis of a simple biomechanical model has suggested that simultaneous EMG bursts may be used to minimize the displacement in the postural joint. We assume that postural anticipation is not a separate process, but a separate peripheral pattern of a single control process that may involve a number of joints and muscles. The observed relation between the EMG patterns is apparently a universal synergy which is preserved in elderly and in Parkinson's disease. We conclude that the postural deficits in Parkinson's disease are not related to a basic deficit in feedforward postural control but to other factors that may include the specificity of maintaining the vertical posture.

729.6

Change in movement kinematics of the discrete arm movement by accuracy demand for the Parkinson's subjects. M.K. Rand, J.K. DeWitt, G.E. Stelmach* Motor Control Lab., Arizona State Univ., Tempe, AZ 85287-0404.

Slowness of movement is one of the most common features of the movement disorder for the Parkinson's disease (PD). The purpose of this study was to investigate the reasons of movement slowness for PD by using tasks where the accuracy demand at the end of the movement varied. Ten Parkinson's and ten elderly subjects performed two types of discrete arm movements in the horizontal plane. All movements consisted of a two segment, shoulder horizontal adduction and elbow extension movement. In the accuracy condition (ACC) the subjects attempted to move from the home position to a 3cm x 3cm target 25cm away. In the non-accuracy (NO-ACC) condition the subjects were asked to move through the target, but not to stop when the target was reached. All subjects were instructed to make precise movements as fast and as straight as possible after an auditory go-signal. Ten trials/condition were recorded. Movement time (MT), peak velocity, time to the peak velocity, and deceleration time (DT) were evaluated. The movements in the ACC condition were analyzed from start to end and the movements in the NO-ACC condition were analyzed from start to the target position.

The Parkinson's subjects showed generally longer MTs compared to the elderly subjects. This feature was pronounced in the ACC condition. This was mainly caused by the prolongation of the DT. Slowness of the movement for PD appears to be caused by either the problem in braking the movement or by increased error correction modulation.

Supported by Flinn foundation.

729.7

ALLEVIATION OF MOTOR SIGN OF PARKINSON DISEASE AFTER STIMULATION OF THE INTERNAL SEGMENT OF THE GLOBUS PALLIDUS : EXPERIMENTAL EVIDENCE IN HUMAN Ch. Gross, A. Rougier, T. Boraud, B. Bioulac*, and J. Julien. Groupe Motricité URA CNRS 1200, Services de Neurologie et Neurochirurgie, Centre Hospitalier et Universitaire de Bordeaux, 33076 BORDEAUX Cedex France.

Parkinsonian rigidity and akinesia are associated to an hyperactivity of the internal Globus Pallidus (GPi). Leitinen & other teams have recently reactualised the old Leskell concept of Pallidotomy. Inhibitory effect of High Frequency Stimulation (HFS) of the Subthalamic nucleus has previously been shown in Monkey and Human. In the present work, we have studied the effect of HFS of GPi in five Parkinsonian Patients, with severe unilateral bradykinesia with associated rigidity. Patients between 40 and 60 years old, were at Hoern and Yarn stage 1 to 3 and suffering from Pharmacological resistant form of the disease. Patients were tested with electrokinetic methods, "tapping" test and UPDRS before and after the electrode implantation in 6 differents situation : one week before the operation 1)with and 2)without Dopatherapy, two weeks after the implantation with Dopatherapy, and 3)with and 4)without stimilation; and without Dopatherapy, and 5)with or 6)without Stimulation. Monopolar electrode was stereotactically implanted using ventriculographic Landmarks from stereotactic atlas and MRI. Results showed that for stimulation sites situated at the base of the GPi, the more medial and the more caudal as possible, HFS of GPi may reduce bradykinesia and rigidity. This indicates that HFS efficacy seems to act on similar territories as the Leitinen Pallidotomy without the irreversibility of a lesion.

729.8

SPATIAL LEARNING DEFICITS IN PARKINSONISM R. J. Arcement* D. J. Ingle and L.J. Cote
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Ingle and Thompson (1993) showed that Parkinson Disease (PD) patients of moderate severity performed poorly on a spatial memory task after walking 4 to 8 meters forwards. Recently Arcement, et. al. replicated the post walking deficit in a new group of PD patients. In the present study we measured localization memory linked to arm movement, rather than to locomotion. Here the blindfolded subjects (S) point to a remembered target (T) on the table before them using the tip of a bent wire held by one hand at the other end. All Ss had the opportunity to learn the location of the bent wire's tip in relation to the hand during a two minute period of touching the tip to various table locations. Testing was done with both the bent wire and the index finger. When 14 PD and 13 controls were tested, the PD Ss were not significantly worse than controls in localizing T (by memory) using their index finger, but were significantly worse in learning to point with an artificial extension of the hand. The control errors averaged 73 mm each while the PD patient errors averaged 96 mm each. We speculate that spatial memory linked to locomotion and to arm movement are mediated by the caudate nucleus and the putamen respectively.

729.9

REVERSAL OF CATALEPSY BY STRESS: INVOLVEMENT OF THE MESOCORTICAL BUT NOT THE MESOLIMBIC SYSTEM. L. Hernandez*, S. Tucci, E. Murzi and T. Baptista. Laboratory of Behavioral Physiology, Medical School, Los Andes University, Mérida 5101, Venezuela.

Stress reverses catalepsy in Parkinsonian patients presumably by releasing dopamine. In an experimental model, i.e. haloperidol induced catalepsy which was reversed by forced swiming (FS), DA activity was assessed by microdialysis of the prefrontal cortex (PFC) or the nucleus accumbens (NAC) in rats. For each region (PFC or NAC) one group (HAL+SWIM) received an ip haloperidol injection (5 mg/Kg) and was forced to swim, another group (HAL) received haloperidol but was not forced to swim and a third group (VEH+SWIM) received vehicle and was forced to swim (six groups total). After haloperidol the rats exhibited catalepsy and dopamine and its metabolites increased. FS increased DA activity in the PFC too. However, the increase was significantly greater in the PFC of the HAL+SWIM than in the HAL or the VEH+SWIM groups ($F(2/96)=28.78$, $p<0.001$). No difference of DA activity in the NAC was observed when the HAL+SWIM vs the HAL group were compared ($F(1/112)=3.01$, NS). Another three groups of rats received microinjections of DA in the PFC during haloperidol induced catalepsy. Intracortical DA injections abolished catalepsy ($F(1/11)= 34.9$, $p<0.001$). These experiments suggest that DA release in the PFC contributes to the reversal of catalepsy by stress.

729.10

LEVODOPA-INDUCED BLOOD FLOW RESPONSES IN MIDBRAIN ARE GREATER IN NORMALS THAN IN PARKINSON'S DISEASE PATIENTS. L.W. Tempel*, T.O. Videen, A.S. Snyder, L. Minnich, J.S. Perlmutter. Departments of Neurology and Radiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We measured regional cerebral blood flow (rCBF) with positron emission tomography (PET) and $H_2{}^{15}O$ before and after acute administration of levodopa (in combination with carbidopa) in 12 patients (5 females) with Parkinson's disease (PD), Hoehn and Yahr stages 1 and 2, and 5 age-matched normal women (NL). All subjects were pretreated with 200 mg of carbidopa about 2 hrs prior to PET. Regional CBF was measured prior to levodopa and 75 minutes after taking p.o. levodopa 150 mg with carbidopa 37.5 mg. Images were transformed into standard stereotactic space. We split both the PD and NL groups into 2 subgroups to permit response identification and subsequent confirmation in each group. Levodopa produced discrete foci of activation in the range of 5 to 15 % above normalized mean global flow. In the PD group, there were confirmed responses in bilateral midbrain, right frontal cortex and left putamen. Right putaminal response only reached $p<0.1$. In the NLs, there were confirmed responses only in right midbrain and right frontal cortex. The relatively low n for NLs limited the power of response confirmation. The bilateral midbrain responses in the NLs were significantly greater than in the PD subjects ($p=0.04$ for MANOVA of bilateral midbrain, bilateral putamen and right frontal cortex responses for NL and PD; post hoc t-tests $p=0.05$ for right and left midbrain). There were no other statistically significant differences between NL and PD. We believe that levodopa-induced blood flow responses provide a powerful tool for investigation of functional changes in dopaminergic neurons and their connections.

729.11

SPECT IMAGING WITH [I-123]ß-CIT DEMONSTRATES STRIATAL DOPAMINE TRANSPORTER LOSS IN HEMI-PARKINSONISM. K.L. Marek*, J.P. Seibyl, B. Sandridge, B. Fussell, E.O. Smith, R.M. Baldwin, S. Zoghbi, P.B.Hoffer, R.B. Innis. Depts Neurology, Psychiatry, Diagnostic Radiology, Yale Univ/VA Med Ctr, New Haven CT

[I-123]ß-CIT (2ß-carboxymethoxy-3ß(4-iodophenyl)tropane) labels monoamine transporters located on the terminals of dopaminergic projections from the substantia nigra to the striatum providing a marker for neurons which degenerate in Parkinson's disease (PD). [I-123]ß-CIT SPECT imaging was performed in 8 idiopathic PD patients with exclusively hemi-Parkinsonian symptoms and in 8 age and sex-matched healthy subjects. All patients had very mild symptoms in the effected side (Hoehn and Yahr stage 1) and had not received treatment with L-Dopa. Data was analyzed relative to two outcome measures: ratio of specific:non-specific striatal activity and striatal uptake expressed as percent of injected dose. Disease stage and severity was assessed by Hoehn and Yahr Staging and UPDRS scales. Specific:non-specific striatal binding ratios in PD patients contralateral ($2.7\pm.9$) to the symptomatic side were reduced to 47% and ipsilateral ($3.9\pm.1.1$) to the symptomatic side were reduced to 68% of HS ratios (5.7 ± 1.1). Striatal activity in PD patients expressed as percent injected dose was reduced to (55%) of HS (with no difference in occipital uptake). . The reduction in striatal activity in the PD patients both in the symptomatic and the'effectively pre-symptomatic' striatum suggests imaging of dopamine transporters with [I-123]ß-CIT may be a useful in vivo marker for early diagnosis of PD and possibly for 'pre-symptomatic' mesencephalic dopaminergic degeneration.

729.12

A NOVEL METHOD FOR ASSESSING DOPAMINERGIC NEURONAL LOSS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by a massive degeneration of dopamine-containing neurons in the midbrain. However, the vulnerability of these neurons is heterogenous both across different dopaminergic subgroups and within the substantia nigra pars compacta (SNpc), the most affected structure in this disease. To determine the exact pattern of cell loss, it is necessary to have landmarks independent of the degenerative process to subdivide the SNpc. We have developed a calbindin D_{28k} (CaBP) immunostaining protocol for this purpose.

Sets of closely spaced transverse sections (40µm thick) from the midbrains of three controls and three parkinsonians were processed for CaBP immunohistochemistry. To permit distribution mapping of dopamine-containing neurons in relation to CaBP staining patterns, sets of adjacent sections were stained for tyrosine hydroxylase immunohistochemistry. We found a heterogenous pattern of CaBP immunoreactivity in the ventral midbrain of the controls. CaBP staining was intense in the SNpc and the SN pars reticulata, and immunostaining appeared to be associated with the neuropil rather than with cell bodies in the SNpc. Within the SNpc, there were conspicuous CaBP-poor zones, which had variable shapes, some being long and thin, others being rounded or branched. Analyzed in serial sections, many of the pale zones seen in individual sections were continuous with one another, forming elements of a branched three-dimensional labyrinth. This organization was consistent from one control to another. Some dopaminergic neurons were located within the CaBP-rich zones, but most of them were densely packed together within the CaBP-poor zones. The CaBP-poor compartments thus can be used to delineate subgroups of dopamine-containing neurons within the SNpc.

The CaBP-positive neuropil staining in the ventral midbrain survived in the PD brains. Moreover, although the CaBP-poor zones were shrunken, their three-dimensional organization was similar to that seen in the control midbrains. We conclude that CaBP immunostaining patterns could be used to calculate very precisely the relative loss of dopamine-containing neurons in the different parts of the SNpc.

Supported by NIH Javits Award RO1NS25529 and Lavoisier Grant (French Foreign Office).

729.13

ALTERED AMOUNT OF DA TRANSPORTER (DAT), TYROSINE HYDROXYLASE (TH), AND THEIR mRNAs IN MIDBRAIN OF PARKINSON, ALZHEIMER-PARKINSON AND ALZHEIMER'S

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We have previously shown that Alzheimer's disease (AD) cases with a Parkinson-like syndrome (AD/Park) show significant (50-80%) losses of [3H]mazindol binding to DAT sites in striatum. However, the majority of cases show normal numbers of DAT sites over midbrain neurons. In contrast, with Parkinson's disease (PD) there are 90% losses in striatum and SNpc. We then examined levels of DA-related proteins and expression of their mRNAs in midbrain neurons of a subset of these same cases. The midbrain of six control (age = 66 ± 13 yrs; PMI =13 ± 6 hr), AD (76 ± 11 yrs; 7 ± 4 hr); AD/Park (78 ± 5 yrs; 12 ± 5 hr) and PD (74 ± 8 yrs; 8 ± 6 hr) cases were sectioned at 16 µm and then processed for TH immunoautoradiography, *in situ* for DAT mRNA (Bannon et al, 1992, PNAS 89: 7095) and *in situ* for TH mRNA (O'Malley et al, 1987, Biochem 26:6910). In normals there is significantly higher TH protein, DAT binding sites, and TH/DAT mRNAs in the Paranigral region of the SN (PN) and the ventral tier of the SNpc than in the dorsal tier of the SNpc. In PD the amount of TH was reduced (-88%) in the ventral tier of SNpc, -62% in dorsal tier of SNpc and -35% in PN. TH was normal in the SNpc and PN of AD and was elevated by 25% in SNpc and 17% in PN of AD/Park cases. TH mRNA was significantly reduced in the SNpc of PD and AD/Park groups but normal in AD. DAT mRNA was significantly reduced in SNpc of PD (-92%) and nonsig in AD/Park (-39%) and AD (-35%). PN was normal in PD (-40%), AD (-14%) and AD/Park (-21%). Whether these markers in midbrain of AD/Park reflects compensatory events prior to cell death (as in PD) or largely independent processes (e.g., protein accumulation in cell bodies) needs to be determined. **Funded by MH 43880, AG 09215.**

729.14

PRESYNAPTIC SEROTONERGIC MARKERS IN THE DORSAL RAPHE IN PARKINSON'S DISEASE: RECEPTOR AUTORADIOGRAPHY AND *IN SITU* HYBRIDIZATION STUDIES. F. Valdeoriola[1], R. Cortés[2], G. Mengod[2], F. Cruz-Sánchez[1], J.M. Palacios[2] and E. Tolosa*[1]. [1]Dept. Neurology, Hosp. Clínic i Provincial, Barcelona E-08036, [2]Dept. Neurochemistry, CID-CSIC, Barcelona E-08034, [3]Laboratorios Almirall, Barcelona E-08024, Spain.

There exists neuropathological and biochemical evidence indicating that the serotonergic system is affected in Parkinson's disease. The alterations observed include the presence of Lewy bodies in 5-HT cells in the dorsal raphe, a loss of more than 50% of large neurons in this nucleus, and a decrease in the levels of 5-HT and its metabolite 5-HIAA in the cortex and basal ganglia. In addition, in these structures, the binding of several ^{3}H-ligands to the 5-HT transporter is also reduced. In contrast, recent autoradiographic studies have shown unaltered densities of ^{3}H-citalopram binding in some raphe nuclei. In the present work we have examined the binding to the 5-HT transporter and the 5-HT_{1A} receptor in the dorsal raphe of control and Parkinsonian patients using ^{3}H-citalopram and ^{3}H-8-OH-DPAT, respectively. The binding of both ligands in the dorsal raphe in Parkinson's disease was not significantly different from control levels. This contrasts with the reported decreases in 5-HT transporter binding in forebrain regions and loss of 5-HT cells in the dorsal raphe in this disease. One explanation would be that the remaining neurons overexpress the 5-HT transporter in order to compensate for cell loss. We thus examined the mRNA encoding the 5-HT transporter by *in situ* hybridization using a ^{35}S-labeled oligonucleotide probe. A strong hybridization signal was observed overlaying cell bodies in the dorsal raphe. In the cases examined, the density of autoradiographic grains per cell was higher in Parkinsonian patients compared to normal subjects. These results might reflect an enhanced expression of 5-HT transporter mRNA. The type/s of cells expressing this mRNA remains to be determined.

729.15

CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVITY IN THE BASAL FOREBRAIN: POSSIBLE RELATIONSHIP TO PARKINSON'S DISEASE ASSOCIATED DEMENTIA. C. Charlton, and J. Mack. Department of Pharmacology, Meharry Medical College, Nashville, TN 37208.

Excess brain methylation caused Parkinson's disease (PD)-like changes in rats. Alzheimer's type dementia (ATD) is often seen with PD, therefore, it is of interest to determine if methylation is involved in PD-associated-ATD (PDATD). Since the major pathology of ATD is the degeneration of acetylcholine (Ach) neurons in the basal forebrain (BF), experiments were designed to determine if the icv injection of S-adenosylmethionine (SAM), the methyl donor, affects choline acetyltransferase (ChAT) immunoreactive neurons in the BF. ChAT-immunoreactivity (I) was determined. Brightly labelled ChAT-I neurons, ipsilateral and contralateral to the injection side, were seen in the BF of control rats. The ipsilateral BF of the SAM injection rats showed no ChAT-I, but pale ChAT-I neurons were seen in the contralateral BF. SAM caused PD-like impairments. Methylation increases in aging and produces neurotoxins, thus methyl-ation may serve an antitrophic role, depleting dopamine terminals and cholinergic neurons in the forebrain, which will precipitate PDATD in predisposed individuals. Supported by NIH RRO-3032, RO1-28432 and RO1-31177.

729.16

LACTOTRANSFERRIN AND LACTOTRANSFERRIN RECEPTOR IMMUNOHISTOCHEMISTRY IN THE MESENCEPHALON OF CONTROL SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE.

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Iron levels are increased in the substantia nigra (SN) of patients with Parkinson's disease (PD). Since this metal catalyzes the formation of highly reactive oxygen free radicals which damage biological molecules, its increase may contribute to the cascade of events involved in nigral neuronal death. A dysregulation of intracellular uptake of this metal by neurons and glial cells may lead to its accumulation in SN. Lactotransferrin (LTf), also called lactoferrin and first discovered in human milk, is supposed to play a role in binding and transport of iron, growth factor activity, antibacterial activity and in immunological processes. LTf and LTf receptor (LTfR) immunostaining have been observed using specific rabbit polyclonal antibodies directed either against LTf or LTfR. Fourty μm thick sections of paraformaldehyde / picric acid fixed midbrains obtained at *post mortem* from control subjects and PD patients were studied. LTf- and LTfR-immunoreactivity were present on perikarya, apical dendrites and axons of neurons in periaqueductal gray substance, nuclei of the oculomotor nerve, ventral tegmental area, SN pars compacta (SNpc) and locus ceruleus. Astrocytes, oligodendrocytes and endothelial cells of microvessels were also immunoreactive. In the SNpc, some LTfR-positive neurons were melanized (dopaminergic) and some were not; some dopaminergic neurons of controls showed low immunoreactivity however. Quantitative computer-assisted image analysis is in progress to compare PD patients and control subjects. These results suggest that LTf and LTf receptors may be involved in iron accumulation occuring in the SN of PD patients. *[Research supported by INSERM, CNRS, ACB, and FdF (grant No 91-5716)]*

MENTAL ILLNESS: DEPRESSION, ANXIETY AND SCHIZOPHRENIA

730.1

OLFACTORY BULBECTOMY (OBX) MODEL OF DEPRESSION IN A PRIMATE: BEHAVIORAL EFFECTS IN VERVETS AND REVERSAL BY ELECTROCONVULSIVE TREATMENT (ECT). A. Sattin*, A.S. Kling, R.L. Lloyd, M.J. Raleigh and M.T. McGuire. Psychiatry Service, Sepulveda VA & UCLA, Sepulveda, CA 91343.

OBX rats simulate aspects of chronic Major Depression in humans, eg, appetitive learning deficits, abnormal sleep, reduced weight gain and increased corticosterone in plasma. These are not caused by anosmia and are usually reversed by antidepressant treatments, including ECT. Vervets are old world monkeys, closely related to humans, that maintain complex social relationships. Individual and inter-individual social behaviors of four adult females, living in stable social groups, were quantified by trained observers, blind to hypothesis and treatments. Two subjects were OBX'd and two controls were craniotomized without additional surgery. Standardized behavioral measures selected to characterize patterns of social interactions with other group members were grooming, proximity to others, aggressing, withdrawal, locomotion and eating. All ss's were assessed during four sequential epochs: 1) Pre-operative, 2) Post-operative, 3) Post-six treatments with anesthesia only: ketamine+I.v. nitroglycerine, to induce hypotension, 4) Post-nine ECT's (under ketamine). All treatments were given in early PM on Mon, Wed & Fri. OBX greatly reduced grooming and proximity and greatly increased withdrawal, all of which were normalized by ECT, but not by anesthesia alone. Aggression increased following OBX, decreased after anesthesia, then increased above pre-op levels after ECT, but aggression didn't change in the sham OBX ss's. Eating decreased after OBX, but ECT increased eating over initial levels in all ss's. Locomotion appeared unaffected by any treatment. If confirmed, these observations will increase the validity of the OBX model of depressive and antidepressant mechanisms. Supported by Research Service, US Dept. of Veterans Affairs.

730.2

A RAT MODEL FOR MANIA: RELATION TO BRAIN AMINES

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We previously have described an animal model for bipolar illness patterned after clinical observations of mood-state-related decreases of erythrocyte Na,K-ATPase pump activity in manic and depressed bipolar patients. We duplicated our initial findings by documenting an ouabain dose-related increase in open field locomotor activity over 30 min. following a 5 µl left lateral intracerebroventricular (ICV) injection of ouabain or artificial cerebrospinal fluid in rats 3 days after ICV cannula placement. Open field activity was increased over controls by ouabain 10^{-4}M, $0.5x10^{-3}$M, and reached statistical significance at 10^{-3}M ($p < 0.05$). Since human mania is associated with cerebrospinal fluid changes in concentrations of amines and their metabolites, we examined amine concentrations in the hippocampi of the animals. The brains of the animals were harvested immediately following open field testing, which was 30 minutes after ICV injection. Animals were sacrificed with decapitation following halothane anesthesia. The hippocampi were dissected out over ice and frozen at -80°C until analysis. Following sonication, the tissues were extracted over perchloric acid 0.1N. Using an HPLC with a reversed phase column (C-18) and a dual electrochemical detector, the following amines were quantified: dopamine, dihydroxyphenylacetic acid (DOPAC), norepinephrine, homovalinic acid (HVA), serotonin (5HT), and 5-hydroxyindolacetic acid (5HIAA). There were no differences in any of the amines between any of the experimental groups, or between the right and left hippocampi. This finding is surprising given the tremendous increase of activity observed in the high dose animals, and that the injections were made exclusively into the left ICV. Other brain structures and neurochemicals need to be examined.

730.3

MAGNETIC RESONANCE IMAGING AND BRAINSTEM EVOKED RESPONSE IN GERIATRIC DEPRESSION B. Kalayam, R Bajulaiye, RC Young* and GS Alexopoulos. The New York Hospital - Cornell Medical Center, 21 Bloomingdale Road, White Plains, NY 10605.

Introduction Rate-dependent latency shift in brainstem auditory evoked response (BAER) is reportedly associated with occult brainstem pathology. Latency increases in BAER in elderly patients with late age of onset of major depression was previously reported by our group. Preliminary findings are now reported that corroborates the latency change with findings on brainstem magnetic resonance imaging (MRI) in nine elderly depressives (66 to 77 yrs age; mean $\pm$ SD = 71.7 $\pm$ 4.2 yrs) evaluated with MRI and BAER. Psychiatric diagnosis was established using a structured clinical interview. Proton density axial images of the brainstem were classified using criteria proposed by the Consortium for Establishment of a Registry in Alzheimer's Disease (CERAD) as *normal*, or with probable *punctate*, or *confluent* white matter changes. BAER was performed using stimulus rates of 11.4/sec and 80.4 clicks/second and the latency shift for wave V was examined. Results Two patients with confluent lesions and one patient with punctate lesions of the lower brainstem had latency shift > 0.4 milliseconds. In these cases depression was of the late-onset type. Among the remaining six patients, latency shift was < 0.4 ms; punctate lesions were detected in two and brainstem imaging was normal in four. Depression was of the early-onset type in five of these six cases. Conclusion The above findings need further investigation using a larger sample. In depressed geriatric patients poorer response to antidepressant drug treatment and relapse in the course of illness may be associated with brain morphologic changes. Brainstem imaging and BAER may prove useful prognostic tests in these patients.

(The study is supported by NIMH grant MH 01051).

730.4

REDUCED STIMULATED MONONUCLEAR LEUKOCYTE (MNL) ADENYLYL CYCLASE (AC) ACTIVITY IS ASSOCIATED WITH ELEVATED $G\alpha_s$ AND $G\alpha_i$ LEVELS IN BIPOLAR DISORDER (BD). J. Warsh*, L.T. Young, P.P. Li, A.Kamble and R.T. Joffe, Clarke Institute of Psychiatry, Toronto, Ontario, Canada, M5T 1R8.

We previously reported higher postmortem cerebral cortical levels of the stimulatory G protein α-subunit, α_s, in BD compared with control subjects (Young et al., J. Neurochem. 61:890, 1993). Levels of α_s and the inhibitory G protein α-subunit, α_i, were also increased in MNLs from BD but not major depressive disorder (MDD) subjects (Young et al., Am. J. Psychiat.151:594, 1994). In the present study, the functional consequences of these G protein changes were assessed in MNL membranes from BD and MDD subjects by determining basal, GTPγS- (10μM), NaF-(10 mM) and forskolin (100μM)-stimulated AC activity. Subjects included depressed BD (N=9; age=31.4±8.4 [mean±SD]; 4f,5m; HAM-D=22.7±1.93) and MDD (N=9; age=35.8±6.8; 7f,2m; HAM-D=18.4±0.77) patients compared with age-(35.2±9.5 and 35.5±7.8, respectively) and sex-matched healthy controls. In BD subjects, MNL membrane GTPγS- and NaF-stimulated AC activity, expressed as a percent of basal values, were significantly reduced (GTPγS=31.8%, paired t=2.36,p<0.05; and NaF=18.7%, t=2.4, p<0.05; respectively versus controls), whereas forskolin-stimulated AC activity showed a non-significant reduction (24.1%, t=2.17, 0.1>p>0.05) and basal AC activity was not different (t=0.8, p=0.4) from that in healthy subjects. In comparison, basal MNL membrane AC activity was significantly reduced in MDD subjects (-26%, t=2.7, p=0.027) compared with matched controls but GTPγS-, NaF- and forskolin-stimulated AC activity did not differ. Thus, despite higher MNL α_s levels in BD compared with healthy subjects, G protein-coupled AC activation is reduced in these cells contrasting with the possible enhanced functional state of this signal transduction process found in postmortem BD cerebral cortex (Young et al. J Neurochem. ibid). This difference, however, may be attributed to more effective compensatory adaptations in inhibitory control of AC activity and/or AC levels in MNLs than in cortex of BD subjects. (Supported by grants from the NAMI Stanley Foundation and MRC Canada).

730.5

VOLUMETRIC STUDIES IN LATE LIFE DEPRESSION USING MRI.
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The purpose of the study was to examine the volumes of ventricular, sulcal and whole brain cerebrospinal fluid (CSF) spaces in subjects with late life depression and to compare these indices to healthy age-matched controls and subjects with probable Alzheimer's disease (DAT). Using a 1.5 tesla GE Signa scanner, we studied 24 subjects with late life depression (15 W, 9 M, Mean age 73.7 SD 7.45), 29 healthy controls (17 W, 12 M, Mean age 67.2 SD 8.45) and 34 subjects with DAT (17 W, 17 M, Mean age 66.4 SD 8.56). All depressed subjects met DSM-III-R criteria for major depressive disorder and had Hamilton depression scores of greater than 15 (Mean 19.7 SD 3.84). All subjects were medically stable and free of significant other neuropsychiatric disease. Proton density and T2 weighted images were used to maximize the contrast between brain and CSF. A semiautomated boundary program and segmentation algorithm were used to differentiate brain tissue from CSF. On normalized measures of ventricular and whole brain CSF, depressed subjects had larger volumes in both hemispheres when compared to the control subjects ($p<0.01$), as did the DAT subjects. When depressed and DAT subjects were compared, subjects with DAT had larger whole brain and right hemisphere CSF volumes and total and right hemisphere sulcal CSF volumes ($p<0.05$). These data suggest that widespread structural brain changes underlie late life depression and may be significant in the pathogenesis of mood disorders in late life.

730.6

Calcium dependency of the release of DA and 5HT from CNS slices from animals subjected to models of depression.
E.H. Jaffé*, V. DeFrías, C. Ibarra. Lab. Neurochemistry, IVIC, Apdo. 21827, Caracas 1020-A.

5HT and DA systems have been implicated in the physiopathology of depression. Previously (Neurosci. Lett. 162:157,1993) we showed an increased K stimulated release of 5HT from hippocampus (Hip) and inhibition of the basal release from Hip, n.accumbens (n.acc) and prefrontal cortex (PFC) of animals subjected to forst swim test and chronic isolation. Here we study the Ca dependency of the release of DA and 5HT from these 3 CNS structures. Slices were incubated in a static chamber system. DA and 5HT release measured by HPLC with electrochemical detection. K (30 mM) stimulated DA release was only significantly higher in n.acc of isolated animals and inhibited with Cd (150μM) a Ca channel blocker. 0Ca-10mM Mg inhibited at the same degree release of control and isolated animals. A similar pattern was seen with the K stimulated release of 5HT from n.acc and Hip which were significantly increased in isolated animals, showing a greater inhibition with Cd than controls. Basal DA release was similar in control or isolated animals from the 3 CNS structures with a strong and significant inhibition of the release in 0Ca/Mg Krebs from isolated animals but not from control. A greater sensitivity to Ca blockade of the Ca dependent DA and 5HT release from isolated animals is suggested.

730.7

EVIDENCE FOR INCREASED CONCENTRATIONS OF NEUROPEPTIDE Y IN PLASMA OF PATIENTS WITH PANIC DISORDER.
S. Bouali*, J.P. Boulenger, I. Jerabec, R. Leduc, F. Jolicoeur and A. Cadieux Departments of Psychiatry and Pharmacology, Faculty of Medicine, University of Sherbrook, Sherbrooke, Quebec, Canada J1H 5N4

Recent experimental evidence suggests that neuropeptide Y (NPY) is involved in the modulation of anxiety-related behavior in rodents. Furthermore, NPY's physiological effects are strongly associated with those of noradrenaline, a neurotransmitter involved in the expression of anxiety symptoms in humans both at the central and at the peripheral levels. The aim of the present study was to assess the relationship between NPY plasma concentration and anxiety levels in both normal volunteers (n=13) and patients (n=13) fulfilling the DSM3-R criteria for panic disorders with or without agoraphobia. Plasma levels of NPY-Like immunoreactivity (NPY-Li) were measured in duplicate using a radioimmunoassay developed in our laboratory with detection limits being 7 pg/100 µl. The first results of this study suggest that, compared to normal controls, the baseline NPY-Li levels of panic disorder patients is increased (75 ± 10 pg/ml vs. 130 ± 8 pg/ml respectively). This difference was statistically significant, $p< 0.05$.

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730.8

SODIUM LACTATE INFUSIONS IN AN ANIMAL MODEL OF PANIC DISORDER: FURTHER STUDIES.
A. Shekhar*, S.R. Keim, J.R. Simon and W.J.McBride, Dept. of Psychiatry, Indiana University Medical Center, Indianapolis, Indiana 46202.

$GABA_A$ receptor blockade in the dorsomedial hypothalamus (DMH) of rats elicits a panic-like response. Our preliminary studies have shown that rats that have inhibition of GABA synthesis in the DMH are susceptible to physiological arousal by lactate infusion similar to patients with panic disorder. To characterize this response further, rats equipped with femoral arterial and venous catheters were chronically infused with L-allylglycine (L-AG, active isomer whose metabolite blocks glutamic acid decarboxylase, GAD) or D-allylglycine (D-AG, inactive isomer) via Alzet mini-pumps implanted unilaterally in the DMH. Infusion of 0.5N sodium lactate (10 ml/Kg, i.v.) elicited significant increases in heart rate and blood pressure after 4, 7 or 14 days only in rats with L-AG pumps and not D-AG pumps. Rats with L-AG pumps also showed increased "anxiety" in the plus-maze and social interaction tests as well as decreased GABA content in the DMH when compared to rats with D-AG pumps. These results further strengthen this animal model of panic disorder. (Supported by MH 45362)

730.9

CARDIOVASCULAR, RESPIRATORY AND PARASYMPATHETIC ACTIVITY IN PANIC DISORDER, POST-TRAUMATIC STRESS DISORDER AND NORMAL CONTROLS.
G.N.M. Gurguis*, J.C. Choate, D. Anti-Otong, R. Andrews, F. Petty, and A. J. Rush.
Dallas VA Medical Center and Department of Psychiatry, UT Southwestern Medical School, Dallas, TX 75216.

Although increased autonomic activity is observed in panic disorder (PD) and post-traumatic stress disorder (PTSD), it is unclear if autonomic pathophysiology is similar or different in these disorders. Heart rate (HR), systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), respiratory rate (RR), indices of respiratory sinus arrhythmia (heart period variance, HPV, heart range, HR, and vagal tone, VT) were monitored while supine and for 10 minutes after standing in 19 normal controls (NC), 18 PD and 16 PTSD patients. There were overall trends for higher resting cardiovascular measures in PD and PTSD. PD patients had higher RR (NC: 15.37 ± 2.5 vs PD: 18.52 ± 3.5, $p<.02$) and a trend for a higher anion gap and lower potassium levels than NC or PTSD patients. In response to standing, 3-way repeated measures ANOVA showed: (1) significant elevations in cardiovascular measures and a drop by 15% in VT in all three groups. (2) PD patients had statistically significantly lower SBP, DBP, and MAP, but normal HR, responses than NC and PTSD patients. (3) PTSD had statistically significant group x time interaction in SBP only while other cardiovascular measures fell on a continuum between NC and PD. (4) PD patients, but not PTSD, had statistically significant group x time interactions in HPV and HR which showed a rebound increase 8 minutes into standing. These observations suggest that abnormal respiratory function may be specific to PD and that the profile of abnormal cardiovascular and parasympathetic responses may be different between PD and PTSD.

730.10

PLASMA PROTEIN PATTERN VARIATIONS IN SCHIZOPHRENIA.
A. Brett Larive, Dale M. VanderPutten, Carl R. Merril*. Laboratory of Biochemical Genetics, NIMH, Washington, DC 20032 and Monoclonetics International Inc., Houston, TX 77027.

Plasma protein patterns in 2-D gels from monozygotic twins discordant for schizophrenia were found to be significantly less alike than the protein patterns of normal monozygotic twins and monozygotic twins concordant for schizophrenia. The ELSIE 5 2-D gel analysis program found significantly ($p < 0.01$) fewer protein spot matches between discordant monozygotic schizophrenic twins than either normal monozygotic twins or concordant monozygotic schizophrenic twins. The relative silver stained intensity of matched plasma proteins was also found to vary to a greater extent between monozygotic twins discordant for schizophrenia. Several polypeptide spots were elevated only in the schizophrenic twin. One of these proteins was also found to be significantly ($p < 0.0001$) elevated in the plasma of unrelated schizophrenic individuals ($n = 75$). Additionally, we found differences in plasma haptoglobin levels between monozygotic twins discordant for schizophrenia suggesting variations in liver metabolism.

730.11

MITOGENIC RESPONSE TO BASIC FIBROBLAST GROWTH FACTOR (bFGF) TO EPIDERMAL GROWTH FACTOR (EGF) OF SKIN FIBROBLASTS FROM FIRST-EPISODE PSYCHOTIC PATIENTS. J. Mahadik, N. S. Shendarkar, R. Scheffer, E. E. Correnti, S. Mukherjee*, S. P. Mahadik. Dept. of Psychiatry & Health Behavior, MCG & VAMC, Augusta, GA 30912.

There is increasing evidence that abnormal brain development is involved in schizophrenia. Among several factors, growth factors play critical roles in brain developmental processes: neuronal proliferation, maintenance, survival and maturation. Since molecular mechanisms of their mitogenic actions are common across cell types, cultured skin fibroblasts were used to investigate such mechanisms. The mitogenic responses of bFGF and EGF was examined in fibroblasts from first-episode, drug-naive psychotic patients (N=10) and normal controls (N=10). Known number of fibroblasts were first synchronized by serum starvation for 24 hr. and then growth factors were added in serum containing medium, and growth was continued. The final number of cells was counted on 7th day. Growth response was determined as % change in cell number. As expected, fibroblasts from normals showed increased mitogenic response (mean% ± sem, bFGF=150.1±9.7, EGF=192.7±11.99). However, the mitogenic response was reduced in fibroblasts from patients (bFGF=90.8±4.64, p<.0001; EGF=144.4± 17.74, p<.04). Further studies on underlying receptor-mediated processes will be presented.

730.12

UPTAKE OF ESSENTIAL FATTY ACIDS IN SKIN FIBROBLASTS FROM SCHIZOPHRENIC PATIENTS. N. S. Shendarkar, S. Mukherjee, J. Mahadik and S. P. Mahadik*. Dept. of Psychiatry and Health Behavior, MCG&VAMC, Augusta, GA 30912.

A generalized abnormal plasma membrane phospholipid (PL) metabolism exists in schizophrenia. Abnormality is predominantly in the distribution of esterified polyunsaturated fatty acids (EPUFAs) of essential fatty acids (EFAs: linoleic, LA and linolenic, ALA). The underlying mechanisms are unclear. The cellular uptake of EFAs and conversion to PUFAs are critical for the quantity and quality of PLs. It is difficult to investigate these processes *in vivo* or using easily available tissues or blood cells *in vitro*. Cultured skin fibroblasts are well suited for this. The uptake of EFAs in fibroblasts was compared in three schizophrenic patients with normal controls matched for age, sex and race. Cultures were synchronized by serum starvation for 24 hr. and then grown in serum containing medium for next 24 hr., and fatty acid uptake was determined as pmoles/mg protein for 30 min. The uptake of both the fatty acids was normal in fibroblasts from patients with LA:ALA ratio of 1:1 (means ± SEM for LA, P=139.5±26.54, N=108.83±22.77; for ALA, P=73.4±8.07, N=54.57±8.39) and 20:1 (LA, P=504.37±192.33, N=317.47±119.49 and for ALA, P=31.3± 11.11, N=15.83±5.81). Data indicate that reported lower levels of membrane fatty acids may be a result of defective conversion into EPUFA and incorporation into functional lipids.

730.13

EVALUATION OF NEURAL-IMMUNE ACTIVATION IN CSF OF PATIENTS WITH SCHIZOPHRENIA. G.R. Heninger*, P. Rao, L.Karper, M.P.Heyes and J. Krystal. Dept. of Psychiatry, Yale Univ. and West Haven Veterans Admin Hosp., New Haven, CT 06510 and Lab Clin. Science, NIMH, Bethesda, MD 20892

There is evidence that infectious and/or abnormal neural-immune mechanisms may be involved in schizophrenia. To evaluate this possibility, 6 measures sensitive to neural-immune activation were assessed in the CSF of 21 male patients (mean age: 41) with schizophrenia (SCH) in comparison to 16 healthy controls (HC) (mean age: 35). Quinolinic acid (QUIN), was measured by GCMS, and interlukins (IL1B, IL2, and IL6), tumor necrosis factor alpha (TNF alpha) and beta 2 microgloublin (B2MG) by double antibody ELISA.

Mean QUIN levels in SCH were increased by 24% over those in HC (18.7 vs 15.0 nM/L respectively, P<.04 Chi square). Mean IL6 and B2MG levels did not differ between SCH and HC (2.4 vs. 2.2Pg/ml and 1.3 vs. 1.4 ug/ml respectively). Levels of IL1B, IL2, and TNF alpha did not differ between SCH and HC since 82vs88; 100vs100, and 71vs81% respectively, had levels below the lower detection limits of the assay.

The relatively small 25% increase in QUIN levels in SCH is less than the 200-500% increase seen in early stage HIV infected patients. The data suggest that a strongly active and abnormal neural-immune process is not occurring in the large majority of these chronic SCH patients. Supported by MH25642, the Stanley Foundation and WHVAMC Schizophrenia Center.

730.14

ALTERED CYCLIC AMP RESPONSE TO PROSTAGLANDIN E_1 AFTER PROTEIN KINASE C ACTIVATION IN EBV-TRANSFORMED B-LYMPHOCYTES FROM SCHIZOPHRENICS. N.Natsukari*, R.J.Wyatt, I.Baker, E.F.Torrey, H.Kulaga and J.M.Masserano. Neuropsychiatry Branch, NIMH Neuroscience Center, Washington, D.C.20032.

Cyclic AMP (cAMP) accumulation was evaluated in EBV-transformed human B-lymphocyte cell lines from normal and schizophrenic individuals. Each cell line showed characteristic individual responses to PGE_1, a prostaglandin agonist, isoproterenol (Iso), a β-adrenergic agonist, phorbol 12-myristate-13 acetate (PMA), an activator of protein kinase C (PKC) and staurosporin (STP), an inhibitor of PKC. PGE_1 significantly elevated cAMP accumulation. Iso produced a slight but non-significant increase in cAMP accumulation. Pretreatment of the cells with PMA (10^{-8} M) significantly enhanced cAMP accumulation following treatment with Iso. STP reduced the potentiation of cAMP accumulation produced by PMA plus Iso (1 μM). In contrast, STP enhanced cAMP accumulation following treatment with PGE_1 (10 μM) plus PMA. Cell lines from controls showed a significantly greater cAMP response following treatment with PGE_1 plus PMA compared with the cell lines from schizophrenic patients. These data suggest that a deficit may exist in the PKC and/or PGE_1 response systems in schizophrenic patients.

730.15

FIRST BREAK PSYCHOSIS AND PLASMA HOMOVANILLIC ACID. R. Scheffer, B.I. Diamond*, R.L. Borison, E.E. Correnti, and S. Mukherjee. Dept. of Psychiatry, Eisenhower Army Hosp. and Med. Coll. of GA, Augusta, GA 30912.

Plasma homovanillic acid (pHVA) levels were examined in 17 patients during their first episode of psychosis before initiating neuroleptic treatment and in 8 normal controls. The effect of neuroleptic treatment was assessed after 3 weeks "doctor's choice" treatment. The mean age of patients was 22.88 yrs and the mean duration of psychosis at entry to protocol was 4.44 days (range 2-10). At six months follow-up, 14 patients met DSM-IIIR criteria for schizophrenia. Baseline pHVA (8.68±2.4 ng/ml) and post-treatment pHVA (8.04±2.7 ng/ml) both were significantly lower in patients than in normal controls (14.5±3.6 ng/ml) (P .001 for both comparisons). Change in pHVA levels was not correlated with change in clinical state after neuroleptic treatment. However, lower baseline pHVA levels were associated with less improvement of both positive (r=.54, P=.05) and negative (r=.65, P=.02) symptoms. These findings are consistent with low pHVA in early age of onset postmortem studies showing chronic presynaptic dopaminergic underactivity in early onset patients. In summary, our findings indicate that, in some patients, pHVA is low at the very onset of psychosis and this is associated with a suboptimal early response to neuroleptic treatment.

730.16

QUANTITATION OF NEUROTENSIN AND METABOLITES IN CSF OF PATIENTS WITH SCHIZOPHRENIA. P.D. Butler[1*], D.J. Printz[2], F. Issa[3], J.M. Gorman[2], R.E. Carraway[4]. Psychiatry Service, DVAMC, NY, NY 10010[1], Dept. Clinical Psychobiology, NY State Psychiatric Instit. NY, NY 10032[2], Neuropsychiatry Branch, NIMH, Washington, DC 20032[3], U. Mass. Med. Center, Worcester, MA 01655[4].

Neurotensin (NT), an endogenous neuroleptic tridecapeptide, and its metabolites were quantitated in CSF collected at 8 AM from patients diagnosed with schizophrenia. CSF was placed into enzyme inhibitors on ice and 8-12 ml was subjected to HPLC and RIA using N- and C-terminal antisera. NT added to CSF was recovered in good yield. Direct assay of CSF (n=12) gave more N-terminal (mean ± SEM; 7.1 ± 1.0 fmol/ml) than C-terminal (< 2 fmol/ml) immunoreactivity. Quantitation after HPLC (n=4) gave the following constituents (fmol/ml corrected for 80% yield; mean ± SEM): NT (0.8 ± 0.2); NT^{1-8} (6.8 ± 0.8); NT^{1-11} (1.6 ± 0.2); NT^{1-12} (0.7 ± 0.2). These results indicate that the levels of HPLC-identified NT in patient CSF are 50-200 fold lower than previously reported (Lindstrom et al. Schizophrenia Res, 1:55-59,1988; Garver et al. Am J. Psychiatry, 148:484-488,1991) and that the metabolite, NT^{1-8}, is the predominant form present. Future studies aim to determine whether patients with schizophrenia metabolize NT differently from controls.

730.17

REGIONAL CEREBRAL GLUCOSE USE IN SCHIZOPHRENIC PATIENTS BEFORE AND AFTER HALOPERIDOL WITHDRAWAL. D.R. Medoff*, H.H. Holcomb, G.K. Thaker, R.F. Dannals, C.A. Tamminga. M.P.R.C., University of Maryland, Baltimore, MD 21228.

Chronic use of antipsychotic drugs produces therapeutic effects on psychosis, concurrent side effects, and neurochemical changes in selected transmitter systems. Much is known about the chronic effect of these drugs on D_2 and $GABA_A$ receptors, dopamine (DA) turnover, and DA neuronal activity. However, the integrated functional effect of chronic antipsychotic drug administration in human brain is less well studied, as are the consequences of its withdrawal. We report here results of a within-subject study of regional cerebral glucose metabolism (rCMRglu) with chronic haloperidol (HAL) in three different states: on HAL treatment, after a 5-day withdrawal, and after 30 days in a drug-free state. Differences between the on-HAL state and the drug-free (30 day) state were found in the caudate and putamen (8% and 7% increase respectively), the thalamus (9% increase), frontal cortex (4% decrease) and cingulate (8% decrease). At the five day withdrawal time, based on our previous studies of supersensitivity phenomena in patients, we had predicted rCMRglu findings consistent with supersensitivity. Examples from representative structures are presented in the Table. After five days of withdrawal, no significant changes from the on-HAL scan were apparent in rCMRglu in the brain areas analyzed. These data fail to provide functional metabolic evidence of withdrawal supersensitivity.

	on HAL	5 days off	30 days OFF
R caud	8.1 (2.9)	8.02 (3.19)	7.5 (1.8)
R thal	7.4 (3.2)	7.50 (3.1)	6.5 (2.4)
R STG	7.6 (3.5)	7.0 (2.7)	8.2 (3.4)

mg/100 gm tissue/min, mean (SD)

730.18

EFFECTS OF THE NMDA ANTAGONIST KETAMINE ON CEREBRAL BLOOD FLOW PATTERNS IN SCHIZOPHRENIC PATIENTS. A.C.Lahti*, H.H. Holcomb, M. Zhao, D. Medoff, R.F. Dannals, C.A. Tamminga. MPRC, University of Maryland, Baltimore, MD 21228 and The Johns Hopkins University, Baltimore, MD 21205

To evaluate glutamatergic transmission in the pathophysiology of schizophrenia, we have studied the action of the NMDA antagonist ketamine in symptomatic inpatients. Research protocols were approved by the University of Maryland IRB. Three subanesthetic doses of ketamine (0.1, 0.3 and 0.5 mg/kg) and placebo were given to schizophrenic patients in a double blind injection study. Ketamine worsened mental status 20 minutes after injection in a dose sensitive fashion, selectively on positive psychotic symptoms. Now we have determined regional cerebral blood flow changes in medicated schizophrenic patients given acute ketamine (0.3 mg/kg). Three ^{15}O water cerebral blood flow PET scans were completed prior to drug administration and seven scans, at timed intervals, after ketamine. Data were analyzed using Statistical Parametric Mapping (Friston, 1991). The three baseline scans were combined and compared with the two most proximal post infusion scans from three subjects. The brain regions significantly affected by ketamine were the cingulate cortex and dorsomedial thalamus. Hippocampal activity elevations were also evident in individuals. Ketamine-induced activity changes in the cingulate and thalamus may partially account for its psychotomimetic effects.

NEURO-ONCOLOGY: THERAPY II

731.1

INTRACEREBRAL TRANSFECTION USING CATIONIC SYNTHETIC LIPID: AN ALTERNATIVE TO VIRAL VECTOR IN C6 GLIOMA "SUICIDE GENE " THERAPY. F. Berger*, JM Vicat, M Lainé, D Crapari, H Chen, G Amalfitano, JF Brunet, L Molin, MF Nissou, JM Verna, AL Benabid. INSERM U 318, BP 217X GRENOBLE 38043 Cedex 09 FRANCE.

Therapeutical trials have begun in human gliomas using retroviral transfection. It confronts us with the ethical problem of the use of vector obtained from infectious agents. For these reasons we investigate direct in vivo transfection using the synthetic vector DOTAP.

we used the "suicide gene" Herpes thymidine kinase (TK). It transforms the antiviral gancyclovir in toxic phosphorylated components and induces a bystander effect which explains that no more than 10 to 20 % of the cells need to be transfected.

DOTAP injected in normal rat brain did not induced any histological dammages.In vitro killing of C6 cells transfected with TK and treated with GC was observed. A single stereotaxic TK transfection induced focal necrosis and no more than 50% volume reduction. Then, we used intratumoral cannula providing intratumoral targetting and repeted transfections. In these conditions we observed dramatic tumor regression. When TK was injected outside the tumor we did not observed histological dammages in the brain itself conforting the absence of toxic effect of TK transfection in non dividing cells.

These results suggests that the DOTAP vector is as efficient as retroviral vector in a "suicide gene therapy" for experimental glioma. It confort a phase I study in humans using repeted transfections through a rickham intra-tumoral delivery.

731.2

COMPLETE REGRESSION OF EXPERIMENTAL BRAIN TUMORS TREATED WITH A HERPES SIMPLEX VIRUS (HSV) MUTANT AND GANCICLOVIR. M. Chase , E.J. Boviatsis, Miguel Sena-Esteves, X.O. Breakefield, and E. A. Chiocca* Molecular Neurogenetics and Neurosurgery, Massachusetts General Hosp.-East, Charlestown, MA 02129.

Transfer of the HSV thymidine kinase (TK) gene into tumors has been shown to confer chemosensitivity to ganciclovir. Retrovirus or adenovirus vectors have been used to achieve TK gene transfer to tumors *in vivo*. These vectors cannot spread throughout a tumor mass due to their inability to replicate. To overcome this limitation, we have employed viral vectors, based on HSV, that selectively replicate in tumor cells in the brain based on complementation of the HSV defect by dividing mammalian cells. We stereotactically inoculated an HSV mutant (designated as hrR3, from Dr. S. Weller, U. Conn.), that possesses an intact TK gene, but has a defective ribonucleotide reductase (RR) gene, into 9L gliosarcomas, previously established into the frontal lobes of rats (n = 41). Control rats received an injection of: 1) an HSV mutant (designated as RH105, obtained from Drs. D. Ho and E. Mocarski, Stanford), that possesses a defective TK gene and an intact RR gene, (n = 21) and 2) medium alone (n = 20). Seven days later, half the animals from each group received intraperitoneal injections of saline or ganciclovir for seven days. Ten out of 21 rats from the hrR3 plus ganciclovir group and 4/20 from the hrR3 plus saline group survived more than 90 days after which they were sacrificed. Rats from the other groups did not survive more than 40 days ($p < 0.001$, Kruskall-Wallis one way ANOVA). Histological analysis revealed the occurrence of extensive tumor necrosis during ganciclovir treatment in the hrR3 group. Selective HSV-mediated gene expression could also be assayed in the tumor before and during ganciclovir treatment by staining for β-galactosidase (both hrR3 and RH105 contain a lacZ gene). These findings indicate that HSV mutant vectors that retain an intact TK gene can confer dramatic tumor sensitivity to ganciclovir.

731.3

A NOVEL HERPES SIMPLEX VIRUS "PIGGY BACK" PACKAGING SYSTEM FOR AMPLICON VECTORS. [1]Peter Pechan, [1]E.Antonio Chiocca, [1]Maureen Chase, [2]Richard Thompson, [1]David P.Corey* and [1]Xandra O.Breakefield. [1]Depts. Neurology and Neurosurgery, Massachusetts General Hospital , Harvard Medical School, Boston MA 02114; [2]Dept. Mol. Genetics, University of Cincinnati Medical Center, Cincinnati, OH 45267.

Herpes virus type 1 (HSV-1) vectors have proven to be efficient at transgene delivery to neurons and other cell types in culture and *in vivo*. In the recombinant virus system transgenes are incorporated directly into the HSV-1 genome. In the amplicon system a plasmid bearing the HSV-1 origin of DNA replication and a packaging signal, as well as a transgene, is packaged into virions through the aid of a helper virus. These two systems can be combined by making propagation of the vector and helper viruses mutually dependent on each other. In this study we are characterizing an amplicon "piggy-back" vector system that uses a viral promoter to express a gene essential for HSV-1 replication, propagated with a mutant virus deleted in the same replication-essential gene. We have constructed amplicon vectors bearing the immediate early ICP4 gene under the control of the SV40 and the glial specific JC virus promoter elements. These promoters can be used to regulate expression of both ICP4 and another gene in one amplicon using an IRES translational read-through element. As a helper virus we have used the ICP4 deletion mutant d120 from Dr. Neal DeLuca, Univ.Pittsburgh, propagated on Vero cells or U87 glioma. The presence of helper virus recombinants was analyzed by PCR and Southern blots of Vero plaque lysates. Current data indicate that this novel packaging system has markedly delayed cytopathic effects as compared to wild type virus, and should prove useful for transgene delivery to neurons. This system provides a basic model which can be modified to include cell-specificity of vector propagation and inclusion of multiple transgene elements.

731.4

REGRESSION OF HUMAN NTERA-2 (NT-2) TUMORS IN NUDE MICE USING A REPLICATION-COMPETENT, NEURO-ATTENUATED HERPES SIMPLEX VIRUS (HSV).

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Herpes virus mutants offer a potential therapeutic alternative to conventional treatment modalities for malignant brain tumors. The rationale for this approach is that these genetically mutant viruses replicate preferentially in tumor cells and not within cells of the nervous system, which are generally post-mitotic. Thus, they selectively lyse tumor cells within the CNS. We have used a neuro-attenuated Herpes Simplex Virus type I (strain 1716) for *in vitro* and *in vivo* studies. *In vitro*, HSV-1716 is efficient at lysing human NTera-2 (a teratocarcinoma cell line) cells and two medulloblastoma lines DAOY and D283. *In vivo*, HSV-1716 regresses human NTera-2 brain tumors in nude mice and prolongs their survival relative to mock treated, tumor bearing mice. We documented the pathology over time associated with tumor formation and HSV treatment using Magnetic Resonance Imaging and immunohistochemical localization of HSV and tumor cells. We have also observed that viral replication is limited to the tumor as predicted. Thus, our results suggest that this is a feasible approach for the treatment of malignant brain tumors.

731.5

Long-term Observations of Thymidine Kinase Deficient HSV-1 Infection in the Rat Brain

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We have been using Herpes simplex virus to deliver foreign genes into the nervous system and to treat brain tumors. As a human pathogenic virus, the HSV-1 in its wild type form has been well known to cause clinical symptoms, including severe encephalitis. We have recently demonstrated the effectiveness of the HSV-1 viral vectors in both gene expression and tumor-cell killing (Jia, McDermott, Goldie, Cynader, Tan, and Tufaro., J.Nat.Canc.Inst.,1994 in press) in the present study, we have investigated pathogenic effects of the viral vectors in animals that survived at least 18 months after treatment. Rats were intracerebrally injected with 1 µl (108 pfu) of KOS-SB, a thymidine kinase defective HSV-1 mutant (tk(-) HSV-1). Animals that survived at least 18 months without showing any sign of illness before sacrificed, and brain tissue was sectioned for histological and immunocytochemical observations. Nissl stains showed a significant enlargement of the lateral ventricles in both hemispheres. Otherwise, the histology of other brain regions appeared normal. Immunocytochemistry with a polyclonal antibody for the HSV-1 virus failed to detect any viral antigen in these long-term survived animals while positive immunoreaction could be seen in brains of animals 7 days after viral injection. In these "short- term" animals, cells in the paraventricular regions showed strong reaction to the antibody staining. Monoclonal antibodies against major histocompatibility complex (MHC) antigens were also used to detect possible immune and inflammatory responses to the viral invention. No immunopositive cells were found in brains of the long term rats. Our results suggest that, although intracerebrally injected tk(-) HSV-1 virus caused mild encephalitis, the damage was limited and long term effects were minor. The failure to detect antigens of both the virus and MHC in long term animals further suggests that any damage observed resulted from an acute response rather than a chronic effect.

731.6

ADENOVIRUS-MEDIATED GENE THERAPY FOR EXPERIMENTAL PRIMARY AND METASTATIC BRAIN TUMORS

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The therapeutic efficacy of adenovirus-mediated transduction and ganciclovir (GCV) administration was tested in models of primary and metastatic brain tumors. A replicative-defective recombinant adenovirus vector (ADV-tk) was created that contained the herpes simplex virus thymidine kinase (HSV-tk) gene controlled by the Rous sarcoma virus LTR. HSV-tk phosphorylates GCV which acts as a chain terminator of DNA synthesis and selectively kills dividing cells. Syngeneic tumors were generated by injection of cultured glioma (9L) or breast tumor cells (MAT-B) cells into the rat caudate. Eight days after tumor cell injection, ADV-tk or a control adenovirus carrying the β-gal gene was injected into the tumors. The animals then were treated twice daily for 6 days with GCV or saline. Twenty days after tumor cell injection, the brains were examined microscopically and tumors were measured. Control rats in both models had large tumors. Rats treated with ADV-tk and GCV had no tumors at the primary site. Necrosis with macrophage and lymphocyte infiltration was present. In survival tests, control rats lived only 22 days after 9L cell injection. Treated rats lived 125 days when they were examined and found to have no residual tumors. These results demonstrate that adenovirus-mediated transfer of the HSV-tk gene confers cytotoxic sensitivity to GCV to tumors *in vivo*.

KEY WORD INDEX

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